THE TRANSPLANTATION AND REPLACEMENT OF THORACIC ORGANS

The present status of biological and mechanical replacement of the heart and lungs

Second Edition

Edited by D.K.C. Cooper, L.W. Miller and E.A. Patterson

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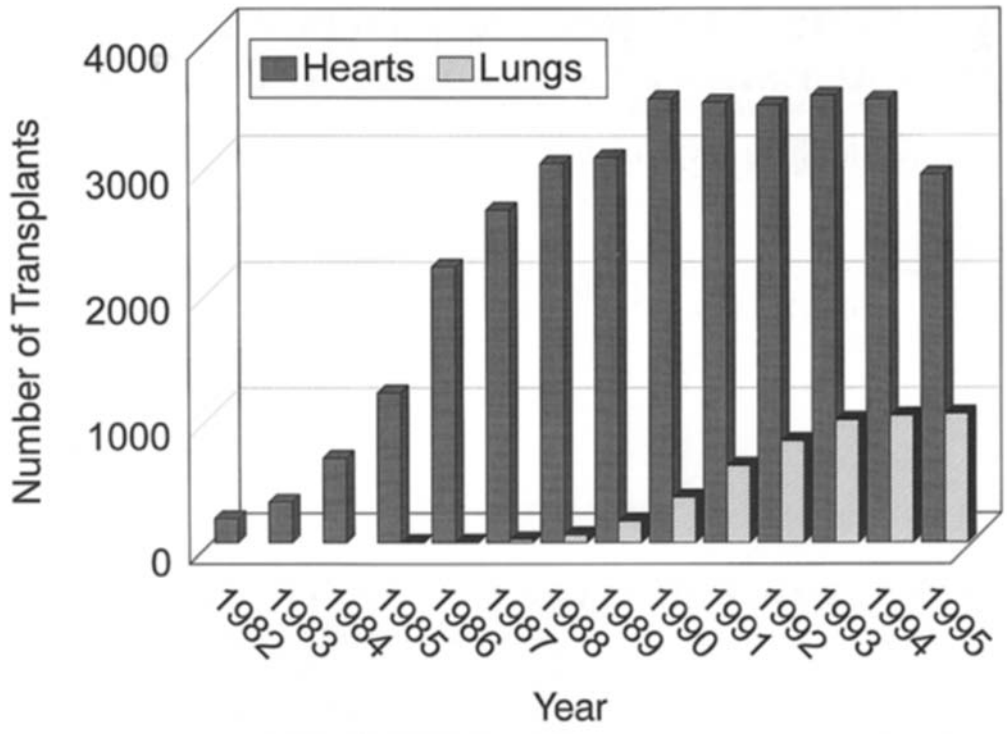
SECOND EDITION



The number of heart and lung (single and double) transplants reported annually to the Registry of the International Society for Heart and Lung Transplantation from 1982 to 1995, inclusive (Courtesy J.D. Hosenpud, MD, Director, ISHLT Registry). The number of heart transplants performed worldwide reached a plateau in 1990 and, indeed, may have declined slightly in 1995 (although the figure for this year is not yet complete). The number of lung transplants performed worldwide has not increased significantly since 1993.

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Second Edition

Edited by

DAVID K.C. COOPER, MA, PhD, MD, MS, FRCS, FACC, FACS,
Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, and
Oklahoma Medical Research Foundation, Oklahoma City, USA;

LESLIE W. MILLER, MD, FACC,
St Louis University Health Sciences Center, St Louis, USA;

and

G. ALEXANDER PATTERSON, MD, FRCS(C), FACS,
Washington University School of Medicine, St Louis, USA

with a Foreword by **Christiaan Barnard**

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Foreword

It is a great pleasure for me to contribute a few words as an introduction to the second edition of this volume, first published in 1990 when it was edited by David Cooper and Dimitri Novitzky. The first edition was, in fact, a greatly expanded version of an even earlier volume *Heart Transplantation*, edited by David Cooper and Robert Lanza and published in 1984. This first work, authored by members of the medical staff of Groote Schuur Hospital and the University of Cape Town Medical School, was, I believe, the first volume reviewing this relatively new field of medicine.

The present volume, therefore, continues the documentation of the development of the transplantation and replacement of intrathoracic organs begun over a decade ago by the editors of the original volume. The pace of advance during the past 10 years has been considerable, as evidenced by the excellent results being achieved by many heart transplant centers and the ever improving results of lung transplantation and the functioning of mechanical cardiac assist devices.

The current editors bring a wealth of expertise and experience to their task, and have blended together absolutely superb contributions by many of the world's experts in their fields. This comprehensive and highly readable volume documents the present 'state of the art' in the field of transplantation and replacement of thoracic organs. It provides an invaluable and unparalleled source of information for those concerned with heart and lung medicine or surgery, and is essential reading for all who wish to keep abreast of developments in this field.

March, 1996

Christiaan N. Barnard
Cape Town, South Africa

Preface

The second edition of this volume reflects the major developments that have taken place in the field of thoracic organ transplantation and replacement since the first edition was published in 1990. With the exception of the 'historical' chapters, every chapter has been extensively rewritten and updated and many new chapters have been added.

In particular, with the rapid growth of single and bilateral lung transplantation, these topics have required considerably more attention and space. In contrast, relatively less space has been devoted to the field of transplantation of the heart and both lungs, where activity has declined significantly in recent years. Similarly, the increasing importance of left ventricular assist devices, and the decreasing clinical activity relating to the total artificial heart, are also reflected in the text.

In a comprehensive work of this nature, some topics are inevitably addressed by more than one author. We make no apology for any such duplication as it almost always relates to topics of great importance, where the opinions of more than one expert are valuable. For example, the subject of infection following thoracic organ transplantation is reviewed by recognized experts in three different chapters which, between them, provide an unparalleled review of this increasingly important field. Several other topics, such as aspects of cardiac allograft vasculopathy and the newer immunosuppressive agents, are also discussed by more than one author, who together provide the reader with comprehensive overviews of these subjects.

Considerable emphasis has been placed on the current experimental and clinical developments taking place in both the transplantation and the mechanical replacement of thoracic organs, thus providing a preview of the clinical advances that are anticipated within the next few years.

In summary, this volume provides by far the most comprehensive review of this subject that has been published to-date. For those entering this field of medicine, this work will provide a sound and extensive basis of information; for those already experienced, we hope it will prove an invaluable source of reference and update for many years to come.

March, 1996

David K.C. Cooper
Oklahoma City, USA
Leslie W. Miller
St. Louis, USA
G. Alexander Patterson
St. Louis, USA

List of Contributing Authors

Anderson, David R., M.A., M.B., B. Chir, F.R.C.S. (Ed).
Division of Cardiothoracic Surgery, Wayne State University School of Medicine, Detroit, Michigan, USA (Currently, Consultant Cardiothoracic Surgeon, Guy's Hospital, London, UK)

Andréassian, Bernard, M.D.
Chief, Thoracic and Vascular Surgery Service, Hôpital Beaujon, Clichy, France

Ardehali, Abbas, M.D.
Chief Resident, Division of Cardiothoracic Surgery, Department of Surgery, UCLA Medical Center, Los Angeles, California, USA

Arkonac, Burak M., M.D.
Postdoctoral Fellow, Department of Cardiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA (Currently, Postdoctoral Fellow, Cardiovascular Biology Laboratory, Harvard School of Public Health, Boston, Massachusetts, USA)

Austin, Lorinda L., R.N., B.S.N.
Worldwide Clinical Manager for Cardiac Assist Venture, Medtronic, Inc., Minneapolis, Minnesota, USA

Aziz, Salim, M.D.
Associate Professor, Division of Cardiothoracic Surgery, University of Washington Medical Center, Seattle, Washington, USA

Barr, Mark L., M.D.
Associate Professor of Surgery, Co-Director, Cardiothoracic Transplantation, and Director, Cardiothoracic Surgical Research, Division of Cardiothoracic Surgery, University of Southern California, Los Angeles, California, USA

Becerra, Eduardo, M.D.
Cardiothoracic Surgeon, Hospital Gustavo Fricke, Viña Del Mar, Chile. Formerly, Senior Registrar, Department of Cardiothoracic Surgery, Groote Schuur Hospital, Cape Town, South Africa

Bolman, R. Morton III, M.D.
C Walton and Richard C Lillehei Professor of Cardiovascular and Thoracic Surgery, Department of Surgery, and Chief, Division of Cardiovascular and Thoracic Surgery, University of Minnesota, Minneapolis, Minnesota, USA

Booth, John V., M.B., Ch.B., F.R.C.A.
Clinical Fellow in Cardiothoracic Anaesthesia, Cardiothoracic Surgical Unit, Papworth Hospital, Papworth Everard, Cambridge, UK

Borovetz, Harvey S., Ph.D.
Professor of Surgery, Biotechnology and Bioengineering Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

Brawner, Nancy J., ACSW.
Clinical Social Worker, Private Practice, Oklahoma City, Oklahoma, USA

Bright, M. Jane, MSW.
Clinical Transplant Social Worker, The Toronto Hospital, Toronto, Ontario, Canada

Chaffin, John S., M.D.
Cardiothoracic Surgeon, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Chen, Jonathan M., M.D.
Postdoctoral Research Scientist, Department of Surgery, Columbia University College of Physicians and Surgeons, New York, New York, USA

Chiu, Ray C-J., M.D., Ph.D., F.R.C.S. (C).
Professor and Chairman, Division of Cardiovascular and Thoracic Surgery, McGill University, The Montreal General Hospital, Montreal, Quebec, Canada

Cinquegrani, Michael P., M.D.
Associate Professor, Co-Director, Cardiac Intervention Program, and Director, Coronary Care Unit, Division of Cardiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Claycomb, William, Ph.D.
Professor of Biochemistry and Molecular Biology, Ochsner Medical Institutions, New Orleans, Louisiana, USA

Collins, Geoffrey M., M.B., M.S., F.R.C.S.
Director of Transplantation, California Pacific Medical Center, San Francisco, California, USA

Cooper, David K.C., M.A., Ph.D., M.D., M.S., F.R.C.S.
Immunologist (Surgery) and Lecturer on Surgery, Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA, and Associate Member, Scientific Staff, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA. Formerly, Cardiothoracic Transplant Surgeon, Director of Research and Education, and Scientist-in-Residence, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Cooper, Joel D., M.D.
Professor of Surgery and Head, Section of Thoracic Surgery, Division of Cardiothoracic Surgery, Washington University School of Medicine, St. Louis, Missouri, USA

Costanzo, Maria Rosa, M.D.
John H. and Margaret V. Krehbiel Professor of Cardiology, and Medical Director, Rush Heart Failure and Cardiac Transplantation Program, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, USA

Couetil, Jean-Paul A., M.D.
Cardiothoracic Surgeon and Director of the Lung Transplantation Program, Department of Cardiovascular Surgery, Hôpital Broussais, Paris, France

Daly, Richard C., M.D.
Assistant Professor of Surgery, Mayo Medical School, and Consultant in Thoracic and Cardiovascular Surgery and Cardiothoracic Transplantation, Mayo Clinic, Rochester, Minnesota, USA

Date, Hiroshi, M.D.
Lung Transplant Fellow, Division of Cardiothoracic Surgery, Washington University School of Medicine, St. Louis, Missouri, USA

Davis, James E., M.D.
Pediatric Cardiothoracic Research Fellow, University of Southern California School of Medicine, Los Angeles, California, USA

Delcarpio, Joseph, Ph.D.
Associate Professor of Anatomy, Oschner Medical Institutions, New Orleans, Louisiana, USA

Demajo, Wilfred A., M.D., F.R.C.A., F.R.C.P. (C).
Assistant Professor of Anaesthesia, The University of Toronto, and Director, Medical/Surgical Intensive Care Unit, The Toronto Hospital, Toronto, Ontario, Canada

Derman, E. Wayne, M.B., Ch.B., B.Sc. (Med) (Hons), Ph.D., F.A.C.S.M.
Senior Lecturer, and Director, Chronic Disease Rehabilitation Program, Sports Science Institute of South Africa, Department of Physiology, University of Cape Town Medical School, Cape Town, South Africa

Derman, Kirsten L., B.Sc. (Med) (Hons), M.Sc. (Med).
Research Assistant, Medical Research Council/University of Cape Town Bioenergetics of Exercise Research Unit, Sports Science Institute of South Africa, Department of Physiology, University of Cape Town Medical School, Cape Town, South Africa

Donahue, Thomas J., M.D.
Associate Professor of Medicine, J.G. Mudd Cardiac Catheterization Laboratory, Division of Cardiology, Department of Internal Medicine, St. Louis University Health Sciences Center, St. Louis, Missouri, USA

Dreyfus, Gilles D., M.D.
Professor of Cardiovascular Surgery, Université Paris V–René Descartes, Hôpital Foch, Suresnes, Paris, France

Drury, John H., M.D.
Clinical Fellow, J.G. Mudd Cardiac Catheterization Laboratory, Division of Cardiology, Department of Internal Medicine, St. Louis University Health Sciences Center, St. Louis, Missouri, USA

du Toit, Ernette D., M.D.
Professor and Head, Provincial Laboratory for Tissue Immunology, University of Cape Town Medical School, Cape Town, South Africa

Edgar, Max M., Ph.D.
Health Science Psychologist, Oklahoma City, Oklahoma, USA

Egan, Thomas M., M.D., M.Sc.
Associate Professor of Surgery, and Director, Lung Transplant Program, Division of Cardiothoracic Surgery, School of Medicine, The University of North Carolina, Chapel Hill, North Carolina, USA

Federspiel, William J., Ph.D.
Assistant Professor of Surgery, Biotechnology and Bioengineering Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

Fishman, Robert, M.D.
Assistant Professor of Medicine, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Stanford University School of Medicine, Palo Alto, California, USA

Frazier, O. Howard, M.D.
Professor of Surgery and Chief, Division of Cardiovascular and Thoracic Surgery, University of Texas – Houston Medical School. Chief, Cardiopulmonary Transplantation, St. Luke's Episcopal Hospital, and Director, Surgical Research, Cullen Cardiovascular Research Laboratories, Texas Heart Institute, Houston, Texas, USA

Frost, Adaani E., M.D.
Associate Professor, Pulmonary Section, Department of Medicine, Baylor College of Medicine, and Medical Director, Pulmonary Transplant Program, Methodist Hospital, Houston, Texas, USA

Fyfe, Alistair I., M.D., Ph.D.
Assistant Professor of Medicine/Cardiology, and Director, Center for Cholesterol and Lipid Management, Division of Cardiology, Department of Medicine, UCLA School of Medicine, Los Angeles, California, USA

Gaissert, Henning A., M.D.
Assistant Professor of Surgery, Brown University, Providence, Rhode Island, USA

Gammie, James S., M.D.
Research Fellow, Division of Cardiothoracic Surgery, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Ghosh, Sunit, M.B., B.S., F.F.A.R.C.S.
Consultant in Cardiothoracic Anaesthesia and Intensive Care, Cardiothoracic Surgical Unit, Papworth Hospital, Papworth Everard, Cambridge, UK

Girgis, Reda E., M.B., B.Ch.
Clinical Assistant Professor of Medicine, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Stanford University School of Medicine, Palo Alto, California, USA

Greer, Kevin A., M.D.
Cardiovascular Research Associate, Division of Cardiothoracic Surgery, Wayne State University School of Medicine, Detroit, Michigan, USA

Haasler, George B., M.D.
Associate Professor, Department of Cardiothoracic Surgery, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Hammond, Elizabeth H., M.D.
Professor of Pathology, University of Utah School of Medicine, and Chairman of Pathology, LDS Hospital, Salt Lake City, Utah, USA

Hardesty, Robert L., M.D.
Professor of Surgery, Division of Thoracic and Cardiovascular Surgery, University of Pittsburgh Medical Center. Presbyterian University Hospital, Pittsburgh, Pennsylvania, USA

Hardy, James D., M.D.
Veterans Administration Distinguished Physician, Professor Emeritus of Surgery, and Formerly, Chairman, Department of Surgery, University of Mississippi Medical Center, Jackson, Mississippi, USA

Hattler, Brach G., M.D., Ph.D.
Professor of Surgery, Division of Thoracic and Cardiovascular Surgery, University of Pittsburgh Medical Center. Presbyterian University Hospital, Pittsburgh, Pennsylvania, USA

Hosenpud, Jeffrey D., M.D.
Professor and Chairman, Division of Cardiology, Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Huddleston, Charles B., M.D.
Assistant Professor of Surgery, and Chief, Pediatric Cardiothoracic Surgery, St. Louis Children's Hospital, Division of Cardiothoracic Surgery, Washington University School of Medicine, St. Louis, Missouri, USA

Imes, Norman K., M.D.
Pulmonologist, Baptist Medical Center, and Co-Director, Lung Transplantation Program, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Jamieson, Stuart W., M.B., B.S., F.R.C.S.
Professor of Surgery, and Head, Division of Cardiothoracic Surgery, University of California San Diego, San Diego, California, USA

Kanoski, Marilyn M., M.S., R.D., L.D.
Clinical Dietitian, Transplant and Critical Care, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Kaplan, Jorge, M.D.
Cardiothoracic Surgeon, Hospital Gustavo Fricke, Viña del Mar, Chile

Keogh, Anne M., M.D., F.R.A.C.P.
Cardiologist in Cardiac Transplantation, St. Vincent's Hospital, and Senior Lecturer, University of New South Wales, Sydney, New South Wales, Australia

Keshavjee, Shaf H., M.D., M.Sc., F.R.C.S. (C).
Assistant Professor of Surgery, Division of Thoracic Surgery, and Director, Thoracic Surgery Research Laboratory, University of Toronto, The Toronto Hospital, Toronto, Ontario, Canada

Klepetko, Walter, M.D.
Professor of Surgery, Department of Cardiothoracic Surgery, University of Vienna, Vienna, Austria

Kobashigawa, Jon A. M.D.
Medical Director, University of California Los Angeles Heart Transplant Program, and Associate Clinical Professor of Medicine (Cardiology), Department of Medicine, UCLA School of Medicine, Los Angeles, California, USA

Kolff, Willem J., M.D., Ph.D.
Distinguished Professor of Medicine and Surgery, Department of Surgery, Division of Artificial Organs, University of Utah, Salt Lake City, Utah, USA

Kriett, Jolene M, M.D.
Associate Professor of Surgery, Division of Cardiothoracic Surgery, University of California San Diego, San Diego, California, USA

Kshettry, Vibhu R., M.D., F.R.C.S.
Assistant Professor, and Co-Director, Thoracic Organ Transplant Program, Division of Cardiovascular and Thoracic Surgery, University of Minnesota, Minneapolis, Minnesota, USA

Laks, Hillel, M.D.
Professor and Chief, Division of Cardiothoracic Surgery, and Director, Heart and Lung Transplantation Program, Department of Surgery, UCLA School of Medicine, Los Angeles, California, USA

Lidsky, Nathan M., M.D.
Chief, Transplant Intensive Care Unit, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Love, Kathryn, M.D., B.M.S., F.R.C.P. (C).
Clinical Associate Professor, Department of Medicine, University of Minnesota, and Section Head of Infectious Diseases, Abbott-Northwestern Unit for Teaching and Research, Abbott-Northwestern Hospital, Minneapolis, Minnesota, USA

Magovern, George J., Sr., M.D.
Professor of Surgery, Division of Thoracic Surgery, Allegheny-Singer Research Institute, Department of Surgery, Allegheny General Hospital, The Medical College of Pennsylvania and Hahnemann University, Pittsburgh, Pennsylvania, USA

Magovern, James A., M.D.
Associate Professor of Surgery, Division of Thoracic Surgery, Allegheny General Hospital, The Medical College of Pennsylvania and Hahnemann University, Pittsburgh, Pennsylvania, USA

Maurer, Janet R., M.D., F.R.C.P. (C).
Head, Section of Lung Transplantation, Department of Pulmonary and Critical Care Medicine, The Cleveland Clinic Foundation, Cleveland, Ohio, USA

McGregor, Christopher G.A., M.B., B.S., F.R.C.S. (Ed and Glas).
Director of Cardiothoracic Transplantation, Section of Thoracic and Cardiovascular Surgery, Mayo Clinic, and Professor of Surgery, Mayo Medical School, Rochester, Minnesota, USA

Michler, Robert E., M.D.
Director, Cardiac Transplant Program and Director, Cardiac Research Laboratory, Division of Cardiothoracic Surgery, Columbia University College of Physicians and Surgeons, New York, New York, USA

Miller, Leslie W., M.D.
Professor of Medicine and Surgery, and Director, Heart Failure/Heart Transplantation Program, Department of Internal Medicine/Division of Cardiology, St. Louis University Health Sciences Center, St. Louis, Missouri, USA

Mischke, Larry, R.N.
Cardiothoracic Transplant Coordinator, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Mott, Brian D., M.D.
Research Fellow, Department of Surgery, McGill University, The Montreal General Hospital, Montreal, Quebec, Canada

Myers, Timothy J., B.S., C.R.A., R.C.P.T.
Coordinator, Clinical Research, Cullen Cardiovascular Research Laboratories, Texas Heart Institute, Houston, Texas, USA

Nash, Eleanor S., M.B., Ch.B., F.R.C.P. (Ed), D.P.M., F.F. Psych. (SA).
Formerly, Associate Professor, Department of Psychiatry, University of Cape Town Medical School, Groote Schuur Hospital, Cape Town, South Africa

Nelson, David P., M.D.
Transplant Physician, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Noakes, Timothy D., M.D., F.A.C.S.M.
Professor and Director, Medical Research Council/University of Cape Town Bioenergetics of Exercise Research Unit, Sports Science Institute of South Africa, Department of Physiology, University of Cape Town Medical School, Cape Town, South Africa

Novick, Richard J., M.D., M.Sc., F.R.C.S. (C).
Associate Professor of Surgery, Division of Cardiovascular and Thoracic Surgery, University of Western Ontario, and Consultant Cardiothoracic Transplant Surgeon, London Health Sciences Centre, University Campus, London, Ontario, Canada

Ohori, N. Paul, M.D.
Assistant Professor of Pathology, Department of Pathology, Montefiore University Hospital, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

Opelz, Gerhard, M.D.
Director, Department of Transplantation Immunology, Institute of Immunology, University of Heidelberg, Heidelberg, Germany

Oudshoorn, Machteld, Ph.D.
Head, Clinical Cellular Immunology Laboratory, Department of Immunohematology and Blood Bank, and Director of the Europdonor Foundation, Leiden University Hospital, Leiden, The Netherlands

Pae, Walter E., Jr., M.D.
Associate Professor of Surgery, Division of Cardiothoracic Surgery, Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA

Panos, Anthony L., M.D., M.Sc., F.R.C.S. (C).
Assistant Professor, Division of Cardiothoracic Surgery, University of Washington Medical Center, Seattle, Washington, USA

Paradis, Irvin L., M.D.
Physician-in-Charge, Lung Transplantation, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA. Formerly, Medical Director, Lung Transplantation, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania, USA

Paris, Wayne D., M.S.W.
Clinical Transplant Social Worker, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Patterson, G. Alexander, M.D., F.R.C.S. (C).
Professor of Surgery, Section of Thoracic Surgery, Barnes Hospital, Washington University Medical Center, St. Louis, Missouri, USA

Penido, Marcia L., L.C.S.W.
Clinical Transplant Social Worker, Cedars-Sinai Medical Center, Los Angeles, California, USA

Penn, Israel, M.D.
Professor of Surgery, Department of Surgery, University of Cincinnati Medical Center and Cincinnati Veterans Administration Medical Center, Cincinnati, Ohio, USA

Pham, Si M., M.D.
Assistant Professor of Surgery, and Director of the Adult Heart Transplant Service, Division of Cardiothoracic Surgery, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Pierson, Richard N., III, M.D.
Assistant Professor of Cardiothoracic Surgery, and Director, Heart and Lung Transplantation, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Pohl, Mary, R.N., B.S.N.
Clinical Trials Nurse Coordinator, Division of Cardiothoracic Surgery, Washington University School of Medicine, St. Louis, Missouri, USA

Portnoy, Vladimir F., M.D., Ph.D., Sc.D.
Scientist, Transplantation Research, California Pacific Medical Center, San Francisco, California, USA. Formerly, Professor of Surgery and Head, Department of Experimental Surgery, Academy of Medical Science Vishnevsky Institute of Surgery, Moscow, USSR

Reddy, Ramchandra C., M.D.
Resident, Division of Thoracic Surgery, Allegheny General Hospital, Pittsburgh, Pennsylvania, USA

Reeder, Gary D., B.S.
Adjunct Assistant Research Professor, Biotechnology and Bioengineering Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

Renlund, Dale G., M.D.
Professor of Medicine, Division of Cardiology, University of Utah Health Sciences Center, and Director, the Utah Transplantation Affiliated Hospitals Cardiac Transplant Program, Salt Lake City, Utah, USA

Robertson, Cina M., A.C.S.W.
Clinical Transplant Social Worker, Barnes Hospital, St. Louis, Missouri, USA

Rose, Alan G., M.D., M. Med. (Path)., F.R.C. Path.
Director, Jesse E. Edwards Registry of Cardiovascular Diseases, St. Paul, Minnesota, USA. Formerly, Wernher and Beit Professor and Head, Department of Pathology, University of Cape Town Medical School and Groote Schuur Hospital, Cape Town, South Africa

Rubin, Robert H., M.D.
Chief of Transplant Infectious Disease, Department of Medicine, Massachusetts General Hospital, Director, Center of Experimental Pharmacology and Therapeutics, Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, and Osborne Chair in Health Sciences and Technology, Boston, Massachusetts, USA

Salomon, Daniel R., M.D.
Director of Transplantation Research, Division of Organ Transplantation, The Scripps Clinic and Research Foundation, and Assistant Member, Department of Molecular and Experimental Medicine, Department of Immunology, The Scripps Research Institute, La Jolla, California, USA

Sanbar, S. Sandy, M.D., Ph.D., J.D., F.C.L.M.
Cardiologist, Baptist Medical Center, Oklahoma City, Oklahoma, USA and Past President, American College of Legal Medicine

Sapirstein, John S., M.D.
Research Fellow, Division of Cardiothoracic Surgery, Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA (Currently, Resident, Department of Surgery, University of Chicago, Chicago, Illinois, USA)

Sawzik, Patricia, Ph.D.
Adjunct Research Professor, Biotechnology and Bioengineering Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

Sayegh, Mohamed H., M.D.
Assistant Professor of Medicine, Harvard Medical School, Department of Immunogenetics and Transplantation, Renal Division, Brigham and Women's Hospital, Boston, Massachusetts, USA

Schäfers, Hans-Joachim, M.D.
Professor of Surgery, Division of Thoracic and Cardiovascular Surgery, Surgical University Clinic of the Saarland, Homburg, Saar, Germany

Shennib, Hani, M.D., F.R.C.S. (C).
Director, Montreal Lung Transplantation Program, and Associate Professor of Surgery, Centre of Immunobiology and Transplantation, McGill University, Montreal, Quebec, Canada

Skotzko, Christine E., M.D.
Assistant Professor, Department of Psychiatry, University of Maryland at Baltimore, Baltimore, Maryland, USA

Smart, Frank W., M.D.
Director of Medical Transplant Services, and Co-Director of Heart Failure and Cardiac Transplantation, Ochsner Multi-Organ Transplant Center, Ochsner Medical Institutions, New Orleans, Louisiana, USA

Smith, Douglas M., M.D., Ph.D.
Director, HLA Laboratory, Department of Pathology, Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma, USA

Speziali, Giovanni, M.D.
Fellow, Thoracic and Cardiovascular Surgery, Mayo Clinic, Rochester, Minnesota, USA

Starnes, Vaughn A., M.D.
Professor and Chief, Division of Cardiothoracic Surgery, and Director, Lung Transplant Program, Children's Hospital, University of Southern California, Los Angeles, California, USA

Stephenson, Larry W., M.D.
Ford-Webber Professor of Surgery, and Chief, Division of Cardiothoracic Surgery, Wayne State University School of Medicine, and Chief, Cardiothoracic Surgery, Harper Hospital and Detroit Medical Center, Detroit, Michigan, USA

Stevenson, Lynne Warner, M.D.
Associate Professor of Medicine, Harvard Medical School, Cardiovascular Division, Brigham and Women's Hospital, Boston, Massachusetts, USA

Stitt, Larry, M.Sc.
Assistant Director, Biostatistical Support Unit, Department of Epidemiology and Biostatistics, University of Western Ontario, London, Ontario, Canada

Taniguchi, Shigeki, M.D.
Cardiothoracic Transplant Clinical and Research Fellow, Oklahoma Transplantation Institute, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Taylor, David O., M.D.
Assistant Professor of Medicine, Division of Cardiology, University of Utah Health Sciences Center, Salt Lake City, Utah, USA

Thaler, Scott J., M.D.
Associate Physician, Infectious Disease Division, Brigham and Women's Hospital, and Instructor in Medicine, Harvard Medical School, Boston, Massachusetts, USA

Theodore, James, M.D.
Pulmonologist and Associate Professor of Medicine, and Medical Director, Heart–Lung and Lung Transplant Program, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Stanford University Medical Center, Palo Alto, California, USA

Thompson, Sharon E., L.S.W.
Clinical Transplant Social Worker, Shands Hospital, Gainesville, Florida, USA

Todd, Thomas R., M.D., F.R.C.S. (C).
Professor and Chairman, Division of Thoracic Surgery, University of Toronto, The Toronto General Hospital, Toronto, Ontario, Canada

Trehan, Sanjeev, M.D.
Fellow, Division of Cardiology, University of Utah Health Sciences Center, Salt Lake City, Utah, USA

Tschernko, Edda, M.D.
Department of Cardiothoracic Anesthesiology, University of Vienna, Vienna, Austria

Tyndall, Kirsten L., Pharm. D.
Clinical Specialist Pharmacist, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Van Meter, Clifford, M.D.
Cardiothoracic Surgeon, and Co-Director of Cardiac Transplantation, Oschner Medical Institutions, New Orleans, Louisiana, USA

Wanke, Theodor, M.D.
Assistant Professor of Pulmonology, Lainz Hospital, Vienna, Austria

Wagner, Florian M., M.D.
Clinical Fellow, Montreal Lung Transplantation Program, McGill University, Montreal, Quebec, Canada

Warner, Ernest G., M.D.
Neurologist, Baptist Medical Center, Oklahoma City, Oklahoma, USA

Wheeldon, Derek R., M.I.Biol.
Senior Research Technician, The Transplant Unit, Papworth Hospital, Papworth Everard, Cambridge, UK

Wicomb, Winston N., Ph.D.
Director, Organ Preservation, Transplantation Research Institute, California Pacific Medical Center, San Francisco, California, USA

Wisser, Wilfried, M.D.
Surgeon, Department of Cardiothoracic Surgery, University of Vienna, Vienna, Austria

Wolford, Thomas L., M.D.
Assistant Professor, J.G. Mudd Cardiac Catheterization Laboratory, Division of Cardiology, Department of Internal Medicine, St. Louis University Health Sciences Center, St. Louis, Missouri, USA

Young, James B., M.D.
Chief, Section of Heart Failure and Cardiac Transplant Medicine, The Cleveland Clinic Foundation, Cleveland, Ohio, USA

Yousem, Samuel A., M.D.
Chief Pathologist and Director of Anatomic Pathology, Montefiore University Hospital, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

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David K.C. Cooper
Oklahoma City, USA
Leslie W. Miller
St. Louis, USA
G. Alexander Patterson
St. Louis, USA

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1
The Concept and Diagnosis of Brain Death

E.G. WARNER

INTRODUCTION

The concept of brain death as opposed to clinical death was first formulated by Mollaret and Goulon in 1959[1]. At the onset this new concept of death produced a considerable amount of discussion, some of it acrimonious, in medical circles. The public was also interested in this new concept of death as distinguished from what it had previously understood.

Up until this time the recognized criteria for diagnosis of clinical death were cessation of respiration and cessation of heartbeat, both usually occurring in close proximity to each other. Thus a new concept, the concept of brain death, when it was first promulgated, was rather revolutionary.

The reason the new concept was essential is because of the effectiveness of resuscitation which is now available after an acute injury or acute organ failure. This has produced a large number of patients who are mechanically ventilated with circulatory parameters stabilized artificially, but without the possibility of recovery of cerebral function. This then is the diagnosis of brain death as opposed to clinical death by the older cardiovascular standards.

This new concept of brain death now signifies the death of the individual, and is accepted throughout most of the world, although there are a few countries which do not recognize this new concept.

From the transplantation standpoint most of the organ donations are from patients who are brain dead. The care of this type of patient has not only ethical importance, but also legal importance. It is the accurate diagnosis and timing of the occurrence of brain death that is essential, not only in procuring organs but in order to continue appropriate perfusion and other techniques necessary to maintain the organs in the best state possible for transplantation. It is not the task of the neurosurgeon or any medical practitioner to provide organs for donation. However, neither should any doctor, on the basis of insufficient evidence or personal prejudice, deny patients in need of transplant surgery their chance for a cure[2,3].

TOTAL BRAIN DEATH VERSUS BRAINSTEM DEATH

At the onset it should be noted that there is a difference in distinguishing between total brain death, which implies irreversible cessation of function of the entire brain, including the brainstem, and failure of the brainstem itself. The importance of this difference varies by geography. In the United States the concept of total brain death is defined as irreversible loss of function of the entire brain including the brainstem. In some other countries the concept of brainstem death is rendered equivalent to brain death.

The etiological factors which produce irreversible brain death are several; these include: (a) supratentorial lesions such as subdural or intracerebral hematomas, subarachnoid hemorrhage, cerebral infarctions, tumors or abscesses; (b) infratentorial masses which directly damage the brainstem, such as brainstem or cerebellar hemorrhage, brainstem infarction, or tumor; (3) hypoxic encephalopathy of a high degree, usually occurring after prolonged asphyxia. Other metabolic factors, such as severe toxic insults or in the setting of severe hepatic necrosis, are not uncommon.

The parameters necessary to diagnose brain death will now be described. These are the descriptions that would be appropriate to clinical evaluation in the United States. In other some countries the use of only brainstem criteria may be adequate.

The end-result of major trauma, whether physical or chemical, which leads to progressive brain swelling, is herniation of the hippocampal gyri with lateral pressure on the brainstem. This eventuates in the loss of brainstem function. Any expanding lesion will increase intracranial pressure to equal systemic arterial pressure, at which point there will be complete arrest of cerebral circulation. Cerebral circulatory arrest can therefore be confidently used as a criterion of irreversible cerebral injury. Functional disintegration, following the conditions described above, leads to cessation of spontaneous respiration. This results in turn in hypoxic cardiac arrest. If gaseous exchange and circulation are maintained artificially, the heart, kidneys and liver may continue to function for some hours or days. However, after brainstem death has occurred, cardiac arrest will usually follow reliably within 1–2 weeks[4].

CONFOUNDING FACTORS IN THE DIAGNOSIS OF BRAIN DEATH

There are a number of factors which may confuse a diagnosis of brain death; these are the following, and must be excluded to

make a proper diagnosis of brain death: (a) hypothermia – this becomes operative, however, only when rectal temperatures are below 32°C; (b) drug intoxication; (c) factors producing severe electrolyte or endocrine disturbances; (d) identification of a structural lesion that might be amenable to surgical treatment, by appropriate imaging techniques; (e) the effect of neuromuscular blocking agents – if these agents have been used, enough time must be given so that they will have dissipated from the blood. To emphasize again, the exact diagnosis (or as close to being exact as possible) must be made as to the etiology of the comatose state.

ESTABLISHING THE DIAGNOSIS OF BRAIN DEATH

There are therefore two major areas of testing which are required to establish the status of brain death. The first is that of coma – total unresponsiveness to any stimuli. The second is the absence of brainstem reflexes, including apnea.

(1) *Coma* – the patient must be totally unresponsive to painful stimuli. This can be tested by supraorbital pressure, sternal pressure, nailbed pressure, or other appropriate means.
(2) *Brainstem death* – measured by absence of brainstem reflexes:
   (a) Absence of pupillary response to light – there must be an adequate level of illumination to make it a valid test. One must exclude previous pupillary abnormalities and/or topical drugs that may be applied to the eyes.
   (b) Absence of corneal reflex – tested by taking a moist piece of cotton or paper tissue and touching the cornea. This should produce a direct response in the eye being tested. It should also produce a consensual response in the opposite eye.
   (c) No oculovestibular reflexes. Irrigation of each ear individually with cold water, using an adequate amount (which is usually defined as 50–60 ml). The patient is allowed to rest for several minutes after the injection. This is then repeated on the opposite side. The head should be elevated 30° during the irrigation on each side. A positive response is deviation of the tonic variety toward the cold stimulation, signifying an intact brainstem. With brainstem death there is no deviation of the eyes with stimulation.
   (d) Absence of oculocephalic reflex: this is absent 'doll's eyes', performed by rapid turning of the head from the neutral or forward position to 81–90° rotation on each side. A similar test may be made with vertical movements of the head. The eye movement is to the opposite side from the deviation of the head. Then, as the movement of the eyes lags behind the head movement, they rapidly adjust to their new position.
   (e) Gag reflex absent after stimulation of the pharynx.
   (f) No cough reflex – this can be determined with bronchial suction.
   (g) Absence of respiratory activity (demonstration of apnea – see below.)

Respiration is driven from a center in the brainstem. If the above brainstem tests are all negative (that is absent), the next procedure to be performed is a test for apnea. Loss of brainstem function produces apnea as well as hypotension.

A large number of factors influence respiratory drive, including P_{CO_2}, pH, etc. The recommendations that will follow are based on the concept of reaching a Pa_{CO_2} level >60 mmHg for maximum drive or stimulation of the respiratory center in the brainstem. Lower levels may be sufficient, but at 60 mmHg there is no question that the respiratory drive should be definitely present if the respiratory center can respond to the hypercarbia. Caution must be used in interpreting these values in patients who have severe COPD or other causes for chronic hypercarbia. In these instances, greater reliance should be placed on other confirmatory tests. Because hypotension may result during the procedure, it is essential that the patient have as normal a blood volume as possible.

One should preoxygenate the patient to a Pa_{O_2} of approximately 200 mmHg. The arterial P_{CO_2} should be within normal limits. Then disconnect the ventilator. Be sure that a diffuse type of oxygenation can take place by giving 6 l/min oxygen via the trachea or placing a fine cannula at the level of the carina. Disconnect the respirator. Generally, if one starts with a P_{CO_2} of 40 mmHg, the value will gradually increase after 6–8 min to a level of approximately 60 mmHg. It will rise probably 3–6 ml/min, but is variable depending on the individual patient. Observe the patient for any respiratory movements that would produce an adequate tidal volume.

At the 8-min interval, check for pH, P_{CO_2} and P_{O_2}. The object here is to be sure that the P_{CO_2} is above 60 mmHg. If no respiratory movements are seen at this level, that is a P_{CO_2} >60 mmHg, then assume that the respiratory drive is adequate and the response of the brainstem is negative. This fits with the diagnosis of brain death. If, however, respiratory movements are observed, then there is still present a response to this level of P_{CO_2}, and the brainstem is not dead. In the event that the patient becomes abruptly hypotensive or significant cardiac arrhythmia develops, the respirator should be restarted promptly and the volume adjusted until the O_2 saturation is >90% (or arterial P_{O_2} >90 mmHg).

To summarize, irreversible apnea is part of the diagnosis of clinical total brain death, particularly involving the responsiveness of the brainstem. Respiration must be absent with a P_{CO_2} of 60 mmHg.

There are other protocols which may be used to ensure adequate respiratory drive to the brainstem occurring in the presence of adequate peripheral oxygenation. At this point there is no agreement on the exact protocol that should be followed in regard to this issue.

COMMENT

Many problems are seen in these critically ill patients in the intensive-care unit (ICU). Particularly troublesome are those patients who have severe facial and head injuries, such that examination of cranial nerves is difficult, if not at times virtually impossible. The presence of confounding drugs, e.g. sedatives, etc., can be evaluated from a thorough history and toxicological analysis. Often these problems, as well as the etiology of the coma, will be clarified with continued observation and laboratory testing[5].

Not infrequently one may have the 'Lazarus reaction' – that is, spinal reflex activity – particularly during apnea testing. If this reaction occurs it may suggest to the observer purposeful movements. Hence, it is our policy to exclude the family from the agonal period so that these reflex actions are not considered as evidence of an error in the diagnosis of brain death. These patients may sit up in bed, swing their arms in front of them, move one limb in a jerking-type action, or even make pseudowalking movements with their legs. Some of these movements may involve chest muscles, but they do not produce adequate movement to enable a respiratory exchange with significant tidal volume.

Since deep tendon reflexes are of spinal origin they may remain present, although they are usually absent; the same applies to Babinski signs.

CONFIRMATORY TESTS

In this particular area there is considerable disagreement between physicians in the USA and those in other parts of the world, particularly in Britain. In the USA the criteria for total brain death include a total lack of function of the entire brain – not only the brainstem but the entire brain. In the UK, however, the argument is made that if one has no evidence of activity in the brainstem, for practical purposes the patient is truly 'brain dead'. Semantically there is, of course, considerable difference here, as the total brain is not necessarily dead when only the brainstem is non-functional. Nonetheless, it is quite true that if all brainstem reflexes are absent there is virtually no chance that the patient will recover. However, given the litigious atmosphere in the USA, we tend to use and advise confirmatory tests. One may use arteriography via a four-vessel (carotid and vertebral arteries) injection. This should show no intracerebral spread of the dye, although it may reach the external carotid circulation. No intracranial arterial dye should reach the external carotids[5].

Other techniques which are used in the USA are somatosensory evoked potentials, transcranial Doppler, isotope angiography, etc. However, none of these has been fully accepted for widespread use. Part of the reason is that they are technically difficult procedures to do in an intensive-care unit with consistent reliable results.

Electroencephalography (EEG), however, has been accepted very widely, and is used in most major hospitals to confirm brain death. The appropriate finding here is an isoelectric EEG. This does not mean that the record is totally isoelectric, but that there is no measurable activity over 2 μV. Standards have been set up for the type of recording necessary, and these must be meticulously and carefully followed. Reference should be made to their publication, which details the exact criteria published by the American EEG Society[7].

Unfortunately, recording EEG in an intensive-care unit can be very difficult. The ICU is electrically an electronic jungle with multiple sources of extraneous electrical potentials. Frequently one may find a given cubical in the ICU unit in which recording of the EEG is absolutely impossible despite every maneuver possible. This has become less common as EEG machines have been improved, but still does occur. In this case the only alternative is to move the patient to another area of the ICU which has less electronic 'noise' in the environment. Careful attention must be paid lest one creates grounds to the patient which could in fact cause an unknown electrical shock. Again, the details of the correct recording of the EEG, and the use of the equipment and instruments, are detailed in American EEG Society guidelines[7].

CHART DOCUMENTATION

The last documentation occurs when all the evidence is present and total brain death is diagnosed. This evidence should then be recorded in an appropriate note in the permanent record. All the major criteria should be listed, and the results of testing noted. In any case when there is a question involved, particularly if there is a question of hypoxic insult or an insult due to extraneous drugs, the testing should be repeated at an interval of no less than 6 hours.

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APPENDIX

Minimum criteria for a diagnosis of brain death

The diagnosis of brain death can only be made if the answer to all the questions is No.

(1) *Cerebral state*
   (a) Is the patient responsive to stimuli (painful, auditory, etc.)?
   (b) Is the cause of coma unknown?
   (c) Does the coma appear to be reversible?
   (d) Have any drugs which may affect ventilation or the level of consciousness been administered during the past 12 hours?

(2) *Brainstem reflexes*
   (a) Do the pupils react to light?
   (b) Do the pupils react to painful stimulation?
   (c) Is the corneal reflex present?
   (d) Are doll's eye movements present?
   (e) Does nystagmus occur when each ear is in turn irrigated with ice-cold water for 1 min?

(f) Is there a gag or a reflex response following bronchial stimulation by a suction catheter passed down the trachea?

(g) Is there spontaneous ventilation?*

(3) *Body temperature*

Is the rectal temperature below 35°C?

**Methods of testing for spontaneous respiration*

(a) If arterial blood-gas analysis can be performed:
  (i) Ventilate the patient with 100% oxygen for 15 minutes.
  (ii) Check $Paco_2$ – must be within normal limits (5.3–6.1 kPa).
  (iii) Disconnect the patient from the ventilator.
  (iv) Administer oxygen (6 l/min) through a catheter in the trachea.
  (v) Check $Paco_2$ – must be greater than 6.7 kPa.

(b) If arterial blood-gas analysis cannot be performed:
  (i) Ventilate the patient with 100% oxygen for 10 min.
  (ii) Ventilate the patient with 5% carbon dioxide for a further 5 min.
  (iii) Disconnect the patient from the ventilator.
  (iv) Administer oxygen (6 l/min) through a catheter in the trachea.

2
Medico-legal Aspects

S.S. SANBAR

INTRODUCTION

The legal and ethical aspects of human organ transplantation vary from country to country, although in broad terms the legal and ethical requirements denote substantial similarities. This chapter provides an overview principally of the statutory requirements relating to heart transplantation.

The major legal issues that pertain to human organ donation include:

(1) consent by donor or by those authorized by law to speak for the donor;
(2) legal tests and doctrines applied to organ donors;
(3) determination of time of death of donor;
(4) professional liability or medical malpractice;
(5) ethical and economic considerations.

INFORMED CONSENT

Competent adults

When dealing with adults of legal age and sound mind, their rights of self-determination and privacy rank supreme and are fundamental. When the transplant donor or donee is a competent adult, his or her consent should be given freely, knowingly and intelligently after being fully and reliably informed regarding the transplant procedure, its material risks, prognosis, and all alternative procedures. The doctor may not induce consent by minimizing the dangers of the surgery, or by misrepresentation or duress.

Donation by a competent adult prior to death

Any person may make such a donation to be implemented after his death: (1) in his will, if he is competent to make such a will; (2) in any document attested to by two competent witnesses; (3) by an oral statement made by the deceased during life in the presence of two persons of at least 18 years of age; and (4) by wearing a prescribed identity tag issued by an approved institution (e.g. driving license). Any such donation may be revoked prior to death by the donor.

In the USA the Uniform Anatomical Gift Act of 1968[1], and amended in 1987[2], allows any individual of sound mind who is over 18 years of age to make a gift during his life by will (to be effective immediately upon death without waiting for probate), or by a card or other document. If the donor is incapable of signing for any reason, including sickness, then the document can be signed on his behalf, if validated by two witnesses. The amended Uniform Anatomical Gift Act of 1987 added provisions for routine inquiry, required requests, presumed consent for Medical Examiner cases, and prohibition of the sale of human organs.

The system of donor cards has the merit of simplicity and portability. A typical example is the Uniform Card developed in the USA following the Uniform Anatomical Gift Act. This card, which can be carried easily in a pocket or wallet, states in simple words the donor's desire to make an anatomical gift to take effect upon death. On the reverse side the card contains provisions for signature, witnessing and personal details. Similar cards are available in several other countries, including Australia, Canada and Britain. In Britain, under the Human Tissues Act, 1961, a patient may carry a signed donor card or record his wishes 'in writing at any time or orally in the presence of two or more witnesses during his last illness'.

Donation by a relative of the deceased

In the absence of specification by the individual while alive (as above), permission to donate organs at death may be obtained from certain specified next of kin of the deceased, i.e. the adult or legally competent spouse, child, parent, brother or sister, provided the deceased donor had not forbidden such a donation. Prior to actual organ removal, any donation by a relative may be revoked by the next of kin who made it. In this context legal competency refers to a person of sound mind who is over the age of 18 or 21 years, depending on the legally specified age of majority for the country.

In the USA, relatives of a deceased may also legally donate by document, telegraph, recorded telephone or other recorded message; in order of legal priority the next of kin are the spouse,

adult children, parents and adult siblings. In many countries 'relative' is not defined.

Donation by an authority empowered to donate after death

The acquisition of hearts in the absence of donation (as above) may be possible in some countries. If a relative authorized at law to consent to a donation cannot be traced, the law of some countries may allow for a designated official to authorize under certain prescriptions the removal of tissue from a deceased person for purposes of a donation, e.g. the District Surgeon in South Africa, and the Coroner in England and Wales. In the USA, however, the law makes no actual reference to the deposition in use of an unclaimed cadaver. A few states allow transplantation of certain organs (such as corneas) if a reasonable effort has been made to trace the relative; authorization must be given by the Medical Examiner.

An adult, mentally competent prisoner may be judicially compelled in the United States to submit to lifesaving treatment, including human organ transplantation, by a declaratory judgement and a temporary restraining order. The judge uses a balancing test weighing the patient's non-absolute constitutional right of privacy and self-determination against the state government's most significant interest – namely, the preservation of life.

Minors and other incompetents

When dealing with minors, mentally retarded patients, or psychiatric patients who are legally incompetent to give a valid consent, several legal problems can arise. As a general rule a court order will almost always be required before organ transplantation may occur. The consent of parents or guardians is also usually required. However, refusal by the parents or guardians of necessary treatment for the incompetent child or mental case may not always be conclusive. In those circumstances involving minors and incompetents the judge applies the doctrine of *parens patriae*, an equitable doctrine which, in essence, permits the juvenile court to act as a parent and attempts to protect the minor from the abuse or neglect by the real parent or guardians.

Cadaver organs

For cadaver donors, where an informed consent by the donor prior to death is unavailable, there are three considerations in obtaining the requisite consent. First, there must be a valid consent from one who is legally capable of granting such a consent (generally, next of kin). Second, the determination of the time of death should be in accordance with the law. Third, the removal of organs from one who has died from a violent cause involves the Medical Examiner, who has jurisdiction over the body. There are certain circumstances where the Medical Examiner may not approve organ donations, including homicide, poisoning, industrial accidents, car accidents involving other persons, and where there is a question of liability.

Unclaimed bodies

For unclaimed bodies, statutes generally require that one should wait 48 hours after the death of the patient, during which time the hospital that is in possession of the body must make a reasonable search for the next of kin. Until such a search is carried out, the body is technically not claimed. In addition, the physician who wishes to use the unclaimed body in a transplant procedure is required to obtain clearance from the Medical Examiner. The physician must document carefully all aspects of the transplant procedure in the medical records in an attempt to avoid future liability.

Anencephalic

Anencephalic infants represent an important potential source of organs. Organs from such infants could meet the bulk of the current demand for infant organs. Organs from stillborns and infants dying from other diseases are not generally suitable for procurement and transplantation.

The first transplant of the heart from an anencephalic infant in the USA occurred in October 1987 at the Loma Linda University Medical Center in California without legal incident. Subsequently, other parents requested that their anencephalic children be used as donors to help other children. It is noteworthy that the anencephalic does not fall under the category of brain-dead. Hence, the brain death statutes are inapplicable under those circumstances. However, in some states an anencephalic infant may fall under the category of either a patient in a 'vegetative state' or the 'terminally ill', or both, as defined by statutes. Without specific federal or state statutes that prohibit anencephalics from donating organs before actual death has occurred, it is conceivable that a State Court may order the organ donation of an anencephalic infant when death is 'imminent', in a matter of hours or days, if the parents, doctors, hospital, clergy, and a court-appointed guardian *ad litem* all approve of the donation.

LEGAL TESTS AND DOCTRINES IN THE UNITED STATES

Simple judicial approval of parental consent

In cases involving intra-familial transplants among minors and incompetents (e.g. mentally retarded and schizophrenics), where both parents agree, judicial approval has generally been granted.

In a 1972 Connecticut case, *Hart v. Brown*[3], the court approved a transplant between two identical 7-year-old twins, considered the medical ramifications, and stated that the parents' motivation and reasoning had met with approval of the guardians *ad litem*, physicians, clergymen, and the court itself.

Best interest test

By statute in Louisiana, medical intervention must be in the 'best interest' of minors and incompetents. A 1975 Louisiana case, *In re Richardson*[4], involved a husband who brought an action against his wife to compel her to consent to surgical removal and transplantation of one of her minor's kidneys for donation to the boy's older sister. Such surgical intervention would invade the minor's right to be free in his person from bodily intrusion, and was not shown to be in the best interest of the minor. In a concurring opinion, a judge stated that before the court might exercise

this awesome authority in this instance, and before it even considered the question of the best interest of the child, certain requirements must be met. He said: 'I am of the opinion that it must be clearly established that the surgical intrusion is urgent, that there are no reasonable alternatives, and that the contingencies are minimal. If those requisites are not met, then the court will not need to address the best-interest of the child issue'.

In 1975 the Supreme Court of Wisconsin reached a similar holding, *In re Guardianship of Pescinski*[5], where a permit was sought for removal of a kidney from a mentally incompetent 39-year-old catatonic schizophrenic to donate to his 38-year-old sister. The Wisconsin Court found that there was absolutely no evidence in this case that any interest of the mentally disturbed brother would be served by the transplant. The court held that where no benefit had been shown to the mentally incompetent, and no consent for the kidney transplant had been given by the incompetent or his guardian *ad litem*, the judge had no power to order such surgery.

Substituted judgement doctrine

Most courts have been using the doctrine of 'substituted judgement', which was first applied in 1816 in an English case. The decision was amplified in 1840 by a second English case, and in 1844 the doctrine of substituted judgement was first applied in the United States in a New York case. In 1945 the United States Supreme Court held that the doctrine of substituted judgement requires that the court substitute itself as nearly as possible for the incompetent, and act with the same motives and considerations as would have moved the incompetent.

In 1969 the doctrine of substituted judgement was first applied in a transplant case, *Strunk v. Strunk*[6]. The mother of a 27-year-old mentally retarded male, with an IQ of approximately 35 and a mental age of approximately 6 years, petitioned the court for authority to proceed with a kidney transplant for the 28-year-old brother who was suffering from chronic renal nephritis. In applying the doctrine of substituted judgement the court did not apply the best interest test. Instead, it applied the 'avoidance-of-detriment' test *vis-à-vis* the donor. The court based its opinion on psychiatric testimony to the effect that the death of the donor's brother would have an extremely traumatic effect on the donor. Stated differently, the benefit to the transplant donor is detriment avoidance, and what the court is trying to avoid is the grave emotional impact on the donor. The latter concept was used by a Massachusetts court in the 1950s when ruling on incompetent candidates for renal transplantation.

Parens patriae doctrine

As a general rule, when parents request permission from the judge to give consent on behalf of minors, e.g. incompetent twins for kidney transplantation, the court applies the equitable doctrine of *parens patriae*, thereby empowering it to permit parental consent on behalf of the minor. On the other hand, when the parents do not request permission of the judge, or even refuse to consent to necessary organ transplantation for minors, the court may resort to the doctrine of *parens patriae* to protect the incompetent.

In Kansas there is statutory authority that allows the judge to delegate powers to a committee of persons who will be appointed by the judge to act for the mentally incompetent under the supervision of the county court. Thus, in addition to its inherent common-law powers, the Kansas court by statute may rightfully act as the *parens patriae* and substitute its judgement to protect and benefit the incompetents who are incapable of protecting themselves.

Guardian *ad litem*

In most cases involving intra-family transplants from incompetent donors, judicial approval has been granted. The court may appoint a guardian *ad litem*, often an attorney, who will be required to argue whether the transplantation procedure is in the incompetent's best interest. The judge then determines as a question of fact whether the parents' exercise of substituted consent is in the incompetent's best interest.

Substantial psychological benefit test

This test was used in a 1979 case, *Little v. Little*[7], from the Court of Civil Appeals of Texas. There, the mother of a 14-year-old daughter with Down's syndrome applied for an order authorizing her to consent to the removal of a kidney from her incompetent, mentally retarded daughter for transplant into her son who was suffering from end-stage kidney disease. The judge appointed an attorney *ad litem* to represent the daughter. The attorney was opposed to transplantation. The mother argued, first, that the daughter with Down's syndrome was the only relative suitable for donating a kidney to her brother; second, that the transplant would provide great and tangible benefit to her; third, that there was no threat to her life; and fourth, that, to the best of the mother's knowledge, the transplant was what the daughter would have wanted for her ill brother. The judge authorized the transplant over the objection of the attorney *ad litem*, using as his yardstick the 'substantial psychological benefit' test. The judge noted that the donor's participation in a procedure that would save her brother's life would be substantially beneficial to the donor from a psychological standpoint.

DETERMINATION OF TIME OR FACT OF DEATH

The 1961 British Human Tissues Act allows removal of tissue once a registered medical practitioner has satisfied himself by personal examination of the body that life is extinct.

The 22nd World Medical Assembly, at the meeting in Sydney, Australia, in 1963, issued a statement that 'the determination of death should be based on clinical judgement supplemented, if necessary, by a number of diagnostic aids, of which electroencephalography is currently most helpful'. In the case of those persons kept alive by artificial means of resuscitation (in use or contemplated), or in which the transplantation of an organ is being considered, it emphasized that the moment of irreversibility of the processes leading to death must be determined, rather than the moment of death. This declaration further states that, while the electroencephalograph is the most useful diagnostic aid, 'no single technological criterion is entirely satisfactory in the present

state of medicine, nor can any one technological procedure be substituted by the overall judgement of the physician'[8].

In the United States the 1978 Uniform Brain Death Act specifies that 'For legal and medical purposes, an individual who has sustained irreversible cessation of all functioning of the brain, including the brain stem, is dead. A determination under this section must be in accordance with reasonable medical standards'. The law is, however, silent on the actual criteria to be used for determining death. The time of death is determined by the physician who attends the death, or, if none, by the physician who certifies the death. By this law, too, this physician may not participate in the procedures for removing or transplanting a part of the deceased's body[9].

In addition to cessation of heart beat and respiration, therefore, brain death is recognized by law. In 1975 a New York court addressed the legal definition of time of death as used in provision of the Anatomical Gift Act. The court held that the term 'death' implies a definition consistent with the generally accepted medical practices of doctors who are primarily concerned with effectuating the purposes of the Anatomical Gift Act. It was noted that the intent of the Act was to provide a systematic procedure of implementing the public policy of New York State, which is to encourage anatomical gifts on death.

Under the Uniform Anatomical Gift Act of 1968, adopted in all 50 USA states, the physician may incur possible liability for making an errant determination of the time of death in the transplant donor, resulting in premature harvesting of the organ(s). (The time of death of the patient must be determined by a 'treating' physician.) However, when the physician removes an organ from a donor patient in 'good faith', the physician is not liable in a civil action under the terms of the Act or an applicable state law. The physician is probably protected from criminal liability, particularly when it involves the question of the determination of the time of death. However, the surgeon who removes the desired organ prematurely may not be protected from a wrongful death action, even though the act may protect the surgeon from the charge of mutilation or mayhem.

Failure to comply with the Anatomical Gift Act is evidence of 'bad faith' *per se*. A physician who makes an honest effort to determine the time of death based on reasonable and well-recognized medical standards, and who acts in the best interest of the patient, would probably be considered to be exhibiting good faith.

In contrast with the United States, most other countries have no specific laws relating to brain death as evidence of the fact of death, and generally rely on acceptable medical criteria. A Working Party on behalf of the Health Departments of Great Britain and Northern Ireland has prepared a quasi-legal Code of Practice, intended for hospital staff and medical administrators relating to 'Cadaveric Organs for Transplantation'. In the section dealing with brain death it states: 'There is no legal definition of death. Death has traditionally been diagnosed by the irreversible cessation of respiration and heart beat. This Working Party accepts the view held by the Conference of Royal Colleges that death can also be diagnosed by the irreversible cessation of brain-stem function – "brain death". In diagnosing brain death, the criteria laid down by the Colleges should be followed.' (The clinical diagnosis of brain death is discussed in detail in Chapter 1).

PURPOSE OF DONATION

In most countries each donation or 'removal' must be for the purposes of medical and dental education, research, or therapy (including use in any other living person), or for any other scientific purpose; such purpose need not be specifically expressed and may include the production of a therapeutic, diagnostic, or prophylactic substance.

THE DONEE

In the USA, state statutes vary with regard to permissible recipients of donated tissue. In general, licensed hospitals, teaching institutions, colleges, medical schools, universities, storage banks, state public health and anatomy boards and institutes approved by the State Department of Health may be donees. Unless the donee has been previously indicated during life by the deceased, the attending physician becomes the donee. If he so desires he can transfer his ownership to another person. Although he is not permitted to participate personally in removing and transplanting organs or parts, he is allowed to communicate with other relevant donees or transplant teams.

AUTHORIZATION FOR THE REMOVAL OF ORGANS

Once a donation has been made in South Africa, a donee specified, and the fact of death certified, the transplant surgeon or a member of the team must request authority from the appropriate medical practitioner (for example, the medical superintendent of the hospital in which the donor is being cared for, or his authorized medical deputy) to remove the donated organ, which removal may only be undertaken by or on the authority of a medical practitioner or dentist. The person authorizing removal must satisfy himself that the body is not required for examination in terms of other legislation which has a higher ranking claim on the body, e.g. the Inquest Act.

Authority to remove a valid donation in the United Kingdom is not essentially different from the above provisions, with the noticeable exception that the person lawfully in possession of the body of a deceased may so authorize, after practicable inquiries, providing that the deceased had not expressed objection to his body being so dealt with, or the surviving spouse or any relative of the deceased expressed objection. Normally, the 'person' lawfully in possession of a dead body is a National Health Service hospital until such time as the body is claimed by the person with the right to possession, i.e. the coroner, the executor, or the next of kin[10].

CONFIDENTIALITY

Disclosure to any other person of any fact whereby the identity of the deceased donor or donee may be established is prohibited by statute in some countries. In the United Kingdom confidentiality is not prescribed, but the staff of hospitals and organ procurement organizations 'must respect the wishes of the donor, the recipient, and the families with respect to anonymity'[10]. There appear to be no specific statutory laws regarding transplantation confidentiality in the USA. However, the constitutionally protected right of

privacy and general statutes governing medical care in the USA preclude unpermitted disclosure.

At common law the privacy of the individual may not normally be intruded upon. The legal and ethical obligations of a medical practitioner to treat patient information as confidential appear to be based also on contract. Experience has shown that breaches in confidentiality in regard to heart transplantation have generally had their origin beyond the medical profession.

IMPORTATION/EXPORTATION OF TISSUE

In most countries the importation or exportation of tissues is subject to permission being obtained from a government authority.

SALE OF TISSUE

An authorized institution or the importer of tissue may receive payment for providing tissue to any person for therapeutic or scientific purposes. If any other person receives payment for such tissue, he shall refund such payment to the person who made it. The object of this and the aforementioned provision is to prohibit trading in tissue, which ethically and scientifically is unacceptable. This prohibition, however, does not prevent a medical practitioner from being paid for his services in the collecting or use of such tissue as a part of therapy.

In the USA the sale of organs is prohibited by the National Organ Transplant Act of 1984, as amended in 1986 by the fiscal 1987 Budget Reconciliation Legislation, setting a uniform policy in this area following a private attempt to establish an organ brokerage firm in Virginia during 1983[11,12]. A reasonable charge for removal costs is allowed. While questions have been raised concerning the constitutionality of the non-sales provision under the 'right of privacy' doctrine, the better position is that the provision is constitutional under the commerce power, and does not properly fall within the sphere of constitutional privacy.

TRANSPLANT MALPRACTICE

With regard to medical malpractice involving transplantation, the reasons for malpractice as a cause of action are the same in transplant cases as in any other medical situation: negligence, lack of informed consent, battery, invasion of privacy, fraud, abandonment, breach of fiduciary duty, etc. To date there have been relatively few cases of medical malpractice involving transplant patients, probably because transplantation is still relatively new and experimental. Also, expectations for success are low, and there has usually been a cultivated personal relationship and good communication between the transplant surgeon and the patient and relative. What suits have occurred have generally turned on the question of informed consent, rather than the applicable standard of care[13].

An area of developing concern, in light of current AIDS malpractice litigation, is that of liability on the part of the physician, the hospital, and the donor's estate for the transplantation of AIDS-infected organs. A cause of action could also arise on the part of the donor's family, if they became involved in an active concealment of the donor's health status prior to death.

This is, in reality, an extension of the general question of strict liability for body tissues in the areas of blood and blood products. While early cases allowed the plaintiff to hold the blood supplier strictly liable for contaminated blood products, the clear trend of later cases has been to reject strict liability, considering blood as a 'service'. This is in keeping with provisions of the Federal Act preventing the sale of organs. Thus, the major impact of the increase in AIDS cases will most likely be to limit the number of organs available for transplant, rather than produce a significant risk of malpractice to the practitioner (in the absence of negligence).

ETHICAL AND ECONOMIC CONSIDERATIONS

The United States' health-care system is subject to an ever-expanding technologic pace plus an insatiable consumer demand for the most advanced medical care; and yet our health care faces a future of limited organ resources and inescapable cost-containment measures[13].

Who shall live? Who shall pay? And who shall decide who will get the next transplant? Not only are there problems in securing organs for transplant, but there are also dilemmas for third-party payers in deciding whether to pay for the costly new transplant procedures. The soaring cost of health care has become a significant factor in determining the zealousness of the physician's search for innovative treatment, be it transplantation or other novel treatment. Although seemingly distasteful to some, cost considerations *vis-à-vis* the patient, the community, the state, the payer, and the physician do influence health-care delivery. Cost–benefit considerations are having an impact on the development of the standard of care.

Economic considerations are also important in determining the performance and acceptance of the innovative procedures and, secondarily, in influencing the standard of medical care. Concerns of the patient, the government, health-care insurers, and the public regarding the escalating cost of health care have resulted in increasing scrutiny of transplants. Affordable types of transplantation may also help determine the standard of care in some cases.

Physicians are becoming sensitized to cost-containment. They find themselves caught between demands to provide the best possible treatment for patients and to keep down the cost of medical care. Fortunately, in many cases the cost-effective use of organ transplantation and good medicine are one and the same. Transplant surgeons appreciate that, as consumers of health care, the patient, the government, and the third-party payer demand accountability.

COMMENT

One can expect that national standards for transplantation, including socioeconomic considerations, will be set by the medical profession and applied by the courts. In addition to (or in lieu of) the standard of care set traditionally by the medical community, the courts may take into consideration legal, ethical, moral and economic considerations. They may, as they have in other situations, develop standards that will be set as a matter of law, or judicially imposed. The problems of informed consent and time-of-death determinations should be completely resolved soon.

Regrettably, affordable medical care is, by definition, selective. When coupled with federal budget constraints and resistance by the third-party payer to cost-shifting by the health-care provider, the net result may be the rationing of health care. This is indeed the dilemma of contemporary and managed medical care, and transplantation is a part of the dilemma.

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3
Donor Organ Availability and Transplant Coordination

J.S. CHAFFIN AND L. MISCHKE

INTRODUCTION

Organ donation is a process that begins with a need. The need is a patient suffering from failure of an essential organ. To satisfy that need, organ procurement organizations (OPO) have developed mechanisms for identification, referral, and equitable distribution of organs that are made available[1]. The primary function of most OPO is education, not only of the general public but, more importantly, of the professional community, regarding the need for organs and tissue for transplantation. Unfortunately, most OPO are still burdened by the lack of suitable donor referrals.

'Harvesting organs' is not a phrase transplant surgeons commonly use, or enjoy hearing used, in public; but, with rare exceptions, we use it routinely in work, journals, and at medical meetings unattended by the press. In today's climate of ever-expanding demand for organs, the mounting pressure to find viable organs is felt by all transplant units. Nowhere is the pressure felt more greatly than by the retrieval team, whose job it is to evaluate, prepare, and 'harvest' the organ(s).

REASONS FOR INADEQUACY OF DONOR ORGAN SUPPLY

Gallup polls have determined that more than 80% of Americans state that they are willing to donate their organs and tissue for transplantation after death. Only about 17%, however, carry organ donor cards[2]. Currently, in the USA, a significant percentage of patients approved for heart or lung transplantation die awaiting a donor organ[3]. If 80% of Americans actually donated post-mortem organs for transplantation we would be closer to supplying our current needs.

Personal experience demonstrates that grieving families frequently welcome an opportunity to consider organ and tissue donation. The knowledge that other lives have been enhanced through the gift of organs is generally a strong solace for a family struggling to accept what they feel is a 'meaningless' death. The reason given by a significant number of those who do not donate, is that either they were not approached, or that the personnel involved in the care of the patient were not aware of the suitability of the patient as a donor of organs. Well-coordinated public and professional education programs must continue to be developed[4].

In 1968 the National Conference of Commissioners on Uniform Law and the American Bar Association drafted the 'Uniform Anatomical Gift Act', an attempt to provide the states within the USA with a model for recognizing and formalizing methods through which individuals or families could make a gift of their organs or those of a relative. The Act authorizes an individual 18 years of age or older, in the presence of two witnesses, to record his wishes regarding organ donation by will, donor card, or other written document, or orally in the presence of two witnesses, and authorizes the next of kin to consent to organ donation in the absence of the deceased's known objection. Space has been made available on the back of all drivers' licenses for recording the wishes of potential organ donors. Less than 10% of those eligible, however, actually mark their license[5].

While it may not seem to be healthy or 'natural' to anticipate one's own death, or the death of a family member, such anticipation is not infrequent – we buy life insurance to provide benefits upon death, we prepare wills and trusts, and even buy cemetery plots in anticipation of death. In a similar manner we must convince the members of the public to accept consent for organ donation as a logical preparatory step for their own demise.

Trust in the system is imperative, and lack of knowledge or understanding of organ donation procedures may instill fear or lack of trust (Table 1). Common sources of concern or mistrust include: (a) lack of awareness of religious or moral propriety of invasion of the body or removal of parts for transplantation; (b) concern over the care of, or proper respect for, a body after donation; (c) concern that organs may be sold for profit of others; (d) fear that signing a donor card might accelerate their own death, and finally (e) fear that organs might be removed from a person before death has occurred[6]. It is the responsibility of the transplant community to alleviate such fears and concerns through proper education. Historically, mankind responds with altruism when called upon to meet needs it understands and trusts.

Table 1 Questions frequently asked by the family of a potential organ donor

Is there anything more that can be done?
Is he really dead?
Why does his heart still beat?
What organs or tissue will be used?
Who will remove the organs?
Who will receive the organs?
What will surgery be like?
How long will surgery take?
Will an autopsy be necessary?
Will the body be picked up by the funeral home?
Will the Medical Examiner be involved?
When can a funeral be held?
How will he look, after donation?
Who will pay the cost of organ donation?
Will anyone know we donated?
Will the media be involved?
Will we be able to make contact with the recipient?
When will we know if the organs were used?

ORGAN PROCUREMENT NETWORKS

Our own personal experience has been largely in the USA, where organization of organ retrieval is now well advanced at a national level. Most of our comments will therefore relate to the system of organ retrieval as it is in the USA, though the underlying principles are relevant in all countries where organ transplantation is performed.

USA

Currently there are 69 separate OPO in the USA, and another eight in Canada, either hospital-based agencies serving the parent hospital or, more commonly, independent organ procurement agencies serving several transplant centers in a given area.

In 1982 the demand became so great for organizations that could expedite procurement, preservation, and distribution of vital organs that the North American Transplant Coordinators Organization (NATCO) established a computer registry called '24-Alert' to facilitate the distribution of organs other than kidneys. This system coordinated more than 80% of the hearts transplanted in the USA from September 1982 through June 1986.

The National Organ Transplant Act, signed into law in 1984, called for the design and implementation of a national computerized network that would include transplant centers, procurement agencies, voluntary health organizations, and the public. The United Network for Organ Sharing (UNOS) now coordinates organ donation nationally, and lists all vital organs required and available, and produces a printout of the listing. This system cannot be activated without a computer terminal[7]. The computer database includes information on all potential recipients, including name, age, sex, weight, blood type, lymphocytotoxic antibody status, medical status, and identification of unacceptable HLA antigens.

Western Europe

Western Europe now has a number of regional organ-matching services, the first of which was set up in the Netherlands, which is now the base of the European Transplantation Service (Eurotransplant)[8]. Most of these units were set up originally for kidney placement. Although most fulfill their functions primarily in their own areas, should there be no suitable local recipient the organ will then be offered to another transplant service. In this way there has been progress towards an international organization. Since 1984 there has been a marked increase in multiple organ donation throughout Europe. Each region retains the right to service its own population first; only if there is no suitable recipient will that organ be offered to another region.

With multiple organ retrieval the potential for chaos in the operating room seems great when perhaps four separate surgical teams, possibly unknown to each other and speaking different languages, may gather for organ removal. One possible solution, pioneered in Cambridge, England, is to provide one surgical team trained to remove and preserve all organs and tissues for transplantation[8]. It is possible that, in the future, such teams will become available throughout the world to expedite multiple organ retrieval.

Elsewhere

In most other countries, such as South Africa, organ retrieval networks are not as yet so well organized. Major transplant centers tend to rely on organs in their immediate vicinity, and on direct referral from physicians at other hospitals who are aware of their requirements. In South Africa, for example, there is no central unified organ retrieval network, though organs are exchanged from center to center depending on need.

ORGANIZATION OF ORGAN RETRIEVAL

The transplant coordinator

With the evolution of transplantation has come a number of new health-care professionals, none more important than the transplant coordinator. In the USA, coordinators are organized under the North American Transplant Coordinators Organization (NATCO) for credentialling and certification. The OPO usually provides the donor coordinators, and the recipient center provides recipient coordinators. The donor coordinator provides expertise in the management of the brain-dead potential donor, and in the procurement, preservation, and distribution of transplantable organs. The recipient coordinator is responsible for coordination of the pretransplant assessment and preparation of the recipient, together with post-transplant care and follow-up.

Transplant coordination

Recipients are listed on computer by their individual criteria through the United Network for Organ Sharing (UNOS). Once an organ has been identified for transplantation, the chain of events may be lengthy but, in practice, the system works efficiently (Figure 1).

A member of the medical or nursing team caring for the potential donor will contact the local organ procurement agency, either directly or through the hospital transplant coordinator. A member of the local procurement agency will generally go to the hospital to

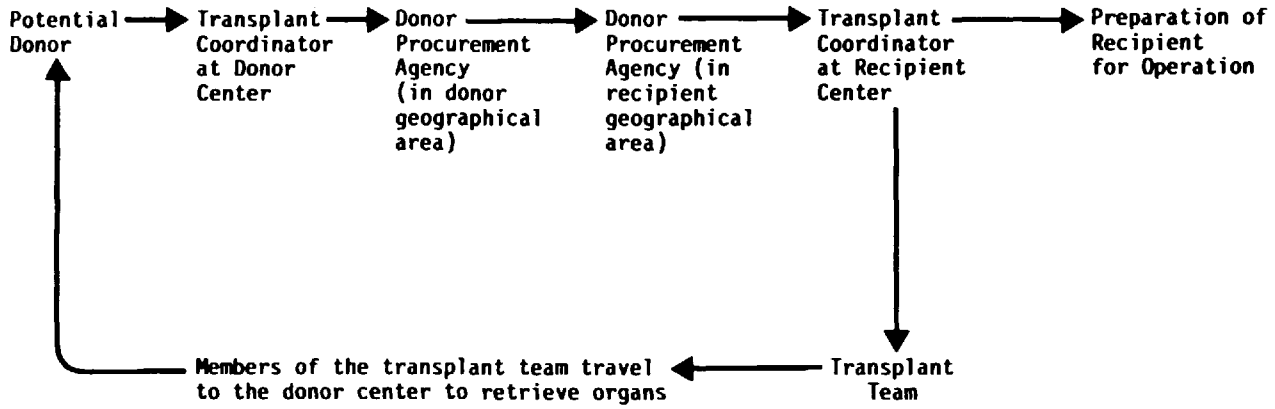

Figure 1 Chain of communication when a thoracic organ donor becomes available

assess the donor personally, and initiate therapy to maintain the donor in as stable a hemodynamic state as possible (Figure 2). A number of essential blood tests will also be requested (Chapter 4).

When basic data, such as blood group, height, weight, and age, are known, the procurement agency will enter this information into the computer, and search for a compatible recipient. With regard to thoracic organs, a potential recipient is generally located within the geographical region in which the donor hospital is situated, but a recipient in a distant region may on occasion be identified as the most suitable.

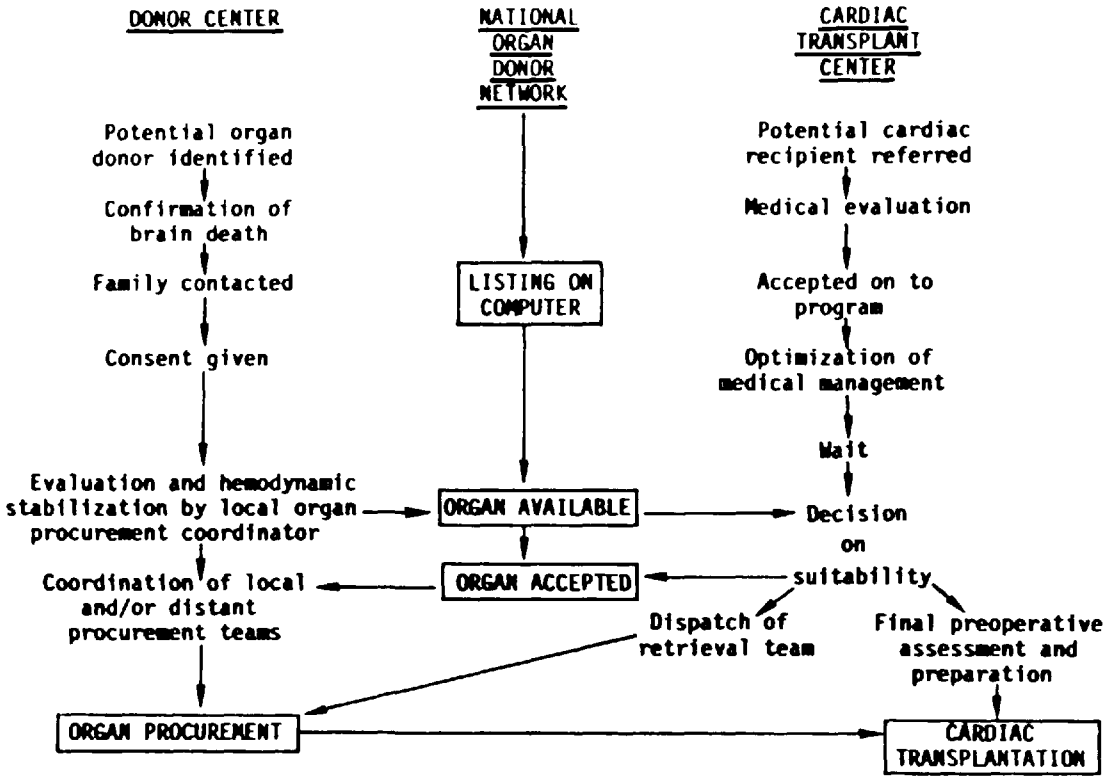

Figure 2 Pretransplant steps involved in the performance of a successful thoracic organ (e.g. heart) transplant

If the recipient is at a distant center, communications will be via the procurement agency serving that area. This agency will, in turn, contact the transplant coordinator or a member of the transplant team in the hospital on whose waiting list the potential recipient is listed. If the donor appears a suitable match for the potential recipient, a number of activities are set in motion.

Transplant coordination at the recipient center

The transplant coordinator at the recipient center organizes the retrieval team (who will travel to the donor center usually with a member of the local donor procurement agency), and the necessary equipment (Table 2). The most expedient transportation (road transport for short range, helicopter for medium range, and private jet for longer distances) is organized. If the potential recipient is not an inpatient he (or she) is notified, arrangements are made to transport him to the hospital, and he is prepared for surgery, which will include any necessary preoperative laboratory tests. All potential recipients waiting as outpatients carry pagers so that they may be easily contacted.

Contact must be made with all appropriate staff (surgeons, anesthesiologist, pump technician, and the operating room personnel) who are advised of the planned transplant procedure. They are advised of the timing, and may require updating at intervals. Contact must be kept with the donor retrieval team to ensure that there will not be an unnecessarily prolonged organ ischemic time. The recipient family is updated frequently by the coordinator during both the pretransplant waiting period and the surgical process. Confidentially as to the donor must be respected and honored.

Transplant coordination at the donor center

Simultaneously, the coordinator at the donor center confirms information on both donor and recipient to ensure acceptable matching of size and blood type. It is not unusual in the USA to have retrieval teams arriving from at least three or four different locations to harvest heart, kidneys, liver, and possibly lungs and/or pancreas. The local coordinator arranges ground transportation from the airport for the teams. It is the donor coordinator's responsibility to ensure that the donor is properly cared for prior to arrival of the retrieval teams, and that on-site arrangements are made for operating-room personnel to assist in the retrieval. This would include anesthesia personnel, as they do not usually travel with the harvest teams. An added burden in the European community might be to ensure a translator is present to overcome any language barrier that might exist between the retrieval teams.

Since we believe that, whenever possible, the heart or lungs should not be ischemic for more than approximately 4 hours, and as 30–60 minutes of this time must be allowed to sew in the transplanted organ, the time of donor aortic cross-clamp should be arranged so that the organ can be delivered to the transplant center within 3 hours. It is essential that close communication be maintained between the retrieval team and the team preparing the recipient, to ensure that the ischemic period is not unnecessarily prolonged. The liver and kidney retrieval teams are usually cooperative, as they appreciate that the heart and lungs have the shortest ischemic survival times of any of the solid organs.

It is vitally important that coordination between the thoracic organ retrieval team(s) and colleagues at the recipient center(s) be well organized at this point, because unnecessary delays of the other retrieval teams do not go unnoticed; a poorly coordinated team may find that it is not invited a second time! Multi-organ procurement etiquette requires being able to work professionally with multiple surgical teams from other programs without creating turbulence. Factors causing friction include arriving late, being unnecessarily demanding with regard to investigations and/or operating room personnel support, and discourteous behavior.

When the procurement team arrives, the surgeon reviews the relevant charts, chest radiographs, and electrocardiogram. The surgeons and/or coordinator call the recipient center to confirm the donor's suitability and coordinate the final stages of preoperative care for the recipient.

Table 2 Equipment required by the heart/lung retrieval team

Supplied generally by donor hospital	*Supplied and transported by retrieval team*
Esophageal temperature probe	Necessary drugs
Blood warmers	Sternal saw
Sterile drape	Sternal retractors – several sizes
Cautery	Special clamps, retractors, or instruments
Suction	Cannulas and administration sets (for cardioplegic/pulmoplegic infusion)
Vascular instruments	Cardioplegic/pulmoplegic solution(s)
Vascular clamps (e.g. Cooley)	PGE1
Pedicle clamps	Cold (4°C) normal saline or ice slush
Satinski clamps	Sterile intestinal bags (for transport of donor organs)
DeBakey forceps	Stapler devices (for trachea, bronchi, SVC)
Umbilical tapes, vessel loops	Portable cool box
Hemoclips	Specimen bottles for lymph nodes
Ties: silk (0000,000,00,0, #1, #2)	
Sutures: silk retention sutures (0)	
Cold (4°C) normal saline or ice slush for irrigation	
Large basin(s)	
Extra poles for supporting intravenous infusion fluids and cardioplegic bags	
Table covers – towels	
Ice and ice bucket	
Heparin	
Inotropic agents	

Before the surgical recovery begins, the surgeons from each of the procurement teams should agree on an order of organ excision which is acceptable to all concerned.

COMMENT

As heart and lung transplantation mature medically, a number of ethical and policy issues remain. A central fact about organ transplantation is the scarcity of organs for transplantation.

The essential need to continue a program of making both the public and the medical profession aware of the requirements of the patient awaiting transplantation has already been discussed. There are, however, several other ways in which the number of organs made available for transplantation could be significantly increased. Several involve possible changes in the laws concerning donation, and some of these will be discussed below. Furthermore, attention must be paid to the efficient use of such organs; unnecessary wasting must be eliminated whenever possible.

'Consent' to donation requirements

The laws governing donation of organs differ from country to country and from state to state. They can basically be divided into three groups: (a) where there are no such laws, (b) required request, and (c) presumed consent.

'*Required request*' implies that the hospital in which a potential organ donor is identified is required by law to ensure that the next-of-kin of the donor is informed of the option of organ donation. The decision as to whether to donate, however, is that of the next-of-kin. Hopefully, this system at least ensures that all potential donors are identified, and that the possibility of organ donation is discussed with their families. Various personnel may be involved in the request for donation. At some centers it is a member of the nursing staff, at others a physician, a hospital chaplain, or an organ donor coordinator. The success of this policy of required request varies greatly from center to center.

The system of family consent for donation from brain-dead, heart-beating cadavers does not result in donation of all of the organs that are medically acceptable for transplantation. It is likely that more hearts and lungs could be retrieved if the need for family consent were modified or even eliminated. The argument in favor of doing this rests on the assumption that the harm that might be inflicted on a (potential donor's) non-consenting family is of less concern than that suffered by a transplant candidate (and his family) who dies from lack of a suitable donor organ.

An alternative is '*presumed consent*', in which the consent of the deceased to donate is assumed unless he/she had previously indicated a formal objection, e.g. in the form of a '*non*-donor' card or by registration on a central computer. Some countries (e.g. Austria, Belgium, Denmark, Finland, France, Norway, and Singapore) currently have such laws, and have seen a significant increase in the supply of organs. Although the law makes it unnecessary, the policy at most centers in such countries is to request family consent, and not to retrieve organs against their wishes. To override strong family objections would almost certainly lead to damaging publicity and impair public relations.

In the USA no state has such a law, although 21 states have some form of presumed consent laws applying to removal of corneas for transplantation, and in Pennysylvania citizens may legally register their objection to donation by attaching a 'non-donor' card to their driver's license. In many states the Medical Examiner can give consent for donation if next-of-kin cannot be located (after reasonable efforts have been made to reach them). In Texas a symbol can be attached to a driver's license that indicates the wish to donate organs without the approval or consent of any family member.

A more radical option would be the elimination of consent altogether, allowing the routine salvage of organs in every suitable brain-dead patient. Routine salvage would treat the deceased's organs or body as a community resource, and permit organs and tissue to be excised as needed. This may not be as radical a solution as it seems. The military 'draft', or the conscription of young people into the armed forces, which is or has been legal in many countries, demonstrates the community's willingness to accept jurisdiction of the live body when an important public purpose – the safety of the community – is at stake.

Relaxation of brain death requirements

Some persons have proposed relaxing the requirement for brain death, which at present includes death of the brainstem, to permit organ retrieval from persons who lack cortical function only[9]. Such a re-definition of death would allow organ retrieval from irreversibly comatose and anencephalic patients. While such a change is not likely to increase significantly the supply of adult thoracic organs, it may prove to be an important factor in the expansion of pediatric heart and lung transplantation.

'Rewarded gifting' – financial incentives to donation

Some form of financial or material incentive to the donor family has been proposed as a way to increase the supply. One plan would enable the donor's family to be rewarded by the State for their humanitarianism in making this valuable gift. Such acknowledgement, by the material rewarding and honoring of families, already occurs in some countries of the Middle East. Offering a small State grant to help pay for funeral expenses, or some form of tax rebate, would be a possible means of financial reward.

Others have suggested more aggressive financial incentives, which would risk making a competitive market of these valuable resources. In the USA, however, it is a federal crime, punishable by 5 years in prison, to 'acquire, receive, or otherwise transfer any human organ for valuable consideration'[10]. Opponents of the 'sale' of organs point out the adverse effect it would have on voluntary organ donation and on donors' families, and its dehumanizing symbolic connotation. Concern has been expressed that a financial inducement would increase the pressure on poor families to donate, but not on wealthier ones, and would therefore lead to discrimination.

Proponents feel that these concerns can be minimized, and are outweighed by the benefits to recipients of a probable increase in the supply of organs. If organ transplant operations are 'sold' by surgeons, inasmuch as the recipient pays the surgeon, the proponents see no harm in 'selling' the organs that make the operations

possible[9]. There are persuasive arguments on both sides, but at present no Western country has passed a law making it legal to offer a financial material incentive to donation.

Modifications in recipient selection

The scarcity of thoracic organs for transplantation inevitably requires a rationing of the organs that do become available. The basis for selecting recipients involves a balance between efficiency and equity in the use of organs. Efficiency is clearly favored at the candidacy evaluation stage, while equity plays a larger role in deciding which candidate on the waiting list receives the organ.

The cardiologist's/pulmonologist's attitude in referring patients for evaluation for candidacy plays an important role in the selection of patients for transplant, and will draw greater scrutiny in the future. The frequency of referral for evaluation varies with the knowledge and attitudes of each individual cardiologist/pulmonologist treating patients with end-stage cardiac or pulmonary disease. Physicians who are unaware of transplant options, or who neglect to refer patients for evaluation, may be denying their patients a viable therapy. Although, to our knowledge, malpractice suits challenging referral decisions have not yet been brought, negligence may soon be claimed in cases of non-referral of patients to transplant centers for evaluation.

The current selection system regarding heart transplantation gives priority to those candidates on the list whose poor physical condition makes them most urgent. These include those who are rejecting a transplant and frequently those who have received a temporary mechanical assist device or artificial heart as a bridge to transplantation. A strict concern for the efficacious use of donated hearts might argue against such an allocation, for the most urgent cases may be less likely to do as well as healthier candidates[11].

While efficiency is important, a strong equity consideration is to avoid abandonment of critically ill patients. Once on the candidate list, one could argue that there is a special commitment not to abandon those in greatest need. Retransplantation after acute rejection of an organ, however, appears to conflict with efficiency by allocating a second organ to a patient who may not have as good a chance of survival as a healthier candidate. Aggressive efforts on behalf of a recipient in acute rejection, however, are viewed by some physicians as essential to demonstrate commitment. Transplantation of a third organ, particularly after acute rejection, would appear to be even more controversial, and policy in this situation is divided between the major centers. Not infrequently these situations are self-limiting, because a donor organ does not become available at the time it is required, particularly during a severe irreversible acute rejection episode.

A similar situation arises with the use of the total artificial heart or ventricular assist device as a bridge to transplantation. Since a significant number of candidates die awaiting transplantation, mechanical hearts and assist devices are used in some programs to act as a bridge until a donor heart becomes available[12]. This actually exacerbates rather than relieves the supply problem for the entire group of potential recipients, although it benefits the individual involved. It increases the number of patients awaiting a transplant, thus increasing the pool of patients from which selection for the next available heart must be made[13]. Moreover, some of these patients will be urgent cases. Although the results of subsequent transplantation in this group are steadily improving, they remain slightly inferior, but the urgent patient will gain priority in the allocation of the scarce resource of the donor heart. This may be considered by some to be a source of inefficiency in the use of donor hearts.

Official designation of transplant centers

Now that heart and lung transplantation have achieved accepted status, a major issue is whether there should be limits on the number of centers doing such transplants. There were 12 centers performing heart transplants in the USA in 1983, but there are well over 100 today, many of which perform relatively few transplants. The situation is developing similarly with lung transplantations.

The Federal Task Force on Organ Transplantation recommended that heart transplants be carried out only at those centers meeting certain criteria, including a minimum volume of 12 transplants a year[11]. Reimbursement through Medicare for both heart and lung transplants limits reimbursement to centers meeting certain requirements for volume and survival. The purposes of permitting only those centers that meet volume and survival criteria to perform thoracic organ transplants include the protection of recipients and the efficient use of scarce organs. Since physicians, patients, and institutions do not have an inherent right to the use of scarce organs, the community is free to limit transplants to designated centers if it deems this limitation essential to the efficient use of this resource.

Physicians in centers unable to meet criteria will argue that access to transplantation in one's own community is a distinct advantage, or that the local community should receive first priority for the organ donations it generates. They should be aware, however, that poor outcomes in centers that do not meet designated standards may be vulnerable to malpractice claims. It is arguably negligent to conduct a transplant program when components that are reasonably deemed essential to good outcome are missing. In any event, it is essential that transplant candidates be informed of a local center's compliance with, or deviation from, accepted standards. In addition, the referring physician should be aware of the standards of a specific center and realize that referral to a center not meeting public criteria for center designation could lead to malpractice claims, and poor patient outcome.

Finally, organ procurement agencies must consider whether it is wise to provide organs to programs that do not meet minimum criteria for safe and efficacious use of donated organs. Presumably, a national network that controlled the distribution of organs would not permit supply to unqualified centers.

The medical and legal constraints on organ donation result in a chronic shortage of hearts and lungs for transplantation. It seems that the demand for organs will always outweigh the supply. How donated organs are distributed, how recipients are selected, etc., are issues of public concern, with demands voiced for public accountability in rationing organs. Medical efficacy plays a major role in selecting recipients, but a variety of equitable and other concerns also enter into the picture. More public scrutiny and

debate about conflicts between efficiency and equity are likely, as is a reduction in the freedom now held by medical professionals to resolve these questions themselves[14].

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4
Selection and Management of the Donor

D.K.C. COOPER AND I.L. PARADIS

INTRODUCTION

The importance of well-functioning donor organs cannot be overemphasized; it is crucial to the success of a heart or lung transplant procedure. Donor organ failure contributes towards a significant number of early deaths in thoracic organ transplant patients today, and is therefore an area where improvements can still be made. Careful selection and management of the potential donor therefore remain essential.

SELECTION OF DONOR HEART OR LUNG

Donor age

Heart

As the incidence of coronary atheroma in Caucasian men increases markedly after the age of 40–45, many groups will exclude men above this age from donation of hearts (and women over the age of 45–50 years) unless fully investigated by cardiac catheterization and coronary angiography. The shortage of donor hearts has become so acute, however, that many centers now consider hearts of both men and women up to the age of 65 years, as long as echocardiography, left ventriculography, coronary angiography, and basic pressure measurements (such as left ventricular end-diastolic pressure or pulmonary capillary wedge pressure) reveal no significant disease. As most donors will be donating multiple organs, including the kidneys, coronary angiography and left ventriculography should be performed using the smallest amount of contrast medium possible, in order not to impair renal function.

Should coronary angiography not be available at the donor center, but it is essential to find a heart for a desperate recipient, some transplant groups believe that the heart could be used if: (a) echocardiography shows normal left ventricular wall movement; (b) direct coronary palpation and inspection reveal no significant signs of atheromatous disease; and (c) no significant ECG changes suggestive of ischemia (preferably with the heart rate increased to >140 beats/min by the infusion of isoproterenol) are present. Though these studies provide only a crude assessment, it is probably justified to use such hearts under urgent circumstances.

Potential donors of certain ethnic groups in some countries may be considered to an older age without the need for coronary angiography. For example, black patients in rural South Africa have an extremely low incidence of coronary atheroma, and may be acceptable as donors up to the age of approximately 60 years without coronary angiography.

A policy of using older hearts for older recipients is now followed by several centers. As early as 1989, Schuler *et al.*[1] provided evidence that donor hearts up to the sixth decade yield satisfactory graft function, even when taken from donors who had not undergone coronary arteriography. Graft function was as good in the recipients of these older hearts as in patients with younger donor hearts. Late complications from reduced ejection fraction and accelerated graft atherosclerosis did not occur more frequently in older donor hearts. Also in 1989, the Houston group similarly showed that there was no increased risk using donors in their 40s compared with younger donors[2]. Both groups urged that consideration should be given to the use of hearts from donors of previously unacceptable age groups, and this has certainly been the trend in recent years, stimulated primarily by the shortage of donor organs.

Recent experience at our own and other centers has shown successful use of hearts from donors in their 60s. However, our own opinion is that coronary arteriography should always be performed in patients of this advanced age group. Despite a good outcome reported at several centers, some caution must be shown as data from the International Society for Heart and Lung Transplantation Registry indicate that increasing age of the donor is associated with a higher recipient mortality (Chapter 43).

Lung

Some centers do not like to utilize lungs from donors who are 50 years or older, and a majority become cautious when donors of 55 years or older are presented. Even if the patient has not been a smoker, there is some loss of lung function in many patients >50 years of age, and this may increase the risk of poor lung function

after transplantation. Multivariate logistic regression analysis of risk factors for 1-year mortality after heart–lung transplantation reveals that a donor older than 40 years is a statistically significant risk factor for survival of the recipient. Similarly, a donor >45 years old is a significant risk factor for death in the first year after double lung transplantation[3]. Some centers would therefore not utilize a donor aged >40 years for transplantation of the combined heart and both lungs (Chapters 67 and 69). The age of the donor (<55 years of age) has not been shown to affect survival after single lung transplantation.

Donor size

Heart

The donor heart must clearly be large enough to support the recipient's circulation immediately after transplantation, but not so large that it is compressed when the chest is closed. The latter circumstance is relatively rare in adults, as most patients undergoing heart transplantation have large native hearts and large pericardial cavities. It is a much more important consideration in children. There are no absolute rules, and successful transplantation has been performed using donors weighing <50% to >150% of the recipient. However, a good working guideline is that the body mass of the donor should not vary from that of the recipient (either larger or smaller) by more than approximately 25–33%. A donor whose body mass is within these limits will usually support the circulation after orthotopic transplantation without difficulty, and will not prove too large or too small.

Chan *et al.*[4] used echocardiographic measurements of left and right ventricular internal dimensions, left ventricular mass, and percentage fractional shortening, and showed there were no significant differences in left ventricular internal dimension when women weighing 40–109 kg were compared with men weighing up to 80 kg. Left ventricular size was not statistically different among men weighing 50–99 kg. No difference was noted in right ventricular size among men and women. Body weight, therefore, does not correlate well with adult cardiac size, and should possibly not be used as an exclusion criterion for a donor heart.

Other factors, therefore, may have to be taken into consideration. The relative heights, muscle masses, and ages of the two subjects are also important factors. The heart from a tall, muscular, yet slim athletic teenager may be functionally satisfactory for a short but much heavier, though relatively inactive, older patient. In contrast, the heart may prove inadequate if the recipient is much taller and has little fat when compared with the donor. If the need of the recipient for transplantation is urgent, and yet the body mass of the donor is >33% less than that of the recipient (i.e. the donor weighs <67% of the potential recipient), then heterotopic heart transplantation should be considered as a possible option (Chapter 37).

Several groups have reported the results of using undersized donor hearts. For example, Blackbourne *et al.*[5] used 28 hearts where the donor-to-recipient body weight ratio was only 0.6–0.8. Hospital survival for non-urgent (UNOS status II) recipients was no different when undersized donors were used than when normal or oversized donors were used. However, in more critically ill patients (UNOS status I) there was a significantly higher mortality when undersized donors were used. This would suggest that, in patients where the risk is already high, the additional risk of utilizing a small heart may reduce survival. However, not all groups have confirmed this increased mortality. The Tucson group showed comparable survival in recipients receiving hearts from donors with a weight difference of >30% (range 30–46%)[6].

In a study from Loyola in 1991[7], the clinical and hemodynamic characteristics of recipients weighing more than their donor (undersized) were compared with those of recipients weighing less than their donor (oversized). Oversizing of donor hearts did not improve the outcome of orthotopic heart transplant recipients who had reversible preoperative pulmonary hypertension. Acceptance of undersized donor hearts was not detrimental to allograft function and recipient survival. This group therefore recommended the use of undersized donor hearts to maximize the use of this critically scarce donor organ.

The Vanderbilt group went one step further and concluded that, in the presence of moderate preoperative pulmonary hypertension, long-term cardiopulmonary function was compromised in recipients of oversized allografts and mildly impaired in recipients of size-matched allografts[8]. In the absence of pulmonary hypertension, cardiopulmonary function was impaired to similar degrees with undersized, size-matched, and oversized allografts. These findings supported the liberal use of undersized hearts for transplantation in patients without moderate pulmonary hypertension and, furthermore, suggested that oversized allografts are not beneficial to long-term cardiopulmonary function in patients with moderate pulmonary hypertension.

By carrying out right-sided cardiac catheterization 3 months after transplantation, Hosenpud *et al.*[9], however, demonstrated that although cardiac output was maintained at levels appropriate for recipient size, patients who had received small hearts relied on an increased heart rate and elevated filling pressures to achieve this end. This suggested that the small cardiac allograft had not adapted to recipient body size by 3 months.

Reviewing all of these studies, it would appear that close matching of donor and recipient sizes is not essential, but probably preferable.

In contrast to orthotopic heart transplantation, a close size match between donor and recipient thoracic cavities is required for transplantation of the heart and both lungs (Chapter 67). Undue compression of the donor lungs within the recipient's thoracic cage results in significant atelectasis and cardiac compression. Although fewer problems result from the use of smaller donor organs, the donor thoracic cavity dimensions should not deviate from those of the recipient by more than approximately 20%[10].

Lung

Selection of a suitable lung is based more on the respective heights of the potential donor and recipient than on the weight. The etiology of the lung disease, however, is of great importance. Patients with chronic obstructive pulmonary disease with emphysema have overexpanded lungs, and therefore can accept a donor lung that is rather larger than would be expected. Therefore, a shorter patient with emphysema could accept a lung from a rather taller donor. However, care has to be taken to ensure that the lung is not too large, or chest movement will be impaired.

A patient with pulmonary fibrosis will have a small thoracic cage and therefore may require a donor lung that is smaller than would be anticipated for the patient's height. Patients with primary pulmonary hypertension often have lungs that are of normal size in relation to height.

A comparison of the measurements taken on chest radiographs usually provides fairly reliable information regarding the relative sizes of the donor and recipient thoracic cavities. Sometimes external measurements of the donor chest (e.g. the submammary thoracic perimeter[11]) are also made, to compare with those of the recipient chest. Given the great variability in muscle and fat mass around the thoracic cage (and the variable size of breasts in women) a comparison of chest radiographs probably provides a more accurate assessment. The chest radiographs to be compared should be taken at a standard distance from the subject (e.g. 1 meter), preferably with both subjects in a supine position. If possible, both anteroposterior and lateral films should be available for comparison.

Measurements on the chest radiograph that have been found useful are illustrated in Figure 1. Alternative measurements that have been used at some centers are: (a) sternal notch to xiphoid, (b) sternal notch to lower rib margin, (c) sternal notch to acromion, (d) chest diameter halfway from sternal notch to lower rib margin, and (e) chest diameter at the level of the lower rib margin. All of these measurements can be readily obtained from the potential recipient during his or her initial evaluation, and from the donor at the time when brain death is established.

When the internal thoracic diameters of the donor are larger than those of the recipient, the donor may need to be excluded from donation for that recipient, although experience is developing in reducing the size of the donor lung when necessary (Chapters 51 and 52). When the internal thoracic diameters of the donor are up to 25% less than those of the recipient, the donor will generally be acceptable.

Some centers no longer rely on comparison of chest and/or chest radiograph measurements, but prefer comparisons of height, weight, and total lung capacity (measured or predicted)[12,13]. The total lung volume of the donor (and the recipient) can be calculated approximately from a knowledge of his/her height and sex (Figure 2)[14]. This is probably the most accurate guideline for the size of lung that could be accepted by any individual recipient.

The *actual* measured total lung volume of the recipient is likely to be increased in the emphysematous patient and restricted in the patient with pulmonary fibrosis. In the case of the emphysematous patient, the optimum size of the donor lung is difficult to determine; as a general guide the *estimated* size of the donor lungs should be approximately 80% of the recipient's *measured* total lung capacity[12,13]. This may necessitate selecting lungs which have a greater volume than that *estimated* for the recipient by the height/sex-based formula.

The experienced Barnes Hospital group from St Louis[15] attempt to provide a prospective recipient with donor lungs that would be an appropriate size for the patient if the thoracic configuration were not altered by his or her lung disease. For single lung recipients they attempt to oversize the donor lung. In contrast, for double lung recipients they make an effort not to oversize, so as to facilitate recipient chest closure. In general, patients with pulmonary fibrosis receive a lung larger than the

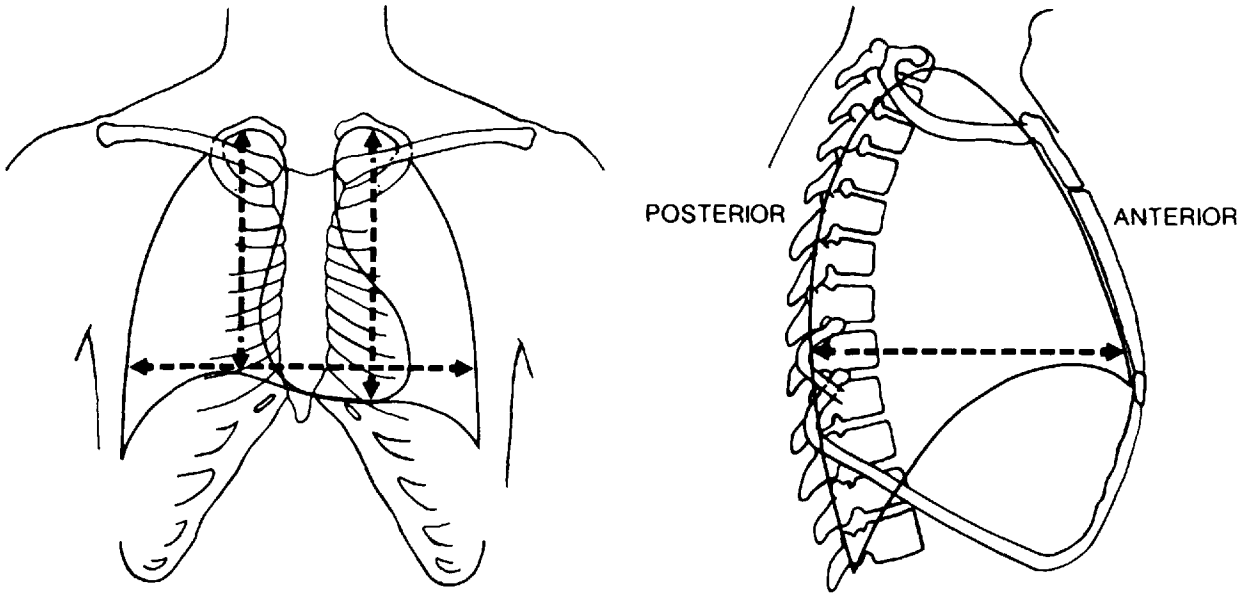

Figure 1 Measurements of anteroposterior and lateral chest radiographs found helpful in assessing relative sizes of donor lungs and recipient thoracic cavity. All measurements are made on chest radiographs ideally taken in the supine position with the camera at a set distance from the radiographic plate. Measurements include: (a) vertical distance from the apex of the pleural cavity to the diaphragm on both right and left sides; (b) the transverse diameter of the widest point of the chest (this is usually near or at the costophrenic angle). If lateral chest radiographs are available (which is rarely the case with the donor) then (c) the anteroposterior diameters from the anterior surface of the vertebral column to the posterior surface of the sternum, and from the posterior curvature of the ribs to the back of the sternum (both of these measurements being made at the midsternal and diaphragmatic levels), can provide additional information.

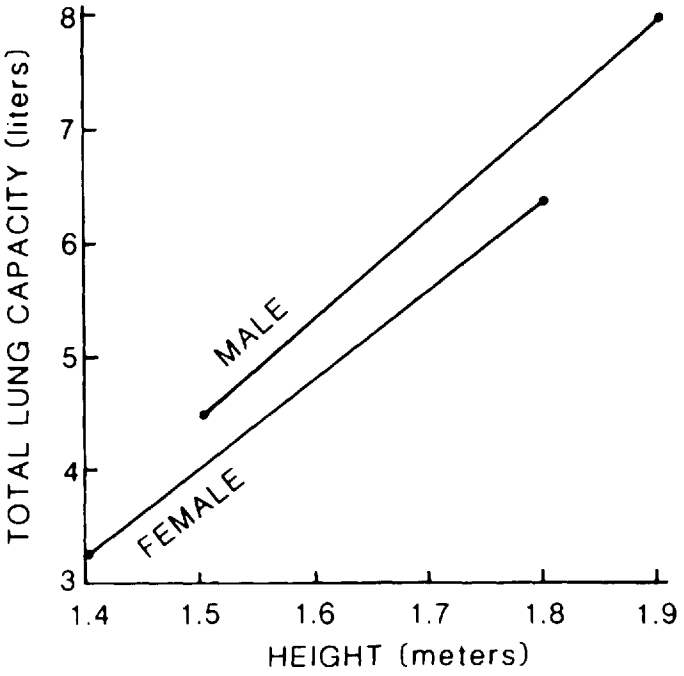

Figure 2 The total lung capacity of the donor can be calculated approximately from the knowledge of his/her height and sex (modified from ref. 13)

native one, and patients with emphysema receive a lung smaller than the native one(s). This rationale is based on observations of mediastinal shift, diaphragm realignment, and chest cavity changes subsequent to lung transplantation. To date this group has observed no difference in outcome or in function related to whether a right- or left-sided single lung implant is placed. For patients with pulmonary hypertension, a single lung that is larger (by 30%) than the size of the native lung is preferred, in order to accommodate the pulmonary blood flow, which is almost all preferentially directed into the allograft due to the elevated vascular resistance in the remaining native lung.

For transplantation of the heart and both lungs a discrepancy of 2–4 liters in pulmonary capacity between donor and recipient can generally be tolerated without the risk of complications. Atelectasis and intrapulmonary shunting have been reported when donor lungs that were too large for that particular recipient were transplanted[13].

Blood group compatibility

It is essential to have ABO blood group compatibility between donor and recipient (Chapter 6). There is an estimated approximate 60% risk of early hyperacute or accelerated acute rejection if ABO incompatibility is present[16]. There are data that suggest that a recipient who receives a heart from an ABO-identical donor (e.g. O to O, or A to A) may survive longer than one who receives a heart from an ABO non-identical, yet compatible, donor (e.g. O to A)[17] (Chapter 6). The shortage of donors is such, however, that most groups will transplant if there is ABO compatibility. Rhesus blood group compatibility is not considered to be of importance.

Presence of lymphocytotoxic antibodies in the recipient

Whenever lymphocytotoxic (LCT) antibodies have been demonstrated to be present in the recipient serum (by prior screening against a panel of lymphocytes), the results of a donor lymphocyte–recipient serum crossmatch should be obtained if at all feasible (see also Chapter 6). Some groups would consider this may not be essential if the level of panel reactive antibodies (PRA) was <10–15%. In the presence of a positive crossmatch (demonstrating antibodies to be present in the recipient serum against the donor cells), there may be a significant risk of hyperacute rejection of the transplanted organ and, unless expert opinion is otherwise (Chapter 6), that donor should not be used for that specific recipient (except possibly if the recipient is likely to die before another donor becomes available, when the risk may be justified).

Exclusion of cardiac or pulmonary disease in the donor

Heart

Patients with pre-existing cardiac disease are obviously unsuitable for heart donation, as are those who have undergone thoracic trauma resulting in contusion of the heart. Previous surgery within the pericardial cavity usually also precludes donation. A history of: (a) severe or long-standing diabetes mellitus, particularly if known to have been poorly controlled or when there is evidence of microvascular disease; (b) long-standing systemic hypertension, particularly if it was known to be resistant to antihypertensive drug therapy or if poorly controlled through the patient's non-compliance; and (c) smoking for many years, may all preclude donation of the heart, or at least indicate the need for very careful assessment of cardiac function, particularly in older donors. For example, features suggestive of ischemia or significant left ventricular hypertrophy on ECG or echocardiography may be sufficient to abandon the heart, or to proceed to coronary angiography and left ventriculography.

The presence of a gross cardiac disorder can generally be excluded by: (a) taking, whenever possible, a clinical history from the patient's relatives or his/her own medical practitioner; (b) careful clinical examination; (c) chest radiography; (d) 12-lead electrocardiography; and (e) echocardiography[18]. However, invasive studies may be required when there is a high suspicion of the presence of cardiac disease from the donor's history. If murmurs or added sounds are not to be missed, it is essential that the clinical examination be performed when a normal arterial pressure is present. Intracranial damage itself may cause ST and T wave changes on the ECG[19,20], which may not be important if all other investigations suggest a normal heart. Hypothermia leads to bradycardia and/or presence of J waves, which are of no pathologic significance, but can be confused with ECG changes suggestive of ischemia.

Echocardiography to determine ventricular wall shortening fraction should also be carried out to give some indication of the quality of myocardial contractility. Septal motion is often paradoxical in brain-dead subjects, though the cause of this is unclear; in our experience its presence does not appear to be associated

with impaired ventricular function in the post-transplant period. It may be unwise, however, to accept a heart where the left ventricular ejection fraction (LVEF) is significantly <50%, particularly if the donor is receiving moderately high doses of an inotropic agent (e.g. dopamine at 12–15 μg/kg per minute). Imaging is not easy in some donors, and transesophageal echocardiography should be considered if transthoracic visualization is not adequate[21].

Individual judgement is required in each case, and the experienced transplant surgeon may accept a heart that has a LVEF <50%. In a study from the University of Virginia at Charlottesville[22], cardiac dysfunction was defined by an ejection fraction <45% and/or a significant segmental wall motion abnormality using standard two-dimensional echocardiographic criteria. These 'marginal' donors were considered acceptable at the time of inspection if: (a) there was no obvious reason for poor function, such as the presence of coronary artery disease or cardiac trauma; (b) the inotropic support requirement was only modest (<10 μg/kg per minute dopamine); (c) hemodynamic parameters were otherwise normal; and (d) the donor heart appeared viable without evidence of cardiac contusion.

More sophisticated tests that assess left ventricular function (such as monitoring of the left ventricular pressure–volume relationship[23]) can be employed, but are more invasive and are frequently unavailable. Measurement of total serum creatinine phosphokinase (CPK) and the CPK-MB isoenzyme should be carried out in cases in which myocardial injury is suspected, and caution should be used in decisions regarding hearts from donors in whom the CPK-MB isoenzyme is extremely elevated. Cardiac troponin I, a sensitive and highly specific marker of myocardial injury[24], is also being explored at some centers in an attempt to detect unsuspected myocardial injury.

Ideally, there should be no history of severe hypotension or cardiac arrest at any time. Recovery from such episodes, however, with return of an adequate blood pressure and diuresis, suggests that myocardial function remains satisfactory. Cardiac arrest requiring a prolonged period (e.g. more than a few minutes) of external cardiac massage, or repeated cardiac arrests, or frequent or intractable episodes of ventricular dysrhythmias, are generally contraindications for use of the heart, unless a long period of stable myocardial function has subsequently been clearly documented.

Many cardiac surgeons believe that the most reliable means of assessing donor heart function is by direct inspection of the organ at the time of procurement, and this is clearly a most important part of donor selection. It is therefore essential that an experienced surgeon should assess each donor heart on an individual basis. Donor heart assessment and retrieval is not a procedure that can safely be left to an unsupervised junior member of the surgical team.

The UCLA group[25] has performed coronary artery bypass surgery on donor hearts with localized atherosclerosis. The surgery has been performed *ex vivo* while the donor heart is still stored in cold saline. Although this has enlarged the donor pool, and has been relatively successful, this procedure is not without its risks and should not be undertaken without very careful consideration.

Lung

Patients with known lung disease are clearly unsuitable for donation. This would include patients with obvious chronic obstructive airway disease or pulmonary fibrosis, as well as lung trauma. Previous surgery within the pleural or pericardial cavities usually precludes donation. The presence of a chest tube (frequently placed to treat a pneumothorax following insertion of a central venous pressure line) is not a contraindication to use of the lung if there is no significant air leak. Patients with infections of the lung, whether chronic or acute (pre- or post-brain death), are also usually excluded. The presence of neurogenic pulmonary edema, a not-uncommon sequel to the hemodynamic changes that take place during brain death, is generally exclusionary. An acutely failing donor heart generally results in poor lung function and therefore may preclude lung donation, but the presence of an acute or chronic cardiac disease (e.g. coronary artery disease) in itself is not a contraindication to lung donation if pulmonary function remains good.

A history of asthma is a borderline contraindication. If severe and prolonged for many years, then this may be an absolute contraindication. Evidence suggests that asthma in the donor leads to asthma in the recipient[26].

Although smoking is not exactly a 'disease', patients who have smoked a total of more than 20 pack-years (i.e. one pack a day for 20 years or equivalent) are generally excluded on the grounds that their lungs may have lost some elastance.

Pulmonary function, as assessed by compliance and gas exchange, may require repeated assessment to ensure that no significant deterioration is occurring before the lungs are excised. With positive end-expiratory pressure (PEEP) of 5 cmH_2O and an F_iO_2 of 0.4 (40% O_2), the Pao_2 should be >100 mmHg. On an F_iO_2 of 1 (100% O_2), the Pao_2 should be >300 mmHg. Lung compliance should exceed 0.1 l/cm H_2O at a tidal volume of 10 ml/kg. In a patient with a non-obstructive endotracheal tube *in situ*, an inspiratory volume of 15 ml/kg should not result in a *static* pressure of more than 20 cmH_2O being measured. This will indicate a relatively normal lung compliance. The measurement should be repeated three times and an average taken. Possibly the simplest way of testing lung compliance is by increasing the ventilatory tidal volume from a minimum 500 ml in an adult by 100 ml increments to 1000 ml; *peak* inspiratory pressure should not rise above 30 mmHg. Airway resistance, though not a routine measurement (on mechanical ventilation), should be <1.6 cm H_2O/l per second[27,28].

In order to achieve or maintain good lung function, fluid restriction is necessary, especially with respect to crystalloid solutions. Close monitoring of central venous pressure (which should be maintained as low as possible compatible with the maintenance of other vital organs), and cautious application of catecholamines are mandatory. Prolonged artificial ventilation, an F_iO_2 exceeding 0.5 (50%), and positive end-expiratory pressures >10 mmHg should all be avoided, if possible.

Due to nosocomial infections, mechanical ventilation in brain-dead subjects for more than 4–7 days will generally preclude use of the lungs for transplantation. The chest radiograph should be clear of major pulmonary infiltrates, including post-traumatic opacifications, suggesting contusion or infection, though some would not decline use of the lungs on these grounds alone if there were no confirmatory evidence of pulmonary damage or infection. Minor areas of infiltrate may not preclude donation[15]. Before the donor operation, frequent aseptic and thorough endotracheal suction and toilet is mandatory, and a broad-spectrum antibiotic should be administered (see below).

The majority of donors have bacterial colonization of the tracheobronchial tree despite normal radiographic appearances. Therefore, specimens should be taken before (optimally, via a bronchoscope) harvesting, to allow immediate Gram stain and cultures[29-31]. The presence of purulent sputum with a large number of organisms on Gram stain, especially if of only one type and if intracellular, indicates the presence of an active infection and may be a contraindication to donation. Some centers, however, might accept such a donor if the offending organism(s) had been identified and specific antibiotic therapy initiated in both donor and recipient. The presence of some neutrophils but no organisms on Gram stain will generally not preclude donation. Postoperative antibiotic treatment can be based on the results of culture. The presence of heavy fungal colonization of the tracheobronchial tree is generally considered a contraindication to donation.

Many believe bronchoscopy to be essential before a final decision is made to accept a lung for transplantation[32]. In 26 otherwise acceptable donors, bronchoscopy was abnormal in 10 (38%) and was sufficiently abnormal in six (23%) to preclude lung donation. The allografts of two recipients whose donor had evidence of aspiration of gastric contents, but normal chest radiograph and gas exchange, failed at 15 and 12 days post-transplantation. The allografts of two recipients whose donors had evidence of aspiration of blood only had uncomplicated post-transplant courses. Certainly, if: (a) the chest radiograph shows any abnormality in the lungs; (b) the endotracheal aspirate is purulent, or significant microorganisms are seen on Gram stain; or (c) there is a possibility of aspiration of gastric contents into the tracheobronchial tree during the course of the donor's care, then fiberoptic bronchoscopy should be performed. Inflammation of the walls of the bronchi with or without the presence of purulent secretions/gastric contents strongly suggests aspiration, and contraindicates use of the lung(s).

Based on bronchoscopy, the absolute contraindications to accepting a donor lung are grossly purulent secretions (especially if voluminous) and the finding of aspirated foreign material or evidence thereof (e.g. severe erythema and tracheobronchitis)[14]. Blood entering the airways is obviously common after head trauma. With the passage of time the blood imparts a distinctly brown coloration to the bronchial secretions, which can be distinguished from pus. If this can be cleared by suctioning, and the bronchial mucosa appears normal, the lung is generally considered suitable for use[15].

The University of Virginia group consider a lung donor to be marginal with a Pa_{O_2} of <350 mmHg on 100% F_iO_2, the presence of infiltrates on chest radiograph, or purulent secretions on bronchoscopy[22]. However, they found these marginal lungs frequently acceptable if the patient had been on a ventilator <24 h and the secretions could easily be cleared by suctioning, even if purulent material was present. A Gram stain revealing bacteria did not exclude a donor unless there was evidence of gross fungal contamination.

The Barnes Hospital group in St Louis considered a lung donor to be marginal with a Pa_{O_2} <300 mmHg on F_iO_2 100%, or when radiographic infiltrates or evidence of trauma were present in either lung. There was, however, no difference in outcome as defined by: (a) $A\text{-}a_{O_2}$ gradients, (b) time on ventilator, and (c) 30-day survival, between marginal and ideal donors[33].

Transferable disease

Neither heart nor lung should be transplanted from donors with transferable disease, such as a malignant lesion (other than a primary tumor of the central nervous system, which generally does not metastasize elsewhere) (Chapter 12) or certain serious infections.

The presence of pyrexia in the hours or days before death may be related to the brain injury itself, and may not necessarily indicate serious infection, although every effort must be made to exclude this possibility. Once brain death has occurred, body temperature usually falls to subnormal levels over the course of a few hours. The length of time that the patient has been ventilated mechanically is equated with an unavoidable degree of pulmonary infection, overt or otherwise, and possibly also with some lung injury; not more than 3 days is desirable, particularly in the case of the lung, and longer than 7 is usually unacceptable even for the heart.

The presence of overt acute pulmonary infection almost certainly precludes donation of the lungs, but does not rule out heart donation. In one study from St Louis, in 43% of cases in which bronchial washings from donor lungs grew at least one organism, similar organisms were isolated from the tracheobronchial tree of the recipient[34]. Six of these recipients (21%) subsequently had invasive pulmonary infection as a result of the organism originally isolated in the donor. The St Louis group recommended that antibiotic coverage in transplant recipients should be initiated on the basis of Gram stain results, and modified on the basis of cultures obtained from the donor lungs. Others also follow this policy[29,31,35].

Infection in the renal tract of the donor is not a contraindication to use of the heart or lungs. Many hearts and some lungs have been transplanted successfully from donors with positive blood cultures; the decision to use an organ from such a donor is a difficult one, and not without risk, but, if the infected organism is known, the recipient can be administered the appropriate antibiotic(s). The policy of many groups in regard to the chance finding of a positive blood culture is liberal; if the patient has clinical features of sepsis, however, the organs should not be excised for transplantation.

Positive human immunodeficiency virus (HIV) antibody serology should preclude transplantation, and this test should clearly be performed before donor organ excision[36]. When positive, if time allows, the test should ideally be repeated, as false-positive results can occur. If the donor is believed to be 'high risk' for HIV positivity (e.g. homosexuals, intravenous drug abusers, hemophiliacs), and yet the HIV antibody serology remains negative, the decision whether to use the organ must be based on the urgency of the potential recipient's condition, after full discussion with the patient and/or his or her family. When making such a decision, the possible risks to operating room, intensive-care unit, and laboratory staffs must also be taken into consideration. Current opinion is that organs from 'high-risk' donors should probably not be used[37]. Transfer of HIV has been reported with kidney, liver and heart transplants with poor survival[38-42]. In five such heart transplants, only two patients had survived with a mean follow-up of 2.75 years.

Blood specimens from the donor are taken for bacterial culture and serologic tests for syphilis, cytomegalovirus, hepatitis B

surface antigen (HBsAg), and hepatitis C serology[36] (which are essential), and Epstein–Barr virus, herpes simplex virus, *Toxoplasma*, and hepatitis A (which may also prove to be valuable). The results, especially for hepatitis B and C, should be available before the organ is excised. If, under unusual circumstances, the transplantation has already taken place when the results become available, the result, if positive, may be of considerable importance in the subsequent care of the recipient.

A thoracic organ from a donor known to be HBsAg-positive should probably not be utilized unless the recipient has previously been vaccinated against hepatitis B. When the need of the recipient is extremely urgent, however, this should possibly not prevent donation, as long as the recipient is covered by a course of gamma-globulin.

Hepatitis C may take several years to cause clinical symptoms, although its progress may be much more rapid in an immunosuppressed subject. The decision to use a thoracic organ from an anti-hepatitis C antibody-positive donor is, therefore, a difficult one, and depends to a great extent on the status of the potential recipient. The recipient almost always seroconverts after transplantation[43]. Even if he or she does, clinical or biochemical features of hepatitis may not develop for several years. We have therefore chosen to transplant such hearts into patients whose life expectancy is short (i.e. days), who are deteriorating in an intensive-care unit despite inotropic, intra-aortic balloon pump with or without ventilator support. Availability of therapy with interferon in the post-transplant period (if the recipient seroconverts) has made us slightly more liberal in the use of hepatitis C-positive donors in such cases. However, the development of clinical hepatitis in the recipient can be fatal, and the decision to risk this eventuality should not be taken without due consideration, which includes full discussion with the patient and his/her family.

With regard to organ transplantation in hepatitis C-positive recipients and the use of hepatitis C-positive organs, Milfred *et al.* carried out a survey of 45 cardiothoracic transplant centers[44]. Seventy-five percent stated that they would list an HCV-seropositive candidate (either directly or by lack of routine screening to exclude such a patient). Only 16% would not accept HCV-seropositive candidates. Nine percent had no policy. Overall, 69% accepted organs from HCV-seropositive donors, at least for selected recipients. Twenty-two percent of the centers said they would accept an HCV-positive donor for any recipient (although a knowledge of the practice at other centers does not support this surprisingly high figure), 45% would select for only HCV-seropositive and/or urgent (UNOS status I) recipients, and 2% would not screen donors and would therefore be unaware of the HCV status of the donor. A total of 27% of the centers would never accept organs from an HCV-seropositive donor, and 4% had no policy towards this issue.

The presence of a positive test for syphilis in the donor need not preclude donation, but it would seem wise to give the recipient a course of antibiotic therapy to prevent transfer of disease. An IgM level suggestive of recent cytomegalovirus infection would generally not preclude use of the heart for transplantation, though an occasional group would not use a CMV IgM- or IgG-positive donor for lung or heart–lung transplantation in a cytomegalovirus-negative recipient.

Toxoplasma has been transplanted with the donor heart[36,45], and unless clinically suspected in the donor is likely not to be diagnosed until after transplantation, sometimes purely on endomyocardial biopsy[45].

Effects of drugs of addiction/substance abuse on the donor organs

Cigarette smoking is probably the most common form of substance abuse and a high percentage of donors will have such a history. This may clearly have a direct effect on the heart or lungs to be used for transplantation, by contributing towards ischemic heart disease and/or emphysema. These matters have been discussed above.

Marijuana/heroin

The smoking of marijuana is also fairly common. If only occasional, it probably is of little significance, either as a contributing factor toward injury to the organ or as an indicator that the donor may be in the high-risk category with regard to HIV or hepatitis. If marijuana use has been heavy, particularly if it has extended over many years, the suspicions of the surgical team must be aroused to the possibility of the intravenous use of other drugs (e.g. heroin) that would increase the possibility of the donor having been exposed to HIV or hepatitis. The history of drug use is frequently inadequate and vague, making decisions of this nature difficult. Physical evidence for intravenous drug abuse (e.g. needle marks in the cubital fossa) should be sought. In the absence of positive serology, and when the clinical history of intravenous drug abuse is certain or strongly suspected, the risks of transfer of an infectious agent to the recipient have to be weighed against the current status of the recipient for whom the organ is intended.

Cocaine

The increasing use of cocaine worldwide – in the USA an estimated 6 million individuals use the drug on a regular basis – leads to the supposition that some donor organs are from unknown cocaine users. Toxicology screening of all donors may more accurately define the incidence of cocaine usage in the donor population. Cocaine produces vasoconstriction, which results in a reduction in coronary artery diameter and coronary blood flow. Cocaine use appears to be associated with a variety of cardiovascular diseases, including ischemic events, myocarditis, and myocardial dysfunction[46–48]. Electrocardiographic and echocardiographic abnormalities are common in long-term cocaine abusers. Several autopsy and endomyocardial biopsy studies in cocaine users have shown contraction band necrosis, myocyte necrosis, and diffuse inflammatory cell infiltration.

Minor *et al.*[46] reported cocaine-induced myocardial infarction in patients with normal coronary arteries. Experimental evidence suggests that cocaine has direct and indirect sympathomimetic effects on vascular smooth muscle, attenuates endothelium vasodilator capacity, exerts a potent depressant effect on cardiac myocytes, and promotes atherogenesis. Cocaine-induced myocardial infarction in patients with normal coronary arteries therefore probably involves adrenergically-mediated increases in myocardial oxygen consumption, vasoconstriction of large epicardial

arteries or small coronary resistance vessels, and coronary thrombosis. Accelerated atherosclerosis and impairment of endothelium vasodilator function may occur after chronic cocaine use.

Warner has reviewed cocaine abuse in some detail[48]. In the USA 7% of adults between 18 and 34 years old had used cocaine during the previous year. In the 1970s and 1980s the development of 'free base' and 'crack' cocaine revolutionized the use of the drug by providing a form that could be smoked. Cocaine can be absorbed through any mucous membrane or injected intravenously. Ninety percent of cocaine users have snorted cocaine, making intranasal insufflation the most common route of use. About one-third of users have smoked the drug, and fewer than 10% have injected it. Free base and crack are the same chemical form of cocaine, but are made using different techniques.

Complications associated with cocaine use include chest pain, myocardial infarction, arrhythmias, cardiomyopathy, myocarditis, pneumothorax, pneumomediastinum, pneumopericardium, pulmonary edema, exacerbation of asthma, pulmonary hemorrhage, bronchiolitis obliterans, and 'crack lung'.

The use of hearts and lungs from donors with a history of cocaine abuse remains controversial. Information regarding the route of administration, purity, quantity, and duration of cocaine exposure is frequently incomplete. Traditionally, hearts from cocaine-using donors have been excluded from procurement. Certainly, if a history of intravenous drug abuse is elicited, such hearts are declined. Routine two-dimensional echocardiography and the inspection of the donor heart by the surgeon at the time of procurement may help to identify and exclude any hearts with significantly depressed global myocardial function.

In a study from Los Angeles, however, 16% of donors had a positive history for non-intravenous cocaine use[49]. No detrimental effects of cocaine usage on donor heart function, recipient survival, hospital stay, or rejection frequency could be detected. Survival rates between this group and those who did not have such a history were similar at 30 days and at 1 year. Freedom-from-rejection rates were also similar at 30 days and 6 months. The investigators concluded that the outcome of patients who received transplanted hearts obtained from non-intravenous cocaine users was favorable, suggesting that the use of such hearts is safe.

Among the Los Angeles patients, none of the initial endomyocardial biopsies carried out within the first 2 weeks after transplantation showed significant inflammatory infiltrates or myocyte necrosis. This finding could possibly be explained by: (a) preselection of hearts without global dysfunction, (b) exclusion of intravenous drug users as donors, and (c) the time elapsed from the last exposure of cocaine until the first biopsy.

Since chronic and irreversible lung dysfunction secondary to cocaine use has not been demonstrated, most groups would not decline a lung from a donor solely on the grounds that he or she was a cocaine user.

Alcohol

A report from Paris investigated the relatively high proportion (approximately 20%) of donors in France who had a history of chronic alcoholism, which was found to be a very detrimental factor to survival of the recipient after heart transplantation[50]. Fifty-four percent of patients who had early cardiac graft dysfunction versus only 12% of patients who had immediate normal graft function had received a graft from an alcoholic donor. Among the early deaths from cardiac dysfunction, 40% of patients had an alcoholic donor. Excluding such alcoholic donors or reserving them for critically ill recipients would seem preferable.

Preclinical alcoholic cardiomyopathy seems to be independent of the duration of chronic alcoholism and of the quantity of cumulative alcohol intake. This latent cardiomyopathy may be unmasked in the recipient because of the particular hemodynamic conditions to which the myocardium is exposed, e.g. myocardial ischemia, elevation of right and left heart filling pressures. The condition can be diagnosed with echocardiography by demonstration of increased ventricular wall thickness and by left ventricular filling impairment without, at this stage, any alteration in the parameters of contractility.

Chronic alcoholism in the donor would not appear to be a contraindication to use of the lungs for transplantation.

Effects of poisoning on the donor organs

There are few reports on the use of thoracic organs from donors who have died of poisoning. In Belgium, 12 poisoned 'brain-dead' patients were considered as organ donors[51]. The toxic substances were various, but included barbiturates, insulin, carbon monoxide, and cyanide. Two heart transplant patients died within 24 h from stroke and acute heart failure, respectively. The Brussels group, although not absolutely excluding organ procurement from a few select donors with acute poisoning, drew attention to the risk of graft damage resulting from some poisons.

Cyanide

Most cyanide poisoning victims do not survive, due to rapid lethal massive cellular anoxia within minutes, particularly if large amounts have been ingested[52]. Reported mortality is as high as 95%. Due to its rapid absorption and paralysis of cellular respiration, the time from ingestion to treatment is critical. Cyanide causes cytotoxic intracellular anoxia. Cellular oxygen utilization is blocked, and anaerobic metabolism with glucose ensues, leading to lactic acidosis. There is consequent respiratory failure due to the high susceptibility of the central nervous system (CNS) to anoxia. The cyanide–cytochrome oxidase complex that is formed, however, is dissociable, with rapid recovery of respiration upon cyanide removal.

Cardiac studies have shown a direct myocardial toxic effect manifested by left ventricular dysfunction and conduction abnormalities. Cyanide causes vascular smooth muscle contraction, arterial vasoconstriction, and accelerated myocardial ischemia. While the myocardium is very susceptible to cyanide anoxia, almost complete dissociation of the cytochrome oxidase enzyme–cyanide complex has been demonstrated.

Intentional cyanide poison victims are not usually considered for organ donation. With rapid antidote administration and close follow-up, however, the cyanide victim with irreversible CNS damage may have organs with reversible cyanide effects that may be suitable for transplantation. A heart has been transplanted from a patient who committed suicide by cyanide ingestion[52]. After transplantation, the recipient was initially on multiple low-dose hemodynamic agents to augment cardiac output, but was successfully weaned off of these on the seventh postoperative day.

Presumably, in such cases of reversal of cyanide effects, the lungs could also be considered for donation.

Carbon monoxide

The potential reversibility of hypoxic organ damage induced by carbon monoxide inhalation depends on the amount of hemoglobin bound to carbon monoxide and on the tolerance of the organ to hypoxemia[53]. It has been suggested that death from carbon monoxide poisoning with a blood carboxyhemoglobin level >20% is an absolute contraindication to use of the heart, and a level <20% is a relative contraindication[21].

Hearts from brain-dead victims of carbon monoxide poisoning have, therefore, generally been reported to be unsuitable for heart transplantation. However, heart transplants have been performed successfully using such donors[54,55]. Carbon monoxide poisoning is therefore not an absolute contraindication to cardiac allograft procurement in the setting of clinical and objective evidence of satisfactory cardiac function.

Iberer *et al.*[55] reported that, despite early post-transplant ECG abnormalities and elevated serum transaminase levels suggesting myocardial damage, after 16 days the ECG reverted to normal. No evidence of ischemic areas or myocardial cell necrosis could be found in any heart biopsies. At the time of reporting, the patient was doing well 4 months post-transplant.

A report from Australia documented two patients who underwent successful transplantation with cardiac allografts from donors who had suffered acute carbon monoxide poisoning[54]. The authors suggest that an ECG, assay of cardiac enzymes, and echocardiography are required to assess the degree of myocardial injury in potential heart donors. Cardiac enzyme elevation suggests severe myocardial injury, but transient elevations may be due to hypoxic injury to other organs. The myocardial effects of acute carbon monoxide poisoning, however, may be delayed. These authors therefore recommend that a short period of observation be incorporated in the overall assessment of the potential heart donor, and suggested that careful consideration of the following variables may minimize any potential risk in using carbon-monoxide-exposed hearts for transplantation: (a) normal ECG and satisfactory echocardiographic findings, (b) minimal elevation of cardiac enzymes, (c) requirement for minimal inotropic support, (d) a short ischemic time, (e) favorable donor-to-recipient weight ratio, and (f) the avoidance of recipients with high pulmonary vascular resistance.

There is one report of successful transplantation of a lung from a donor with carbon monoxide poisoning[56], but presumably the same cautious guidelines apply.

Anticipated donor organ cold ischemic period

Most surgeons would prefer to have the donor organ (heart or lung) reperfused by recipient blood within a maximum of 4 hours after aortic crossclamping in the donor (Chapter 23). If the anticipated cold ischemic period is likely to be much longer than 4 h (e.g. because of the long distance between donor and recipient centers) the surgical team may decide to decline acceptance of the donor on these grounds alone, particularly if: (a) the donor has other 'borderline' characteristics or (b) the recipient has other factors that may complicate the procedure. There is a natural tendency on humanitarian grounds to accept a long ischemic time (with or without a borderline donor) for critically ill potential recipients (e.g. those on ventilators with or without intra-aortic balloon pump with or without high doses of inotropic therapy) for whom there is doubt that a subsequent donor will become available before the patient dies. Such as decision, though understandable, may substantially increase the risk of failure or major complication of the operative procedure, and it may be prudent to decline such a donor.

Although the surgeon's primary concern is to the patient most in need of the transplant, he or she must never fail to remember the many other patients awaiting organ transplantation throughout the world. Failure of a transplant from an accumulation of risk factors may have prevented successful transplantation in another, more suitable, recipient. A poor decision in such circumstances may be associated with three deaths: (a) borderline donor, (b) high-risk recipient, and (c) the low-risk recipient who may subsequently die through lack of a suitable donor organ.

Cold ischemic times of >4 h have frequently been reported, but are associated with an increased risk of early donor organ failure (Chapters 43 and 44). The pediatric heart appears to be less susceptible to cold ischemia, and cold ischemic intervals of >9 h have been documented.

Perhaps surprisingly, in view of its delicate structure, the adult lung may be more tolerant of cold ischemia than the heart, as cold ischemic times <8 h have been reported[57]. As a general guideline, however, the risk of early organ failure is likely to increase if ischemic times of >4–5 h are incurred.

Comment

In an important review of 1719 consecutive primary cardiac transplants performed at 27 institutions, Young *et al.*[58] documented that risk factors for death of the recipient included: (a) old donor age ($p = 0.001$); (b) smaller donor body surface area (female donor heart placed into larger male patient) ($p = 0.003$); (c) greater donor inotropic support ($p = 0.01$); (d) donor diabetes mellitus ($p = 0.01$); (e) longer ischemic time ($p = 0.0003$); (f) diffuse donor heart wall motion abnormalities by echocardiography ($p = 0.06$); and (g), for pediatric donors, death from causes other than closed-head trauma ($p = 0.02$). The 30-day mortality rate was 7%, but increased to 11% when donor age exceeded 50 years, and was 12% when inotropic support exceeded 20 μg/kg per minute dopamine plus dobutamine, and 22% with diffuse echocardiographic wall motion abnormalities. The interaction of donor risk factors was such that the heart of the smaller female donor given high-dose inotropes placed into a larger male recipient produced a predicted 30-day mortality rate of 26%, and the heart of a 25-year-old male donor given high-dose inotropes with diffuse echocardiographic wall motion abnormalities transplanted into a 50-year old male recipient led to a predicted 30-day mortality rate of 17%. The authors concluded that their analysis supported cautious extension of criteria for donor acceptance but with an anticipated greater risk in the presence of diffuse echocardiographic wall motion abnormalities and long ischemic time, particularly in older donors given inotropic support.

Such data based on a large number of lung transplant patients are not yet available.

THE EFFECTS OF THE AGONAL PERIOD AND BRAIN DEATH ON MYOCARDIAL AND PULMONARY FUNCTION AND STRUCTURE IN THE DONOR

The major source of donor hearts has been, and would appear to continue to be, persons dying of head injury or spontaneous intracranial hemorrhage. The adverse effect of brain dysfunction on the heart was demonstrated as early as 1954[59–63]. Electrocardiographic changes have been reported clinically in association with subarachnoid hemorrhage, intracranial infections, and cerebral tumors. Subendocardial hemorrhage and even myocardial necrosis have been reported in association with intracranial lesions. Electrocardiographic changes can be produced in animals by midbrain stimulation, and chronic stimulation produces myocardial necrosis; excessive sympathetic discharge may be etiologically responsible.

The pathological sequence of events that leads to brain death (i.e. the mode of death) varies to some extent depending on the cause of death (e.g. exsanguination, asphyxia, etc.). There is experimental evidence that the mode of death influences the extent of myocardial injury. For example, experimental studies have indicated that hearts arrested by exsanguination of the animal are easier to resuscitate than those arrested by asphyxia[64–66], and have therefore presumably suffered less injury and/or depletion of energy stores.

Studies in both experimental animals and potential clinical donors, which have been reviewed elsewhere[67,68], have suggested that brain death has major histopathological and functional effects[69–77].

Two major effects of brain death have been observed in experimental studies. The first is a series of electrocardiographic, hemodynamic, and histopathological changes which take place during and immediately following the agonal period[69,70,72,74,76]. The second consists of significant changes in circulating levels of certain hormones, which in turn result in major changes in body metabolism[69–71,75]. The time-course and nature of these events may be greatly modified under clinical circumstances.

'Autonomic (sympathetic, catecholamine) storm'

A sudden increase in intracranial pressure or the sudden onset of ischemia of the brain leads to a series of major pathophysiological changes which may collectively be referred to as the 'autonomic storm'[69,71,72,74]. Though there is a brief initial period of excessive parasympathetic activity, evidenced by a marked bradycardia, most of the effects are brought about by the sympathetic nervous system[71,72,74]; the terms 'sympathetic storm' or 'catecholamine storm' have therefore also been used to describe these events.

There is a large increase in circulating and endogenous catecholamines in the early few minutes after induction of brain death, which is associated with an increase in myocardial activity, together with the appearance on the EKG of ventricular arrhythmias[69]. Within 5 min of a sudden rise in intracranial pressure, circulating epinephrine, norepinephrine, and dopamine concentrations rise markedly, though they return to control levels within 10–15 min[69].

Electrocardiographic effects

When intracranial pressure is increased acutely (or the brain made acutely ischemic), an immediate bradycardia, progressing to sinus standstill with occasional junctional escape beats and a short period of atrioventricular dissociation, is rapidly followed by a sinus tachycardia and, subsequently, ventricular ectopic activity of multifocal origin[69] (Figure 3). A further period of sinus tachycardia follows but, on this occasion, with marked ischemic changes.

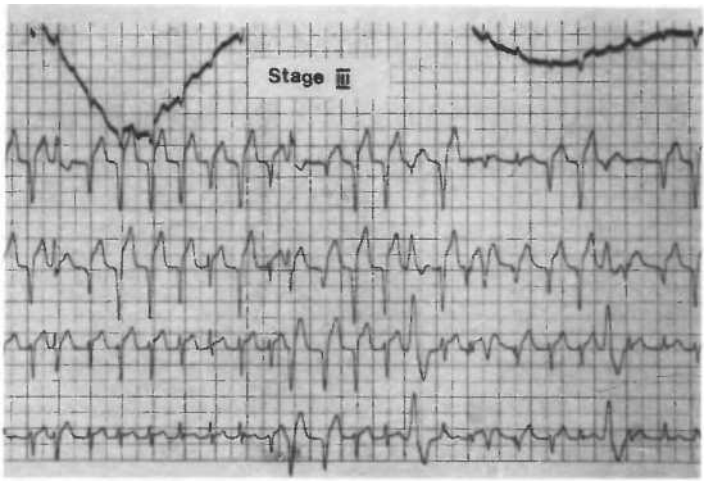

Figure 3 Electrocardiogram taken during development of brain death in an experimental animal showing multifocal ventricular extrasystoles

Factors which contribute to cardiac ischemia possibly include endogenous catecholamine release, spasm of coronary arteries (indicated by contraction band necrosis in the major coronary arterial walls), and a high systemic vascular resistance (SVR) from increased sympathetic activity which increases afterload and results in acute temporary myocardial oxygen supply–demand imbalance. This ischemia not infrequently results in myocardial histopathological damage, especially in the subendocardium, though this is rarely significant enough to exclude use of the heart for transplantation.

Finally, there is a reduction in rate to the pre-agonal level or below, with a regular rhythm. Fifty percent of experimental animals, however, continue to show abnormalities of the QRS complex and/or ST segment, including pseudo-infarct patterns. These ECG changes rarely indicate severe ischemic injury, and are believed to be due to loss of energy within the cell.

Hemodynamic effects

The hemodynamic changes observed in such experimental animals reflect the body's attempts to compensate for the intracranial changes taking place during 'coning' (Cushing's reflex)[71]. The classic picture is outlined below, but this may be greatly modified in the clinical situation.

Significant and often massive increases in SVR and mean arterial pressure (MAP) occur (Figures 4 and 5), and are almost cer-

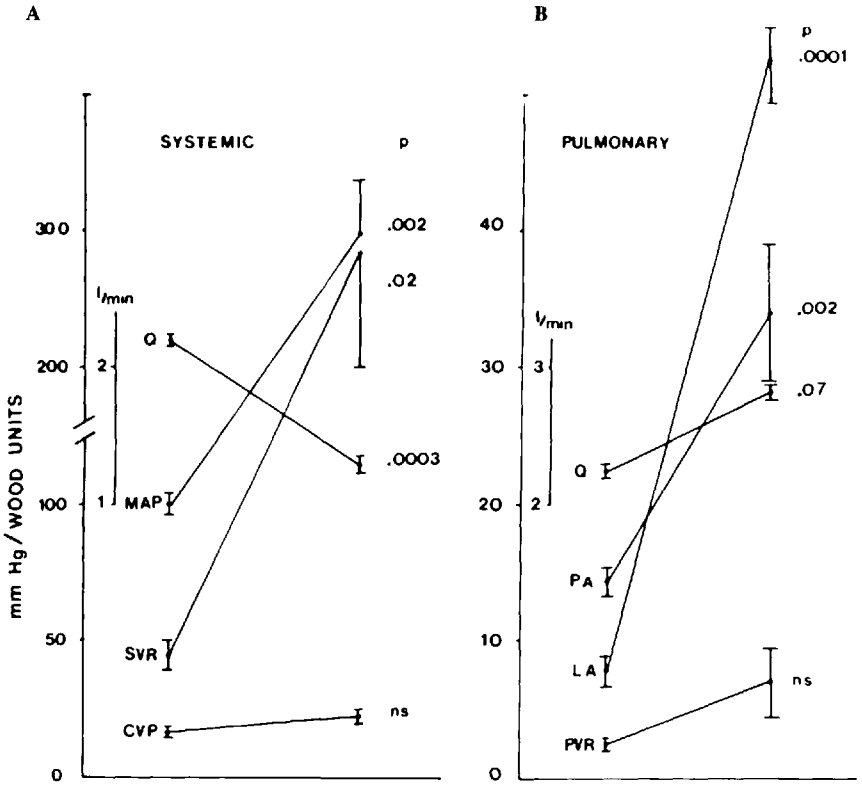

Figure 4 Mean changes in systemic and pulmonary hemodynamic data during the induction of brain death in eight baboons. The left hand graph shows changes in systemic hemodynamics between control levels (**A**) and those recorded at the peak of systemic vascular resistance (**B**). (MAP = mean arterial pressure (mmHg); SVR = systemic vascular resistance (Wood units); CVP = central venous pressure (mmHg); Q = aortic blood flow (l/min).) The changes in MAP, SVR and Q reached statistical significance. The right-hand graph shows changes in pulmonary hemodynamics between control levels (**A**) and those recorded at peak of systemic vascular resistance (**B**). (PA = pulmonary artery pressure (mmHg); LA = left atrial pressure (mmHg); PVR = pulmonary vascular resistance (Wood units); Q = pulmonary artery blood flow (l/min).) The changes in PA and LA reached statistical significance

tainly the direct result of a great increase in sympathetic nervous activity, which produces an extreme degree of peripheral vasoconstriction. Blood is therefore redistributed into the capacitance vessels, leading to a rapid accumulation within the great veins and right atrium. Due to a combination of low pulmonary vascular resistance (PVR), high pulmonary vessel compliance, and pulmonary capillary reserve recruitment, associated with a higher degree of right ventricular compliance compared to the left ventricle, the right ventricle is able to adjust to this increased venous return, and increase its output, demonstrated by an increase in pulmonary artery flow compared with aortic flow at this time[72].

The left atrial pressure may actually exceed the pulmonary artery pressure for a matter of seconds during the period of peak peripheral vasoconstriction[72]. This remarkable and surprising observation implies that the pulmonary capillary blood flow temporarily ceases entirely. The extreme rise in SVR which occurs during the agonal period almost certainly results in transient mitral regurgitation following temporary failure of the left ventricle. It would seem likely that it is during this period that disruption of the normal pulmonary capillary anatomy can occur. As the peak left atrial pressure far exceeds the normal hydrostatic pulmonary capillary pressure, capillary integrity within the lungs may be disrupted, resulting in pulmonary edema with high protein content and interstitial hemorrhage.

Histopathological effects

In approximately 75% of experimental animals undergoing brain death, histopathological changes develop in the left ventricular wall[69,71]. These consist of contraction bands, focal coagulative necrosis, and myocytolysis, with edema formation and interstitial mononuclear cell infiltration (Figure 6). Contraction band necrosis may even develop in conduction tissue and in coronary arterial smooth muscle.

In addition, over a third of the animals show pulmonary edema with an exudate rich in proteins (Figure 7); there is also evidence of capillary endothelial damage and, on occasion, diffuse hemorrhage, both in the alveolar walls and into the alveolar spaces[72].

All of these changes are believed to be the result of the catecholamine excess which occurs during the development of brain death, and are thought to be related to cytosolic calcium overload. Similar myocardial structural damage has been clearly documented in human potential organ donors[73]. Various combinations of the three forms of acute myocardial necrosis have been described; namely contraction bands, coagulative myocytolysis, and coagulative necrosis (colliquative myocytolysis). Focal mononuclear cellular infiltration has also been described surrounding areas of myocyte necrosis.

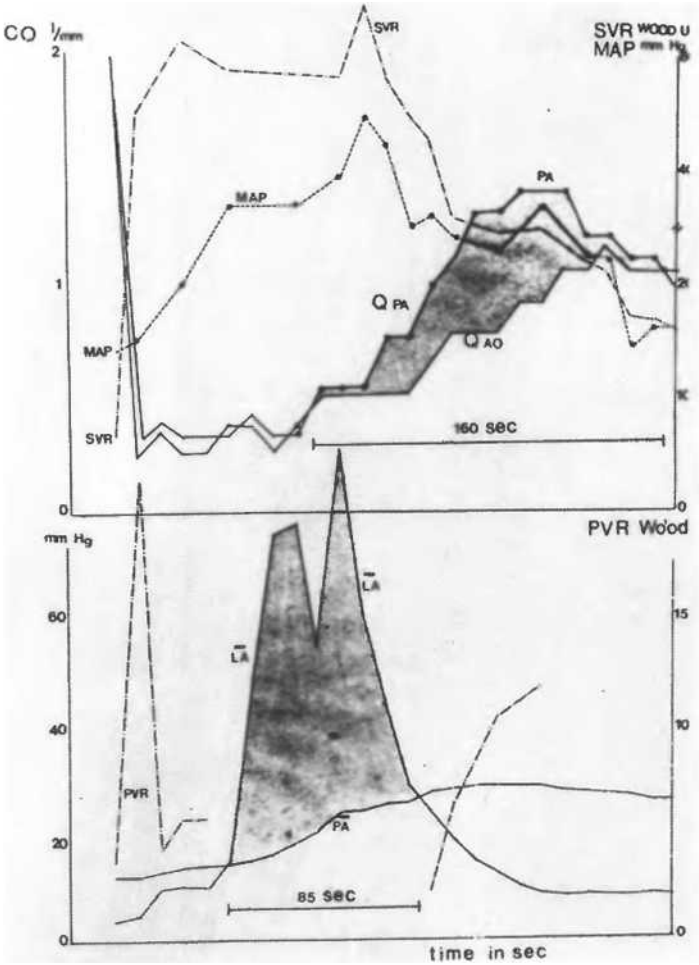

Figure 5 Systemic and pulmonary hemodynamic data during induction of brain death in a baboon. The upper graph shows changes in systemic vascular resistance (SVR) (Wood units), mean arterial pressure (MAP) (mmHg), pulmonary artery blood flow (PA) (l/min) and aortic blood flow (AO) (l/min). The discrepancy between pulmonary artery and aortic blood flows (shaded area) represents the period and extent of blood pooling within the lungs; in this case blood pooling extended for a period of 160 seconds. In the lower graph, changes in mean left atrial pressure (LA) (mmHg), mean pulmonary artery (PA) (mmHg) and pulmonary vascular resistance (PVR) (Wood units) are shown. The shaded area represents the period of 85 seconds during which the left atrial pressure exceeded the pulmonary artery pressure

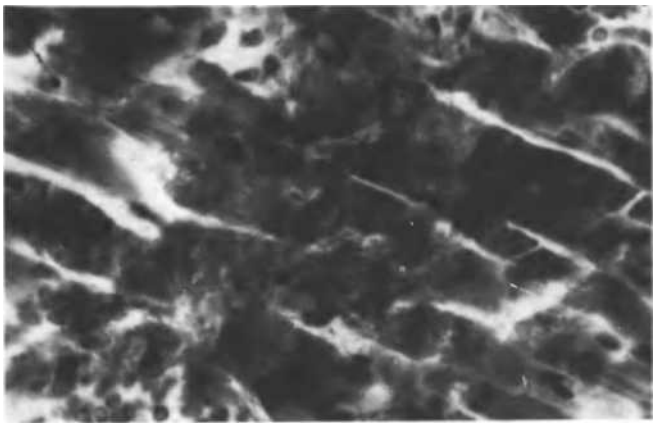

Figure 6 Light micrograph section of myocardium showing widespread contraction bands and edema (H&E × 310)

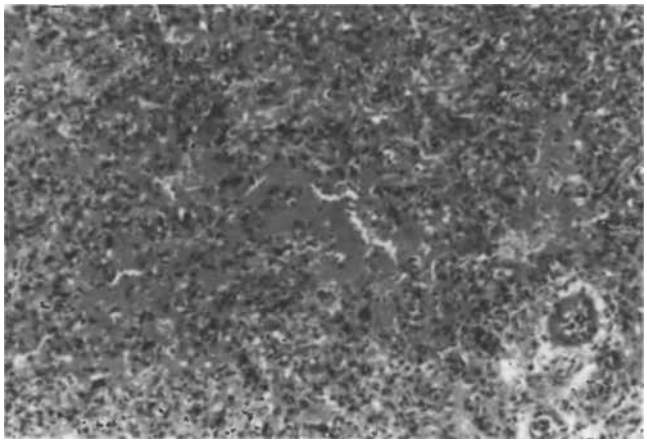

Figure 7 Light micrograph of lung following induction of brain death. Alveolar wall hemorrhage due to disruption of capillary integrity can be seen, as well as considerable pulmonary edema. The heavy eosinophilic staining of the edema fluid demonstrates it to be an exudate rich in protein (H&E × 100)

A majority of human donor hearts show mild (or occasionally more severe) degrees of myocardial injury (Figure 8), such as subendocardial hemorrhage, presumably once again due to the high catecholamine output during the period of brain injury and the development of brain death. Changes of ischemic injury incurred at the time of brain death can sometimes be seen in the myocardium at the time of the first post-transplant endomyocardial biopsy.

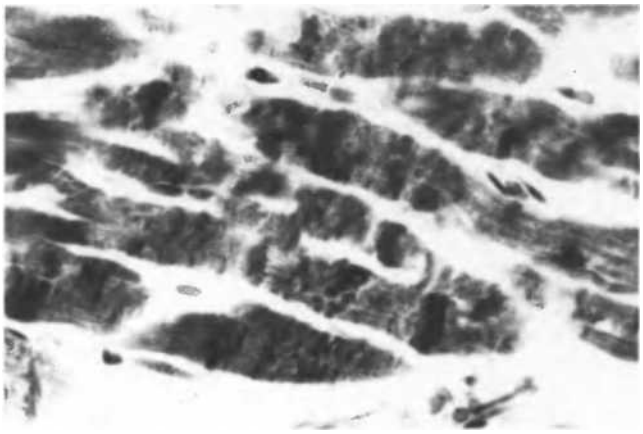

Figure 8 Light micrograph of a human donor heart which failed 2 hours after operation, showing extensive contraction bands (H&E × 320)

Endocrine changes and metabolic responses

This subject remains one of considerable controversy, and has recently been reviewed in some detail[78]. There is considerable

variation in the findings of different groups who have been interested in this potentially important field.

In the baboon, the thyroid hormones plasma free triiodothyronine (T3) and thyroxine (T4), together with plasma cortisol and insulin, fall significantly within a few hours of the onset of brain death[71]. Antidiuretic hormone also disappears from the plasma within a few hours. These changes are associated with a reduction in myocardial energy stores (adenosine triphosphate (ATP), creatine phosphate (CP) and glycogen), as well as a significant increase in myocardial lactate[69,70,79]. These myocardial tissue changes suggest that there is impairment of aerobic metabolism following brain death.

The functional testing of hearts taken from brain-dead animals demonstrates a deterioration in myocardial function, as evidenced by significant reductions in cardiac output, stroke volume, and left ventricular pressure[70,79]. There is also evidence that brain death leads to functional deterioration of the kidney[80].

Plasma free T3 levels have also been shown by most investigators (but not all) to be reduced in human potential donors, and there would appear to be some correlation between the level of T3 and the time interval that has elapsed since brain death took place[77]. Cortisol and insulin have also been shown to be in the low normal range.

Further experimental work in the baboon, studying the kinetics following single bolus injection of 14-carbon labeled metabolites (glucose, pyruvate, and palmitate) has indicated that there is a major change in metabolic oxidative processes following brain death, involving the entire animal[75]. The rates of glucose, pyruvate and palmitate utilization were found to be markedly reduced, and there was an accumulation of lactate and free fatty acids in the plasma. These findings indicated a change from aerobic to anaerobic metabolism affecting the body as a whole, and correlated well with the previous findings relating to metabolism in the heart and kidney alone. Evidence was provided that high-energy phosphates are rapidly depleted under this changed metabolic environment, almost certainly leading to deterioration in function of all organs. These observations, however, and their interpretation, remain controversial, although recent confirmatory evidence has been provided[81].

MANAGEMENT OF THE DONOR

Care of the donor can be a time-consuming activity. If the patient is to be maintained in an ideal state for organ donation, as much care has to be taken over his management as would be given to any patient in an intensive-care unit. When brain death has been confirmed, interest in the care of the 'patient' by the primary medical and nursing personnel may wane, and support by the staff of the organ procurement agency and/or retrieval teams is often called for.

Some donors are in a less-than-optimal hemodynamic/pulmonary state when first seen, and initially the organ(s) may not seem suitable for donation. In many cases, however, intensive monitoring and management of the donor leads to improvement in status, resulting in excision of organs of good quality[82,83]. In contrast, however, particularly in the case of the lungs, organs that initially appear acceptable may show signs of deterioration despite intensive care, excluding them from use. It is important, therefore, to repeat important tests (e.g. the Pa_{O_2} on an Fi_{O_2} of 40% or 100%) at intervals throughout the period of donor management[84]. If the donor no longer fulfills the necessary criteria, the organ(s) may need to be abandoned.

As the majority of brain-dead donors are donors of multiple organs, the aim of management has to be to achieve a balance so that no organ is functionally improved at the expense of another. For example, the heart may function better if the central venous pressure is increased by intravenous fluid infusion, but this may be detrimental to the lungs. Increased inotropic support may therefore be preferable in such a situation.

Mechanical ventilation will already be employed, and blood gases are maintained within the normal range. The donor will be maintained on a (physiological) PEEP of 5 cmH_2O with the lowest possible Fi_{O_2} sufficient to maintain an oxygen saturation of 90–95%. Maintaining an Fi_{O_2} of 100% for several hours, if necessary, is probably not deleterious to subsequent lung function. If the lungs are to be donated, frequent tracheobronchial suction is required to prevent secretions from collecting. At the earliest opportunity, tracheobronchial aspirate should be sent for Gram stain and aerobic and fungal culture.

A urinary catheter may already be *in situ*; if not, one is inserted. A central venous pressure (CVP) monitoring catheter is essential if the volemic state of the patient is to be well controlled. A Swan–Ganz catheter is not generally required, but it may prove helpful in donors who continue to show signs of hemodynamic instability. An arterial pressure line is an advantage, but is not essential if monitoring by sphygmomanometer cuff is satisfactory; its presence, however, facilitates the frequent estimation of arterial blood gases. (The femoral artery and vein, or jugular vein, can be utilized for easy access for arterial and CVP monitoring.) At least one, and preferably two, other peripheral venous infusions are set up for fluid and drug administration. Care is taken to introduce all vascular and urinary catheters under sterile conditions, especially if the groin is used for vascular access.

As a result of pituitary injury, brain-dead patients frequently pass large quantities of urine (diabetes insipidus), and rapidly become hypovolemic and hypotensive if fluid is not replaced. Fluid, preferably warmed to prevent hypothermia, is administered in the form of electrolyte solution or colloid. If the patient has bled significantly, e.g. from a head or other injury, whole blood (or packed cells) is given to maintain the hemoglobin >8 g/dl. The serum sodium may rise to high levels in patients with impairment of production of antidiuretic hormone, and the administration of sodium chloride as a replacement fluid is therefore avoided. Potassium is lost in the urine and may require frequent monitoring and replacement on a large scale; 30 mEq potassium chloride are added to each liter of intravenous fluid given. Further supplements of 15–40 mmol/l administered in 30–100 ml of intravenous fluid over periods of 15–60 min may be necessary to maintain the serum potassium level above 3.5 mmol/l.

A systemic MAP of between 60 and 80 mmHg (with a systolic pressure of ± 100 mmHg) would appear sufficient to provide adequate coronary flow. Such a pressure may be best obtained by a combination of fluids, to maintain a moderate preload, and an intravenous infusion of vasopressin (or small increments of intramuscular vasopressin) to increase the afterload. Excessive increases in either preload or afterload may be damaging to the myocardium, and particularly to the lungs. Heart function should

be satisfactory with a central venous pressure of 5–10 mmHg but, if the lungs are to be procured, every effort should be made to maintain good cardiac function and an acceptable systolic blood pressure with a CVP that does not exceed 5 mmHg. Fluid overload must be prevented. If the kidneys are also to be excised for the purpose of transplantation, as is usually the case, an MAP of much below 60 mmHg may prove inadequate to maintain renal perfusion, and the CVP may need to be slightly increased.

If fluid replacement (including blood, if necessary) sufficient to maintain a CVP of 5–10 mmHg (or less, if the lungs are to be utilized) does not maintain an adequate systolic blood pressure, inotropic support should be initiated. Dopamine is the usual inotrope of choice, and should be used at the lowest dose necessary to maintain the systolic blood pressure at approximately 100 mmHg. Doses of <10 μg/kg per minute are usually sufficient. If a rate of >15 μg/kg per minute is required, the acceptability of the heart for donation needs to be reviewed.

If urinary output is excessive, making adequate fluid replacement difficult, vasopressin given intravenously or intramuscularly is of value in reducing this loss. Since vasopressin at concentrations higher than those required for antidiuresis results in peripheral (including renal) vasoconstriction, great care is required in its administration if the kidneys are to remain suitable for donation. (Similarly, phenylephrine is contraindicated, although neither of these agents is harmful if the heart or lungs alone are to be donated.) The intramuscular administration of vasopressin can be particularly effective, only small doses being required (0.1–0.25 U/kg), repeated as necessary. For intravenous infusion, vasopressin (100 U/250 ml normal saline), administered initially at approximately 0.25 ml (0.1 U/min) usually results in a decrease in urine flow and/or increase in blood pressure.

Desmopressin (dDAVP), a synthetic analog of arginine vasopressin (AVP), has enhanced antidiuretic potency, diminished pressor activity, and a prolonged half-life and duration of action compared to the natural hormone AVP[85,86]. It also has a greatly reduced splanchnic vasoconstrictor effect. It is therefore preferred by some centers for the control of diabetes insipidus. Doses of 2 μg i.v. for up to two doses have been found satisfactory[86].

Brain-dead patients lose thermoregulation and rapidly cool to low temperatures if not actively warmed with an electric warming blanket. Although a mild degree of hypothermia may, in fact, be beneficial to the preservation of organs in a satisfactory condition, ventricular fibrillation can occur at temperatures below 30°C. Our policy has been to maintain the central temperature at approximately 35°C.

If the Pa_{O_2} and Pa_{CO_2} are maintained within normal limits by mechanical ventilation, and if the central venous and arterial pressures are also maintained within the desired range, acid–base balance may remain within normal limits. If acidosis occurs, as a result of a combination of increasing anaerobic metabolism and peripheral vasoconstriction associated with hypothermia, sodium bicarbonate should be administered to correct the base deficit (base deficit × body weight (kg) × 0.3/2 = ml 8.4% sodium bicarbonate).

A suitable wide-spectrum, non-nephrotoxic antibiotic (e.g. a cephalosporin) is administered at regular intervals until the donor is taken to the operating room for organ excision. Some pulmonologists prefer more aggressive antibiotic cover, depending on the results of Gram stain of the sputum. A combination of vancomycin (1–1.5 g as a single dose) and ampicillin/sulbactam (Unasyn) (3 g repeated q. 6 h) provides very wide cover. If the donor is known to be allergic to penicillin, a combination of aztreonam (2 g i.v.) and clindamycin (900 mg i.v.) given 8-hourly is effective.

By the measures outlined above, the heart and lungs of most brain-dead donors can be maintained in a viable state for several hours, occasionally up to 24 h. In our experience, however, increasing instability of the circulation is the rule, and every effort should be made to organize the transplant operation as soon as possible.

If there is undue delay in retrieval of organs it is wise, if not mandatory, to repeat the chest radiograph and oxygen challenge in potential lung donors. The appearance of a new infiltrate or edema on chest radiograph or a significant reduction in P_{O_2} (even if still >300 mmHg) should be reviewed with concern, and the decision to utilize the lungs may need to be reconsidered.

Hormonal therapy

Noting both the deterioration in cardiac function and depletion of myocardial energy stores that can occur after brain death, consideration has been given as to whether these effects resulted from the depletion in circulating hormones, such as T3, cortisol, and insulin, and, furthermore, could be reversed by replacement therapy.

Experimental observations

Brain-dead experimental animals treated with these hormones showed a return toward control level in respect to cardiac output, though left ventricular pressure remained slightly reduced[79]. Myocardial ATP, CP, glycogen (which had been depleted) and lactate (which had been increased) did, however, return to control values[79]. Similarly, there was a return to normal renal function following hormonal therapy to the brain-dead animal[80].

When T3 was administered to a brain-dead baboon (2 μg at hourly intervals), there was a dramatic increase in the rate of metabolite (glucose, pyruvate, and palmitate) utilization, and reductions in plasma lactate and free fatty acids[75]. These changes indicate stimulation of aerobic metabolism in the body as a whole, resulting in a reversal from anaerobic to aerobic metabolism in the brain-dead animal. These observations correlated well with the earlier studies which showed replacement of myocardial energy stores and improvement in myocardial function.

Clinical observations

In an initial study, a group of potential donors was treated with intravenous T3 (2 μg), cortisol (100 mg), and insulin (10–20 international units) when first seen, and the therapy repeated at hourly intervals, depending on the condition of the donor and his response to the treatment, until the heart was excised. Observations in this group of potential donors were compared with those in potential donors who did not receive any form of hormonal therapy[77]. The hormonally-treated group showed a marked improvement in cardiac performance, as measured by significant

increases in MAP and heart rate and a fall in CVP, despite a significant reduction in inotropic requirements. This was in contradistinction to the donors who did not receive hormonal therapy, in whom there was no improvement in cardiac function, despite a significant increase in the level of inotropic support. In the hormonally treated donors there was a reduction in the bicarbonate requirement, and falls in serum lactate and pyruvate; in those who did not receive hormonal therapy the need for bicarbonate administration increased by 100% over the same period of time.

Nineteen percent of the donor hearts in the untreated group were eventually considered unsuitable for subsequent transplantation on the basis of poor or deteriorating hemodynamic performance, whereas all of the hormonally treated donors were considered suitable for transplantation, and all showed immediate good function following transplantation, and good long-term performance except where affected by acute or chronic rejection.

A further larger study showed a similar good response. The optimum dosage of T3, which is considered the most important of the replacement hormones, remains uncertain, but may be more than the 2 μg/h given in the above studies. Unpublished data from the Papworth group in the UK suggest that, to achieve and maintain normal blood levels of T3, an initial bolus of 4 μg is required, followed by an infusion of 4 μg/h (Wheeldon, D., personal communication).

It should be stressed that hormonal therapy remains controversial, and is not yet fully accepted by the transplant community[21,78]. There are increasing data, however, to suggest that it may prove a physiological way of maintaining donor organs in a viable state before excision and transplantation.

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5
Selection and Evaluation of the Potential Thoracic Organ Transplant Recipient – General Considerations

D.K.C. COOPER, N.K. IMES AND D.P. NELSON

INTRODUCTION

Selection of appropriate recipients for heart or lung transplantation may be the most important factor determining long-term survival, yet selection remains difficult and guidelines vary to some extent from one institution to another[1–3]. With increasing experience and success, there has been a relaxation in selection criteria in heart transplant candidates, with both older and younger patients and insulin-requiring diabetics now being offered transplantation. Similarly, with increasing experience in lung transplantation, selection criteria are gradually being modified. Greater experience with the new immunosuppressive regimens that are steadily becoming available, including tacrolimus (FK506) and mycophenolate mofetil, is likely to further broaden the criteria for transplantation.

EVALUATION OF THE POTENTIAL RECIPIENT

Screening of the recipient begins with a full history and physical examination. At this stage any major contraindication may already be evident. Some patients will be rejected at this early, informal evaluation, as they are seen to be completely unsuitable. Usually this is on the grounds of extremely advanced age or prohibitive, coexistent disease in other organ systems.

Once having passed this informal assessment, candidates undergo systematic and extensive medical screening. If full data on the cardiac or pulmonary status have not already been obtained, a thorough assessment is completed. Once the patient is judged to be a candidate for transplantation on the basis of his/her cardiac or pulmonary clinical status, then further screening is performed to ensure that: (a) the patient is likely to withstand the rigors of surgery, (b) major contraindications to the use of immunosuppression are not present, and (c) there is no condition that will prevent satisfactory post-transplant rehabilitation or long-term survival.

This screening process is obviously guided by good clinical judgement, and the sequence of testing may be altered because of clinical suspicions generated by the history or physical examination. For example, if a lung transplant candidate is suspected of having ischemic heart disease, a coronary arteriogram should be done early in the evaluation. The early identification of disqualifying cardiac disease might result in a significant monetary saving, since other expensive tests will then not be necessary.

General screening tests (Table 1) include urinalysis, chest radiography, a complete blood count, biochemical blood screening of liver and kidney function, glucose metabolism (including glycosylated hemoglobin), calcium, thyroid, and coagulation status. Serum cholesterol, triglycerides and prealbumin levels are helpful as baseline values, since they may be altered by the transplant process and subsequent drug therapy. Serologic tests for HIV, HTLV1 and 2, and hepatitis B (surface antigen) and C (antibody and RNA) are performed during the early evaluation of patients and may reveal disqualifying disease to be present. A rapid plasma reagin (RPR) test for syphilis should also be performed. Serologic testing, such as for CMV and toxoplasmosis, may be delayed until late in the evaluation process, as this information will assist in post-transplant management but will not preclude candidate selection.

Other screening tests include a 24-hour urine collection for creatinine clearance and protein, skin tests (PPD for tuberculosis with suitable control, and *Candida*), and any bacteriological studies that might be indicated by the history and physical examination. All cardiac transplant patients should have pulmonary function studies unless this is precluded by the status of the patient (e.g. if on a ventilator). At our own center we assess the vertebral bone density in all patients >40 years of age as a baseline for following the development or treatment of osteoporosis post-transplant[4].

Doppler ultrasound studies of the carotid arteries and abdominal aorta–iliofemoral systems are performed in all patients over the age of 40 years, and in younger patients with a history of ischemic heart disease or considered to be at risk for peripheral vascular disease. If Doppler ultrasound demonstrates more than mild arterial disease we progress to arteriography and/or aortography, for reasons that will be discussed later.

Further investigation of major systems, such as gastrointestinal endoscopy and intravenous pyelography, are performed when indicated by the history, physical examination, or laboratory data.

Table 1 Suggested general evaluation of the potential thoracic organ recipient

General data
Comprehensive history and physical examination
Blood chemistry determinations, including renal and liver function panels, TSH
Complete blood count, differential white blood count, platelet count, prothrombin time, partial thromboplastin time, fibrinogen
Urinalysis
Stool for guaiac examination ×3*
24-hour collection of urine for creatinine clearance, total protein
Chest radiography
Pulmonary function testing
Psychological questionnaire (e.g. MMPI)
Mammography*
Papanicolaou smear*
Lung ventilation–perfusion scanning*
Vertebral bone densitometry*
Doppler ultrasound of peripheral arteries*
Sputum cytology*
Consultations*
Nutritional status and diet history
Psychiatry
Physical therapy
Social services
Dental (+ dental radiography)
Otorhinolaryngology
Essential cardiovascular data
Electrocardiography
Radionuclide ventriculography*
Echocardiography*
Right heart catheterization
Left heart catheterization*
Endomyocardial biopsy*
Essential immunologic data (see also chapter 6)
Blood type and antibody screening
Human leukocyte antigen (HLA) typing (may be performed at the time of transplant)
Screening of panel of reactive (lymphocytotoxic) antibodies
Essential infectious diseases data
Serology for:
Hepatitis HBsAg (HBsAb, HBcAb), HcAb, HcRNA
Human immunodeficiency virus (HIV)
HTLV1 and 2
Cytomegalovirus (CMV) IgM and IgG antibody
Toxoplasmosis
EB viral capsid IgG and IgM antibody*
RPR
Lyme titers*
Urine culture and sensitivity*
Stool for ova and parasites × 3*
Skin testing for tuberculosis (PPD) and *Candida*

* If indicated by history, age, or physical examination.

In older women (>40 years) mammography should probably be performed, if not done within the previous 12-month period.

Selected patient groups, such as those with cystic fibrosis, may require extensive organ system evaluation and testing for resistant bacteria (such as *Pseudomonas cepacia*) and fungi. The presence of chronic sinusitis may require consultation with an otorhinolaryngologist in an effort to control sinus infection.

Psychological evaluation is performed on all patients, with particular attention being paid to: (a) a history of noncompliance with medical advice or therapy, (b) substance abuse, or (c) overt psychiatric illness. Patients complete a psychological questionnaire (e.g. MMPI), and psychiatric evaluation is requested in patients where doubt exists regarding their compliance or psychiatric health. A dietary consultation is necessary in obese or cachectic patients, to assess and modify eating behavior.

Based on the results of these investigations, specific therapy may be indicated to improve the treatment of the patient's disease, or the patient may be deemed unsuitable for transplantation. If the patient cannot be improved by medical measures and no contraindication is detected, then the patient becomes a candidate for transplantation and awaits a suitable donor.

WHICH PATIENT WILL BENEFIT FROM HEART OR LUNG TRANSPLANTATION?

The substantial improvement in survival that has occurred following thoracic organ transplantation in the past decade is principally the result of improvement in survival during the first 3 postoperative months. This reflects improvement not only in management but also in patient selection. Retrospective analysis has aided recognition of recipient-related factors that influence survival after transplantation (see Chapters 43, 44, 65 and 69).

The major limiting factor in thoracic organ transplantation remains availability of donor organs and, therefore, careful consideration should be given to providing this scarce resource to the most likely candidates to survive long-term and enjoy a sufficient improvement in quality of life.

Contraindications

There are a number of 'absolute' and 'relative' contraindications to heart or lung transplantation (Table 2). However, what was an 'absolute' contraindication 10, or even 5, years ago may be only a 'relative' contraindication today[5–10]. The weight given to these 'relative' contraindications will differ between different transplant centers, depending on the availability of donor organs, the number of recipients waiting, and prior experience.

Active infection

Patients with active infection must generally be excluded because of the risk of exacerbation by postoperative immunosuppression unless the infection is confined solely (or predominantly) to the organ(s) being transplanted (for example, bilateral lung transplantation for cystic fibrosis). In some cases, however, when the infecting organism is known and specific therapy is effecting a satisfactory response, it may be acceptable to proceed with transplantation if the cardiac or pulmonary status of the patient is critical. The high risk of death from the underlying cardiac/pulmonary disorder may outweigh the risk of transplantation carried out in the presence of persisting infection.

The presence of antibiotic-resistant organisms, particularly in candidates for lung transplantation, may render the patient permanently unsuitable for transplantation, or unsuitable until the microorganism is replaced by susceptible organisms. For example, resistant *Pseudomonas cepacia* in patients with cystic fibrosis has resulted in a high percentage of transplant failures (Chapter 61). The policy of many groups is to attempt, by reduced and judicious use of antibiotics, to decrease the drug resistance of

Table 2 Contraindications to heart or lung transplantation

'Absolute'	*'Relative'*
Active infection	Advanced age
Untreated malignancy	Poorly controlled insulin-requiring diabetes mellitus with microvascular disease
Coexisting systemic illness that may severely limit life expectancy	*Active* peptic ulcer disease
Irreversible and severe dysfunction of any other major organ (kidney, liver)	Significant peripheral vascular or cerebrovascular disease
	Drug addiction, alcoholism, mental illness, or psychosocial instability
	Severe obesity or cachexia
	Non-compliance with medical care
	Unresolved pulmonary infarction (may not be an absolute contraindication to lung transplantation if this lung is to be removed and if there is no further risk of pulmonary embolism)
	Acute diverticulitis
	Severe osteoporosis

the organism before transplantation is carried out[11]. The objective is to have a candidate with an organism in his/her sputum that is sensitive to at least two antibiotics. Under such circumstances, many groups feel it is safe to proceed with the organ transplant. Fungal colonization of the airways with *Aspergillus* requires eradication prior to transplantation.

Serologic evidence of HIV or active hepatitis B are also generally accepted as contraindications to thoracic organ transplantation (Chapters 11, 32 and 57). The situation is less clear with regard to the hepatitis C-positive candidate, although, if active hepatitis C infection is present, transplantation is almost certainly excluded, unless the patient tolerates and responds to a course of interferon therapy.

Pre-existing malignancy

Pre-existing malignancy may progress rapidly in the immunocompromised patient (Chapter 12). A history of successfully treated malignancy may represent a relative contraindication as immunosuppression may impair the body's ability to control residual malignant cells. Many centers consider an absence of symptoms or signs of malignant disease for 5 years or longer to be an adequate period of time to permit transplantation, but more specific evaluation related to the malignancy (e.g. the exclusion of 'silent' distant metastases) may be warranted before making a final decision.

The disease for which transplantation is being considered may, in fact, be related to therapy given for the original malignant condition (e.g. chemotherapy-induced cardiomyopathy or radiation-induced pulmonary fibrosis). If there is no evidence of recurrence of the malignancy, transplantation is not contraindicated in these cases.

Coexisting systemic disease

The decision to reject a transplant candidate under the broad general category of coexisting systemic illness is clearly subjective. The availability of donor organs and prior experiences may determine the ultimate decision regarding the possibility of transplantation in patients afflicted with disease in other organ systems. For example, many transplant physicians may exclude patients with collagen vascular disease. As a general rule, pre-existing conditions that will significantly reduce early post-transplant survival, or that will adversely affect the long-term ability of the patient to withstand the side effects or infections secondary to immune suppression, should be considered contraindications to transplantation.

Dysfunction of other major organ systems

Dysfunction of a major organ system (e.g. renal or hepatic) may be secondary to the end-stage cardiopulmonary disease present in the thoracic transplant candidate. It is frequently difficult to determine whether this dysfunction will be partially or completely reversible once myocardial or pulmonary function has returned to normal after transplantation. Correction or improvement of the underlying circulatory state by heart transplantation will result in variable recovery of function of such organs as the kidneys or liver. Therefore, pretransplant evaluation must include every effort to determine the reversibility of the impaired organ's function. For example, pulmonary diseases such as emphysema and chronic bronchitis may be extremely difficult to evaluate in the presence of left ventricular failure, but if significant lung disease is present, heart transplantation may be contraindicated. Furthermore, cyclosporin and azathioprine may adversely affect kidney and liver function, respectively, so great care must be taken to ensure that significant irreversible renal and/or hepatic function are not already present. Accurate assessment of reversibility is not always possible, but diligent evaluation may discover disease which precludes transplantation.

In patients being considered as candidates for lung transplantation, a full cardiac evaluation is usually indicated in those patients over 40 years of age, or in those felt to be at risk for coronary artery disease at a younger age. Most emphysema patients will have a long smoking history, which will increase the risk of cardiovascular disease. Left and right heart catheterization with visualization of the coronaries and left ventricle, as well as pressure measurements, is warranted. Significant coronary artery disease

or left ventricular dysfunction will almost certainly exclude the patient from further consideration. A considerable degree of *right* ventricular failure, however, has clearly been demonstrated to be reversible after lung transplantation, particularly when the primary pulmonary disease was associated with pulmonary hypertension.

However, if right ventricular failure or pulmonary hypertension has been severe and long-standing, permanent injury to the liver may have occurred (leading to cirrhosis), which is a contraindication to thoracic organ transplantation because of the risk of developing coagulopathy, encephalopathy and/or liver failure in the post-transplant period[11,12].

Advanced age

The first heart transplants performed were in older patients. Using conventional immunosuppressive therapy with azathioprine and methylprednisolone, the early experience revealed that mortality rose in patients over the age of 40 years. More recently, even before cyclosporin became available, improved survival was achieved in carefully selected patients over the age of 50 years[13]. There are now many reports of successful transplants in older patients, even into the late 60s[14–17]. Indeed, there is decreased frequency of rejection episodes in this group[18], which is attributed to an age-associated decline in immune function. Obviously this might well represent a survival advantage to this group. Most physicians now believe that absolute age limits are no longer applicable. Attention must be paid to the general condition of the patient, and physiologic age considered in preference to chronologic age of the patient. One early study suggested that the likelihood that a patient over age 55 is a suitable recipient decreases as age increases, and reaches zero at age 67[10]. With improved selection and post-transplant management this is probably no longer true, but it does draw attention to the fact that older patients have to be assessed particularly carefully.

In the selection of lung transplant candidates (where overall survival is currently less than after heart transplantation) most groups are still relatively conservative with regard to age criteria. Patients with emphysema and interstitial pulmonary fibrosis are generally considered up to the age of 60–65 years, but rarely later. Patients with cystic fibrosis are in any case generally much younger when they present with the need for lung transplantation. Transplant candidates with primary pulmonary hypertension are also usually younger, and a higher risk of early post-transplant complications may make patients >50 years of age less suitable for lung transplantation. The operation that is to be undertaken may also influence the decision. Many groups will offer single lung transplantation to suitable patients with a physiologic age <65 years, double lung transplantation to those <60 years, and heart–lung transplantation to those <50 years of age[11].

Once again, however, the medical team must keep in mind the shortage of donor organs, and carefully consider the allocation of an organ to an 'elderly' patient when there are many young patients on the waiting list who, hopefully, may gain many more years of benefit from the transplanted organ.

Unresolved pulmonary infarction

A heart transplant candidate with recent unresolved pulmonary infarction should probably be excluded because of the risks of: (a) cavitation and secondary infection, (b) increased pulmonary vascular resistance, and (c) further embolic disease. This is a temporary condition and, when resolution and scarring have occurred, the patient once again becomes eligible. If the patient is to be given the best chance of survival, most centers would wish to delay transplantation for 2–6 weeks whenever possible. Anticoagulation should be provided during this period, to minimize the risk of further emboli from venous thrombosis.

A major pulmonary embolus may dramatically raise pulmonary artery pressure and pulmonary vascular resistance from incomplete clot lysis, and it is wise to re-evaluate the pulmonary circulation again when the infarction has resolved before proceeding to heart transplantation.

Recent pulmonary infarction is a less significant contraindication in potential lung transplant candidates if it has occurred in the lung to be replaced. However, transplantation should again be delayed until it is certain that no deep vein thrombosis remains that could prove a post-transplant source of further emboli.

Insulin-requiring diabetes mellitus

A generally accepted contraindication to heart transplantation in the pre-cyclosporin era was insulin-requiring diabetes mellitus, because of the post-transplant exacerbation of diabetes by high-dose corticosteroids. Therapy with cyclosporin and azathioprine alone (or with added low-dose corticosteroids) has been successful, thus allowing heart or lung transplants in patients with diabetes mellitus. Although insulin-requiring diabetics without evidence of end-organ disease have shown heart transplant survival comparable to non-diabetics, transplant outcome has not been carefully studied in patients with limited diabetic complications. Some programs exclude diabetics with any significant diabetic complications, including microvascular disease (e.g. retinopathy, nephropathy, neuropathy) in view of the likelihood of post-transplant impaired renal function complicating cyclosporin or tacrolimus therapy. Other centers, including our own, assess such patients on a case-by-case basis. Unstable brittle diabetes should probably exclude transplantation. Those with type II diabetes and impaired glucose tolerance are warned that they may require insulin therapy when taking corticosteroids, especially during the early post-transplant period.

Active peptic ulcer disease

Active peptic ulcer disease is a contraindication because of the risk of bleeding, perforation, and infection in the immunosuppressed patient. This, too, is temporary, and healed ulceration is not a contraindication. With the plethora of currently available agents to suppress gastric acid secretion, and to treat *H. pylori* infection, active peptic ulcer disease rarely precludes heart or lung transplantation today. Healing of ulceration should ideally be confirmed by endoscopy before proceeding to transplantation.

Peripheral or cerebrovascular disease

The presence of significant peripheral or cerebrovascular disease will prevent the transplant patient from obtaining maximum benefit from the procedure, and increase the short-term risk of complications.

We believe that carotid ultrasound, followed by arteriography in selected cases, is important to assess the risk of cerebrovascular events at the time of transplantation. We have had a policy of performing carotid endarterectomy as an elective procedure pretransplantation in a number of patients. Similarly, particularly in patients with previous cardiac surgery, in whom the need to cannulate the femoral artery and vein to initiate cardiopulmonary bypass is a possibility, we believe it is essential to have eliminated the presence of significant atheromatous disease in the aorta–iliofemoral systems.

Apart from these above considerations, it has been our experience that patients with widespread peripheral atheromatous disease, even if only moderate, do not do well long-term after heart transplantation. Complications such as gastrointestinal ischemia and renal dysfunction have not been common. It is perhaps too early to come to a conclusion with regard to the influence of peripheral vascular disease on long-term survival after lung transplantation, but it is likely that its presence will lead to increased morbidity and decreased quality of life.

Obesity/cachexia

The overweight heart or lung transplant candidate creates a difficult selection problem for which there is no clear-cut solution. When obesity is gross (e.g. >25% above ideal weight), we believe transplantation should not be offered until a considerable amount of weight has been lost. The patient's name may be placed on the waiting list, but with the clear understanding that transplantation will not proceed unless significant weight reduction (to within 10–15% of ideal body weight) is achieved.

This policy can be justified by the considerations that: (a) the patient in cardiac or respiratory failure may improve symptomatically with weight loss, possibly even to the point of being able to postpone (or, in rare cases, avoid) transplantation; (b) post-transplant mobilization and rehabilitation will likely be much more rapid in the non-obese patient; (c) obesity may make the technical aspects of the transplant surgery more difficult; (d) obese patients have greater problems with postoperative pneumonia, atelectasis, hypoventilation, thrombophlebitis, and physical rehabilitation[19,20]; (e) the corticosteroids, that form part of the immunosuppressive regimen at most centers, tend to increase the patient's appetite and thus are likely to increase weight further post-transplantation; and (f) in the large, obese patient, e.g. patients >6 ft (1.83 m) in height and >250 lb (113 kg), it may be exceedingly difficult to obtain a donor heart large enough to support the patient adequately in the immediate post-transplant period. Varying results have been reported of the influence of obesity on transplant and post-transplant mortality and morbidity, but extreme obesity clearly complicates many aspects of patient care, and is generally considered to increase morbidity and possibly mortality.

This nutritional policy can be followed and is generally well understood by the transplant candidate, unless the patient's clinical condition rapidly deteriorates to the point that intensive care unit support is required and organ transplantation becomes an emergency. It is extremely difficult to deny a patient (particularly a young patient) heart or lung transplantation purely on the basis of obesity but, if gross (e.g. >50% in excess of ideal body weight), refusal to transplant may be necessary. Furthermore, when such an urgent situation arises, a suitably large donor may not become available.

When obesity is less extreme, we still believe that every effort should be made towards a voluntary reduction in weight. This will require excellent nutritional advice, frequent outpatient follow-up, and a modest program of exercise if the cardiac or respiratory status allows this. The most important factor in the success of such a weight-reducing program, however, is patients' self-discipline and compliance.

Cachexia (e.g. <80% of ideal body weight) related to the poor cardiac status of the patient is today relatively rare, and therefore an unusual reason for not progressing with heart transplantation. If present to any degree, however, every effort should be made to improve the patient's nutritional status before transplantation (Chapter 16). Malnourished patients are at a greater risk for such postoperative complications as poor wound healing, infections, and greater difficulty in physical rehabilitation.

Obesity is a less common finding in patients presenting for lung transplantation due to emphysema, whereas cachexia may be a major problem. Extreme weight loss and malnutrition may occasionally prove a contraindication to lung transplantation, but usually can be reversed to some extent by food supplements, nasogastric tube feeding, or intravenous hyperalimentation (Chapter 16).

Prolonged mechanical ventilation of a lung transplant candidate is a contraindication to proceed to transplantation because of its effect on the state of the patient's (a) general nutrition, (b) muscle strength, and (c) propensity to infection. After transplantation these patients have great difficulty in developing the strength to breathe spontaneously and cough effectively[8]. The recovery of lung recipients who were ventilator-dependent for <2 weeks, however, has been reported to be similar to that of non-ventilator-dependent recipients[21].

Psychological instability/substance abuse/non-compliance

The psychosocial criteria regarding patient selection, including substance abuse and non-compliance, are, by their very nature, difficult to define, and are to some extent subjective. Inevitably they arouse more controversy than the relatively straightforward medical criteria[22–24].

Patients currently addicted to drugs (including narcotics, tranquilizers, and stimulants), who consume excessive amounts of alcohol, or continue to smoke, are not suitable candidates for heart or lung transplantation. Patients with psychiatric illnesses that are predicted to preclude a reasonable expectation of desirable outcome after transplantation are also considered to be poor candidates, and require very careful assessment and consideration. The drug-addicted or alcoholic patient is unlikely to comply during the postoperative period with a complex drug regimen and regular attendance for follow-up visits. This noncompliance increases the risk of transplant rejection, side-effects of drugs, and likelihood of infections.

The policies of thoracic transplant centers towards these problems vary considerably. Most centers insist on a period of abstinence from addictive substances, including alcohol, before transplantation can be performed. However, the length of abstinence required, and the degree to which it is confirmed by the

performance of blood or urine testing, vary considerably between transplant centers.

Our own policy has been not to offer transplantation to patients with a long history of addiction, who are unlikely to remain abstinent and compliant post-transplantation. With selected patients, in whom the history of addiction has been less clear or prolonged, we will accept them if compliance with a 6-month period of abstinence can be documented. In nearly all cases we have insisted on careful monitoring and screening of the patient's compliance by the transplant social worker or psychiatrist. Frequently, patients with problems relating to alcohol have been referred to organizations such as Alcoholics Anonymous for help at this stage (and in the post-transplant period).

Compliance with a non-smoking policy may be difficult to assess and monitor. Random testing for cotinine, a breakdown product of nicotine, can be carried out[11,25] to assist in monitoring compliance. Although we always impress upon all patients the need to refrain from smoking, it may prove difficult to absolutely deny an otherwise acceptable cardiac patient a lifesaving procedure such as transplantation solely on the grounds that he or she continues to smoke an occasional cigarette, or even two or three cigarettes, each day. Ideally, the medical team should probably be as strict with regard to smoking as with regard to alcohol or substance abuse, particularly in patients in whom smoking has contributed to the disease process. In reality, however, we have found it difficult to be quite so rigid, and undoubtedly have allowed some patients to undergo heart transplantation when we were aware, or suspected, that they continued to smoke intermittently. Other centers may well feel differently, and would be justified in denying this scarce resource of a donor organ to a patient who persisted with this destructive behaviour.

Lung transplant candidates, however, are absolutely excluded from consideration if they continue to smoke, primarily because it makes assessment of the allograft post-transplant infinitely more difficult[11]. In the non-smoker long-term allograft dysfunction can be caused only by infection, rejection, or complications at the airway anastomosis. In the smoker, allograft dysfunction can also be caused by bronchospasm, airway irritation, infectious bronchitis, and/or respiratory bronchiolitis. In single lung recipients these effects can occur in the allograft and in the native lung.

Perhaps more than most major surgical procedures, the success of heart or lung transplantation depends on the patient's ability to understand fully the lifelong treatment and follow-up program that is an essential part of his or her management. A strong supportive family may be of great value in seeing the patient through the perioperative and early post-transplant periods, but the patient must ultimately take responsibility for his or her own well-being.

In particular, the patient's will and ability to comply with medical therapy, and to undergo possibly repeated admissions and unpleasant procedures (biopsies and bronchoscopies) after transplantation, are difficult to assess. These are best evaluated by a team consisting of the physician responsible for the care of the patient, a psychiatrist, and a social worker experienced in the problems associated with heart or lung transplantation. Not infrequently, an initial evaluation that a patient is unsuitable for transplantation because of inadequate family support or emotional instability is shown to be incorrect (and vice-versa). Observation and evaluation over a period of time may show that some such patients, initially judged to be unsuitable, are indeed capable of adhering to complex medical regimens involving multiple diagnostic tests, clinical visits, and medications.

This is, in fact, one of the few advantages of the current delay in obtaining a suitable donor organ for an individual patient. This period of time (frequently several months) allows a continuing assessment of the patient's compliance with medical advice and family support. A patient who proves noncompliant during this period (e.g. by not taking medication regularly, by failing to attend outpatient appointments, or by failing to make any effort to lose weight, etc.) is likely to prove noncompliant post-transplantation.

Noncompliance of this nature in the post-transplant period has proved to be a major factor in morbidity and mortality in patients at some centers[26–28]. Our impression is that it is perhaps particularly seen in younger patients (those <30–40 years of age) who may be less mature or have less experience in managing a chronic medical condition than do older patients. In addition, our selection of younger patients is possibly not so rigorous, and for compassionate reasons we are more inclined to offer transplantation despite the presence of modest noncompliance concerns.

Financial considerations

In countries where there is no universal health-care insurance, such as the USA, an important practical matter is to ensure that the patient has sufficient financial resources to pay for the costs of organ transplantation. In *all* countries, however, it is important to ascertain that the patient will be able to find resources for expenses not covered by the state, private health insurance, or some other medical aid agency. While the state or health insurance organization, or the success of fundraising, may bear the cost of the operation and postoperative care, there may be many additional and 'hidden' costs, such as chronic drug therapy and the cost of travel to and from the transplant center.

In the USA, for example, the Federal medical healthcare system (Medicare), which is available to only a relatively small section of the population, does not currently cover the cost of medications after the first 2–3 years post-transplantation. This may well leave the patient in a difficult or nearly impossible financial situation. Some relief can be obtained by arranging for drugs to be provided free of charge (if a case can be made) through relevant pharmaceutical companies' indigent patient programs (e.g. NORD – the National Organization for Rare Diseases).

COMMENT

Organ transplantation has provided physicians with a successful form of therapy for end-stage heart or lung failure. At the same time, however, it has posed difficult questions related to allocation of scarce resources (both organs and economic resources), selection of patients for such therapy, and maintenance of the dying patient. In view of the inadequacy of the availability of donor thoracic organs, great care has to be taken to ensure that patients selected for these procedures are those most likely to benefit long-term. Patients with significant medical or psychosocial contraindications, or who have proven themselves to be noncompliant, should, in general, be excluded.

In summary, the ideal thoracic organ transplant recipient should have severe cardiac or pulmonary failure untreatable by conventional surgery or medical therapy. He (or she) should be free of any condition likely to predispose to the complications of immunosuppression, be psychologically stable and compliant, and have sufficient resources to support self and family through the perioperative period prior to full rehabilitation.

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6
Pretransplant Immunological Considerations

E.D. DU TOIT, M. OUDSHOORN AND D.M. SMITH

INTRODUCTION

Consideration of a patient's state of prior sensitization and the effects of histocompatibility antigen matching are critical to avoid antibody-mediated rejection and improve the long-term outcome of transplantation. Since there are many reviews of transplantation immunology in the literature and the mechanism of allograft destruction is outlined in Chapter 7, this chapter will concentrate only on those immunological considerations that affect pretransplant histocompatibility testing and recipient selection for thoracic organ transplantation. When applicable, prospects for developing new procedures to circumvent antibodies in the recipient will be discussed, as will new technologies in histocompatibility testing. The discussion will be divided into four topics: (a) the importance of donor–recipient ABO and Rh blood group compatibility, (b) human leukocyte antigen (HLA) typing and the role of HLA matching, (c) evaluation of recipients for prior sensitization and the importance of the lymphocytotoxic crossmatch, and (d) the effect of pretransplant blood transfusion in recipients of cardiac allografts.

As there is considerably more information in all of these fields relating to kidney and, to a lesser extent, heart transplantation, rather than lung transplantation, emphasis will inevitably be placed on renal and cardiac, rather than pulmonary, transplantation. Increasingly, what has been demonstrated to influence renal allograft survival has subsequently been found to be of importance with regard to cardiac allograft survival. There is no reason to believe that, when sufficient data have been accumulated, the same will not be the case for lung allografts.

RED BLOOD CELL GROUPS

ABO groups

ABO blood group antigens are carbohydrate structures that are components of glycoproteins and glycolipids found on cell surfaces throughout the blood and tissues of the body. Antibodies to these antigens are consistently found in the serum of individuals that lack one or more of these antigens. These antibodies appear before the age of 6 months and persist throughout life. Sensitization occurs due to the presence of these antigens on bacterial flora. (Indeed, animals that are maintained germ-free from birth do not develop similar antibodies.) Each person's antibody titer is highly variable and may diminish with age or chronic illness.

ABO blood group compatibility between donor and recipient has traditionally been regarded as a prerequisite in patients undergoing organ transplantation. ABO incompatibility frequently leads to rapid destruction of the allograft[1,2]. A or B antigens present on the vascular endothelium of the allograft are targets for antibodies and complement in the recipient's serum[3] and rejection may be immediate[4]. A number of factors contribute towards renewed attempts to break the ABO barrier, such as (a) the shortage of cadaver organs, (b) the over-representation of patients with O blood group on transplant waiting lists, (c) difficulties in finding ABO-compatible grafts for presensitized patients, and (d) the importance of maximal utilization of potential living-related donors in kidney, liver, and, to a small extent, lung transplantation.

Doubt about the importance of ABO compatibility arose following the observation that a proportion of ABO-incompatible liver transplants continue to function[5] (and raise the hope that the ABO barrier may be overcome). In a recent retrospective study of 30 patients receiving emergency liver transplants it was concluded that the use of ABO-incompatible grafts is justified in emergency cases when no other donor is available, but should only be used in patients with O blood group[6]. Farges and coworkers showed that the 5-year patient and graft survival rates were 50% and 20%, respectively, in 43 ABO-incompatible liver transplants[7]. Various immunomodulatory management techniques have been used in ABO-incompatible liver transplantation and have included the use of prophylactic antilymphocyte globulin or OKT3 therapy, pretransplant splenectomy, pre- and posttransplant plasmapheresis, and immunosuppressive therapy with FK506[8,9]. Using these techniques for the management of liver transplants from ABO-incompatible living-related maternal and paternal donors resulted in a 1-year patient survival rate of 77%[8].

Opelz, in 59 cases, verified that ABO-incompatible cadaver kidney transplants showed poor results[10] and a similar poor graft survival was observed in a subset of these patients treated with cyclosporin. However, there have been occasional reports of successful renal transplants involving major ABO incompatibility[4]. Most of these have been achieved by immunological modification of recipients, including immunoadsorption, plasmapheresis and splenectomy[11,12]. In a recent study, 10 ABO-incompatible live-related renal transplants were performed after immunoadsorption to reduce anti-A and anti-B IgM titers in the recipients. The graft survival rate at 6 months to 2 years was 83%[13,14]. The few successful renal transplants performed across the ABO barrier without modification of the recipients have been explained by low initial titers of isoagglutinins in the recipient[15], or the weak expression of A antigens, as seen particularly in the case of blood group A_2 donors into O recipients[16]. Although ABO-incompatible kidney transplantation must still be considered experimental, its relative success to date, combined with the desperate need for more organs, warrants its continuation, especially if done under the close scrutiny of the transplant community[17].

In a survey by Cooper, the incidence of irreversible hyperacute or accelerated acute rejection in patients receiving incompatible ABO hearts was approximately 60%[18]. The results of a study on the expression of blood group antigens on the mesothelial cells on the surface of the epicardium and the cardiovascular endothelium indicated that there is some variation in the antigen expression between individuals. This may explain why approximately one-third of organ transplants are successful even when breaching the ABO blood group barrier[19].

Experimental techniques continue to be explored. In hyperimmunized baboons, Cooper and co-workers[20] showed that the continuous intravenous infusion of specific A or B trisaccharides (pre- and post-transplant for several days) inhibited rejection of ABO-incompatible cardiac allografts, and that this protection continued in some cases even after the infusions were discontinued. This form of therapy may permit cadaveric organ allotransplantation across the ABO blood group barrier in the human.

Data collected from the Collaborative Heart Transplant Study by Opelz show no influence of recipient–donor ABO blood type on graft survival[21]. However, several individual transplant centers have shown that group O recipients survive longer after renal[22,23] and cardiac[24] transplantation than non-O recipients, particularly in the presence of HLA-DR mismatching. Early in the series of cardiac transplants at Groote Schuur Hospital in Cape Town (with patients immunosuppressed with azathioprine, corticosteroids, and antithymocyte globulin), poor survival of recipients with the B antigen (blood groups B and AB) was observed[25]. It was presumed that those with blood group B might elicit a greater immune response. This presumption, however, remains controversial[26,27]. There is some evidence that patients receiving ABO-identical hearts (e.g. A to A) have significantly improved survival and less fatal rejection episodes than those receiving ABO-nonidentical hearts (e.g. O to A)[27–29]. Similar results have been clearly documented in large studies of renal transplants[30]. Opelz's multicenter data, however, showed no evidence that ABO-compatible (as opposed to ABO-identical) cardiac transplants have an inferior success rate[21].

Rh group

Rh antigens in transfused blood are strong immunogens but they are not significant tissue antigens and are therefore not generally considered important for heart allograft rejection[31], although a few studies have found improved renal allograft survival in Rh-positive recipients when compared with Rh-negative recipients[32]. Furthermore, there appears to be a low rate of Rh immunization in patients receiving cyclosporin immunosuppression[33], but caution is still advised in young Rh-negative women who may still wish to bear children, and in patients who may have been previously sensitized to Rh antigens by transfusion or by pregnancy[34].

THE HUMAN LEUKOCYTE ANTIGEN (HLA) SYSTEM

The most important antigens involved in tissue rejection are named the major histocompatibility antigens. The genes encoding these antigens constitute the major histocompatibility complex (MHC), known in humans as the human leukocyte antigen (HLA) system. The MHC has been identified in all vertebrates and consists of a number of closely linked genetic loci that are usually inherited as a unit (haplotypes).

The HLA system is the human homologue of an evolutionarily ancient system of major histocompatibility complex (MHC) proteins[35]. They serve to hold and display peptides on the surface of cells so that receptors on T lymphocytes may recognize them as being derived from self or foreign antigens[36,37]. They are essential to the immune system's ability to recognize intracellular infections such as viruses and in providing T-lymphocyte 'help' for the primary antibody response to foreign proteins. The central role of these MHC proteins in the immune response and their widespread tissue expression make their effect on graft survival as important as all other polymorphic protein antigens (minor histocompatibility antigens) combined.

The major histocompatibility antigens can be divided into two groups: (a) the MHC class I antigens composed of an MHC encoded α-chain associated with β_2-microglobulin and (b) the MHC class II antigens composed of two molecules, the α-chain and the β-chain, both of which are encoded in the MHC region[35].

Genes of the major histocompatibility complex

The MHC genes are located on the short arm of chromosome 6 (Figure 1) with the HLA class I genes located telomeric to the HLA class II genes[38]. The distance between the HLA-A locus and the HLA-B locus, and the distance between the HLA-B locus and the first HLA class II locus (HLA-DR), is about 1 centimorgan. This means that there is only a 1% chance that any child will inherit a haplotype having a crossover between the A and B loci, or between the B and DR loci of their maternal or paternal chromosomes. It is therefore sometimes helpful to type other family members in difficult cases.

To date, 37 closely linked HLA genes have been described and are designated HLA-A, B, C, E, F, G, H, J, K, L, DRA, DRB1, DRB2, DRB3, DRB4, DRB5, DRB6, DRB7, DRB8, DRB9, DQA1, DQB1, DQA2, DQB2, DQB3, DOB, DMA, DMB, DNA,

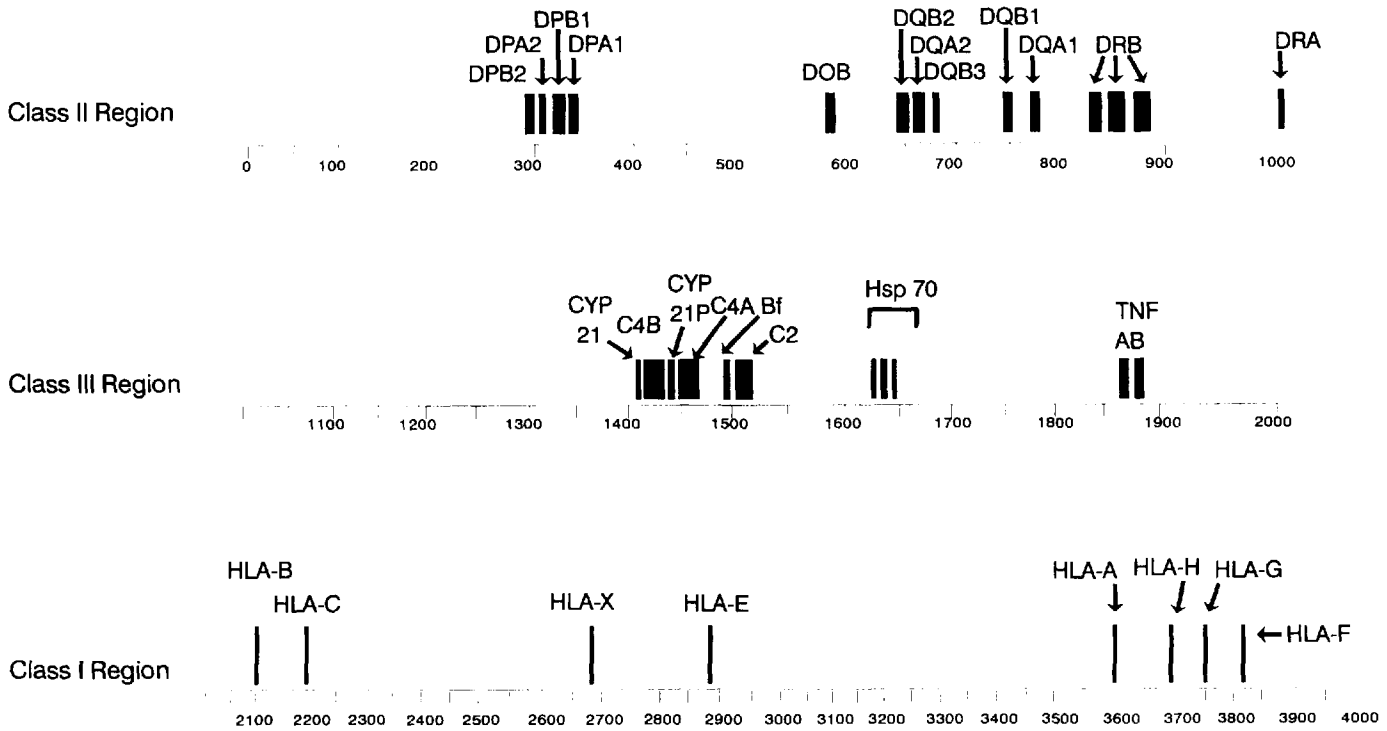

Figure 1 A partial map of the major histocompatibility complex on chromosome 6 (scale = kilobases). HLA-A–H = class I heavy chain genes, DRA–DPA = class II α-chain genes, DRB–DPB = class II β-chains, TNF = tumor necrosis factor α and β genes, Hsp 70 = heat shock proteins, Cyp 21 = 21 hydroxylase genes, C2–C4 = complement components. (Adapted from ref. 38)

DPA1, DPB1, DPA2, DPB2, TAP1, TAP2, LMP2 and LMP7[39]. Some of these genes are pseudogenes and some of them are not known to be expressed, thus not all of these genes are involved in immune functions or transplant rejection[40].

The A, B, and C antigens are composed of an α-chain and β_2-microglobulin. Only the α-chain gene is located within the MHC. The HLA-A, B and C α-chains are highly polymorphic and expressed on most nucleated cells of the body. They are therefore known as the classical HLA class I antigens. There are three other expressed non-classical class I genes: the E, F, and G loci. These genes show limited polymorphism and tissue distribution and have not been shown to have any role in transplantation[41]. The HLA-G antigen has only been found on trophoblast cells of the placenta and may have a role in maternal–fetal tolerance[42,43]. There are also many HLA class I pseudogenes. These are partial copies of HLA class I genes that have arisen during evolution by gene duplication but which have defects (deletions, frame-shift mutations, premature stop codons, etc.) which prevent the transcription of messenger RNA, or translation into functional protein.

The HLA-DR antigens are coded for by a family of very tightly linked genes. The first gene codes for the HLA-DR α-chain. The HLA-DR α-chain forms a dimer with a HLA-DR β-chain. The highly polymorphic HLA-DRB1 gene codes for the β-chain of HLA-DR1–18 antigens. The HLA-DRB5, DRB3, and DRB4 genes code for the HLA-DR51, DR52, and DR53 antigens, respectively. The HLA-DR genes are so tightly linked that one can rely on certain associations, such as the association of HLA-DR3 with HLA-DR52. These associations can be helpful clues in determining an individual's HLA type.

The HLA-DQ antigens are coded for by two genes for the HLA-DQ α-chain and two genes for the HLA-DQ β-chain. The HLA-DQ and HLA-DR loci are also tightly linked to one another. This linkage can make it difficult to distinguish the effects of HLA-DR matching from those of HLA-DQ matching on graft survival. At least one study has suggested that HLA-DQ mismatches may have an adverse impact on cardiac graft survival.

Within the MHC are also many other genes that have important functions in the immune system, including antigen processing. These include the TAP genes which encode a transport protein that moves peptides from the cytoplasm to the endocytoplasmic reticulum, where they can form complexes with HLA proteins[36]. The LMP genes code for a protease which cuts cytoplasmic proteins into peptides of the right size to complex with HLA proteins[36]. Additional genes, of which the function is uncertain, are also included in the class II region (DMA, DNA, DMB, and DOB), as well as several pseudogenes.

HLA-A, B and C antigens (MHC class I)

HLA-A, B and C antigens are expressed on the surface of virtually all nucleated cells. To determine an individual's HLA-A, B and C phenotype, the lymphocytes (T lymphocytes or whole blood lymphocytes) are incubated with a large panel of antisera with known HLA specificities, and then with rabbit complement. These antisera are derived mostly from the serum of multiparous

women, and interpretation may be complicated by cross-reactivity or unrecognized specificities in these antisera. The lymphocytes are lysed if they bear the antigen recognized by the antisera. For example, if the antiserum known to contain antibodies against HLA-A1 lyses the lymphocytes, then those lymphocytes are coded as HLA-A1. Some monoclonal antibodies are now available and, when enough specificities are developed, these may replace antisera.

It has recently become possible to type the HLA-A, B and C genes using molecular techniques[44–48], but to date HLA-A, B and C antigens are not routinely typed by molecular methods. In the research setting these methods have already shown that existing HLA serologic types may be broken down into many additional subtypes. DNA typing methods are currently being used in the clinical laboratory, primarily for HLA class II antigen typing (and therefore DNA typing methods will be discussed further under the section on MHC class II antigens). As DNA testing methodology improves, HLA class I antigens will probably also be determined by DNA typing methods.

At the HLA-A locus there are 57 known alleles (different genes that may occupy the same position or locus on a specific chromosome), at the HLA-B 118 alleles, and at the HLA-C 35. Each allele produces a gene product which is expressed on the cell surface as an antigen. Table 1 shows only those specificities which can be detected using serological methods[39]. Each individual has two chromosomes 6, one inherited from each parent, and therefore has two of the 57 HLA-A antigens (for example, A1 and A2), two of the 118 HLA-B antigens, and two of the 35 HLA-C antigens. If the recipient and donor are not HLA-identical, the immune response of the recipient following organ transplantation

Table 1 Complete listing of recognized HLA serological specificities (1995)[39]

HLA-A	*HLA-B*	*HLA-C*	*HLA-DR*	*HLA-DQ*
A1	B7	Cw1	DR1	DQ5(1)
A2	B703	Cw2	DR103	DQ6(1)
A203	B8	Cw9(3)	DR15(2)	DQ2
A210	B13	Cw10(3)	DR16(2)	DQ7(3)
A3	B64(14)	Cw4	DR17(3)	DQ8(3)
A11	B65(14)	Cw5	DR18(3)	DQ9(3)
A23(9)	B62(15)	Cw6	DR4	DQ4
A24(9)	B63(15)	Cw7	DR11(5)	
A2403	B75(15)	Cw8	DR12(5)	
A25(10)	B76(15)		DR13(6)	
A26(10)	B77(15)		DR14(6)	
A29(19)	B71(70)		DR1403	
A30(19)	B72(70)		DR1404	
A31(19)	B18		DR7	
A32(19)	B27		DR8	
A33(19)	B35		DR9	
A34(10)	B37		DR10	
A36	B38(16)			
A43	B3901		DR51	
A66(10)	B3902		DR52	
A68(28)	B39(16)		DR53	
A69(28)	B60(40)			
A74(19)	B61(40)			
	B4005			
	B41			
	B42			
	B44(12)			
	B45(12)			
	B46			
	B47			
	B48			
	B49(21)			
	B50(21)			
	B51(5)			
	B5102			
	B5103			
	B52(5)			
	B53			
	B54(22)			
	B55(22)			
	B56(22)			
	B57(17)			
	B58(17)			
	B59			
	B67			
	B73			
	B7801			

is directed primarily against those antigens on the surface of the donor organ that are not shared by the two individuals.

The exact role of HLA-A, B and C matching between recipient and donor in allograft survival remains controversial, but analyzes from large regional and national centers in Europe and North America have found a consistent correlation between HLA-A and/or B matching and improved renal graft survival[49–51] (Chapter 44). The survival rate of recipients of well-matched renal allografts is approximately 10–20% higher than that of recipients of poorly matched allografts. According to some, matching for 'splits' (subgroups) of HLA-A, B antigens resulted in better long-term graft survival than when transplants were matched for a 'broad' antigen[52,53]. It should be noted that matching for HLA-B, DR or DR alone (see MHC class II) seems to be most beneficial in improving graft survival. Several reports have demonstrated no evidence for an improved outcome of cardiac allograft survival with HLA-A and B matching[28,54–58]. However, in a study of 164 consecutive cyclosporin-treated cardiac transplant patients, the Stanford group found that the degree of matching for HLA antigens at the A and B loci correlated with long-term survival in their patients[59]. The group at Houston also found that a better donor/recipient HLA matching was associated with improved graft and patient survival[60]. In addition they, like several others, showed that the incidence and severity of graft rejection episodes were diminished in the well-matched patient group[58,60–62]. Preliminary studies have shown that death from allograft vasculopathy after a heart transplant is associated with a higher degree of HLA mismatches[63]. The clinical relevance of HLA matching in heart transplantation is (as is the case in kidney transplantation) most noticeable when matched for HLA-B and DR or DR alone (see MHC class II)[52,56,62,64–66].

Analysis of the effect of HLA-C matching on renal allograft did not show any significant difference in transplant outcome between well-matched and poorly matched donor–recipient groups[54,67]. This may be due to the fact that there is a high frequency of undefined HLA-C 'blank' phenotype when using serological methods, presumably due to the lower expression of HLA-C antigens on the cell surface compared to that of HLA-A and B antigens. Recently, HLA-C typing by molecular techniques has become a reality[48], and may throw a different light on the role of HLA-C antigens as transplantation determinants. In a renal transplant recipient, mismatched for a subtype of HLA-B22 as well as the HLA-C locus, Baan *et al.* demonstrated that the *in vitro* response by the kidney-derived lymphocytes was stronger against the HLA-C locus than the HLA-B locus product[68]. This confirmed the immunological competence of the HLA-C antigens in a clinical setting.

HLA-DR, DQ and DP antigens (MHC class II)

The MHC class II region contains all the genes encoding the known HLA class II molecules: HLA-DR, DQ and DP. The HLA class II antigens have a more limited tissue distribution than do the class I antigens. HLA class II antigens are expressed on the surface of B lymphocytes and on antigen-presenting cells (e.g. macrophages, dendritic cells, etc.) and thymic epithelium, all of which are involved in interaction with helper T lymphocytes during normal immune responses. They are also expressed on other cell types, including vascular endothelial cells, thymic epithelium, and Langerhans cells. Many other cells which do not express class II antigens under normal conditions may be induced to express them. One example would be T lymphocytes which express class II antigens when activated[69]. Also, cytokines, such as γ-interferon, are known to induce HLA class II antigen expression on epithelial or endothelial cells. Thus, increased expression of class II antigens has been seen on renal epithelial cells which are involved in a rejection episode[70].

The expression of HLA class II antigens on antigen-presenting cells reflects their importance in the process of presenting antigens to CD4+ helper T cells. Class II antigens are thought to preferentially present peptides that are derived from proteins that are phagocytosed by antigen-presenting cells[37]. In contrast, class I antigens preferentially present peptides derived from cytoplasmic proteins (e.g. self and viral peptides) to CD8+ cytotoxic T cells.

Serologic typing of HLA-DR and DQ antigens is performed in the same manner as HLA class I typing, except that purified B cells are used instead of whole blood lymphocytes, which are about 80% T cells (which do not bear class II antigens). HLA-DP typing is rarely used for patient selection in clinical laboratories because there is little evidence that DP matching affects graft outcome, and because until recently typing had to be determined by complicated cell culture assays. More recently, monoclonal antibodies for HLA-DP typing have become available, as have DNA techniques.

Many laboratories are using DNA testing to determine the class II antigen types for some or all of their patients. Some of the advantages of DNA-based typing are:

(1) It does not depend on antigen expression, purified B cells, or cell viability, which makes it more reliable with fewer blank alleles and less repeat testing.
(2) It is more precise. Many of the amino acid differences between alleles occur in areas of the protein that are not accessible to antibodies, and are therefore not recognized by antisera. Although subtle, these differences can be recognized by the T cells responsible for cell-mediated rejection. Many such subtypes of serologic types have been identified.
(3) Unlike antisera, which are in short supply and expensive, DNA typing reagents can be synthesized in unlimited quantities. However, not all information gained from DNA typing may be clinically useful.

The most common DNA-based typing methods used in clinical laboratories are polymerase chain reaction-sequence specific oligonucleotide probe (PCR-SSOP), PCR restriction fragment length polymorphisms (PCR-RFLP)[71], and PCR-sequence specific primers (PCR-SSP)[72–75]. The main disadvantage of DNA-based typing is that polymerase chain reaction-based tests are very sensitive to contamination by DNA from previous specimens. Thus, great care must be taken in designing the facilities and procedures to prevent such contamination. Fewer laboratories are performing class I typing by DNA-based methods because it is more complicated. There are more HLA class I alleles that must be distinguished and the polymorphic areas of the HLA class I alleles are spread across multiple exons. However, DNA testing technologies are evolving rapidly and many more laboratories will be using DNA-based typing even for HLA-A, B, C typing within a few years.

Matching for HLA-DR was first shown to influence the outcome of cadaveric renal transplantation by Ting and Morris[76] and Persijn *et al.*[77] in 1978. It has been said by Ting that HLA-DR incompatibility between donor and recipient may well be the major stimulus for the generation of the immune response against a transplanted kidney[78]. The positive effect of matching for HLA-DR has been confirmed by numerous single and multicenter studies[79–81].

Data from the Collaborative Transplant Study showed that many of the serological HLA-DR typings were incorrect when compared to the DNA-RFLP method, and graft survival was significantly improved ($p < 0.02$) in the HLA-A, B, DR DNA-matched grafts (87%) compared to the mismatched grafts (69%)[82,83]. Even centers which had previously found no association, or only a weak association (presumably due to incorrect HLA-DR typing), now find an association between the results of matching and kidney graft survival when using molecular methods to type for HLA-DR[84,85].

The question whether matching by genomic DRB1 typing (i.e. typing for alleles which cannot be determined by serology) may lead to further improvement of graft survival in HLA-DR matched combinations were studied by Leivestad *et al.* and Ichikawa *et al.*[86,87]. They found that genomic DRB1 matching did indeed further improve the clinical course of serologically (DR1–10) matched renal grafts. This is in contrast to the findings of Opelz *et al.*, who found no such effect[83]. The importance of HLA-DRB1 amino acid residue matching between recipient and donor cadaveric renal transplantation was investigated by the group of Tsuji from Japan[88]. They found that rejection correlated with the incompatibility of the DRB1 amino acid residue on the β-pleated sheet rather than on the α-helix. Especially the frequency of mismatches in the second variable region on the β-pleated sheet was significantly higher in the rejection-positive group as compared with the rejection-negative group.

There are many studies indicating that matching for HLA-DR improves cardiac allograft survival[28,64,66,89,90]. HLA-DR matching also appears to reduce the incidence and severity of rejection episodes in cardiac transplant recipients[58,64,90–94]. The role of matching for HLA-DRB1 in heart transplantation has hardly been evaluated. Poli *et al.*[95] found no HLA-DRB1 matching effect on heart transplantation outcome. However, HLA class II matching also appeared to influence survival after combined heart–lung transplantation[96].

Few data are available on the relevance of matching for HLA-DQ antigens in organ transplantation, due partly to the fact that not all centers type for HLA-DQ and partly to the strong linkage disequilibrium of HLA-DQ with DR. There are studies which have shown a beneficial effect of HLA-DQ compatibility on the survival of cadaveric renal allografts[97–100]. However, the study of Bushell *et al.* showed no significant effect on renal graft function or outcome in a small group of patients with HLA-DQ mismatches in the presence of compatibility for HLA-DR[101]. That the effect of HLA-DQ compatibility in organ transplantation still has to be elucidated is most markedly demonstrated by the fact that one group even found a negative effect of HLA-DQ compatibility on the survival of haplo-identical living-related kidney grafts[102]. A study conducted by the Pittsburg group on 349 cardiac transplant patients demonstrated an increase in the risk of earlier-onset cellular rejection and lower rejection-free survival when recipient/donor pairs were incompatible for HLA-DQ[61].

The role of HLA-DP in the outcome of heart and kidney transplantation has hardly been investigated. In a single-center report on renal graft survival no effect of HLA-DP matching was found[103]. This group also investigated the effect of HLA-DPB1 mismatches in a group of zero-mismatched HLA-A, B and DR renal transplants, and again found no effect of HLA-DP matching on graft survival[104]. However, in a study on HLA-DP matching in HLA-haplo-identical living-related kidney transplants it was demonstrated that DPB compatibility increased the long-term graft survival rate[105].

Is it time to allocate hearts on the basis of HLA matching?

A report from the Collaborative Transplant Study analyzing 8331 patients who underwent primary cardiac transplantation in 104 centers shows an impressive correlation between matching for HLA-A, B and DR and graft survival after 3 years[106] (Chapter 44). Matching for HLA-DR alone was also associated with a beneficial effect, but not as marked as that of matching for HLA-A, B and DR. The time constraints surrounding donor heart preservation have made prospective HLA matching impossible. Opelz and Wujciak[106], however, suggested that the time has come to begin transplanting hearts on the basis of prospective HLA matching. This could be done by typing all potential recipients in advance and typing the potential donor (before the heart is removed) with the newly developed rapid DNA typing techniques. They estimated that the creation of a pool of 1000 recipients would allow approximately one-third of patients to receive a heart with no or only one HLA-A, B or DR mismatch. In a comment on this study, Morris supported the view of Opelz and Wujciak in saying that evidence is slowly accumulating to support prospective HLA matching, including crossmatching in sensitized recipients, in order to select well-matched recipients for cardiac transplantation[107].

Do we have to look at matching for HLA antigens differently?

An interesting new view on HLA matching in renal transplantation has been proposed by Terasaki's group[108]. They were able to identify permissible mismatches based on an extensive analysis of the UCLA renal transplantation data. Permissible antigens were defined as those mismatches that led to immunologic failure in less than 15% of cases. They found that cadaver donor kidney transplants, judged to have received a permissible mismatch of the A, B or DR loci, had graft survival rates equivalent to grafts where there were no A, B, DR mismatches. These data were supported by the Eurotransplant data[109]. The data are preliminary but, if confirmed, could greatly increase the number of well-matched grafts.

SEROLOGIC HISTOCOMPATIBILITY TESTING

Patients who require a transplant may have preformed (allogeneic) antibodies due to their previous exposure to sensitization sources: (a) blood transfusion, (b) organ transplantation and/or (c) pregnancy. Since the original discovery of hyperacute rejection

of kidney allografts in patients with preformed HLA antibodies, lymphocytotoxic crossmatches have been performed before all kidney transplantations[110]. Currently, hyperacute rejections are exceedingly rare. Thus, conventional crossmatching techniques are adequate to nearly eliminate the risk for such complications[111].

It has long been established in kidney transplantation that antibody-mediated graft rejection is most often caused by preformed antibodies to HLA antigens[110]. These antibodies, identified by testing for reactivity with a panel of T and/or B lymphocytes which have been previously HLA typed, are known as panel-reactive antibodies (PRA). A lymphocytotoxic crossmatch identifies antibodies reactive with the donor's lymphocytes.

However, it is not generally accepted that crossmatches have to be performed before allogeneic liver, heart or lung transplants, since it has not been unequivocally shown that these organs can succumb to hyperacute rejection[61,112]. The debate arises, in part, due to a lack of standardization in assay techniques and an incomplete description of the antibodies responsible for the positive crossmatch[113]. More recent data indicate that the presence of donor-specific HLA antibodies is associated with an increased risk of immunological complications following both liver[114] and heart transplantation[115]. The absence of HLA-specific antibodies does not guarantee that hyperacute rejection will not occur. Hyperacute rejection has been reported in patients without evidence of a positive PRA and in patients with a positive PRA but who had a negative donor lymphocyte crossmatch[112]. Also, not all patients who have had a positive crossmatch will have hyperacute rejection[116]. However, the presence of donor HLA class I-specific IgG antibodies should be considered a high risk factor for early graft failure. In a study by Smith *et al.*[117] the actuarial 1-year survival of 258 recipients with negative T lymphocyte crossmatches was 73% compared to 28% for seven recipients with a positive crossmatch. Ratkovec *et al.* also analyzed 328 cardiac allografts and showed that rejection occurred earlier, and survival was poorer, in recipients with a positive crossmatch[113].

Data from the International Society for Heart and Lung Transplantation Registry showed that in retransplantation the incidence of a positive PRA rises to 20% from 2% in primary transplants, and the incidence of a positive T lymphocyte crossmatch rises to 17%[118]. Patients retransplanted with a positive crossmatch had an actuarial 1-year survival of 33% compared to 72% for recipients with negative crossmatches[118]. Reports of uneventful transplantation of patients who were found retrospectively to have positive crossmatches have generally stated that the crossmatches were weak or doubtfully positive, were IgM, or had no specificity for the donor's HLA antigens. The interpretation of crossmatch results at the time of transplant is made much easier and more reliable if pre-existing antibodies have been well characterized prior to transplant.

Panel-reactive antibodies

The panel-reactive antibody (PRA) screen is used to determine the degree of humoral sensitization of a patient awaiting transplantation. When a patient becomes a candidate for thoracic organ transplantation, a fresh serum sample should be screened against a panel of at least 30 HLA typed T lymphocytes. If the screen is positive, the serum should be tested against a larger panel in order to identify the HLA specificity of the antibodies (i.e. to detect preformed HLA antibodies in the recipient). These antibodies are often multispecific, but often these specificities fall into one of several well-characterized crossreactive groups[119]. A history of the patient's prior transfusions, pregnancies, miscarriages, or transplants should be obtained, and this information given to the laboratory. A positive PRA in a patient with no history of a sensitizing event should be carefully scrutinized.

Patients who show reactivity with a high percentage of the panel, without a well-defined specificity, should also be tested for the presence of an autoantibody. The characteristics of an autoantibody are that it: (a) reacts with the patient's own cells, (b) reacts with many of the panel cells without a clear specificity, and (c) is entirely of the IgM class. A crossmatch with the patient's own cells will determine if the antibody is autoreactive, and treatment of the serum with a reducing agent such as dithiothreotol (DTT) will remove any IgM antibodies[120]. If the serum is autoreactive and DTT treatment removes that reactivity, then the PRA should be repeated with DTT-treated serum to determine if there is also a true alloantibody previously hidden by the autoantibody. If an autoantibody is present, then at the time of transplant the crossmatch should be performed using DTT-treated serum.

Patients who have high PRA results and who are transplanted with a crossmatch-negative donor may still have an increased risk of rejection. The influence of preformed non-donor-specific T cell lymphocytotoxic antibodies (that is, antibodies that have been demonstrated against a panel of T lymphocytes only, but not specifically against donor T cells) on the subsequent survival of an allograft remains uncertain. There is some evidence that patients with broadly reactive antibodies (antibodies with a high frequency of lymphocytotoxicity against a random panel) have impaired renal[121–123], heart–lung[96], and cardiac[116] graft survival rates. In a histological analysis of 2564 endomyocardial biopsies in 349 cardiac transplant patients, Zerbe and co-workers showed that sensitized patients with panel-reactive lymphocytotoxic antibodies >10% experienced more histological rejection than non-sensitized patients[61].

Should the recipient be shown to have preformed lymphocytotoxic antibodies against T cells, difficulty may be met in finding a compatible donor for that patient. If there are no preformed antibodies, selection of a suitable donor should in all likelihood be easy. Most cardiac transplant centers require a negative prospective donor-specific lymphocyte crossmatch in 'sensitized' recipients with elevated PRA values[112,113,116,124]. We believe that a donor-specific lymphocytotoxic crossmatch should be performed whenever preformed lymphocytotoxic antibodies (HLA class I IgG) have been identified, and that it is preferable in cases where no antibodies have been detected.

The difficulty in obtaining donor blood or lymph nodes prior to organ harvesting from distant donors, and the very limited acceptable cold ischemia times, may make this impractical. In that case the patient may be limited to the use of organs from local donors, which may make it very difficult to find a suitable donor organ. Many centers for this reason set thresholds of PRA of >10% or 15% for requiring a prospective crossmatch.

If the patient receives a blood transfusion after his/her initial antibody screen, it is extremely important that the patient be tested again approximately 1 month after the transfusion. If this

screen is positive, the patient should be followed monthly for 3–6 months because the specificity often becomes narrower, or sometimes disappears. Retesting patients after transfusion is probably the most often overlooked procedure, but if not done may have catastrophic consequences, since those patients with a negative PRA are usually transplanted before the results of lymphocytotoxic crossmatching become known.

Lymphocyte crossmatching

Lymphocyte crossmatching is performed to detect donor-specific anti-HLA antibodies in the serum of the transplant recipient. Most cardiac transplant centers require a negative prospective donor-specific lymphocyte crossmatch in 'sensitized' recipients with elevated PRA values[112,113,116,124]. HLA class I antigens are found on most nucleated cells in the body, including the graft endothelial cells, which are the principal target of hyperacute rejection. HLA class II antigens are not normally expressed on endothelial cells so the presence of antibodies to these antigens is probably rarely associated with hyperacute rejection.

The crossmatch method that has been widely used is the cytotoxicity method, using either donor splenic or whole blood lymphocytes (>80% T cells which do not express HLA class II antigens), or purified populations of T and B lymphocytes. This method only detects complement-fixing antibodies, i.e. of the IgM class and of some IgG subclasses (IgG1, IgG3). New and more sensitive methods have recently been introduced for crossmatching (see below) which, in addition to being more sensitive, are not dependent on complement binding and therefore can be used to detect non-complement-fixing IgG2, IgG4 and IgA antibodies. The augmented antiglobulin test or the flow cytometric assays are much more sensitive than the conventional cytotoxicity tests. Antibodies demonstrable only by these more sensitive crossmatch methods have been found in patients with early non-functioning kidney allografts, as well as patients with early acute rejection. Recent reports have also shown that certain non-HLA antibodies may be harmful to the transplanted allogenic kidney graft[111]. Endothelial cell-specific antibodies have been identified in patients with early immunological complications. These antibodies can be identified using fluorescent activated cell sorter analysis. The importance of a low-level allosensitization only demonstrated using the more sensitive crossmatch methods is still uncertain[111].

Several modifications of the standard complement-mediated lymphocytotoxicity assay have been developed, and different laboratories may use one or more of these techniques. The final crossmatch should be done using a technique that has increased sensitivity over the standard NIH method[125].

Standard NIH method

Donor lymphocytes are isolated from the donor's blood, lymph nodes or spleen and incubated for 30 minutes with the donor's serum, undiluted and diluted. Baby rabbit complement is then added and incubated for 60 minutes. The reaction is stopped by the addition of EDTA and a vital dye (eosin or trypan blue), and the reactions are scored from 1 to 8+ based on the percentage of cells killed.

Extended incubation method

The standard method is used except that the incubation times are extended up to 60 min with the patient's serum and 2 hours with complement. This increases the sensitivity of the crossmatch but may also increase the incidence of non-specific cytotoxicity.

Amos modified method

The standard method is used except that the cells are washed once after incubation with the patient's serum, before adding complement. This removes any 'anti-complementary activity' that may be present in the serum, prior to incubation with complement, and thus increases the sensitivity of the assay.

Anti-human globulin method

The standard method is used except that an antiglobulin reagent (usually a polyclonal anti-human kappa light chain antiserum) is added after incubation with the patient's serum. This modification increases the sensitivity of the crossmatch for antibodies that bind complement poorly.

Flow cytometric crossmatch

This method uses fluorescently tagged secondary antibodies (anti-IgG or anti-IgM) and a flow cytometer to analyze reactivity with individual cell types. This can be useful in determining the specificity of recipient antibodies and in enhancing the sensitivity of the crossmatch.

The patient's initial serum and a current specimen collected within 48 hours prior to transplant should be tested, along with any sera collected following a sensitizing event or showing a peak in PRA activity. If a positive crossmatch is seen, then the immunoglobulin class should be determined by incubating the serum with a reducing agent such as DTT. An IgG antibody in a current serum should be considered a contraindication to transplantation. However, in cases of extreme urgency some patients have been transplanted using plasmapheresis and immunosuppression to remove most of the serum antibody[126].

The significance of an IgM antibody in a current serum is more controversial. In some studies of primary transplants a positive crossmatch has not been predictive of hyperacute rejection[58,61,112]. In most cases these have been described as weak or doubtfully positive due to IgM. In those patients who also had a positive PRA, the specificity of the antibodies was not to the donor's HLA type. This is consistent with the experience in renal transplantation where the presence of IgM antibodies only was not predictive of hyperacute rejection[120]. If the patient has recently been transfused, then the presence of IgM antibody alone may be indicative of a primary antibody response, and thus may be of more significance. Patients receiving secondary grafts are at increased risk of graft rejection. Although there are no data on the significance of IgM antibodies in this group, one might be more reluctant to discount such an antibody in a patient about to undergo a second transplant.

A positive crossmatch with a past serum, when the current serum is negative, may not be predictive of graft survival in primary transplants. Cardella and co-workers claimed, and re-

cently affirmed, that kidney transplantation in recipients who had a positive historic-serum donor-specific reaction, but negative current-serum crossmatch, could safely be transplanted[127]. However, others reported a reduced survival in patients transplanted where the same conditions pertained[111]. Similar data are not available for recipients of cardiac or pulmonary transplants.

Although the importance of T cell lymphocytotoxic antibodies in the subsequent destruction of an allograft is accepted, that of B cell lymphocytotoxic antibodies remains uncertain[128]. As a result of the uncertain clinical significance, the B cell crossmatch is not universally performed before renal transplantation. It should be noted that reactivity with B lymphocytes can be caused by autoreactive antibodies, by other non-HLA specific antibodies, or by low concentration of class-I-specific antibodies, rather than the commonly assumed presence of HLA class-II-specific antibodies[111].

The role of B cell antibodies in clinical transplantation has received much attention following the first reports of a successful graft outcome in the presence of a positive B cell lymphocytotoxic crossmatch[129,130]. Donor-specific B lymphocytotoxic antibodies, when present in a potential recipient before transplantation, have been variously reported to: (a) lead to early graft rejection[131–133], (b) bear no relationship to subsequent outcome[134,135], and (c) correlate with improved graft survival[136]. It has been established, however, that preformed donor-specific B cell antibodies do not lead to immediate or early allograft failure from hyperacute or accelerated graft rejection, which may occur when preformed donor-specific T cell lymphocytotoxic antibodies are present in the serum[137,138]. Isolated cases have been reported[139], and recently Bunke *et al.*[140] investigated the effect of a positive B cell crossmatch in 69 patients receiving orthotopic cardiac transplants. Six patients in this group who had a positive B cell crossmatch and a negative T cell crossmatch demonstrated an increased incidence and increased severity of rejection episodes in the first 6 months post-transplant.

The evidence that antibody formation following rejection of a renal allograft confers a less favorable prognosis on subsequent graft survival is supported by our own observations in one patient following cardiac retransplantation[141]. This patient developed strong multispecific antibodies against T lymphocytes after rejection at 5 weeks of an HLA non-identical heterotopic cardiac transplant. The heterotopic allograft was excised, and the patient remained alive supported by his own heart. After some months the antibodies could not be detected, although they recurred following a test transfusion of 500 ml of HLA non-identical blood. When another donor heart became available, the donor-specific T cell lymphocytotoxic crossmatch test using both stored and fresh recipient sera was negative. A second heterotopic heart transplant was performed, but the donor heart failed within 5 days, following the onset of a severe irreversible acute rejection episode. At that time, a 32-fold increase in lymphocytotoxic antibodies against donor T cells was demonstrated in the patient's blood.

The following suggestions were made at the recent workshop[111] on the clinical relevance of new crossmatching techniques:

(1) As no consensus could be reached at the workshop, many speakers advocated future controlled trials to clearly establish the clinical importance of the new crossmatch techniques.
(2) It was suggested that panel-reactive antibodies should be characterized in all patients who are to undergo kidney transplantation in terms of the presence of autoreactive antibodies, non-HLA antibodies, and Ig complexes, and that the HLA specificity of the PRA should be defined.
(3) It was proposed that, if sera from alloimmunized patients contain panel-reactive cytotoxic HLA class I-specific antibodies, crossmatches should be performed using a sensitive assay for reactivity against donor cells.
(4) Crossmatches should be performed with donor T lymphocytes using current serum as well as historic serum with the highest reactivity.

The workshop report also stated that crossmatches are not performed before liver and heart transplantations, in most centers. However, inferior survival of grafts performed against a positive crossmatch caused by HLA antibodies was reported in both categories of patients. The importance of HLA-specific alloantibodies in recipients of liver and heart allografts has to be further studied.

PRETRANSPLANT BLOOD TRANSFUSIONS

Prior to 1973, blood transfusions were avoided whenever possible in potential transplant recipients in order to minimize the risk of presensitization to HLA antigens. It was thought that the development of cytotoxic antibodies to HLA alloantigens would reduce the number of potential donors compatible with the particular patient[142,143]. In 1973, however, it was demonstrated in a large renal transplant series that transplant recipients who had never received blood transfusions before transplantation had a significantly lower allograft survival rate than their counterparts who had been transfused[144]. These results have been confirmed by most renal transplant centers[145–148], though the magnitude of the effect varies markedly from center to center. Clinical data on pretransplant transfusions in cardiac allograft recipients also indicate a beneficial effect[149,150].

The beneficial effect of pretransplantation blood transfusion in cyclosporin-treated patients is controversial. Several studies have reported that, in patients transplanted without prior transfusion, graft survival is no longer substantially lower than that in transfused patients[55,151–153], while others still report a beneficial effect[154]. As mentioned by Cecka *et al.*[154], these controversial reports (coupled with the public awareness that recipients of blood transfusions may be at higher risk for acquired immunodeficiency syndrome (AIDS) and other viral infections) present a serious impediment to the physician who must justify recommending blood transfusions to a potential transplant recipient.

The impact of HLA sensitization on a patient needing a cardiac transplant is also much greater than the impact on a patient needing a renal transplant. The renal transplant candidate may have to remain on dialysis for a longer period of time, but in a cardiac transplant patient this may greatly limit the pool of potential donors, and therefore greatly decrease the chance of finding a suitable donor.

Although one major center has reported that favourable renal allograft outcome is directly related to the number of pretransplant transfusions received[155], other groups have not confirmed this. On the contrary, they reported that a single transfusion is as

effective as several transfusions, and lessens the risk of the potential recipient developing antibodies against HLA antigens[156,157]. The number of transfusions needed for optimal effect might also depend on the specificity of the recipient's HLA antigens[158]. Virtually every study indicated that the transfusion effect must contain leukocytes to induce the graft-protecting effect.

The influence of HLA compatibility on the blood transfusion effect has also been studied to determine whether the sharing of one genotypically identical HLA haplotype between the recipient and the donor-specific transfusion might have something to do with these excellent results. Little is known about the effect of HLA class I matching between unrelated blood transfusion donor and recipient. Nube *et al.*[159] observed a beneficial effect of such a matched pretransplant blood transfusion on graft survival, whereas Albert *et al.*[160] were unable to confirm these findings. A study by Lagaaij *et al.* has shown a significant positive effect of one HLA-DR antigen-matched blood transfusion compared to completely HLA-DR mismatched blood transfusions on the survival of heart and kidney allografts[161]. The beneficial effect was said to be due to the absence of increased CTLp reactivity in the one HLA-DR antigen-matched group[162]. Middleton and colleagues demonstrated that the incidence of rejection episodes was significantly reduced in patients who received blood matched for one HLA-DR antigen compared with patients who received random blood[163]. Similar results were seen by Bayle *et al.* in a group of naive patients (no previous pregnancies or blood transfusions) who received haplo- or semi-identical transfusions[164]. This group of patients did not develop anti-HLA antibodies, and had significantly less acute rejection episodes when compared to a group of naive patients who received three random transfusions. Data of Van Twuyver and colleagues suggest that the beneficial effect of donor-specific blood transfusions before transplantation may be explained by the induction of specific transplantation tolerance which may be due to the presence of mixed chimerism[165].

In general, there are three mechanisms that might explain the induction of specific transplantation tolerance[166]: (a) clonal deletion, which implies the elimination of all T cells bearing receptors that are reactive with the specific transplantation antigens of the donor; (b) clonal anergy, which is defined as the inactivation but not elimination of alloreactive cells; and (c) active immunologic suppression, which implies the existence of a specific suppressor cell population capable of inhibiting alloreactive cells.

COMMENT

In summary, although ABO-incompatible transplantation is still considered experimental, the shortage of donors possibly warrants cautious continuation of such trials, despite the limited success rate. This should be combined with immunological modification of the recipient and should be continued only under the close scrutiny of the transplant community. There is evidence to show that HLA compatibility and, in particular, matching for HLA-B, DR or DR alone, seem to be beneficial in improving graft survival. With the advent of HLA class I typing by molecular techniques, new light may be thrown on the role of the HLA-C antigens as transplantation antigens. Investigations into permissible mismatches should be pursued, since this is likely to provide a greater number of successful transplants. Patients with high levels of circulating T-lymphocytotoxic antibodies are at higher risk of losing graft function from acute rejection, despite a negative donor-specific crossmatch at the time of transplantation. However, the importance of B cell lymphocytotoxic antibodies remains uncertain.

The policies and procedures of each thoracic organ transplant center may vary according to the program director's assessment of the available studies. The following recommendations are based upon the authors' assessment of the studies reviewed above.

(1) ABO compatibility between recipient and donor should be confirmed (unless a formal clinical trial has been approved).
(2) All patients to be listed for thoracic organ transplantation should be screened for anti-HLA class I antibodies against a panel of at least 30 HLA-typed T lymphocytes (PRA).
   (a) If the PRA is negative, it is extremely important to have policies and procedures that will assure notification of the transplant team and laboratory if the patient is transfused, or has another potentially sensitizing event (e.g. pregnancy or transplant). If a patient is transfused, he/she should be followed for at least 1 month post-transfusion, to determine whether new antibodies have developed. If new antibodies develop, the patient should be followed monthly until the specificities have been stable for several months (if the patient's clinical status makes this possible).
   (b) If the PRA is positive: (i) the patient's history of transfusions, pregnancies or prior transplantation should be obtained or reviewed; (ii) the serum should be tested against a larger panel of cells (e.g. 60) in order to identify the HLA specificity of the antibody; (iii) the immunoglobulin class of the antibody should be identified by treating the serum with DTT (IgM vs IgG); (iv) if the PRA is high (>50%), has no identifiable HLA specificity, or is entirely IgM, then a crossmatch against autologous lymphocytes should be performed to identify any autoantibody.
(3) All patients who have evidence of prior sensitization to HLA class I antigens should have a T lymphocyte crossmatch performed prior to transplantation, whenever possible. It is acceptable to have a policy that sets a threshold PRA value (usually <10–15%) below which a transplant will be performed before a crossmatch is completed, if prior crossmatching is not feasible (e.g. distant donor).
   (a) A positive crossmatch on a current specimen should be considered a relatively strong contraindication to transplantation. If medical necessity requires immediate transplantation, then some form of antibody reduction treatment should be performed.
   (b) Autoantibodies alone are not a contraindication to transplant.
   (c) There is insufficient evidence in cardiac or pulmonary transplantation to recommend whether a patient with a currently negative but historically positive crossmatch should be transplanted. Evidence from renal transplantation may support transplantation in patients who have not previously been transplanted.

(4) If logistics allow, one should strive to match HLA antigens of the donor and recipient, with preference given to good DR or B/DR matches. There is increasing evidence of improved long-term survival of well-matched grafts, but often medical necessity outweighs HLA matching considerations.
(5) Avoid transfusion of potential transplant candidates whenever possible, and use leukocyte-depleted blood products when transfusion is necessary. The risk of HLA sensitization outweighs any effect of transfusions on graft survival when using modern immunosuppression.

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7
Immunobiology of Allograft Destruction

M.H. SAYEGH AND D.R. SALOMON

INTRODUCTION

The past decade has been marked by a veritable explosion in our understanding of the immune response to alloantigens and the mechanisms of graft rejection. We can now consider both cellular and molecular events that directly impact on the function and survival of transplanted organs. The immune response that once may have seemed a relatively simple set of cell–cell interactions has now been expanded into such marvelous detail that the resulting complexity is a major and daunting challenge for both clinicians and scientists.

For example, we can start with one cell and describe transcription of multiple specific cytokine genes whose secreted products connect with cytokine receptors, resulting in transmembrane signalling connecting these events to a complex cytoplasmic network of enzymes. These in turn signal across the nuclear membrane via DNA binding proteins to regulate the transcription of another set of genes determining cellular behavior. As a result the universe in which we view the immune response now extends from our original vantage point at the cell–cell level to encompass events taking place in the cell cytoplasm and nucleus.

A major theme of this chapter is that we cannot escape the implications of biological complexity of the immune response for the practice of clinical transplantation. On the contrary, we must create an organization for this new material that is relevant to the decisions we make affecting patient care, including the choice of new therapies, and that will survive as a framework for our understanding of new information in this rapidly expanding field. We will consider three questions in this chapter:

1. What is the molecular basis of the immune response?
   (a) the major histocompatibility complex (MHC)
   (b) mechanisms of allorecognition
   (c) mechanisms of T cell activation
2. What are the mechanisms of graft rejection?
   (a) acute and chronic rejection
   (b) cell-mediated and humoral-mediated injury mechanisms
   (c) the adhesion paradigm in rejection
3. Will tolerance ever be a clinical reality?
   (a) definition and mechanisms of tolerance
   (b) strategies to induce tolerance

WHAT IS THE MOLECULAR BASIS OF THE IMMUNE RESPONSE?

The major histocompatibility complex

The MHC molecules are the principal targets of the immune response to allografts[1], although minor or tissue-specific antigens may be other targets. In humans the MHC is located on the short arm of chromosome number 6; it encodes for class I and class II MHC molecules which are expressed on the surface of cells. Class I molecules are on all nucleated T cells and consist of two non-covalently bound polypeptide chains. The heavy (44 kDa) chain is inserted into the plasma membrane, and consists of three domains: α_3 is the closest to the cell membrane and has homology to immunoglobulins, while α_1 and α_2 contain the antigenic portions. The light (12 kDa) chain is called β_2-microglobulin, encoded by a gene on chromosome 15.

There are three class I heavy chain loci: HLA-A, B, and C. The first MHC molecule to be crystallized was HLA-A2[2]. X-ray diffraction studies show that the two membrane distal domains, α_1 and α_2, form a groove along the top surface of the molecule facing away from the cell membrane. The margins of the groove are formed by α-helices, and the base is floored by a series of eight parallel β-strands. The groove, approximately 25 Å long and 10 Å wide, contains a peptide fragment, which is eight or nine amino acids long[3]. The origin of the peptides is the intracellular pool of polypeptides derived from metabolic turnover of housekeeping proteins or intracellular infections, such as viruses. Interestingly, the genes controlling this process are located in the same region as the class II MHC[4].

Class II molecules are expressed on B lymphocytes, monocytes/macrophages, dendritic cells, and some activated T lymphocytes. Endothelial and epithelial cells can express class II MHC upon activation. Class II molecules consist of two non-covalently associated glycosylated polypeptides, both of which are inserted into the cell membrane: α (34 kDa) and β (28 kDa). Each of these chains has two domains with the polymorphic (antigenic) regions on the outer, N-terminal, domains. There are three class II loci: HLA-DP, DQ, and DR. The crystal structure of class II HLA-DR1[5] shows a remarkable similarity to class I, the main

difference being the longer size of the peptides (13–26 residues)[6,7]. Peptides bound to class II molecules are derived from proteolysis in acidic endosomal compartments and represent endocytosed exogenous proteins or micro-organisms.

Mechanisms of allorecognition

T cell recognition of allo-MHC is the primary and central event which initiates allograft rejection[1,8,9]. The two fundamental questions in allorecognition are: first, why is the frequency of alloreactive T cells so high? second, how can positively selected self MHC-restricted T cells recognize foreign antigens as well as allo-MHC?

Lechler and Batchelor, in the early 1980s[10,11], proposed that there are two 'routes' of alloimmunization: one stimulated by MHC antigens on graft passenger cells, and the other by donor MHC antigens after processing and presentation by host antigen-presenting cells (APC). Recent work confirms at least two distinct pathways of allorecognition[8,9]. In the 'direct' pathway T cells recognize intact allo-MHC molecules on the surface of donor or stimulator cells. This recognition is probably by 'molecular mimicry' where T cells recognize *allo-MHC* because it resembles *self-MHC* at the molecular level of three-dimensional structure[12]. Peptides, derived from endogenous proteins, including MHC molecules, bound into the groove of the MHC appear to play an important role in direct allorecognition. In the 'indirect' pathway T cells recognize alloantigen which has been processed and presented as allopeptides in the groove of host MHC by self-APC. Mounting evidence indicates that this indirect pathway may also play an important role in mediating the rejection process.

Direct and indirect allorecognition are not mutually exclusive pathways. Each is mediated by different sets of T cell clones and may both be involved simultaneously (Figure 1). Direct allorecognition clearly accounts for the cytotoxic T cell function targeted against graft cells, while both direct and indirect allorecognition synergize to initiate and amplify T helper cell function. Activated $CD4^+$ T helper cells secrete cytokines and provide the necessary signals for the growth and maturation of effector mechanisms of allograft rejection including $CD4^+$ lymphocytes mediating delayed-type hypersensitivity (DTH), cytotoxic $CD8^+$ T lymphocytes, and antibody-producing B lymphocytes (see below).

It has been suggested that early acute allograft rejection is predominantly mediated by the direct pathway, since the graft contains a significant number of donor-derived passenger APC which are capable of providing the necessary co-stimulatory signals for full T cell activation (see below). The indirect pathway of allorecognition, on the other hand, may play the dominant role in chronic allograft rejection. However, the relative contributions of direct versus indirect allorecognition to acute and chronic rejection remain unclear, and studies to elucidate these mechanisms are necessary for the development of better strategies to prevent chronic rejection. Finally, in xenotransplantation there is evidence for both direct as well as indirect presentation of xeno-MHC, although the contribution of these pathways to xenograft rejection is currently unknown.

T cell activation, co-stimulation and adhesion

The first step in T cell activation is the binding of the TCR to the MHC molecule. The TCR is a two-chain protein complex (a

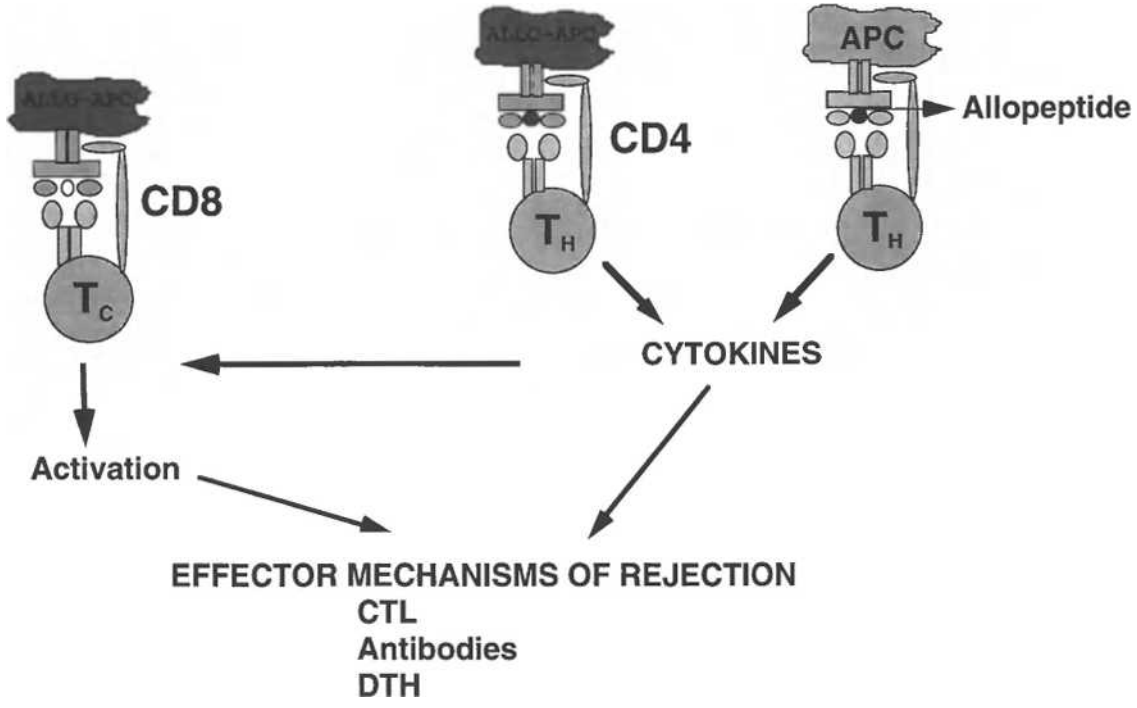

Figure 1 Direct and indirect allorecognition pathways are related

heterodimer) which is anchored in the T cell's membrane at one end (C-terminal) while forming a complex antigen-binding site with its outermost portion. The amino acid sequences of the two TCR chains, α and β, determine the antigen specificity of the two-chain complex. In turn the TCR is connected to the CD3 complex, which consists of at least five molecules all of which are required for signal transduction after the TCR successfully engages its specific antigen. Because the TCR cannot be expressed on the T cell surface without the CD3 complex, we refer to the whole group as the *TCR/CD3 complex*.

Concept 1: The repertoire of antigens capable of triggering T cell activation is determined during T cell development in the thymus

The earliest steps in T cell development occur in the thymus and involve the random rearrangement of specific variable regions on the TCR α and β chain genes to produce millions of different TCR, each of which is expressed on a given T cell clone. In the next stage the thymus selects those TCR rearrangements that are capable of recognizing self-MHC molecules with good affinity and eliminates the remaining inefficient T cell clones. This is called *positive selection*. In the third step the thymus destroys all the T cell clones that recognize self peptide antigens presented by self-MHC. Otherwise, if these autoreactive T cell clones escaped the thymus they could attack the patient's own tissue and cause autoimmune disease. This is called *negative selection*. The end-result of both positive and negative selection is that less than 5% of the T cell progenitors originally produced in the thymus complete the full cycle of development and leave the thymus to function in the peripheral immune system. The rest die through a process of programmed cell death called *apoptosis*.

As discussed above, the implication of this active thymic selection process for transplantation is that circulating mature T cells are very capable of efficiently binding and responding to antigenic peptides presented by self-MHC molecules (indirect allorecognition) and allo-MHC molecules (direct allorecognition)[8,9]. Whether direct and indirect T cell recognition of allo-antigen differ in activation signals is not presently known.

Concept 2: Adhesion and co-stimulation are necessary and regulate T cell activation

A fundamental feature of the immune response is that the actual affinity of TCR binding to the antigenic peptide/MHC complex of APC is too low to create stable adhesion and T cell activation. Thus, by design, successful TCR engagement requires the simultaneous engagement of one or more secondary adhesion or co-stimulatory molecules to stabilize the cell–cell interaction and provide the necessary signal for full T cell activation.

We have known for quite some time that the CD4 and CD8 molecules define the T helper and cytotoxic T cell subsets, respectively. Two relatively recent advances help explain the significance of this differential expression.

First, it was discovered that the CD4 molecule binds selectively to the MHC class II molecule while CD8 binds to the MHC class I molecule. Thus, these CD4 and CD8 accessory molecules are *adhesion molecules*. In fact, they are members of the *immunoglobulin gene superfamily* which includes adhesion molecules such as ICAM-1 and VCAM-1. This CD4/CD8-mediated adhesion to either MHC class II or I molecules means that $CD4^+$ helper cells must be restricted by antigens presented in the context of class II molecules, and $CD8^+$ cytotoxic T cells must be restricted to antigens presented by MHC class I.

Second, it turns out that endogenous antigens, including self peptides and viral proteins produced within the cell by ongoing replication of virus, are preferentially presented in the context of MHC class I. In contrast, exogenous antigens engulfed and processed by macrophages, such as viral proteins obtained from dying or injured cells or transplant antigens from the organ donor, are preferentially presented by MHC class II molecules. Therefore, the differential expression of CD4 and CD8 has an enormous regulatory significance for the generation of immune responses. This molecular view of helper and cytotoxic T cell activation is also being used to design some novel immunosuppressive strategies based on creating peptide analogs that selectively inhibit either CD4 or CD8 binding to the MHC molecules[45,46].

A perfect example of how complex our understanding of the immune system has become is the evolution of the initially simple concept of accessory molecules described above for CD4 and CD8 molecules. The *first* level of increased complexity is that the T cell expresses multiple molecules besides CD4 and CD8 which can act a receptors for ligands which are capable of stabilizing its interaction with antigen-presenting or target cells. A partial list of T cell surface molecules and their ligands is given in Table 1. Each molecule is capable of mediating a physical adhesion of two cells by binding the respective ligand on the opposite cell surface. This adhesion is the first step in stabilizing the recognition of antigen by the TCR/CD3 complex. The *second* level of complexity is that most of these molecules are constitutively expressed on all T cell surfaces and can bind their ligands without any further cell activation. Thus, T cell activation mediated by TCR/CD3 complex engagement of antigen can simultaneously involve multiple molecules, not just CD4 or CD8. The *third* level of complexity is that there may be a difference between molecules primarily responsible for *adhesion* and those mediating *co-stimulation*. In its strictest definition, co-stimulation may be viewed as signals which prevent *anergy*. Anergy is a state in which the T cell can recognize the antigen via the TCR/CD3 complex yet not initiate its activation cascade. For example, the CD28/CD80 (or B7) pathway is a co-stimulatory pathway. In fact, strategies targeted at blocking CD28-B7 co-stimulation have been effective in suppressing T-cell-mediated immune responses in autoimmune as

Table 1 Adhesion molecules and ligands

Receptor	*Ligand*
LFA-1 (CD11a)	ICAM-1 (CD54)
LFA-1 (CD11a)	ICAM-2 (CD102)
CD2	LFA-3 (CD58)
CD28	B7-1 (CD80)
CD28	B7-2 (CD86)
VLA4 ($\alpha4\beta1$)	VCAM-1 (CD106)
L-selectin (CD62)	MadCam-1
$\alpha4\beta7$	MadCam-1
PECAM (CD31)	CD31
CD31	$\alpha v\beta3$ (VNR)
CD45	Glycolipids

well as transplantation models (see below). In addition, inhibiting LFA1/ICAM1 or CD2/CD58 interactions can also result in potent suppression of the immune response.

These complexities give rise to several questions. If all these molecules are involved in adhesion/co-stimulation, how can we block the immune response by inhibiting one pair? Are there primary and secondary interactions? Do these molecules share signals so that some form of synergy is created? Are there differences in signals for different T cell subsets? Do different immune mechanisms engage different molecules so that blockade of these signal might have one effect on transplant rejection and another on antiviral cytotoxicity or autoimmunity? Are the signals delivered in some specific order so that we could design a strategy to block only the last step in the cascade? Clearly, these questions are directly relevant to the potential future use of these blocking reagents in transplantation therapy.

Concept 3: Cell–cell adhesion/co-stimulation and TCR/CD3 complex engagement triggers a cascade of cytoplasmic enzymes leading to T cell activation

Once the T cell has successfully recognized the target antigen presented by the appropriate MHC molecule the next steps in T cell activation occur in the cytoplasm. In fact, our current view of cytoplasmic signalling is now as complex as the network of T cell subsets, cytokines, and surface receptors determining the course of the immune response on the cell's exterior. Table 2 is a list of currently known cytoplasmic signalling intermediates adapted from a recent review[47].

Table 2 Intracytoplasmic signalling molecules associated with cell membrane receptors and cell activation pathways

Kinases	*SH2–SH3 signalling*	*GTPases*	*Phospholipid mediators*
FAK	Crk	Ras	PIP-5K
Src	Grb2	Rho	cPLA2
Fgr	PI-3K	mSOS1	Arachidonic acid
Csk	PLC	C3G	5-lipoxygenase
PKC		RasGAP	
MAP kinase			

From ref. 47

As shown in Figure 2, the cytoplasmic tails of the different co-stimulatory adhesion molecules, including CD4 and CD8, serve as connecting points for a very complex array of intracytoplasmic enzymes. Our current thinking is that the engagement of the adhesion molecule with its ligand on another cell surface results in a conformational change that is transmitted through the protein to the cytoplasmic tail of the molecule. This conformational change reveals a binding site for the first of several cytoplasmic signalling molecules which triggers a signal cascade much the way we think of cytokines or clotting factor cascades in the extracellular space. This process of molecular activation can also connect the adhesion molecule to the cell's cytoskeletal frame-

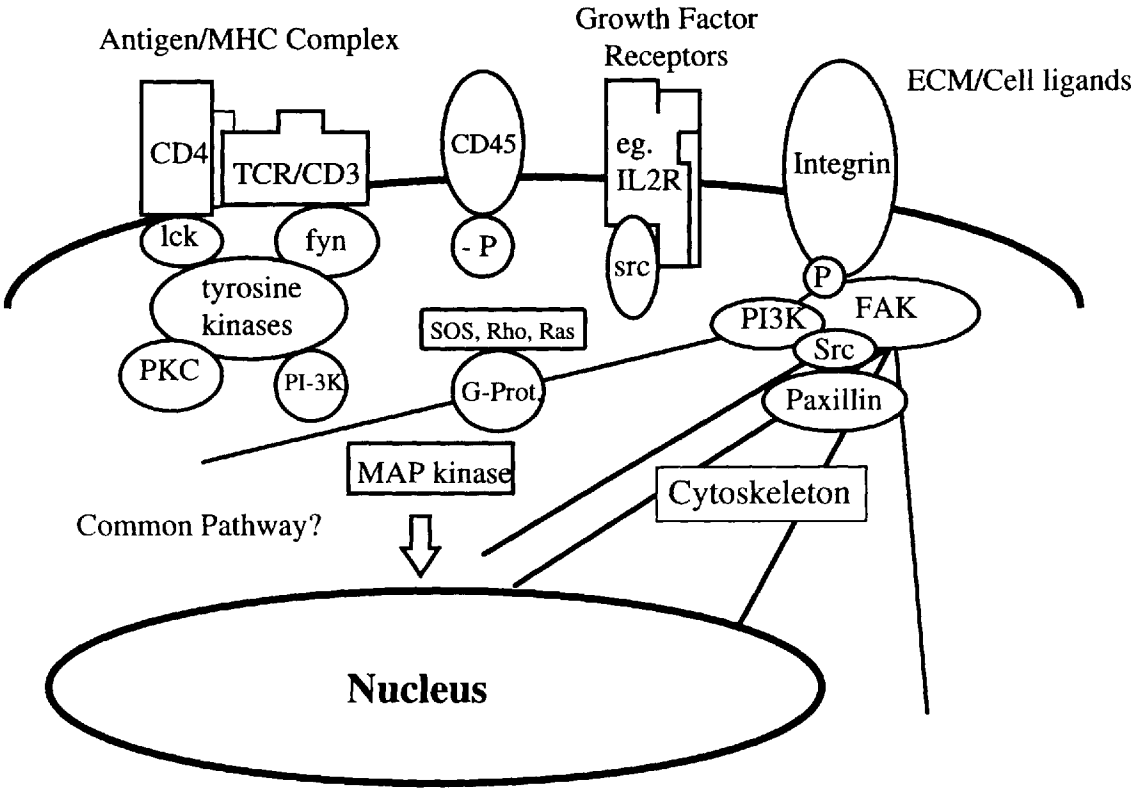

Figure 2 Intracellular cytoplasmic signals

work, a process that is necessary for cell migration and that also determines cell shape.

The TCR/CD3 complex is also directly involved in the generation of cytoplasmic signals; in fact it is the CD3 molecule complex that is responsible for this signalling. A number of cytoplasmic tyrosine kinases such as src, lck and fyn can bind to the CD3 complex, and are activated when the TCR engages its antigen on the cell's exterior surface. Moreover, the close physical location of the co-stimulatory CD4 or CD8 molecule, which is binding to the same MHC molecule as the TCR binding its antigen, may serve to create a bridge between kinases bound to the cytoplasmic tails of the CD3 complex and kinases such as lck bound to the CD4 or CD8 molecules.

Concept 4: Cytoplasmic signalling leads to activation of nuclear transcription factors

The final stage in the molecular events of T cell activation is gene transcription. For example, we know that when a $CD4^+$ helper T cell recognizes an alloantigen during transplant rejection it releases a number of inflammatory cytokines, such as IL-2 and INF-γ. Similarly, when a $CD8^+$ cytotoxic T cell is activated it increases the cell content of an enzyme called granzyme and the molecular complex called perforin, both of which are necessary for killing a target cell. These events in both CD4 and CD8 T cell activation require gene transcription.

Gene transcription is regulated by the cell through the cytoplasmic activation of a number of DNA-binding proteins called *transcription factors*. When a transcription factor is activated in the cytoplasm it can cross the nuclear membrane where it binds to specific regions of the genes usually located upstream of the coding regions and called *promoter sites*. These promoter sites are the control boxes for gene transcription.

While at first glance the idea of transcription factors and promoter regions may seem very esoteric for transplant clinicians, they actually directly determine the function of our most effective immunosuppressive drugs, cyclosporin and FK506 (tacrolimus). When either of these drugs binds to the two cytoplasmic carrier proteins, cyclophilin or FK-binding protein, the resulting complex inhibits another set of cytoplasmic enzymes which prevent the activation of transcription factors, including NFkB and NF-AT. In turn, the lack of the activated transcription factors in the nucleus blocks transcription of the IL-2 and INF-γ genes. The immunosuppressive efficacy of both cyclosporin and FK506 depends on the effective suppression of cytokine gene transcription.

Concept 5: New immunosuppressive strategies will evolve from our knowledge of the molecular basis of T cell activation

We stated in the introduction that a major purpose of this chapter was to create an organization for understanding the immune response at the molecular level that would be useful for following the new developments in immunology.

First, as noted in Concept 4, the mechanisms of action of our two key immunosuppressive drugs are directly explained by alterations in cytoplasmic signalling altering transcription factor function at the gene level.

Second, our discussion of co-stimulation and adhesion molecules explains the immunosuppressive mechanism of a number of new reagents under development for clinical transplantation. For example, a number of monoclonal antibodies to block adhesion molecules are currently in clinical trials, such as anti-ICAM-1, anti-FA-1, and anti-CD45 antibodies. Another generation of new reagents is just being developed, including soluble, molecular-engineered recombinant fusion proteins such as CTLA4-Ig and CD2-Ig which block the CD28/CD80 and CD2/CD58 pathways of activation, respectively (Table 1). We have mentioned that peptide analogs designed to selectively block CD4 or CD8 adhesion are being developed, and anti-CD4 and anti-CD3 monoclonal antibodies are already well tested.

Third, the high complexity of the intracytoplasmic signal cascade has also created numerous possibilities for new drug design. For example, the new immunosuppressive, rapamycin (sirolimus), appears to work by interfering with cytoplasmic signals generated by cytokine receptors and required for cell activation and proliferation. One possible mechanism of immunosuppression with mycophenolate mofetil may be alterations in the synthesis of GTP, a high-energy phosphate intermediate implicated in a number of cytoplasmic and cell membrane kinase activities.

Finally, whole new possibilities for direct immunosuppression will be created as we extend our understanding of the molecular structure and regulated function of nuclear transcription factors and their ability to bind the specific promoter regions of the cytokine genes in the nucleus.

WHAT ARE THE MECHANISMS OF GRAFT REJECTION?

Acute and chronic rejection defined

It is important to understand that acute and chronic rejection can be defined in clinical terms, pathological terms, or immunological terms. However, once defined by one set of terms it is critical to keep all subsequent observations and conclusions in the correct context. The danger is when discussions move back and forth across this field without recognizing the fundamental differences in these definitions.

Clinical rejection is defined by the post-transplant timing of diagnosis, the rate of progression, and by specific disturbances in graft function. Typically, an *acute* cardiac rejection episode occurs in the first 6 months after transplantation and may be associated with increased pulmonary artery pressure, increased ventricular wall thickness or decreased compliance measured by ultrasound. Fortunately, cardiac dysfunction, such as a significantly decreased ejection fraction, or clinical evidence of congestive cardiac failure is rarely seen. In fact, the majority of acute rejection episodes after heart transplantation are based on pathological terms and are just correlated with the clinical situation. In contrast, acute rejection after lung transplantation may present with clinical dysfunction and signs, including dyspnea, and radiographic changes of interstitial inflammation. The clinical aspects of acute rejection in both heart and lung transplantation will be described in great detail in subsequent chapters. A defining feature of clinical *acute* rejection is the sense of urgency that a failure to treat aggressively and successfully can result in the rapid progression to a fulminant rejection and acute deterioration in heart or lung function.

Chronic rejection after heart transplantation typically occurs after 1 year, is often associated with discrete coronary artery lesions identified by angiography, and may also present with significant cardiac dysfunction or electrocardiographic signs of ischemia. The rate of progression is typically slow, measured in months or even years. However, if organ function is diminished at the time of presentation clinically, or if significant myocardial ischemia is present, the patient's clinical situation can deteriorate very rapidly. The progression of chronic lung transplant rejection is often marked by more clinical symptoms and a more rapid downhill course.

Pathological rejection is defined with biopsy material typically viewed by standard light histology, though the use of immunofluorescent staining has also been described. Clearly, the use and interpretation of the routine endomyocardial biopsy in heart transplantation have been one of the major advances in the field. Subsequent chapters will describe the pathological criteria for acute and chronic rejection. The key point to make in the present discussion is that finding pathological changes consistent with organ injury does not predict clinical presentation, and is only roughly correlated with subsequent clinical course.

A second point is that the endomyocardial biopsy provides material from the muscular portion of the right ventricular wall, but provides very little in the way of larger vascular structures for analysis. Thus, the biopsy is a good window into the interstitial cellular infiltrates and injury typically associated with acute rejection. However, it provides little or no information on the larger arterial structures, such as the coronary arteries, which are the focus of the vascular injury defining chronic rejection. These differences are also true for the transbronchial biopsy done in lung transplantation, where there is little sampling of larger vascular structures. Ironically, as a result of these biopsy limitations, our understanding of the progression of chronic rejection at the tissue level is very limited despite the fact that chronic rejection is probably the single greatest challenge to long-term successful transplantation that we face today.

Immunological rejection is the most likely rejection definition to cause confusion. The complexity described above for T cell activation creates multiple immunological mechanisms to test in rejection. For example, the expression of cell proliferative activity in culture with donor-specific APC, the presence of killer cell activity, elevations of cell activation markers such as the IL-2 receptor and adhesion molecules, the release of specific T cell cytokines such as IL-2 or IFN-γ or inflammatory cytokines such as IL-6 and TNF-α, and/or the transcription of the genes for these cytokines or their receptors can all be measured in the laboratory and interpreted as evidence of acute or chronic rejection. Unfortunately, a large number of these immunological parameters will be positive in every patient post-transplantation, and those that are negative may simply reflect the effect of the immunosuppressive drug therapy being routinely administered.

Little is known to correlate the immunological mechanisms directly with the clinical and pathological definitions of rejection. This has resulted in a controversy over whether there is a unique immunological mechanism responsible for chronic rejection, or whether it is merely the progression of low-grade or episodic acute rejection. Thus, despite little or no evidence for a unique immunological mechanism in chronic rejection, the distinct clinical and pathological features have led many to conclude that there must be a separate process. We are skeptical that this is the case, and rather believe that chronic rejection is merely the expression of a lower-grade and ongoing immune response to the transplanted organ. There is no problem if these arguments are used to generate scientific studies to test various theories. However, there is a problem when clinicians determine immunosuppressive therapies and test new drugs based on a conviction that acute and chronic rejection mechanisms are fundamentally separate events.

The summary point is that we must be careful to determine how rejection is defined in any given study or theory, whether it be a laboratory culture, an animal model or a clinical study. While all these approaches are exciting and valuable to our growing understanding of rejection mechanisms, we must remain critical and keep the differences in context. Our ultimate achievement will be when we understand the mechanisms of rejection well enough to successfully integrate the separate clinical, pathological and immunological manifestations into a single working definition directly relevant to patient care, prognosis, and the development and testing of new immunosuppressive strategies.

Cell-mediated and humoral-mediated injury mechanisms

Rejection, acute or chronic, involves tissue injury directed to the transplanted organ and caused by several mechanisms of immune-mediated cell damage. This injury can be cell mediated or humoral mediated.

Cell-mediated Injury

The classic acute cellular rejection seen in the cardiac or lung interstitium is cell mediated. This cell-mediated immune response is initiated by $CD4^+$ T cell recognition of donor antigens. The predominant effector cells are $CD4^+$ T cells and macrophages mediating DTH responses and $CD8^+$ cytotoxic T cells. Stabilization of target binding of T cells is accomplished by adhesion molecules, such as LFA-1 and CD2, on the T cell surface to ICAM-1 and CD58 on the target cell surface. In the case of $CD8^+$ T cells, successful adhesion to the donor target cell results in T cell activation and the insertion of the perforin molecular complex into the donor cell's membrane. Cytolytic enzymes such as granzyme are released by the activated killer cell. The end-result is cell injury leading to death, which is seen on the biopsy as T cells in proximity to injured or dying myocytes or bronchial epithelium. This distinction is important for pathologists, since the mere presence of T lymphocytes in the organ interstitium without evidence of cell injury or inflammation does not constitute evidence of rejection.

For the immunologist the presence of T cells without injury has always been an interesting challenge to explain. Some think that it represents the success of baseline immunosuppression to prevent tissue injury while not being able to prevent activated T cells from entering the transplanted organ. However, others interpret these local T cell infiltrates as specific modulator or suppressor cells which are capable of inhibiting the immune response and injury mediated by the killer T cells. This theory has been given a lot of attention recently, based on the discovery that $CD4^+$ T helper cells can be divided into two opposing subsets based on the pattern of cytokines that they produce. One subset, called Th1, produce primarily IL-2 and INF-γ, while the second subset, called Th2,

produce primarily IL-4, IL-10 and IL-13. There is evidence from animal studies that rejection is a Th1-mediated phenomenon, while Th2 cytokines are relatively suppressive and have been associated with a state of tolerance to allografts (see below). Thus, the balance of Th1 and Th2 cytokines in a transplanted organ may be one determinant of whether rejection occurs or not. Human studies to confirm these observations in animals are required, since they may impact on decisions regarding optimal therapy based on understanding immunological mechanisms of rejection.

The word 'humoral' is defined in the dictionary as pertaining to the body's 'humors'. Immunologists are referring to antibodies and inflammatory cytokines. Transplantation clinicians usually refer to humoral mechanisms in the context of chronic rejection and vascular injury. However, it must be remembered that antibody-mediated rejection can destroy a vascularized transplant in minutes, by causing such profound vascular injury that the vessel thromboses and the organ is acutely ischemic. This hyperacute rejection process is a critical issue in xenotransplantation where natural antibodies recognizing species-specific vascular surface antigens are present.

Antibodies are produced by B cells and plasma cells which receive help from activated $CD4^+$ T cells. The binding site of an antibody is determined by gene rearrangements similar to the rearrangements which produce the TCR specificities in the thymus. A naive B cell may become sensitized to a new antigen when it is presented by the same antigen-presenting cells involved in $CD4^+$ T cell activation. Alternatively, mature B cells can use the antibodies expressed on their cell surface to present antigen to naive B cells and even T cells. The B cells' ability to bind antigen via antibody is very high, so that adhesion molecules are not required to stabilize the binding complex the way they are required for T cell activation. On the other hand, it is important to emphasize that the regulation of B cell immunity does require co-stimulatory signals, and so a rapidly growing number of unique B cell molecules have been identified recently, such as CD23, CD21 and CD40. Therefore, the potential of developing new strategies to manipulate the B cell immune response in transplantation is as great as for the new T cell reagents described above.

Another fascinating problem is to understand the molecular basis of controlling B cell function in the cytoplasm and nucleus. While we have potent T cell immunosuppression with cyclosporin and FK506, we do not have similar agents for suppressing B cell activation. Nonetheless, our current understanding of B cell biology clearly includes the roles of various cytoplasmic kinases and nuclear transcription factors in complex networks very similar to the T cell. It will be a major advance in immunosuppression when the problem of B cell activation is solved by development of a new drug or reagent.

When antibodies engage their antigen target, typically on vascular surfaces but also on the surfaces of epithelial cells in the transplant, they bind and activate complement components. The complement cascade ends with the insertion of a molecular complex into the target cell membrane that is the equivalent of the killer T cell's perforin complex. Complement activation also results in the recruitment of inflammatory cells like neutrophils and macrophages, which secrete various inflammatory cytokines such as IL-1, IL-6 and TNF-α. These cytokines can cause direct cell injury. For example, TNF (tumor necrosis factor) can cause epithelial cell death, and IL-1 can cause myocardial cell dysfunction as well as fever and peripheral vasodilatation. It is likely that such cytokine-mediated injury is a major determinant of fulminant heart transplant failure in some patients with severe acute rejection in which the degree of myocyte necrosis seen in the biopsy cannot explain the profound clinical state. The cytokines can also recruit other inflammatory cells, including T cells, into the site. Finally, the activation of complement, antibody-mediated endothelial injury, and inflammatory cytokine production can all result in local activation of the clotting cascade.

The adhesion paradigm in rejection

We have already described the role of cell–cell adhesion molecules in T cell binding to antigen-presenting and target cells. The adhesion molecules serve to stabilize the TCR/CD3 complex binding to MHC/peptide antigen complexes and may provide co-stimulatory signals involved in T cell activation. However, the role of adhesion molecules in transplantation is even more complex. While antigen recognition and T cell activation are the critical first step in the rejection cascade, the next major challenge is to regulate the traffic of activated T cells from the peripheral lymphoid organs (lymph nodes and spleen) via the peripheral blood to the transplanted organ where rejection must occur. We now understand that this process is also adhesion molecule dependent.

First, we must emphasize that the primary barrier between the transplanted organ and the host immune system is the vascular endothelium. In a vascularized transplant such as the heart or lung, the endothelium is basically from the donor. We have known for a long time that the donor endothelium expresses MHC class I molecules and will increase the expression level and express MHC class II antigens if activated by inflammatory cytokines. The significance of this cytokine-dependent up-regulation of donor alloantigens on the endothelial surfaces of the graft was recognized as generating a 'vicious cycle' where early rejection events were amplified.

The new information is that the donor endothelium will also express a number of ligands for the activated adhesion molecules on the T cells. As described in Table 1, there are multiple adhesion molecule and ligand pairs that could participate in T cell adhesion to the vascular surface of the graft. Moreover, the same inflammatory cytokines that up-regulate MHC molecule expression can also up-regulate adhesion ligand expression. Thus, the role of these inflammatory cytokines in amplifying the rejection process also involves the adhesion molecule paradigm. Three of the best-studied endothelial ligands are intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and a group of glyco-sialylated mucins (also called addressins) which bind the T cell adhesion molecules, LFA-1, VLA4, and L-selectin, respectively. These three endothelial ligands cooperate in a physical process that initially slows down and then stops the activated T cells and leukocytes as they flow through the vascular bed of the graft.

The *adhesion molecule paradigm* explains that a T cell activated by the recognition of alloantigen in a lymph node or the spleen will express the adhesion molecules on its surface in an activated state. T cells that do not recognize the transplant as foreign are left out of the process. The activated T cells circulate

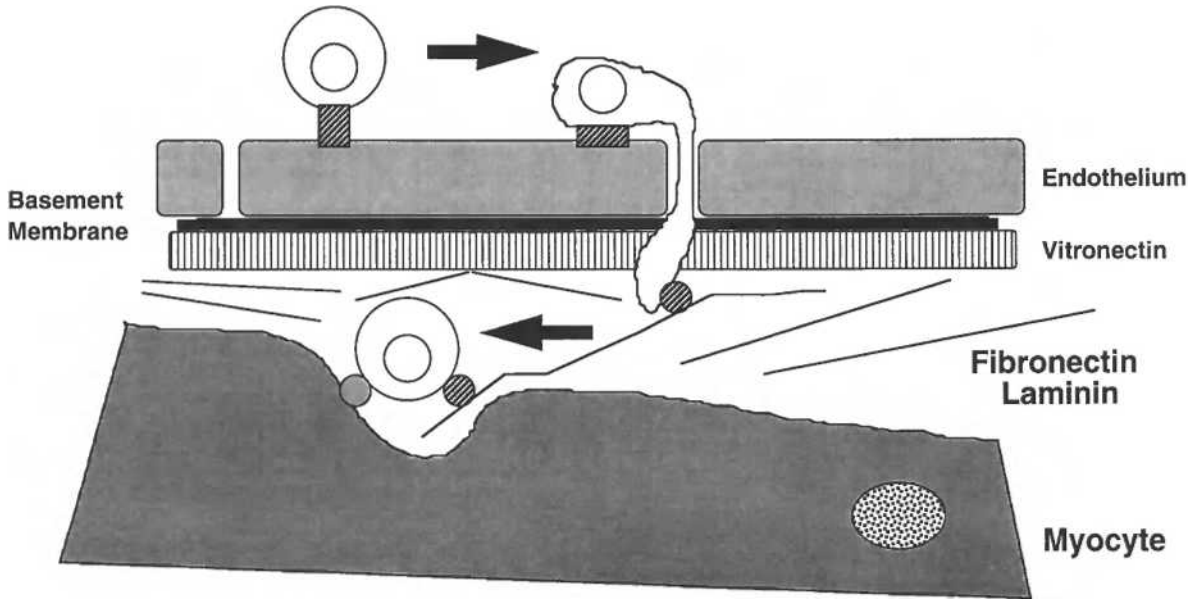

Figure 3 T lymphocyte crosses the vascular barrier and follows ECM fibrils to attack the myocyte: classic cell-mediated rejection

through the peripheral blood to the transplant where they come in contact with endothelial ligands, as described above, and this results in the adherence of these activated T cells to the graft endothelium. The activated T cells then use the combination of alloantigen recognition on the donor endothelium and adhesion molecule/ligand binding to further activate and migrate physically across the endothelial barrier and into the interstitium of the transplant, where rejection occurs. Figure 3 is a picture of such a process in which an activated lymphocyte is shown binding to the endothelial surface of the graft while a second lymphocyte, which has already bound and migrated into the interstitium, is shown attacking a myocyte (i.e. acute rejection).

The pharmaceutical and biotechnology companies have already discovered the potential significance of the adhesion molecule paradigm in the design of new agents to prevent and treat rejection. Clinical trials with a monoclonal anti-ICAM-1 antibody for induction therapy are now under way in kidney transplant patients. An anti-LFA1 antibody has been developed and some preliminary clinical trials started. Anti-VCAM-1 antibodies and anti-VLA4 antibodies have been shown to prolong cardiac allograft survival in animal transplant models. Finally, a whole new generation of novel peptide analog inhibitors of adhesion molecules are now being developed for clinical testing in the next few years.

WILL TOLERANCE EVER BE A CLINICAL REALITY?

Mechanisms of tolerance

The availability of TCR transgenic as well as specific knockout animals has made it possible to study the cellular and molecular mechanisms of self tolerance. The thymus plays the major role in development of self tolerance[13,14]. Precursor T lymphocytes reach the thymus early in fetal life, although there is a continuous input of prothymocytes throughout life. Immature T cells reside in the cortex, and lack the TCR, CD4, and CD8 molecules. These cells undergo population growth, rearrangement of TCR genes, and surface expression of CD4 and CD8 molecules ('double-positive' thymocytes). $TCR^+CD4^+CD8^+$ T cells interact with thymic APC (epithelial cells and bone-marrow-derived macrophages/dendritic cells) which express self-MHC molecules, and undergo selection processes which ultimately shape the T cell repertoire toward self-MHC restriction and self tolerance. Self tolerance is mediated by 'negative selection' by deletion of autoreactive T cell clones.

Surviving TCR^+ T cells move into the medulla, increase the density of TCR expression to that of peripheral T cells, and before leaving the thymus lose one of the co-stimulatory accessory molecules, thus becoming single-positive ($CD4^+CD8^-$ T cells or $CD4^-CD8^+$ T cells) T cells. At the same time 'positively selected' medullary thymocytes acquire MHC-class-II-restricted helper ($CD4^+$) or MHC-class-I-restricted cytotoxic ($CD8^+$) functions, and migrate to the periphery as immunocompetent T cells (see above). There are two types of thymic APC, bone-marrow-derived macrophages/dendritic cells and epithelial cells, and there is evidence that each type of thymic APC has a different function in the induction of self tolerance, and in T cell repertoire selection. Positive selection is thought to be mediated by thymic epithelial cells, and negative selection is mediated by the bone-marrow-derived cells.

Transplantation tolerance is defined as a state of immunologic unresponsiveness to the antigens of the graft without immunosuppression[15,16]. Operatively, tolerance is defined as a reduction in the immunocompetence of lymphocytes responding to the specific antigen. Transplantation tolerance involves several mechanisms (Table 3), and it is perhaps best to classify these mechanisms as 'central' or 'peripheral'. Central tolerance involves thymic deletional mechanisms analogous to self tolerance.

Central tolerance can be induced in experimental animals by creating mixed bone marrow chimeras[16]. This can be achieved by

Table 3 Mechanisms of tolerance

1.	Clonal deletion
2.	Clonal anergy
3.	Regulatory or suppressor cells (infectious tolerance)
4.	Immune deviation (Th1/Th2): ? Role of suppressive cytokines (IL-4, IL-10, TGF-β)
5.	Veto cells: phenotypically distinct cells which inactivate/delete alloreactive T cells
6.	Microchimerism: persistent donor cells in the recipient

infusing a mixture of donor plus syngeneic bone marrow coupled with some kind of a myeloablative regimen. The resultant animals become specifically tolerant to donor alloantigen, presumably by deletion of alloreactive T cells in the thymus. This can also be achieved by using bone marrow infusion with immunosuppressive drugs, although the mechanisms involved with such strategies may not be deletional. Other mechanisms which have been described with such strategies include 'microchimerism' or 'veto cells'. Microchimerism is the persistence of small numbers of donor-derived cells in the recipient[17]. Microchimerism has been reported to be associated with long-term acceptance of allografts in experimental animals as well as in humans, although it has not been established whether the persistence of donor cells is in fact responsible for induction or maintenance of tolerance. It is possible that the persistence of donor cells in the recipient is the result rather than the cause of the tolerant state. Finally, veto cells are cells with a unique phenotype which have been demonstrated to inactivate/delete alloreactive T cells[18,19]. Veto cells have been described in some transplantation models involving donor bone marrow infusion, although the mechanisms of how T cells recognize the veto cell leading to T cell inactivation/deletion remain unknown. Furthermore, whether veto cells mediate the observed association between graft acceptance and microchimerism has not been established.

Mechanisms of peripheral tolerance, on the other hand, involve 'anergy' and/or 'regulatory or suppressor cells'. Anergy is a state of functional inactivation in which antigen-specific T lymphocytes are present, but are unable to respond. Two types of anergy-related mechanisms have been described: T cell anergy which can be reversed by exogenous cytokines, such as IL-2, or 'dense' anergy which is not reversed by cytokines. Biochemical analysis indicates that increased intracellular calcium is associated with T cell anergy. Anergic T cells remain viable but are unresponsive for a minimum of several weeks both *in vitro* and *in vivo*. The fate and function of anergic T cells *in vivo* remain undetermined; however, evidence from experimental models suggests that anergic T cells can be reactivated by infections, presumably cytokine-mediated. Recent evidence also indicates that anergy can be accompanied by variable degrees of deletion, and that anergic T cells may become apoptotic. These observations suggest that anergy may be a reversible state, and that the therapeutic use of tolerance strategies involving mechanisms of T cell anergy alone, although potentially useful, may not lead to permanent tolerance. Strategies which promote peripheral deletion of anergic T cells may be more desirable.

The second major mechanism to explain peripheral tolerance is the identification of 'regulatory' cells which exert antigen-specific suppression of the immune response. The presence of 'regulatory' cells has been demonstrated *in vitro* by suppressor assays as well as *in vivo* by adoptive transfer experiments leading to a state of 'infectious' tolerance, whereby T cells from a tolerant animal can actively transfer tolerance to a naive animal[20]. Although suppressor phenomena have been clearly demonstrated, the suppressor cells themselves have proven difficult to grow and hence to characterize. In addition, the mechanism of action of suppressor cells is still poorly understood, although recent data suggest that, at least in some cases, the tolerogenic effects may be mediated by suppressive cytokines such as TGF-β, for example[21].

A related area which may explain the role of 'regulatory' cells in tolerance is the Th1/Th2 paradigm (see above)[22,23]. Studies of T cell clones show that CD4+ T helper cells are not homogeneous; for example CD4+ T helper cells can be subdivided by their pattern of cytokine production[24] into Th1 cells, which produce mainly IFN-γ and IL-2, and Th2 cells, which produce IL-4, IL-10, and IL-13. Allograft rejection is a predominantly Th1-mediated process, as suggested by up-regulation of mostly Th1 cytokines in rejecting allografts. Th1/Th2 helper T cell subsets have mutually antagonistic activities. IL-4 blocks up-regulation of IL-2 receptor expression and IL-2-dependent proliferation of T cells, suppresses production of IFN-γ by human mononuclear cells, and inhibits up-regulation of endothelial cell expression of ICAM-1 and other adhesion molecules in response to inflammatory stimuli. IL-10 inhibits the production of IFN-γ and IL-2 by Th1 clones. In some experimental transplantation models, intragraft analysis demonstrates that tolerant grafts have decreased expression of Th1 and up-regulation of Th2 cytokines[25].

Such a state of 'immune deviation' toward a predominantly Th2-cell function has also been described to be associated with tolerance in experimental models of autoimmunity[21]. The exact mechanisms responsible for steering the immune response toward Th2, and whether the tolerant state is actually mediated by Th2 cytokines, remain to be determined. The use of cytokine knockout animals, cytokine fusion proteins, and cells transfected to secrete a particular cytokine will help better understanding of these mechanisms, and may give rise to novel strategies for suppressing the immune response to allografts.

Clinically relevant tolerance strategies

Although the induction of transplantation tolerance has been readily achieved in experimental animal models, only few cases of tolerance have been described in humans[26,27]. The use of immunosuppressive drugs or monoclonal antibodies directed at specific T cell surface receptors to induce transplantation tolerance has been extensively studied in animal models of organ transplantation. It is obvious, however, that such strategies alone have not been successful in humans. The use of donor antigens with or without initial immunosuppression, on the other hand, may provide better strategies that can be clinically relevant. In addition, some recent advances in our understanding of mechanisms of lymphocyte activation and tolerance may lead to development of potentially clinically applicable strategies to induce specific tolerance in humans.

Donor antigens

Early experiments on the induction of neonatal tolerance proved that introduction of allogeneic cells or tissue at a 'critical period'

early in development would produce tolerance[28]. Studies have shown that neonatal tolerance is largely due to 'clonal deletion'. Thus, T cells reactive with alloantigen are deleted during their selection in the thymus, presumably by the same mechanisms that delete self-reactive T cells. In adults, administration of donor alloantigen with or without some form of transient immunosuppression has been shown to be effective in inducing tolerance to allografts in several experimental animal models.

Several factors have been found to play a role in the induction and maintenance of the tolerant state. These include the type of donor cells, the dose of alloantigen, the timing of administration of alloantigen, the route of administration, and the immunosuppressive protocol. In regard to the route of administration the intravenous, intraportal, oral, and intrathymic routes have been generally tolerogenic, while the subcutaneous route is typically immunogenic. Intrathymic injection of donor cells, soluble MHC, or MHC allopeptides has been recently shown to induce specific tolerance in several experimental transplantation models, although the mechanisms have not been fully elucidated[29,30]. The applicability of such a strategy in large animals is currently being tested.

Finally, recent data indicate that in certain models it is possible to induce tolerance by administering recipient cells transfected with donor MHC[31,32], thus obviating the need for administration of donor cells. Strategies utilizing donor alloantigen administration which have been investigated (or are currently under investigation in multicenter studies) in humans include donor-specific blood transfusions (from living or cadaver donors), one HLA-haplotype or DR-matched blood transfusion[33,34], and donor bone marrow infusion (Table 4). Initial studies in human renal transplant recipients using donor bone marrow infusion are encouraging[35], and a multicenter study is currently under way.

A related area is the use of MHC-derived peptides to immunomodulate the alloimmune response to allografts; synthetic class I and class II MHC peptides derived from highly conserved regions have potent immunomodulatory properties, although they appear to act by different mechanisms[36,37]. For class I MHC peptides, T cell unresponsiveness is precisely associated with induction of a calcium flux and binding of peptide to members of the hsp70 group of molecules. There are *in vivo* data in murine transplantation models that such peptides can prolong allograft survival or even induce tolerance[38], and initial clinical pilot trials in humans are under way. The mechanisms of action of class II MHC peptides remain unclear, although they may prove to be useful immunotherapeutic agents and warrant further investigation in experimental transplantation as well as in autoimmune models[39].

Table 4 Clinically relevant strategies for induction of transplantation tolerance

1. Donor-specific blood transfusion
2. One HLA-haplotype/DR-matched blood transfusion
3. Donor bone marrow infusion
4. Co-stimulatory blockade
5. MHC-derived peptides

Blocking co-stimulation

As discussed above, recent evidence also confirms that T cells require two distinct signals for activation. The first signal is provided by the engagement of the TCR with the MHC plus peptide complex on APC, and the second co-stimulatory signal is provided by engagement of one or more T cell surface receptors with their ligands on APC. Signalling through the TCR alone, without a co-stimulatory signal, leads to a prolonged state of T cell anergy. Increasing evidence suggests that interaction of CD28 on T cells with its ligands, B7-1 or B7-2, on APC appears to be the most important co-stimulatory pathway for the response to alloantigens[40–42]. Recently, strategies targeted at blocking CD28-B7 co-stimulatory T cell activation have been shown to be very effective in inducing tolerance in experimental transplantation models, and there are plans to try such strategies in humans[43].

CTLA4Ig, developed by Linsley *et al.*[44], is a recombinant fusion protein which contains the extracellular domain of human CTLA-4 (a gene highly homologous to CD28) fused to a human IgG_1 heavy chain. CTLA4Ig has a 20–100-fold higher affinity for B7 than does CD28, and acts as a competitive inhibitor of CD28 binding to B7-1 or B7-2. Human CTLA4Ig efficiently binds to human, mouse, and rat B7 molecules, and inhibits the immune response *in vitro* and *in vivo*, including induction of tolerance to allografts and suppression of autoimmune disease in experimental animal models. Plans are also under way to test the safety and efficacy of this molecule in humans.

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8
Maintenance Immunosuppressive Drug Therapy and Potential Major Complications

K.L. TYNDALL AND D.K.C. COOPER

INTRODUCTION

The various pharmacological immunosuppressive agents available to those involved in organ transplantation have been discussed by a number of authors, and detailed accounts of their structure and mode of action can be found elsewhere. A comprehensive review of the newer agents presently in early clinical use, or in clinical or experimental development, is presented elsewhere in this volume (Chapter 70). The vast majority of centers today, however, utilize triple-drug maintenance therapy with cyclosporin (CsA), azathioprine (AZA), and corticosteroids. CsA-Neoral is beginning to take the place of CsA, and will eventually supersede it. Tacrolimus (FK506) has been introduced in place of CsA in a few centers (Chapters 10 and 70). (It is important to note that CsA and tacrolimus should not be given in combination, due to their severe nephrotoxic effect.) Cyclophosphamide is sometimes used to replace AZA, and mycophenolate mofetil is beginning to replace AZA in a small number of centers (Chapter 70). In addition, some centers include induction cytolytic therapy with an anti-T-cell polyclonal (an antithymocyte (ATG)/antilymphocyte (ALG) globulin) or monoclonal (OKT3) antibody.

Several areas of controversy currently persist and need to be addressed. These include: (a) Is induction therapy with cytolytic agents necessary or beneficial? If so, is ALG/ATG or OKT3 to be preferred? (b) Can corticosteroid therapy be withdrawn completely and, if so, at what stage of the post-transplant progress of the patient? (c) Are any of the newer agents, e.g. tacrolimus or mycophenolate mofetil, preferable to drugs such as CsA and AZA, respectively?

None of these areas will be discussed in any detail in this chapter. The first two of the above topics are discussed in Chapter 9 and the third topic in Chapters 10 and 70. There are increasing data with regard to subjects (a) and (b), but not yet sufficient evidence to know the conclusive answer to the question raised in topic (c). Few heart or lung transplant groups have as yet any significant experience with any of the newer agents.

As a preliminary to the discussion of these matters, however, some basic data on the use of the standard maintenance and induction immunosuppressive agents that are available today, and with which significant experience has been accumulated, will be provided in this chapter.

CYCLOSPORIN (CsA)

CsA was initially isolated from the fermentation broth of a soil fungus, *Trichoderma polysporum Rifai*[1]. The cyclosporins have a narrow spectrum of antibiotic activity[2], reducing the growth rate of a few yeasts and fungi. Full reviews of the immunosuppressive properties of this drug, which were first documented by Borel (Figure 12, Chapter 18), have been published previously[3–5]. The drug is lipid-soluble, metabolized in the liver and excreted mainly in the bile as metabolites. The immunosuppressive effect of CsA is much more specific than that of corticosteroids, AZA, or cyclophosphamide. The critical phase in the maturation of T-helper cells is the synthesis of lymphokines; CsA blocks this synthesis[5], primarily by inhibition of interleukin-2, a growth factor for T lymphocytes (Chapter 7). This action effectively prevents the development of mature cytotoxic T cells, and also prevents release of both gamma-interferon (macrophage-activating factor) and B cell activating factors.

CsA has little or no effect on antigen-presenting cells, and does not result in bone marrow suppression. Its prime value is in the prevention of rejection by shutting down the effector limb. CsA decreases T-helper cell activity, though T-suppressor cell activity remains at a normal level. The drug has no direct effect on macrophage function[6].

Administration and dosage

Ideally, CsA should be begun before operation (i.e. before exposure of the antigens to the T cells). The initial dose, administered orally, is modified depending on the physical state of the patient. A patient in good condition (no overt signs of cardiac failure, good renal and hepatic function) may receive 4–6 mg/kg body weight. A patient in extremely poor condition (severe cardiac failure with secondary renal and/or hepatic failure) should

receive no or very little CsA before operation. Rather, immunosuppression should be induced with the other available drugs until postoperative progress has been assessed. A perioperative period of hypotension or severe liver dysfunction in a patient receiving CsA may result in increased nephrotoxicity, which may greatly complicate management.

Whenever in doubt, it has been our experience that it is wiser to administer less rather than more CsA in the pre- or early post-transplant period. The drug level in the blood or serum should be monitored daily (or more frequently) and the dose gradually increased (by perhaps 1 or 2 mg/kg per day) until a safe therapeutic range has been reached. Immunosuppression can be obtained adequately for the first few post-transplant days without CsA, by administering a combination of AZA, corticosteroids, and a polyclonal or monoclonal anti-T-cell antibody while the CsA level is being slowly increased.

In regimens in which induction ATG or OKT3 is not used, the dose of CsA must be carefully modified in the light of the renal function of the patient. The University of Minnesota group, who have obtained excellent results using such a regimen[7], calculate the initial pretransplant dose of CsA on the basis of the serum creatinine (Table 1). No further CsA is administered for 24 hours, at which time a second dose equal to half of the initial dose is administered, again taking into consideration any change in serum creatinine. This dose is given twice daily until kidney function is normal (or near-normal), at which time the dosage is adjusted to obtain a whole blood trough level by high-pressure liquid chromatography (HPLC) of 200 ng/ml. Our own experience has indicated that a rather higher level (250–300 ng/ml) has to be maintained in lung transplant recipients if rejection is to be prevented.

Table 1 University of Minnesota triple-drug immunosuppressive protocol for heart transplant patients*

Cyclosporin (oral)
Pretransplant
6 mg/kg if serum creatinine <1.5 mg/dl (<133 μmol/l)
4 mg/kg if serum creatinine 1.5–2.0 mg/dl (133–177 μmol/l)
2 mg/kg if serum creatinine >2 mg/dl (>177 μmol/l)

Early post-transplant: 3 mg/kg, 2 mg/kg, or 1 mg/kg twice daily, depending on serum creatinine level

When renal function satisfactory: dosage adjusted to maintain whole blood CsA level (by HPLC) at 200 ng/ml*

6 months post-transplant: reduce blood level to 150 ng/ml*
12 months post-transplant: reduce blood level to 100–125 ng/ml*

Azathioprine (i.v. or oral)
Pretransplant: 2.5 mg/kg
Post-transplant: 2.5 mg/kg per day (maintain WBC >4000 cells/mm^3)

Corticosteroids
Methylprednisolone
500 mg i.v. during cardiopulmonary bypass
125 mg i.v. every 8 hours for 24 hours

Prednisone: 1 mg/kg per day (divided into four doses/day) for 3 days, reducing at 3-day intervals to approximately 0.4 mg/kg per day at 12 days. Thereafter monthly reductions (giving the drug in two divided doses/day) until a dosage of 0.125–0.15 mg/kg per day is reached after 6 months. This dose is maintained indefinitely.

*Levels approximately 25–50% higher are required in lung transplant recipients.
Based on refs 7 and 8.

For oral administration the drug comes in liquid or gelatin capsule form. If the patient is unable to take oral drugs, then CsA can be given by continuous intravenous (i.v.) infusion, but in much reduced dosage 1 mg hourly (*not* 1 mg/kg hourly). For this purpose the drug is diluted in normal saline or 5% dextrose (250 mg CsA in 250 ml) and administered continuously to reduce its renal toxic effect. Both the oral and i.v. forms can induce oliguria from toxicity on the proximal renal tubules. Though absorption of the drug when given orally is usual (though erratic) in patients recovering from cardiac transplantation, achievement of a stable therapeutic blood level may be difficult. Combined i.v. and oral administration of the drug for the first few days is preferable until the patient is eating and drinking normally, at which time oral therapy alone is continued. Using this regimen we have had excellent results with a low incidence of acute rejection, and yet little serious nephrotoxicity[8,9]. Intramuscular administration is not recommended, since the drug is poorly absorbed.

Monitoring of drug levels

Whether CsA is given orally or i.v., it is essential to measure the blood levels frequently (at least daily) until the dosage has been adjusted to achieve the correct therapeutic blood level. Blood levels are then estimated at intervals of a few days for one further month and on a less frequent basis thereafter. When CsA is being given orally, blood should be drawn immediately before administration of the drug to give a 'trough' level. If being given continuously only by the i.v. route, the blood level will clearly vary little throughout the 24-hour period.

CsA levels can be measured by radioimmunoassay (RIA)[10,11], fluorescent polarization immunoassay (TDx)[12], or by a HPLC technique[13]. As several metabolites of CsA are measured by RIA and TDx, but not by HPLC, the therapeutic range differs depending on the technique used, and is generally 50–75% lower when measured by HPLC. The therapeutic activity and significance of some of the metabolites are uncertain, and, therefore, the method of choice remains in doubt. The most commonly used method today is possibly TDx, which offers rapid sample turn-around times without putting great demands on the laboratory[12]. Some groups believe, however, that only the parent CsA should be measured, without the metabolites, and therefore the HPLC technique should be the method of choice.

Immunoassay methodology involves labeling an antibody with either a radioactive or a fluorescent molecule; the antibody, which can be monoclonal or polyclonal, is then bound to the CsA. The monoclonal form shows little or no cross-reactivity with metabolites of CsA[14], and the results correlate well with those obtained by HPLC. Monoclonal techniques therefore offer a simpler alternative to HPLC.

A number of other drugs affect the blood level of CsA (Table 2)[15], in particular ketoconazole and erythromycin, which increase its concentration, and phenytoin, phenobarbitone, and rifampicin, which decrease its concentration. Major changes in CsA dosage may be required if these other drugs are being administered simultaneously. Diltiazem, which increases CsA levels, is of particular interest as it may be useful in the treatment of CsA-induced systemic hypertension[16]. There is also some evidence that it may be associated with a reduced incidence of graft vasculopathy[17].

Table 2 Selected drugs which influence blood levels and toxicity of cyclosporin*

Increase blood levels Potentiate nephrotoxicity	*Decrease blood levels*
Diltiazem	Phenobarbitone
Aminoglycosides (gentamicin)	
Erythromycin	Phenytoin
Amphotericin B	
Ketoconazole	Rifampicin
Melphalan	
Corticosteroids	
Sulfonamides/cotrimoxazole	Sulphadimidine + trimethoprim (i.v. only)
Metoclopramide	
Trimethoprim	

*Only well-substantiated drugs have been included. Data provided by Sandoz Pharmaceuticals, USA. An extensive survey has been conducted by Lake[15].

The level of CsA that is considered therapeutic varies considerably from center to center, depending on the other therapy being given. For example, if large daily doses of ALG are being administered during the early post-transplant period, the patient may remain very adequately immunosuppressed whilst receiving only small doses of CsA. If no ALG or AZA are being administered, then the patient may require a high CsA level if acute rejection is to be prevented. Each center should ascertain for itself its own therapeutic and toxic ranges of CsA. The whole blood level is approximately 3–5 times that of the plasma. When HPLC is used to measure CsA level, whole blood trough levels of 200 ng/ml are generally considered adequate in the early post-transplant period, reducing to 150 ng/ml at 6 months and 100–125 ng/ml at 1 year[7–9].

Maintenance oral CsA therapy should consist of twice-daily doses (rarely it may be necessary to give three doses per day) sufficient to maintain the desired therapeutic level in the blood or plasma. When triple therapy (CsA, AZA and corticosteroids) is being administered, initial CsA dosage is generally between 4 and 10 mg/kg per day.

Major side-effects and complications

The major complication of immunosuppressive therapy, no matter what drugs are used, remains infection. However, other side-effects/complications are commonly seen with the use of these agents. The potential complications of the use of CsA are outlined briefly below and also discussed in Chapter 13. Some of these side-effects may not result solely from CsA therapy; for example, corticosteroids play a role in the systemic hypertension and hypercholesterolemia seen in the immunosuppressed patient.

Nephrotoxicity

Like many fungal antibiotics, CsA is nephrotoxic. The initial loading dose should be judged after considering the patient's general condition. In patients with already diminished renal function secondary to poor renal blood flow resulting from a low cardiac output, the initial dose of CsA should be withheld until the patient's renal function is clearly recovering after operation[7,8] (Table 1). A short period of hypotension during induction of anesthesia or during the operative procedure or early postoperative period, in the presence of a high blood level of CsA, can result in oliguria or anuria. Renal function usually recovers spontaneously as the CsA blood level falls, but even temporary dysfunction can be a major complicating factor in the early post-transplant period.

In view of the toxic effect of CsA on the proximal tubules, careful monitoring of the blood urea, serum creatinine, urine electrolytes, and creatinine clearance is essential. The most sensitive test would appear to be the creatinine clearance, which falls when toxicity occurs, though this does not differentiate CsA toxicity from acute tubular necrosis. For practical purposes, however, the serum creatinine provides an approximate but valuable guide to CsA nephrotoxicity. Certain other drugs, if given concomitantly, increase the risk of nephrotoxicity (Table 2).

Renal function usually improves following reduction of CsA dosage or, when impairment of function is severe, with complete discontinuation of the drug for a short period. Furosemide is also helpful in reversing the accompanying oliguria. Short-term hemodialysis may be required on rare occasions[8].

Systemic hypertension

Persistent hypertension has been noted as a major side-effect of CsA administration. Within the first year a majority of patients may be expected to have significant hypertension requiring treatment. Its onset may be gradual over days, weeks, or months, or at times precipitous. There appears to be no direct association between post-transplant hypertension and nephrotoxicity.

When rapid reduction in blood pressure is required, sublingual nifedipine (10 mg) is generally effective. Long-term therapy of hypertension in transplant patients is similar to standard antihypertensive treatment. Attention needs to be paid to such contributing factors as obesity and lack of exercise. Diuretics are used if needed for control of volume. We have found calcium channel antagonists (e.g. diltiazem, verapamil), central alpha-receptor stimulators (e.g. guanfacine, clonidine), and peripheral alpha-receptor-blocking drugs (e.g. prazocin, terazocin) to be useful, alone or in any combination. Angiotensin-converting-enzyme (ACE)-inhibiting medications (e.g. captopril, enalapril, lisinopril) may prove helpful additions to the antihypertensive regimen, though in some patients they have little effect. Beta-receptor blockers are less desirable, because of their depressive effect on myocardial function. In some cases hypertension is severe and requires the combination of two or more medications in full doses. As corticosteroids may play a role in the development of hypertension, gradual withdrawal of steroids should be considered if possible (Chapter 9).

Occasionally, supine hypertension and orthostatic hypotension are encountered, particularly in older patients. Treatment is empirical. In our hands diltiazem, starting with small (30 mg) doses, has been effective, perhaps because of the mild increase in circulating volume which accompanies this therapy. Treatment of the sitting/supine hypertension is not always possible without severe exacerbation of the orthostatic hypotension. Fortunately, the problem of orthostatic hypotension appears to diminish spontaneously with time.

Neuromuscular effects and neurotoxicity

Tremors, muscular weakness, and muscle cramps, particularly in the legs, are not uncommon[18–21], and appear to occur more frequently in association with a low serum magnesium[22,23]. Hypomagnesemia is common in transplant patients, possibly due to the renal effects of CsA, and may lead to increased cardiac irritability. We routinely monitor serum magnesium and prescribe oral replacement therapy at a rate of 500–1500 mg magnesium daily (in the form of magnesium oxide). The goal is to maintain the serum magnesium at 0.8 mmol/l (1.5 mEq/l, 1.8 mg/dl) or greater. Tremor also improves when corticosteroid dosage is reduced, and muscle cramps improve when diuretic doses are reduced.

A neurotoxic effect of CsA, which occurs in patients with abnormally low serum levels of cholesterol, was first described following liver transplantation[24]. Clinical features consist of seizures, confusion, cortical blindness, quadriplegia, and coma. Computerized tomographic scanning and magnetic resonance imaging studies disclose a severe diffuse disorder of the white matter. All central nervous system effects and radiographic findings may be reversed by discontinuation or reduction of the dose of CsA.

We have seen severe neurological damage occur following transplantation of the heart and both lungs in a patient who had a persistently low serum total cholesterol level[25]. It has since become our policy to reduce the dosage of CsA in patients with abnormally low serum levels of cholesterol, which is usually seen in debilitated patients during the early weeks after transplantation. A substantial portion of the whole blood or serum content of CsA is carried in the low-density lipoprotein (LDL) fraction of blood. It may be that when the LDL cholesterol is low, there is a higher proportion of CsA free in the serum.

Hyperlipidemia

In many patients, irrespective of their underlying pathologic condition for which transplantation was undertaken, there is a significant post-transplant increase in serum cholesterol and triglycerides[26,27]; this is believed to be related to CsA and/or corticosteroid therapy. The hyperlipidemia can develop at any time during the first year, or even later, after transplantation.

These patients should initially be encouraged to adhere strictly to a diet which is low in cholesterol. Weight loss and an exercise program are encouraged. Gradual withdrawal of corticosteroids should be considered. If these measures fail to reduce cholesterol and triglyceride levels to within normal limits within 3 months, we prescribe a single cholesterol-lowering drug. We were initially reluctant to use lovastatin because of the reported risk of rhabdomyolysis when this drug is administered to patients receiving CsA[28,29]. Further experience from many centers, however, suggests that this drug can be safely prescribed in low doses (20–40 mg/day) and is effective in reducing total and LDL cholesterol levels in transplant patients. An excellent response has also been documented to another HNG-CoA reductase inhibitor, pravastatin, which has also been demonstrated to have some correlation with a reduced incidence of severe rejection[30].

Other side-effects

A large number of other side-effects of CsA have been described (Table 3). In particular, the role of CsA in the development of osteoporosis post-transplantation is receiving increasing attention[31]. This topic is discussed under the late complications of immunosuppressive therapy (Chapter 13).

Table 3 Major potential complications from immunosuppressive drug therapy

1. *Cyclosporin (CsA)*
   - Nephrotoxicity
   - Hypertension
   - Neurotoxicity
   - ? Hyperlipidemia
   - Others (including hypertrichosis, gingival hyperplasia, tremor, hepatic dysfunction)
2. *Tacrolimus (FK506)*
   - Nephrotoxicity
   - Neurotoxicity
   - Hyperlipidemia
   - Endocrine disorders (including glucose intolerance/diabetes mellitus)
   - Gastrointestinal disorders
   - Hair loss
3. *Azathioprine (AZA)*
   - Hematopoietic disorders (including leukopenia, thrombocytopenia, and anemia)
   - Gastrointestinal disorders (including nausea and vomiting, hepatitis, and biliary stasis)
   - Others (including skin rashes, alopecia, fever, arthralgias, diarrhea, steatorrhea, and negative nitrogen balance)
4. *Cyclophosphamide (CPP)*
   - Hematopoietic disorders (including leukopenia, thrombocytopenia, and anemia)
   - Gastrointestinal disorders (including anorexia, nausea, and vomiting)
   - Genito-urinary disorders (including sterile hemorrhagic cystitis)
   - Gonadal suppression
   - Pulmonary disorders (including interstitial pulmonary fibrosis)
5. *Methotrexate (MTx)*
   - Hematopoietic disorders (including leukopenia, thrombocytopenia, and anemia)
   - Gastrointestinal disorders (including anorexia, nausea, and vomiting)
   - Hepatic dysfunction (fibrosis and cirrhosis, dose-related)
6. *Corticosteroids*
   - Gastrointestinal disorders (including peptic ulcer, pancreatitis, and ulcerative esophagitis)
   - Musculoskeletal disorders (including osteoporosis, vertebral compression fractures, pathological bone fractures, aseptic necrosis, muscle weakness, steroid myopathy, and loss of muscle mass)
   - Endocrine disorders (including development of Cushingoid state, suppression of growth in children, menstrual irregularities, decreased carbohydrate tolerance, and manifestations of latent diabetes mellitus, impotence)
   - Metabolic disorders (including fluid and electrolyte disturbance and negative nitrogen balance, hyperlipidemia)
   - Neurological disorders (including psychiatric complications and convulsions)
   - Ophthalmic disorders (including cataracts, increased intraocular pressure, glaucoma and exophthalmos)
   - Dermatological disorders (including acne, spontaneous hemorrhage, striae)
7. *Antithymocyte globulin (ATG/ALG)*
   - Anaphylactic shock
   - Others (including musculoskeletal pains, rash, fever, chills, and bronchospasm)
8. *OKT3*
   - Fevers, chills
   - Aseptic meningitis
   - Meningoencephalitis

CYCLOSPORIN–NEORAL (CsA-NEORAL)

CsA-Neoral is a microemulsion preconcentrate comprising the drug, CsA, together with a surfactant, a lipophilic solvent, a hydrophilic solvent, and a hydrophilic cosolvent. The active form of the drug has not been altered. CsA-Neoral has been developed in order to reduce or eliminate problems associated with the erratic absorption characteristics of CsA[32]. Due to the high lipophilicity of CsA, its absorption is influenced by the rate of bile flow, gastrointestinal motility, and concomitant food intake[33–35]. There is also a wide inter- and intra-individual variation in CsA absorption. With CsA-Neoral there is a more consistent absorption profile, with less influence by concomitant food intake and diurnal rhythm[36–38], and also less influence by bile flow and pancreatin[39]. Improved oral absorption of CsA in the microemulsion formulation has resulted in reduced inter- and intra-individual variation of CsA pharmacokinetics, with a more consistent and predictable concentration–time profile[38,40,41].

When CsA is administered in its original form (Sandimmune R), an oil-in-water emulsion forms in aqueous gastrointestinal fluids, with CsA mainly distributed in lipid droplets, which must be dispersed by pancreatin and bile to form micelles for drug absorption. Upon contact with aqueous fluids, CsA-Neoral forms a microemulsion, without the actions of bile, enzymes, or small intestinal secretions, and becomes rapidly available for absorption. It is this property which effectively increases the rate and extent of absorption[36,37] and also decreases the inter- and intra-individual variability of CsA absorption[38].

Clinical trials have been performed to study pharmacokinetics, absorption, bioavailability, safety, toxicity, and tolerability of Neoral in normal healthy volunteers[42]. Pharmacokinetic studies and clinical trials have also been conducted in stable renal transplant patients, and in *de novo* renal, liver and heart transplant recipients. Oral absorption of CsA is improved with the Neoral formulation in healthy volunteers and in established transplant patients[40,42–46]. In normal volunteers a one-to-one conversion from CsA to CsA-Neoral resulted in an increased bioavailability of 30%, an increase in maximum concentration (C_{max}) of an average of 60%, and a significant reduction in the time to reach C_{max} (T_{max})[42]. In patient studies, the extent of absorption was greater for CsA-Neoral when compared to CsA, and the increased bioavailability was achieved with less variability[43–45]. The correlation between trough concentration and area under the curve (AUC) was stronger with CsA-Neoral than with CsA, and there was less variability with this parameter[40]. Studies have shown Neoral to be well tolerated, with no significant difference in incidence or severity of adverse events[38,45,47].

Conversion from CsA to CsA-Neoral has been studied in healthy volunteers[42] and in stable renal transplant patients[41]. It appears that comparable CsA trough concentrations can be achieved by maintaining CsA-Neoral at the same CsA dose (a one-to-one conversion). Concomitant with this conversion, there was a significant increase in C_{max} and AUC, due to absorption-related differences between the formulations, but there were no associated problems with tolerability, or changes in blood pressure or clinical laboratory parameters.

Use of CsA-Neoral has been studied in cardiac transplant patients, with similar improvement in pharmacokinetic profiles. In this patient population, CsA-Neoral proved to be superior to that of CsA alone with less variability in AUC, T_{max} and C_{max}[43–45], as well as a noted improvement with respect to the correlation between AUC and corresponding trough levels in some patients. In patients with pre-existing malabsorption the absorption of CsA-Neoral appeared to be independent of bile flow and food intake, with an improved tolerance[44]. In all of these studies, overall tolerability was reported as good, with no significant adverse events attributed to CsA-Neoral, in spite of the increase in AUC and C_{max}, and no deterioration in renal function, or worsening of other pre-existing abnormalities.

Neoral is available as an oral solution and as a soft gelatin capsule. Although it appears that a one-to-one conversion for changing from CsA to CsA-Neoral will be a reasonable approach, ongoing trials should be referred to when making dose conversion determinations. Furthermore, it is recommended that frequent trough level monitoring and dose adjustment be done during the first few weeks of the conversion, due to the possibility of enhanced systemic exposure. Patients may benefit from the possibility for dose reduction with CsA-Neoral and improved drug safety due to fewer variations in drug exposure. The improved intra-individual reproducibility of CsA trough concentrations with CsA-Neoral should facilitate dosage optimization, with more reliable CsA dose prediction and safer CsA therapy.

TACROLIMUS (FK506)

Tacrolimus (FK506) was isolated in 1984, from the fermentation broth of a strain of *Streptomyces*, from a soil sample obtained from Tsukuba, Ibaraki Prefecture, Japan[48]. The strain is designated *Streptomyces tsukubaensis*, referring to the soil from which the organism was isolated. Tacrolimus is a novel 23-member macrolide with a chemical structure entirely different from that of CsA[49]. The immunosuppressive effects of this drug were discovered by Kino and colleagues in 1984, and full reviews have been published elsewhere[50–54].

Tacrolimus is absorbed from the gastrointestinal tract after oral administration with a mean bioavailability of about 20%, although variability in transplant patients is considerable. Tacrolimus is highly lipophilic and is distributed disproportionately between red blood cells and plasma. Its distribution in plasma is significantly different from that of CsA, as the drug binds mainly to serum albumin and α_1-acid glycoprotein. It undergoes hepatic metabolism with biliary excretion of most of the metabolites[55–58]. Like CsA, tacrolimus exhibits a narrow therapeutic index.

Tacrolimus affects the immune response through inhibition of interleukin (IL)-2 synthesis, thereby suppressing T cell-mediated immunity[58–61]. It exerts inhibitory effects on T cells similarly to CsA, as both agents inhibit the proliferation and generation of cytotoxic T lymphocytes in mixed lymphocyte culture (MLC)[62]. At the transcriptional level, tacrolimus inhibits the synthesis of interleukin (IL)-2 mRNA as well as mRNA of IL-3 and IL-4 and other factors up-regulated during T cell activation, including colony-stimulating factors, tumor necrosis factor-alpha, and interferons -gamma and -alpha[63–66]. It has also been shown to inhibit some B cell functions[67]. While tacrolimus inhibits T-cell-dependent antibody responses, its inhibition occurs in the early phase of T cell activation; it is therefore unable to inhibit the activity

of mature cytotoxic T cells[66]. The immunosuppressive activity of tacrolimus is up to 100 times more potent than that of CsA. A further discussion of the immunosuppressive properties of the drug is provided in Chapter 70.

Administration and dosage

Dosing principles and blood level monitoring have been studied in healthy volunteers, and in liver and kidney transplant patients[55,58,68,69]. Studies following cardiac transplantation, however, are still limited. The pharmacokinetics of tacrolimus can vary greatly between patients and within a patient over time[56]. Correlations between blood or plasma concentrations and toxicity or rejection episodes exist, but are not well defined; therefore, guidelines for target trough levels should be used in combination with clinical assessment and other laboratory parameters. Early clinical trials have targeted a plasma trough concentration in the range 0.5–2 ng/ml (5–20 μg/l) in clinically stable transplant patients.

Immunosuppressive therapy with tacrolimus is usually initiated within 6–12 hours after transplantation. Although experience in thoracic organ transplantation is limited, the University of Pittsburgh group has prospectively studied the use of the drug as primary immunosuppression in cardiac transplant recipients[70,71] (Chapter 10). In early trials, tacrolimus immunosuppression was initiated as a continuous i.v. infusion, at a dose of 0.05 mg/kg per day, for a period of 24–48 hours. As soon as gastrointestinal function returned, an oral dose was begun at 0.3 mg/kg per day, divided in twice-daily doses. Dosing adjustments were made according to whole blood trough levels, clinical status of the patient, renal and hepatic function, and rejection history.

Administration of i.v. steroids with the tacrolimus immunosuppressive regimen is begun in the operating room with a methylprednisolone dose of 7–10 mg/kg, with a further three doses (for a total of 5 mg/kg) in the first 24 hours post-transplantation[72]. Thereafter, when the patient is tolerating oral intake, oral prednisone is given at 0.15 mg/kg per day. Steroid-tapering, according to the University of Pittsburgh protocol, is begun at the twelfth post-transplant week and is based on freedom from rejection. Steroid-tapering protocols vary per institution and have been discussed in this chapter (with regard to CsA immunosuppressive regimen) and are further discussed in Chapter 9.

In the Pittsburgh immunosuppression protocol the use of AZA is reserved as adjuvant immunosuppressive therapy: (a) for cases of rejection or (b) to allow for lower tacrolimus dosage in patients with impaired renal function. OKT3 is used only in cases of severe rejection associated with hemodynamic compromise.

Experience with tacrolimus after pulmonary transplantation is very limited. A prospective randomized trial comparing tacrolimus to CsA has been conducted at the University of Pittsburgh[73]. In the study protocol, tacrolimus was administered as a continuous infusion (when the recipient was hemodynamically stable) at a dose of 0.025 mg/kg per day. Once tolerating oral intake, tacrolimus was given in two divided doses, each of 0.15 mg/kg per day, and adjusted according to target trough levels. There were fewer episodes of acute cellular rejection in the tacrolimus group during the early postoperative period; the prevalence of infection and survival were similar in the two groups.

Optimal dosing of tacrolimus has not yet been fully determined and clinical trials are still in progress. Management during the immediate postoperative period is tending away from the use of i.v. tacrolimus, and immunosuppression is being initiated with lower oral doses, in the range 0.1–0.15 mg/kg per day. If the i.v. route of administration is used, overlap of the i.v. and oral dose is generally not recommended, in order to avoid the possibility of reaching toxic blood levels. Adequate levels can usually be achieved with the oral route alone. In contrast to CsA, bile flow is not required for tacrolimus absorption, nor is absorption decreased in patients with liver dysfunction[56]. The drug plasma/blood level should be monitored daily and the dosage adjusted gradually until a safe therapeutic level has been reached. Dosage adjustments are also dependent on the clinical status of the patient, renal and hepatic function, and rejection history.

Similar to CsA regimens, patients in extremely poor condition (severe cardiac failure with secondary renal and/or hepatic failure) may be managed by delaying initial dosing of tacrolimus, or by induction with other available agents (e.g. ATG, OKT3), until postoperative progress has been assessed.

For oral administration, tacrolimus is available as a hard gelatin capsule, which can be swallowed whole or pulled apart for sublingual administration of the contents. The sublingual route of administration has resulted in therapeutic levels at dosages equivalent to or lower than the oral dose. It is preferable to administer the drug by the oral route, even in the absence of normal gastrointestinal function, as nephrotoxicity is more likely with the i.v. route of administration. If adequate levels are not achieved with oral or sublingual administration, the drug may be given i.v. at a dosage three to four times lower than the oral dosage. The i.v. dose must be diluted in normal saline or 5% dextrose and administered as a continuous infusion, to reduce renal toxicity.

Monitoring of drug levels

There are several factors which necessitate routine monitoring of plasma/blood tacrolimus levels, in order to provide an acceptable level of immunosuppression without toxicity. The drug, whether administered i.v. or orally, is a very potent compound with a significant nephrotoxic potential[56,74,75]. Pharmacokinetic studies have shown that there is a larger inter- and intra-individual variation of kinetic characteristics in transplant patients[75].

Dosage adjustments are made according to graft function, drug tolerance, adverse effects of immunosuppression, and plasma/blood levels of the drug. During the initial adjustment period, levels are checked daily in order to rapidly achieve a therapeutic level and avoid toxicity. There is a correlation between dose and the resulting trough plasma/blood level[55], which suggests that the trough is a good indicator of systemic exposure and may be used for therapeutic drug monitoring. When tacrolimus is administered orally, blood samples should be drawn immediately before administration of the drug to determine the 'trough' level. When administered as a continuous i.v. infusion, timing of the blood level is less relevant, as drug concentration will not vary appreciably.

Several assays are available for measurement of tacrolimus concentration in blood and plasma. Because the drug is primarily bound to red blood cells in whole blood, the whole-blood assay

may be more dependable than the plasma assay[76,77]. The enzyme-linked immunosorbent assay (ELISA) for measuring tacrolimus concentrations was first reported by Tamura *et al.*[78]. The drug can also be measured in whole blood by: (a) radioreceptor assay (RRA)[79], (b) high-pressure liquid chromatography with mass spectrometry (HPLC-MS)[80], (c) the Abbott IMx method[81], and (d) in biological fluids by a combined HPLC-ELISA method[82].

The ELISA is a manual enzyme immunoassay method based on CH_2Cl_2 extraction and has been used for therapeutic drug monitoring in clinical trials[78,83]. This method is highly sensitive, but is nonspecific, because the polyclonal antibody employed cross-reacts with the drug metabolites.

The IMx is a semiautomated technique based on the principle of microparticulate enzyme immunoassay (MEIA), and is used for monitoring tacrolimus in whole blood only[81]. It uses the same antibody as the ELISA method and is therefore nonspecific. The method is sensitive, but its limit of quantification is currently 5 ng/ml, which may not be sufficient for monitoring patients on low-dose maintenance therapy. IMx has a rapid turnaround time and is currently the most widely used method for quantifying levels in transplant patients.

The most accurate, sensitive, and specific assay for determining tacrolimus concentrations in blood is HPLC coupled with mass spectrometry (HPLC-MS)[80], which can separately quantify the parent compound and its metabolites. However, the usefulness of the HPLC-MS method is limited by lack of available instrumentation at most transplant centers, and difficulty in analyzing a large volume of samples on a routine basis.

There are wide variations in the pharmacokinetics of tacrolimus, which means that for a given dose there will be a wide variation in plasma concentration. In addition, like CsA, tacrolimus exhibits a narrow therapeutic index. Currently, the most commonly used assay is the Abbott IMx, due to its rapid turnaround time, semiautomation, and ease of use by laboratory personnel. In addition, the importance of tacrolimus metabolism is still poorly understood and the significance of the drug's metabolites and their corresponding cross-reactivities should be considered when interpreting drug levels.

There is potential for drug–drug interactions in transplant patients, as a number of drugs affect the blood level of tacrolimus[55]. Because tacrolimus is metabolized by the P450-3A4 enzyme system, concomitant use of drugs which inhibit or induce cytochrome P450-3A4 will influence its metabolism. The therapeutic significance of such drug interactions has not yet been demonstrated, and it is advisable to avoid concomitant use of drugs which have the potential to interact with tacrolimus metabolism. If administration of such agents is unavoidable, tacrolimus blood levels should be monitored more closely and dosage adjustments made as appropriate.

Major side-effects and complications

The most significant adverse events associated with tacrolimus therapy are nephrotoxicity, systemic hypertension, neurotoxicity, and new-onset diabetes mellitus. Nephrotoxicity is worsened in the presence of ischemia, use of other nephrotoxic agents (including CsA), and administration in the early post-transplantation period, particularly by the i.v. route. It can be controlled to some degree but, along with hypertension, it remains a serious problem in the transplantation setting. As both tacrolimus and CsA are nephrotoxic, they must not be used together.

Nephrotoxicity is well documented in all transplant settings[84–87], partly because the optimal dosage range for use of tacrolimus has not yet been well defined. The proposed mechanism for transient decline in renal function is efferent arteriole vasoconstriction leading to diminished glomerular filtration, with subsequent tubular dysfunction[85]. Hyperkalemia, secondary to renal tubular acidosis, is also common in patients receiving tacrolimus, and can be controlled with fludrocortisone. The use of i.v. tacrolimus as primary and rescue therapy after cardiac transplantation has been associated with significant nephrotoxicity requiring dosage reduction. However, an apparent advantage over CsA is the number of recipients who become free of antihypertensive agents[70,71].

Neurotoxicity with tacrolimus ranges from mild symptoms, such as tremors, insomnia, and headaches, to more severe symptoms, including incapacitating headaches, dysarthria, seizures, and coma[86]. It appears to be related to high levels of the drug, and symptoms often resolve with dosage reduction. Many of the neurological side-effects are observed in the immediate post-transplantation period, and resolve over time. However, in some cases patients are intolerant of the neurological symptoms and may need to be converted to CsA therapy.

Glucose intolerance has been associated with tacrolimus-based immunosuppression, and new-onset diabetes mellitus has occurred in liver transplant recipients[87]. Although a number of patients have required long-term insulin therapy, it has been suggested that the diabetogenic effects may subside with time.

Gastrointestinal disturbances, ranging from mild cramps to severe diarrhoea, occur frequently with tacrolimus therapy, but appear to respond to dose reduction and abate over time. Other less significant toxicities include hypercalcemia, hyperphosphatemia, and hair loss. Hyperlipidemia/hypercholesterolemia is similar to that reported with CsA.

Very recently there have been reports of myocardial hypertrophy in patients receiving tacrolimus (Lawrence, I.D., personal communication). In the majority of cases the observation is a thickening of the ventricular walls and/or interventricular septum on echocardiography, the pathophysiology of which is unclear. In the few instances in which histology is available, no evidence has been seen of disorganization of myocyte structure. The condition appears to be reversible following dose reduction or discontinuation of tacrolimus therapy. Of a total of 42 cases seen in the USA, only one occurred in a patient with a heart transplant, and none has yet been reported in patients with lung transplants. The majority of cases have occurred in patients who have undergone liver transplantation. Myocardial hypertrophy has been observed as early as 3 days and as late as 25 months from the initiation of tacrolimus therapy. Maximum trough concentrations in whole blood have ranged from 7.8 to 103 ng/ml. From clinical trials of tacrolimus, the incidence of clinically manifested myocardial hypertrophy is estimated to be 0.2%.

AZATHIOPRINE (AZA)

AZA is one of the large group of antimetabolite compounds that compete for and block specific receptors, thus affecting DNA and

RNA synthesis, and interfering with protein synthesis[88]. It reduces or prevents the rapid cell division that is an important part of the immune system response, thus blunting the ability of the host to generate cytotoxic T cells. Though it is active on B cells, this activity is much less than on T cells, which accounts for its suppression of cell-mediated rejection with less effect on antibody production. AZA was introduced into experimental and clinical practice with regard to renal transplantation by Calne (Figure 8, Chapter 18) in 1961[89]. Specifically, it is a purine antagonist that is similar in structure to 6-mercaptopurine. AZA is useful in the prevention of acute rejection rather than the reversal of established rejection. It is rather ineffective when used as a sole immunosuppressant following human renal transplantation[90,91], but has not been used alone after cardiac transplantation.

Administration and dosage

It is usual policy to begin AZA before operation with a loading dose of 2.0–2.5 mg/kg orally or i.v. After transplantation it is given initially i.v. and subsequently orally at the same dose (or the maximal tolerable levels judged by the absence of bone marrow and hepatic toxicity). In our experience the average maintenance dose for adults ranges between 0.5 and 2.5 mg/kg per day; the total white blood count should be monitored at intervals and maintained in the range 5000–7000 cells/mm^3.

Major side-effects and complications

AZA is an easy and generally safe drug to use, and is relatively free from serious complication. AZA's main toxic effect is on the bone marrow, which results in leukopenia, thrombocytopenia and, occasionally, anemia, though leukopenia is rarely severe enough to prove a problem. Following withdrawal or reduction of the drug, recovery of the bone marrow is usually rapid. Employment of agents such as granulocyte colony-stimulating factor (GCSF) is rarely necessary. AZA not infrequently results in minor abnormalities of liver function, but rarely leads to clinical hepatic dysfunction[92]. Withdrawal of AZA or substitution with cyclophosphamide is recommended in such cases. Its role in the occurrence of pancreatitis (see below) remains uncertain.

Spontaneous hemorrhages into the skin (ecchymoses) are commonly seen when AZA is used either alone or, particularly, in combination with a corticosteroid; similar spontaneous bleeding can occur with corticosteroids alone.

CYCLOPHOSPHAMIDE (CPP)

In patients who show refractory or repeated acute rejection despite triple or quadruple drug therapy, or in those in whom AZA is associated with hepatic dysfunction, we have found cyclophosphamide (CPP) to be a useful agent[93]. In view of its potential to cause severe bone marrow depression, however, it must be used cautiously.

CPP, an alkylating agent, has a more marked effect on B cells than does AZA[88]. The alkyl groups attach to DNA, interfere with its integrity, and thereby produce significant cytotoxic effects.

A low dose (0.5–1.5 mg/kg per day) of this drug is generally sufficient to maintain a total white blood count within the desired range, 5000–7000 cells/mm^3. Unlike AZA, CPP use may result in a precipitous fall in white blood count, resulting in a severe, life-threatening neutropenia (WBC <1000/mm^3), which may persist for several days before spontaneous recovery occurs. Neutropenia is, however, a less serious problem today than it was even a few years ago, in view of the availability of GCSF and GMCSF therapy.

Hemorrhagic aseptic cystitis may be a complication of CPP therapy, necessitating its withdrawal.

METHOTREXATE (MTx)

Methotrexate (MTx) is the 4-amino, N^{10}-methyl analog of folic acid which competitively inhibits dihydrofolate reductase, and is a potent cytostatic agent, which exerts inhibitory effects on cellular and humoral immunity[88,94–98]. It has also been shown to affect B cells by decreasing their ability to proliferate and secrete antibodies[99]. MTx functions as an antimetabolite and was initially used in the treatment of a variety of malignancies, as well as psoriasis and rheumatoid arthritis. More recently it has been used as adjunctive treatment of acute refractory cardiac allograft rejection[100,101], and for the treatment of persistent mild rejection[102,103]. Its role in maintenance immunosuppressive therapy in patients with thoracic organ transplants is therefore small. Future prospects for MTx include the possibility of prolonging allograft survival with combination low-dose CsA/MTx therapy[104], or the use of MTx for donor pretreatment, again in an attempt to prolong allograft survival[105].

Administration, dosage and monitoring

Clinical trials have utilized dosing regimens based on a once-weekly schedule (for 1–9 weeks), with the weekly dose, given orally, determined by total white blood cell count[100,101,103,106,107], but usually in the range of 5–25 mg/week. In these trials corticosteroid doses were also decreased as tolerated. The white blood count needs to be monitored regularly. Transient leukopenia occurs in almost all patients, but MTx is otherwise well tolerated.

In a limited number of cases, MTx successfully resolved mild-to-moderate rejection where corticosteroid treatment had failed (Chapter 30). The drug can also be used successfully as an adjunctive agent in conventional immunosuppressive regimens: (a) for patients in whom AZA has been discontinued or (b) to allow reduction in the dose of steroids[101].

It takes some time for its effect to develop, and therefore it is *not* valuable in *severe* acute rejection, where a response is required rapidly. In the future its ability to suppress B cell function may prove valuable in xenotransplantation (Chapter 80).

Major side-effects and complications

Adverse events associated with MTx therapy include leukopenia, hepatic fibrosis and cirrhosis (dose-related[108,109], and an increased risk of infection[110]. Leukopenia is by far the most common and potentially serious adverse event, and a clearer understanding of

MTx's ability to suppress the bone marrow is needed before it gains wider use. Although MTx-induced bone marrow suppression is uniformly reversible, it has resulted in deaths from infection.

CORTICOSTEROIDS

Steroids were introduced into clinical renal transplant practice in 1963[111]. It is the glucocorticoids, rather than the mineralocorticoids, which possess immunosuppressive activity.

Prednisone or prednisolone have been most commonly used in transplantation. They inhibit a variety of intracellular enzymes that depress protein, RNA, and DNA synthesis. There is extensive death of small lymphocytes in the blood, thymus, lymph nodes and spleen, though the mechanism for this effect is not well understood, but is possibly mediated by an increase in cytoplasmic calcium. Cell-mediated immunity is depressed in most species.

Much of the efficiency of corticosteroid therapy is related to nonspecific immunosuppressive and anti-inflammatory effects, and to the inhibition of migration of immune cells to the graft. There is also evidence they: (a) impair antigen recognition, (b) interfere with macrophage function, and (c), at very high doses, interfere with some membrane functions of lymphocytes.

Administration and dosage

There are many different regimens for administering maintenance steroids to transplant patients. Our own regimen consists of 500 mg methylprednisolone sodium succinate given i.v. during operation before reperfusion of the donor heart, and further i.v. boluses of 125 mg 8-hourly for the first postoperative 24 hours. Thereafter, if the patient is absorbing oral fluids, an oral prednisone dose of 1 mg/kg per day is given, divided into two doses. The dosage is slowly reduced according to the University of Minnesota protocol (Table 1)[7]. If the patient is not absorbing oral fluids, i.v. methylprednisolone is continued (at the equivalent dosage) until such time that prednisone can be started.

For an 80 kg patient, for example, the total daily dosage would be 80 mg initially, reducing by 10 mg every 3 days until a dosage of 30 mg/day was achieved. This would be maintained for 1 month. By 6 months the daily dosage would have been reduced to 10 mg/day, where it would remain for a further 6 months. In stable patients we would then reduce dosage to 8 mg/day.

Most centers use prednisone or prednisolone in similar dosage (1 mg prednisone being the equivalent of 0.8 mg methylprednisolone). Prednisone is converted to methylprednisolone in the liver, and is a much less expensive drug. Unless there are features of liver dysfunction, the patient should be switched to prednisone in the early post-transplant period. Policies regarding reduction in dosage or withdrawal vary from center to center and are discussed in Chapter 9. When ATG or OKT3 is part of the initial immunosuppressive regimen, low doses of corticosteroids can be administered in the initial post-transplant period, and some groups will give only 0.2–0.3 mg/kg per day.

There is increasing evidence that steroids can be withdrawn entirely in patients with heart transplants, particularly in the patient who has shown no signs of rejection within the previous 6 months (Chapter 9). Some groups have been more aggressive and have demonstrated that in approximately 50% of patients it is possible to discontinue the drug completely within the first few weeks or months after transplantation. In patients who have been receiving more than approximately 5 mg/day for some long period of time, it is essential to reduce the drug cautiously (by approximately 1 mg/month) to ensure that the patient's own corticosteroid production can provide the body's basic needs.

Our own policy until recently was to maintain prednisone indefinitely at 0.1 mg/kg per day. However, we have recently entered the majority of our patients in a multicenter trial, where they are being randomized into: (a) a slow-withdrawal group or (b) a no-withdrawal group. The results of this trial are not yet available for discussion (Opelz, G., personal communication).

More caution in withdrawing steroids is required in patients with lung transplants, as experience in this field is very much less.

Major side-effects and complications

As stated above, it is sometimes difficult to attribute a post-transplant complication to any specific drug, but corticosteroid therapy is believed to contribute to several post-transplant problems (Table 3). These include systemic hypertension, diabetes mellitus, osteoporosis and aseptic necrosis of bone, impotence and sexual dysfunction, growth retardation and delayed onset of puberty in children, gastrointestinal tract complications, and lenticular cataracts. Psychiatric complications are also considered to be associated with corticosteroid therapy in some patients. These complications are discussed in Chapter 13, but brief mention will be made of some that can be of concern in the early post-transplant period.

Diabetes mellitus

We have not considered uncomplicated diabetes to be a contraindication to heart or lung transplantation, though we have not accepted the patient with significant diabetic complications, such as microvascular disease or neurotrophic foot ulcers. Some degree of impairment of glucose tolerance is seen in virtually all patients at some point in the transplantation process.

In its simplest form, hyperglycemia is seen in the first few days following transplantation during high-dose methylprednisolone therapy. It is seen again during the treatment of an acute rejection episode with methylprednisolone pulse therapy. In these circumstances the hyperglycemia usually lasts only a few days. Without treatment, plasma glucose levels greater than 16.7 mmol/l (300 mg/dl) are commonly encountered. In the unproven belief that it is beneficial to maintain a near-normal metabolic milieu, we routinely monitor and treat this hyperglycemia with the goal of maintaining glucose levels closer to 5.6 mmol/l (100 mg/dl), and certainly below 8.3 mmol/l (150 mg/dl). Granulocyte chemotaxis may be impaired at higher plasma glucose levels.

Some patients presenting for thoracic organ transplantation will have a pre-existing degree of impaired glucose tolerance or even frank diabetes. In particular, those with long-standing congestive heart failure and its associated cachexia will have impaired tolerance. Typically, the pretransplant glycosylated hemoglobin

ranges from 6.5% to 10% in this group. (Non-diabetics typically have a glycosylated hemoglobin of from 4.5% to 5.5%). Following transplantation, the improved nutritional status of the patient, with restored vigor and muscular activity, is associated with resolution of the impaired glucose tolerance. Usually these patients require tapering doses of oral hypoglycemics in the first few post-transplant weeks only. Thereafter, they may display no sign of impaired tolerance or diabetes.

Many patients will present for transplantation with familial type II diabetes. The degree to which they display this diabetes will depend on several factors, which include: (a) relative body masses of muscle and adipose, (b) diet, and (c) activity. Their diabetes can often be controlled with proper diet and activity. These persons are profoundly affected by pharmacological doses of corticosteroids. Until the corticosteroid dose is reduced to physiological levels, or eliminated entirely, treatment for diabetes will be required. In the long-term follow-up of such patients after reduction of corticosteroid dosage, those who follow the prescribed diet and activity instructions rarely require pharmacological treatment of their diabetes. Many patients, however, are unable or unwilling to follow such instructions, and require prolonged treatment, usually with oral agents.

At our own center we have transplanted very few patients with uncomplicated type I diabetes. They have done well, but have required large doses of insulin (<300 units daily) during the periods when they were taking corticosteroids in high or moderately high doses.

Gastrointestinal tract complications

Several gastrointestinal diseases are not uncommon in the transplant patient. The severity and risks of these diseases are generally increased by corticosteroid therapy and, after heterotopic heart transplantation, by the addition of anticoagulation and antiplatelet therapy.

The first and most formidable of potential gastrointestinal tract complications is bleeding from the stomach or duodenum, which may result from the stress of surgery and/or prolonged high-dose steroid therapy. In general, endoscopic examination reveals the bleeding to be due to hemorrhagic gastritis or peptic ulceration.

Therapy for acute upper gastrointestinal tract bleeding is more complicated and hazardous in immunosuppressed patients, whether the treatment be by surgery or endoscopic thermal coagulation. We believe, therefore, that it is imperative to investigate and, where possible, treat all digestive diseases before transplantation. Upper or lower gastrointestinal endoscopy is today a benign procedure that can be tolerated by all except the most severely ill patients, and should not be postponed if indicated. When acid peptic disease is found, it is treated aggressively pretransplant by a suitable H_2 blocker.

After transplantation all patients receive H_2 blockers, the rationale for this being that the stress of the post-transplant period and steroid therapy may increase the patient's susceptibility to develop ulcer disease. Therapy is continued at least until corticosteroids have been discontinued. The wisdom of treating peptic ulcer disease prophylactically is well supported by a review of the literature, since the complications of active severe ulceration, perforation, and gastrointestinal hemorrhage have been reported by a large number of transplant centers[112-115].

Pancreatitis

The cause of post-transplant pancreatitis remains uncertain. Several etiologies have been implicated; these include drugs (corticosteroids, AZA, furosemide, epinephrine, and alcohol)[116-120], infections (cytomegalovirus, viral hepatitis, mumps, Coxsackie and enteroviruses)[121-124], autoimmune disorders[125,126], ischemia[127] and biliary tract or peptic ulcer disease[128]. The University of Arizona reported a 6% incidence in cardiac transplant patients within the first 3 months[112]. Incidences of up to 7% with a mortality rate of more than 50% have been reported from several renal transplant centers. Though clinically severe acute pancreatitis is relatively uncommon after organ transplantation, a low-grade, asymptomatic pancreatitis has been noted in 49% of heart transplant patients coming to necropsy[129]. Similarly, at Stanford, evidence of pancreatitis was found at necropsy in 70% of cardiac transplant patients[128].

Psychiatric complications

The incidence of psychiatric complications in patients receiving steroid therapy has been reported to be between 4% and 36%[130,131]. Suicide has been successfully attempted by transplant patients. In the early experience of the University of Arizona, 12% of the cardiac transplant patients developed major psychiatric complications[112] (Chapter 14). It is, however, difficult to determine whether such disorders are drug related or occur as a result of the stress of the post-transplant period or other factors. It seems likely that a combination of factors exists in many cases.

Symptoms vary in severity from insomnia, nervousness and mood changes, to manic or depressive symptoms, or agitation with paranoid ideation. Depression may on occasion be related to the changes in physical appearance that may follow prolonged steroid therapy, e.g. Cushingoid features, hirsutism; fortunately, with the low dosages used today, extreme changes in physical appearance are rare.

Other side-effects

The list of other complications of corticosteroid therapy is long (Table 3). Fluid and salt retention can lead to hypertension and edema formation. Diuretic therapy can usually control these clinical features. Increased appetite and weight gain are common and may lead to obesity if not controlled by a strict diet; increased deposits of fat are seen particularly over the trunk. Changes in menstrual cycle, sexual activity, acne, night sweats, myopathy, joint pains, and spontaneous petechial hemorrhages in the skin are also common.

POLYCLONAL OR MONOCLONAL ANTI-T-CELL ANTIBODIES

Antilymphocyte (or antithymocyte) polyclonal globulin

The immunosuppressive effect of antilymphocyte globulin (ALG) was first demonstrated in the rat skin graft model[132]. The properties of ALG depend to a large extent on its method of preparation. It can be prepared against a wide variety of anti-

gens, including those on thoracic duct or blood lymphocytes and thymocytes. ALG is prepared by immunizing, most commonly, horses, rabbits, or goats with human lymphocytes or thymocytes. A clear description of the steps involved in its preparation is given by Touraine *et al.*[133]. The dosage varies widely, depending on the preparation used. Several commercial preparations are currently available; there are variations in potency between them.

Administration, dosage and monitoring

ALG was probably first used in cardiac transplantation by several groups in 1968. Until 1989 we used ALG routinely as induction therapy. ALG was administered initially during operation, once the patient was on cardiopulmonary bypass; we adopted this policy following rapid circulatory deterioration from an anaphylactic-like reaction in two patients who were receiving ALG immediately before induction of anesthesia, even though skin testing showed no sensitivity to the drug. The dose is estimated as that necessary to reduce the circulating T lymphocytes to the therapeutic range of 50–150 cells/mm^3. The drug is usually given intravenously, with a usual loading dose of approximately 5–15 mg IgG/kg, depending on the initial T cell count. The drug can be given intramuscularly, but repeated injections are painful and can cause local inflammation and pain[134].

The T-11 lymphocytes are counted daily by flow cytometry using monoclonal antibodies (or by the sheep red cell rosetting test), and the dosage of ALG estimated accordingly. (The percentage lymphocyte count gives an approximate value for the T-11 lymphocytes as these form approximately 50% of the total lymphocytes. If the absolute lymphocyte numbers are kept in the region of 200–400 cells/mm^3, this probably represents an adequate reduction in T cell activity). ALG is given in doses as necessary (usually 5–10 mg IgG/kg per day) to maintain the T lymphocytes at the desired level; the daily dosage may vary widely from patient to patient and from day to day. The drug is diluted in 100–200 ml normal saline or 5% dextrose, and is usually given as a single daily infusion over a period of 4 hours. When severe acute rejection is occurring, and the T lymphocytes multiplying rapidly, it is on occasion necessary to count the T lymphocytes every 8 or 12 hours and administer ALG 8- or 12-hourly.

In several centers ALG is administered prophylactically daily until such time that the trough CsA level is considered adequate and consistent. This usually takes only a few days, rarely requiring more than 1 week of ALG therapy; at this time ALG is discontinued. It is introduced again only when required in the management of an acute rejection episode.

Major side-effects and complications

There is a small risk of anaphylactic shock following its administration, whether this be intravenous or intramuscular. At Stanford, approximately 2% of patients exhibited frank anaphylaxis with the intramuscular use of ATG[135]. The risk of anaphylaxis may be reduced by administering a test dose subcutaneously and observing for a severe histamine skin reaction, and by administering steroids and an antihistamine to the patient before the ATG dose is given.

Patients who experience anaphylactic shock may require urgent steroid and epinephrine therapy, vasopressor support, and mechanically assisted respiration. They may, however, cautiously receive further ATG on subsequent occasions; in our experience this reaction may be related to the batch of ATG used, rather than to the ATG *per se*. Should the complication occur again, ATG produced in a second species of animal (e.g. rabbit or goat rather than horse) may be found not to provoke the reaction, and may be used safely.

A small proportion of patients receiving ATG develop a combination of symptoms, which include rash, fever, chills, back and joint paint, and, less frequently, bronchospasm. During acute rejection episodes the chills and fever can be minimized by giving intravenous steroid therapy prior to the intravenous administration of ATG. Bronchospasm is treated as for anaphylaxis. Aspirin and antihistamines are useful in the treatment of joint pain and fever, and rash, respectively.

We have seen the clinical syndrome of serum sickness (fever, hepatomegaly, splenomegaly and lymphadenopathy, associated with upper abdominal discomfort and pain) during intravenous ATG administration in two patients; it resolved after discontinuation of therapy.

In our experience allergic reactions to ALG have been uncommon, but it is general policy to give a prophylactic antihistamine such as promethazine hydrochloride (12.5–25 mg) intravenously immediately before drug infusion.

In 1989, concerned by the increasing incidence of cytomegalovirus (CMV) infection in our patients during the first few post-transplant months, we discontinued the initial course of ATG, immunosuppressing the patient with the University of Minnesota regimen of CsA, AZA, and corticosteroids only (Table 1). The incidence and severity of CMV disease have been significantly reduced[136]. We therefore now reserve ALG only for those patients in whom it may be hazardous to administer CsA (e.g. in those with greatly impaired renal function) and for those undergoing a severe rejection episode unresponsive to intravenous corticosteroid therapy alone. This topic is discussed further in Chapter 9.

Similar effects are observed after therapy with antithymocyte globulin (ATG) or with antilymphocyte globulin (ALG), though the response is theoretically more specific. Only T cells are supposedly affected when ATG is administered, but both T and B cells are involved when ALG is administered. This is not always so in practice, as ATG often depletes all lymphocytes, not just T cells.

Monoclonal antibody (OKT3)

Monoclonal anti-T-cell antibodies are directed to, and block, the recognition of antigen by the recipient circulating T cells. Receptors are found on all circulating human T cells[137], and monoclonal antibodies can be raised against any T cell subset. The modes of action of monoclonal antibodies include removal of T cells from the circulation by complement-mediated lysis or by the reticuloendothelial system, and coating of cell surface antigens[138,139].

Immunosuppression with monoclonal antibodies is clearly more specific than with ATG or ALG. Monoclonal antibodies

have the advantages (over ATG and ALG) of better consistency in preparation, greater ease in monitoring serum levels, and administration of less foreign protein. As the immune response is so complex, however, there may be advantages of ATG or ALG in destroying or inhibiting a wider spectrum of lymphocytes.

OKT3, a murine monoclonal antibody of the IgG-2a series, is a pan-T cell suppressor. It can only be given intravenously. OKT3 causes an immediate but temporary decline in circulating T cells which persists while the antibody is being administered. OKT3 blocks all known T cell functions. A rapid and concomitant decrease in the number of T3 (CD3), T4 (CD4), and T8 (CD8) cells occurs within minutes of administration of OKT3. There is some recovery in the numbers of T4 and T8 cells within the first week of administration, though the T3 cells remain undetectable in the peripheral blood. Recovery of T3 cells to pretreatment levels occurs within a week once administration is discontinued.

OKT3 monoclonal antibody directly binds to the cell surface antigen, leading to loss of the cell's recognition apparatus[140]; these T cells can therefore play no further part in the acute rejection process. The initial decline in the number of T cells following institution of OKT3 therapy is probably brought about by opsonization of the cell surface, and removal of the cells by the reticuloendothelial system of the host[141]. Many monoclonal antibodies (other than OKT3) have been, or are currently being developed (Chapter 71).

Experience with OKT3 has rapidly accumulated, and several programs now use it to treat rejection unresponsive to conventional therapy (Chapter 30). A smaller number of centers use it in the early postoperative period as a prophylaxis to prevent rejection. The various indications for its use are, therefore: (a) treatment of refractory rejection; (b) treatment of life-threatening rejection when it is elected not to wait to assess the full response of conventional corticosteroid therapy; (c) rejection prophylaxis.

Administration, dosage and monitoring

It is recommended that 5 mg of OKT3 be administered daily for 10–14 days, each dose being given i.v. over 1 minute. This dose may require adjustment to maintain the T3 lymphocyte subset at about 20 cells/mm^3. To prevent excessive immunosuppression (and thus increase the risks of infection and lymphoproliferative disease) most groups suggest that, during therapy, concomitant immunosuppressive therapy should be decreased to approximately half the maintenance level. Others advocate discontinuing concomitant immunosuppressive therapy entirely. Normal maintenance immunosuppressive doses (of CsA, etc.) should be resumed 3 days prior to completion of OKT3 therapy.

Serum levels of OKT3 can be measured by an ELISA (enzyme-linked immunosorbent serum assay). A mean trough level of 0.9 μg/ml (achieved with the recommended dose) has been shown to block T cell effector functions *in vitro*.

Premedication with acetaminophen 650 mg orally, methylprednisolone 1 mg/kg i.v., and diphenhydramine 100 mg i.v. 30 min prior to administration of OKT3 is given to minimize allergic reactions. It is recommended that 100 mg of i.v. hydrocortisone be given intravenously 30 min after the injection of OKT3; further acetaminophen and antihistamines are optional.

One of the transplant groups to have considerable experience with OKT3 is the Utah group[141–143]. Their initial studies of its use prophylactically suggested improved patient and graft survival when compared with patients receiving ATG as prophylaxis during the same period of time. However, the sample size was not sufficient to demonstrate a reduction in mortality or graft loss in the OKT3-treated patients. More recent studies from this and other groups have suggested that OKT3 does not impart any increased benefit, and that its use is associated with an increased rate of infection, and a significant incidence of early vascular (humoral) graft rejection[144] (Chapter 28). These complications are particularly common if the prophylactic course is continued for 21 days.

IgM anti-OKT3 appears in approximately 21% of patients receiving this agent, while IgG anti-OKT3 is seen in 86%, and IgE anti-OKT3 in 29%, occurring approximately 20 days after treatment is begun. The numbers of T3 cells should be carefully monitored and, if they increase despite OKT3 therapy, the dosage of the monoclonal antibody should be increased at least 2–3 times. Patients who develop large quantities of IgG anti-OKT3 antibodies, and therefore fail to respond to OKT3 therapy, may not be candidates for another course of OKT3 treatment.

Anti-idiotypic antibody development, which is a prominent feature of the immune response to OKT3, and probably other monoclonal antibodies, can lead to a block in their therapeutic effectiveness and can arise despite intense immunosuppression[145]. This response may require the use of different idiotypes for prolonged or repeated courses of therapy, and may prove a major obstacle.

Major side-effects and complications

The side-effects of OKT3 include fever (which occurs in 73% of patients), chills, dyspnea, chest pain, vomiting, wheezing, nausea, tremor, and diarrhea. Aseptic meningitis, exhibited by fever, headache, neck stiffness and photophobia, has been reported.

Because of the risk (less than 2%) of fatal pulmonary edema, OKT3 is contraindicated in patients with fluid overload, as determined by pulmonary congestion on a chest radiograph and by a weight gain of more than 3% within the week prior to the planned OKT3 therapy. It is recommended that a Swan–Ganz catheter should be inserted before treatment is begun, and that if the pulmonary capillary wedge pressure is seen to be unduly high, OKT3 therapy should be avoided. The Utah group has shown, however, that OKT3 is generally well tolerated in the setting of cardiac transplantation[141].

COMMENT

With maintenance triple-drug therapy (CsA (or CsA-Neoral), AZA, and steroids), the development of severe acute rejection is today fairly unusual, and has been seen in only 2% of endomyocardial biopsies at our center during the past 5 years. This combination of drugs is, therefore, potent and efficient, and care has to be taken to avoid over-immunosuppression. Changes suggestive of mild or moderate acute rejection remain relatively common, but frequently do not warrant extra immunosuppressive therapy.

The efficacy of CsA has allowed steroid dosage to be significantly reduced, and in an increasing number of patients it

can be discontinued entirely. This is a major advance that reduces the debilitating effects and complications associated with long-term steroid therapy. The use of triple-drug therapy has not yet been shown definitively to reduce the incidence of graft vasculopathy (chronic rejection), which still occurs in a significant percentage of patients (Chapter 35), although our impression is that its onset is delayed when compared with 10 years ago.

Optimal combinations of immunosuppressive agents can be arrived at rationally and effectively only by a full understanding of the site and mode of action of each agent. As our knowledge of the various mechanisms that play a role in allograft destruction increases, so it should become possible to devise more sophisticated methods of blocking the sequence of events outlined in Chapter 7. The clinical employment of such agents may eventually enable graft destruction to be prevented, and yet allow the body to maintain all or most of its other host defense mechanisms.

Long-term immunosuppressive therapy is clearly associated with a large number of side-effects and complications. Some of these can be avoided or minimized by careful selection of the patient, or by pretransplant prophylactic treatment (e.g. for peptic ulcer), and others by post-transplant prophylactic medication. Avoidance of all such complications, however, cannot be ensured, and the potential risks must be considered when the potential recipient is initially assessed for organ transplantation.

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9
Trends in Immunosuppressive Therapy With Regard to Cytolytic Induction Therapy and Corticosteroid Withdrawal

J. KOBASHIGAWA

IS CYTOLYTIC INDUCTION THERAPY BENEFICIAL?

A cytolytic agent (e.g. antilymphocyte serum) administered immediately after heart transplantation (HTx) is known as 'induction' therapy and is given in the hope of inducing graft tolerance[1–4]. The benefit of this form of therapy remains controversial, as no randomized studies have been performed. Forms of antilymphocyte sera include murine monoclonal antibody (OKT3), rabbit and equine antithymocyte globulin (ATG), and antilymphocyte globulin (ALG).

In general, all cytolytic induction therapies appear to have similar effects. Single-center studies have reported that cytolytic therapy appears to prolong the time to the first acute cellular rejection episode; however, the total number of rejection episodes within the first year after HTx is not reduced when compared with the number in patients receiving triple-drug immunosuppression alone (cyclosporin, prednisone and azathioprine, without cytolytic therapy)[5–7]. Barr and colleagues[6] reviewed 52 HTx patients, one-half treated with OKT3 for 14 days immediately postoperatively and one-half treated with triple-drug immunosuppression alone. Although time to first rejection was longer in the OKT3 group, there was similar rejection frequency, freedom from rejection, and survival in the two groups. Prieto *et al.*[18] showed similar results in a review of 82 HTx patients (35 treated with OKT3 versus 47 treated with triple-drug immunosuppression). A large multi-institutional study[9], involving 911 HTx patients, revealed that there was no benefit in the use of cytolytic therapy over triple-drug immunosuppression with regard to time of onset of first rejection or cumulative frequency of rejection episodes. However, this study involved many different antilymphocyte preparations, which might have masked the efficacy of any one agent.

The effectiveness of one form of cytolytic therapy over another has been compared in numerous studies[10–13]. These comparison studies are difficult to interpret, as many different doses and lengths of cytolytic therapy have been used. A non-randomized, retrospective study by Ladowski *et al.*[14] reported on 79 patients treated with equine ATG for 4 days (34 patients), Minnesota ALG for 10 days (15 patients), or OKT3 for 14 days (30 patients). Although there appeared to be an advantage of equine ATG in leading to a lower rejection incidence, there was no survival advantage. Furthermore, this was the only group to receive preoperative cyclosporin, which complicates interpretation of the results. Copeland *et al.*[15] demonstrated superior results in regard to survival, rejection, and infection with the use of rabbit ATG in 155 HTx patients; however, the 'controls' were the contemporary results of other programs.

A large multicenter database suggested that there may be an increase in infectious complications (cytomegalovirus) with the use of cytolytic induction therapy[16,17]. Review of 1553 HTx patients from 26 programs revealed, through multivariate analysis, that there was earlier development of cytomegalovirus infection in patients who receive cytolytic induction therapy. However, mortality due to cytomegalovirus was not increased[17]. OKT3 murine monoclonal antibody is the most common form of cytolytic induction therapy used, and has been associated with a higher incidence of cytomegalovirus infection[18] and, more recently, with the development of lymphoproliferative disease[19]. The use of OKT3 may also result in the development of human anti-mouse antibodies, which has been associated with adverse outcomes after HTx[20,21]. Specifically, vascular damage can be produced by immune complexes that contain host antibodies against highly immunogenic proteins, such as antithymocyte globulin or murine monoclonal antibodies[22,23]. Overall, however, due to the lack of randomized trials, controversy still exists as to the increased risks in HTx patients treated with OKT3 induction therapy[24].

Many centers use cytolytic induction therapy in short courses to allow delay of initiation of cyclosporin therapy, thus avoiding acute cyclosporin nephrotoxicity in patients at high risk for immediate or early-postoperative kidney failure. Kormos and colleagues[25] initially reported success in using rabbit ATG for 3 days and starting cyclosporin 24 h after surgery, to avoid postoperative renal dysfunction. Similarly, Barr *et al.*[6] successfully used a short course of OKT3 (5 days) to avoid renal dysfunction in high-risk HTx patients.

More recently, some HTx centers have employed short courses of cytolytic therapy in the hope of achieving graft tolerance and avoiding the complications associated with longer courses of

therapy. Menkis *et al.*[26] randomized 39 HTx patients to a short course of OKT3 (7 days) or to Minnesota ALG (7 days). There was no difference between the two groups in either cumulative rejection episodes or time to first rejection. A study by Alonso-Pulpon *et al.*[27] evaluated varying doses of OKT3 in 39 HTx patients; 23 were treated with 5 mg OKT3 and 16 with 2.5 mg OKT3, both for 7 days duration; there was no difference in the incidences of rejection or infection, or in the total methylprednisolone dose given.

Our HTx program at the University of California at Los Angeles (UCLA) Medical Center performed a randomized trial of short-course OKT3 therapy in 30 HTx patients[28]. Fifteen received a 5-day course of OKT3 while 15 received routine triple-drug immunosuppression alone. No patient had a baseline serum creatinine level of >1.5 mg/dl. At 6 months follow-up there was no difference between the two groups in: (a) the number of rejection episodes per patient; (b) the number of patients who were free of rejection; (c) the time to first rejection (25±29 days in the triple drug immunosuppression group compared to 57±68 days in the OKT3 group (p=0.1)); (d) renal function, determined by serum creatinine levels (no patient in either group required hemodialysis); and (e) infectious complications, including cytomegalovirus infection. Overall, there did not appear to be a benefit in the use of short-course OKT3 induction over triple-drug immunosuppression. However, the benefit of OKT3 induction, in which longer courses are used (e.g. a 14-day course), is yet to be determined.

COMMENT

The benefit of antilymphocyte sera administered immediately after HTx, known as 'induction therapy', remains controversial as no randomized studies have been performed. However, there are an increasing number of reports that suggest the use of OKT3 monoclonal antibody in induction therapy may have deleterious long-term effects in causing a higher incidence of cytomegalovirus infection and post-transplant lymphoproliferative disease. There may be a role for the use of early cytolytic therapy in patients at high risk for early postoperative kidney failure by allowing initiation of cyclosporin therapy to be delayed. It is unlikely that large multicenter studies will be performed comparing cytolytic induction therapy to triple-drug immunosuppression as the risks of cytolytic therapy have recently become more apparent and several new immunosuppressive medications are becoming available.

CORTICOSTEROID-FREE IMMUNOSUPPRESSION

The discontinuation of corticosteroid therapy (to allow steroid-free immunosuppression) has been attempted immediately and late (>6 months) after HTx, to avoid the complications related with such therapy[29–36]. Potential complications of steroid therapy are numerous, and include osteoporosis, avascular bone necrosis, myopathy, increased incidence of infection, salt and water retention, hypertension, gastrointestinal complications, hyperlipidemia, exacerbation of diabetes, weight gain, cosmetic alterations (such as hirsutism and Cushingoid habitus), and growth retardation in children (Chapter 8). In addition, corticosteroids may be atherogenic and potentially contribute to the development of transplant coronary artery disease (graft vasculopathy).

The objective of corticosteroid withdrawal is to improve the overall health and quality of life of HTx recipients by eliminating its long-term complications. One can decrease a patient's exposure to steroid complications by maintaining low prednisone doses or by complete withdrawal. There has been concern regarding the risks of steroid withdrawal, which may lead to inadequate immunosuppression and an increased risk of rejection. Therefore, there may be a need for long-term surveillance by endomyocardial biopsies. There is also the question of whether increased doses of other immunosuppressive agents (cyclosporin and azathioprine) are necessary to avoid rejection. Corticosteroid withdrawal symptoms may be quite significant, and include arthralgia, myalgia, fatigue, and headaches, and in some cases may require reinstitution of steroids. Finally, the role of corticosteroids in the development of transplant coronary artery disease (graft vasculopathy) remains unclear. As this is probably an immune-mediated process, steroids could play a role in its prevention. On the other hand, steroid therapy is known to be associated with elevated lipid levels and, therefore, may be atherogenic.

Approach to corticosteroid withdrawal

There are currently two approaches to corticosteroid withdrawal in HTx recipients: (a) it has been attempted immediately (1–30 days) after HTx, usually in programs that use cytolytic induction therapy[29–31]; (b) the other approach has been to wean steroids late (>3 months) after HTx; cytolytic induction therapy has usually not been used[32–35]. The apparent advantage of late steroid withdrawal relates to the opportunity of selecting for weaning those patients who have experienced either no or few rejection episodes. This may indicate that these patients show less immunologic response to their graft, and thus may be more likely to undergo successful steroid weaning without late rejection. However, no established superiority of either withdrawal approach has been documented, as there have been no prospective randomized trials comparing these two approaches. Even the benefits of steroid withdrawal have not been conclusively established.

Corticosteroid withdrawal protocols

Protocols

The early corticosteroid withdrawal protocols weaned steroids in a tapering dose schedule over 1–2 weeks after HTx. At the University of Utah Heart Transplant Program patients could experience up to three episodes of rejection before they were considered protocol failures and placed back on maintenance steroids[31].

Late corticosteroid weaning is routinely attempted at UCLA beginning 6 months after HTx[32]. Criteria for corticosteroid weaning include patients who: (a) have survived at least 6 months since HTx, (b) have not experienced even moderate rejection during the prior 3 months, (c) have not experienced more than two episodes of treated rejection, and/or (d) have experienced no hemodynamic compromise through rejection.

By 6 months, patients are usually at a baseline corticosteroid dose of 5 mg prednisone/day. They are weaned slowly by decreasing the daily prednisone dose by 1 mg each month.

Endomyocardial biopsies are performed monthly during the weaning period and 2 months after discontinuation of prednisone. Those patients who cannot be successfully weaned are placed back on 5 mg prednisone daily. The attempt to wean is repeated at least twice before considering a patient a protocol failure.

Results

Corticosteroid withdrawal attempted within the first 30 days post-HTx has a success rate of 40–61%[29,31,34]. Withdrawal attempted later than 3 months has a success rate of 73–92%[32,33,35]. Early (<30 days) withdrawal is attempted in *all* patients (not just those with a low incidence of rejection), which probably accounts for the lower success rate. Overall, similar numbers of patients are being weaned off corticosteroids by each approach (early versus late) since weaning is attempted in a smaller (but select) percentage of patients >3 months after HTx. In the UCLA Heart Transplant Program approximately 60% of patients are eventually weaned from steroids.

Factors that appear to affect the success of corticosteroid weaning include: (a) donor–recipient HLA-DR matching, (b) recipient gender, and (c) number of previous rejection episodes. At UCLA, donor and recipient HLA-DR antigens were compared in 75 patients[37]. Thirty of 31 patients (97%) with at least one HLA-DR match were successfully weaned from prednisone, compared with 33 of 44 (75%) with no HLA-DR matches ($p<0.05$). However, similar observations were not found in 62 patients evaluated at the St Louis University Heart Transplant Program.

Women are at higher risk of osteoporosis and, therefore, it is particularly desirable to reduce cumulative doses of steroids in female HTx patients. However, steroid withdrawal success rates in women have been reported to be only 13–18% in the HTx programs at St Louis, Utah, and UCLA (unpublished data). These findings are consistent with the fact that female HTx recipients have higher incidences of acute graft rejection and early mortality compared to male patients[38]. It is unclear whether the newer immunosuppressive agents will allow a higher success rate of steroid withdrawal in female patients.

The history relating to previous rejection episodes has been a good predictor of the success of steroid withdrawal in patients weaned late after HTx. At St Louis University the risk of developing a rejection episode after steroid withdrawal was almost 2-fold greater in patients who had experienced a previous rejection episode (while taking corticosteroids) when compared to patients who had experienced no prior rejection episode[33].

Benefits of corticosteroid withdrawal

Hyperlipidemia occurs in 60–80% of HTx patients[39]. Some investigators have shown a correlation between hyperlipidemia and transplant coronary artery disease (graft vasculopathy); others have been unable to confirm this finding. The development of hyperlipidemia after HTx is multifactorial, with corticosteroids being one of the contributing factors. At the University of Utah, corticosteroid withdrawal had a significant impact on serum cholesterol levels[40]. Beginning 3 months after HTx, the cholesterol levels for 51 patients maintained on a mean prednisone dose of 12.4±1.2 mg/day ranged from 262±8 to 272±8 mg/dl. This contrasted with the corticosteroid-free group, in which the cholesterol level ranged from 199±8 to 211±9 mg/dl. At UCLA the cholesterol levels (a) at the time of corticosteroid weaning and (b) 1 year later, showed an average decrease of 14±31 mg/dl in those successfully weaned, while the steroid-dependent group showed a rise in cholesterol of 20±41 mg/dl[37].

Corticosteroid withdrawal has been shown to decrease the need for antihypertensive medications. At UCLA, at the time of attempted steroid withdrawal, 80% of the patients (subsequently successfully withdrawn from corticosteroids) were taking antihypertensive medications[37]. One year later this number decreased to 73%. Of the patients who could not be weaned off corticosteroids (and were maintained with prednisone 5 mg/day) 54% were taking antihypertensives at baseline, and this number increased to 67% one year later. Similarly, at St Vincent's Hospital in Australia, after 4 years of follow-up, patients still receiving steroids required significantly more antihypertensive medications than those no longer receiving steroids[34].

Weight gain is common after HTx and is associated with steroid therapy. Corticosteroid-free immunosuppression has been reported to ameliorate weight gain[37,41]. At UCLA, after 1 year of follow-up, patients on steroid-free immunosuppression had an average 1±4 kg weight *reduction* compared to a 4±9 kg weight *increase* in those dependent on steroid therapy[37].

The University of Utah program evaluated 178 HTx patients to determine whether those on corticosteroid-free immunosuppression had a lower incidence of gastrointestinal complications[42]. Twenty-six patients on maintenance steroids developed 30 major abdominal complications whereas no steroid-free patient developed such a complication.

Corticosteroid withdrawal in children

The chronic use of corticosteroids in children may retard growth[43,44]. The effect of steroid-sparing regimens was retrospectively reviewed in 42 infants who underwent HTx at <6 months of age at Loma Linda University[43]. Ten of 15 infants who had abnormal growth were receiving steroids; maintenance steroids were subsequently discontinued in six, four of whom experienced rebound growth. Within 6 months of steroid withdrawal, three of the four patients moved from below the 5th percentile for growth to the 95th percentile or higher.

Safety of corticosteroid withdrawal

There is concern that corticosteroid withdrawal may lead to an increased development of transplant coronary artery disease. Steroid-free therapy may provide an adequate level of immunosuppression to prevent acute allograft rejection, but may not be sufficient to prevent immune-mediated endothelial injury in the coronary arteries. Available data from single-center studies suggest that steroid withdrawal does not cause an increase in the development of graft vasculopathy[33,45]. Unpublished data from the UCLA program reveal a *lower* incidence of angiography-defined transplant coronary artery disease in those patients successfully weaned from steroids. As patients who are able to be weaned from steroids may be immunologically privileged, then it is logical that they may be less prone to develop graft vasculopathy.

Data from several institutions reveal comparable or improved patient survival in those weaned from steroids[33–37]. Furthermore, data from Utah indicate that cardiac allograft function appears to be normal in those patients weaned from steroids[46].

Cyclosporin and azathioprine dosages are not routinely increased in patients who have undergone steroid withdrawal. It is important, however, that medication compliance be maintained, as the risk of rejection may be greater if the cyclosporin level becomes subtherapeutic.

COMMENT

Corticosteroid withdrawal, whether performed early or late after HTx, appears successful in the majority. Significant advantages in regard to weight, hyperlipidemia, and hypertension may be achieved. Steroid withdrawal does not appear to impact adversely on survival, development of transplant coronary artery disease, or on hemodynamics. Finally, through the avoidance of the complications of long-term steroid therapy, it is likely that corticosteroid-free immunosuppressive protocols will have a positive impact on the quality of life of HTx patients[47].

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10
Tacrolimus (FK506) in Thoracic Organ Transplantation

J.S. GAMMIE AND S.M. PHAM

INTRODUCTION

Tacrolimus (FK506) was discovered as the result of a systematic screening program to look for an improved immunosuppressant. In the mid-1980s Goto and associates from the Exploratory Research Laboratories of Fujisawa Pharmaceutical Co. Ltd, Japan, discovered a macrolide compound that had potent immunosuppressive properties[1–3]. This compound, then named FK506, was extracted from the fermentation broth of the soil fungus *Streptomyces tsukubaensis*. Further preclinical studies on the immunosuppressive properties of this compound on organ transplantation were carried out intensively at Chiba University, Japan[4–9], Cambridge University, UK[10–12], and the University of Pittsburgh, USA[13–18]. The initial results of FK506 as an effective immunosuppressant in organ transplantation were presented at the first International Symposium on FK506 in June 1987, in Göteborg, Sweden, and were subsequently published in *Transplantation Proceedings* (Vol. 19, Suppl. 6, 1987).

The first clinical trial using FK506 in organ transplantation began at the University of Pittsburgh in February 1989[19]. In this trial, FK506 was used as a 'rescue' agent for liver transplant recipients experiencing refractory rejection on cyclosporin-based regimens. Encouraged by the initial results, clinical trials of FK506 as a primary immunosuppressant in liver, kidney, heart and lung transplantation were initiated later that year at the same institution[20–23]. Recent national and international trials have helped to define the role for this important immunosuppressant[24–27]. In 1994, FK506 was approved by the FDA in the USA for use in liver transplant recipients. Medicare and major third-party payors have approved the use of tacrolimus for approved transplant procedures. The generic name of FK506 is now *tacrolimus*, and it is marketed under the trade name Prograf® (Fujisawa USA, Deerfield, IL).

In this chapter we will review the pharmacology and clinical use of tacrolimus in heart and lung transplantation.

Chemistry

Tacrolimus, a macrolide lactone, is a member of the macrolide antibiotics whose chemical structure consists of a hemiketal-masked α,β-diketoamide incorporated in a 23-member ring (Figure 1). Other drugs belonging to the family of macrolides include erythromycin, oleandomycin, and rapamycin. Tacrolimus is a large molecule with a molecular weight of 822.05 daltons, and is highly lipophilic. Because it is practically insoluble in water, tacrolimus is dissolved in cremaphor for intravenous administration.

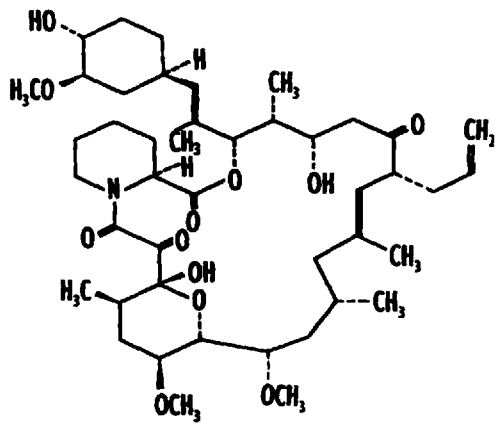

Figure 1 Chemical structure of tacrolimus

Mechanisms of action

Despite having different chemical structures, tacrolimus and cyclosporin (CsA) have similar mechanisms of action. In a broad sense these immunosuppressants work by inhibiting the T-cell activation that normally occurs in response to the presentation of antigens to the T-cell receptor (TcR)–CD3 complex on the surface of the T cell. They block the transcription of early-phase T-cell activation genes, including those genes encoding interleukin (IL)-2, IL-3, IL-4, IL-5, granulocytes–macrophage colony-stimulating factor (GM-CSF), tumor necrotis factor-α (TNF-α), and interferon (IFN)[28–31].

Tacrolimus and CsA initially bind to their respective cytosolic target proteins (known as immunophilins), cyclophilin and FK506-binding proteins (FKBP)[30]. The cyclophilin–CsA and the FKBP–FK506 complexes, in turn, bind to the calcium- and calmodulin-dependent cellular signaling protein calcineurin[31]. Calcineurin is a serine/threonine phosphatase that plays a key role in the transfer of information from the cell surface to the nucleus, ultimately resulting in the transcription of various cytokines (predominantly IL-2), which in turn leads to T-cell activation. The transfer of signals from the cytoplasm to the nucleus, to activate the transcription of the IL-2 gene, requires the nuclear factor of activated T-cell protein (NF-AT). NF-AT consists of two subunits, one located in the cytoplasm and one in the nucleus. Following stimulation of the T-cell receptor, the cytoplasmic subunit of the NF-AT is modulated by calcineurin and transported into the nucleus, where it combines with its nuclear counterpart to function as a transcription factor for the IL-2 gene[32]. The tacrolimus–FKBP complex binds to calcineurin and blocks the modulation of the cytoplasmic subunit of the NF-AT, resulting in the inhibition of IL-2 gene transcription and activation of T cells[33].

Bioavailability

Following oral administration tacrolimus is erratically and incompletely absorbed. Mean bioavailability is 27%, although there is significant inter- and intra-patient variability[34]. Time to peak plasma concentration ranges from 1 to 3 hours. In contrast to cyclosporin, and of some advantage in the liver transplant population, tacrolimus does not require an intact enterohepatic circulation for absorption. Fasting enhances the rate and extent of the absorption of tacrolimus. The mean maximal blood concentration in fasted patients is twice that observed in fed patients, and the time to maximal concentration is 1 as opposed to 3 hours[35]. However, the total drug bioavailability (area under the curve, AUC) is not significantly affected by fasting. Hence, it is suggested that tacrolimus be administered at least 1 hour before, or 2 hours after, a meal. Conversion between intravenous and oral dosages of tacrolimus is based on a 1:5 ratio.

Distribution

Volume of distribution of tacrolimus is 1300 liters, suggesting extensive extravascular uptake. Tacrolimus is rapidly partitioned into red blood cells, with a whole blood to plasma ratio of 15:1, and is more than 99% bound to plasma proteins (albumin and α_1-acid glycoprotein).

Metabolism

Tacrolimus is metabolized by the microsomal P-450 IIIA enzyme system in the liver and intestine, with less than 5% excreted unmetabolized in the stool[35]. Less than 1% of the drug is eliminated renally. Tacrolimus is not dialyzable. The main metabolic pathway includes 13- and 15-*O*-demethylation and 12-hydroxylation, with at least nine resultant metabolites, some of which retain immunosuppressant properties. Elimination is biphasic, with an overall half-life ranging from 5.5 to 16.6 hours (mean 8.7 hours).

Table 1 Drugs potentially interacting with the metabolism of tacrolimus*

Decrease ← Metabolism → Increase Increase ← Blood level → Decrease	
Erythromycin	Rifampicin
Troleandomycin	Phenobarbital
Clotrimazole	Phenytoin
Ketoconazole	Carbamazepine
Fluconazole	
Cyclosporin	
Diltiazem	
Verapamil	
Nifedipine	
Ethinylestradiol	
Methylprednisolone	
Midazolam	

* Adapted from ref. 35.

Because tacrolimus is metabolized by the cytochrome P-450 IIIA system, its metabolism is subject to influence by agents which induce or inhibit the P-450 system (Table 1)[34]. Drugs commonly used in transplant recipients that reduce the metabolism of tacrolimus (and thus increase its blood level) include erythromycin, clotrimazole, ketokonazole, fluconazole, and calcium channel blockers. Phenobarbital, phenytoin, and rifampicin, on the other hand, will reduce the blood level of tacrolimus.

Of clinical interest, and compatible with the observed augmented toxicities when cyclosporin and tacrolimus are combined, has been the recognition that tacrolimus inhibits the cytochrome P-450 IIIA enzyme oxidase, resulting in sustained CsA levels.

Blood level monitoring

It has been demonstrated that pharmacokinetic parameters for tacrolimus vary substantially between individual patients, and at different times in the same patient. There is also significant inter- and intra-patient variability of bioavailability and clearance, with the net result being a poor correlation between tacrolimus dose and blood levels[36]. Since dose alone cannot be used to predict overall drug exposure (area under the curve, AUC), a parameter that correlates strongly with the efficacy and adverse effects of the drug, frequent monitoring of drug levels is essential.

All tacrolimus assays are based on the same monoclonal antibody. Initial clinical studies in Pittsburgh and elsewhere were performed based on plasma trough level, using an enzyme-linked immunosorbent assay (ELISA)[37]. Plasma target levels were between 0.5 and 2.0 ng/ml. More recently a microparticle enzyme immunoassay (IMx) has been used[38] to measure whole blood level, with target levels between 5 and 30 ng/ml. Advantages of this assay include more rapid turn-around time (1–3 vs 10–20 hours), greater sensitivity, and lack of temperature dependence (tacrolimus partitions into red cells as temperature drops).

Side-effects

Given that tacrolimus and CsA share a similar mechanism of action, it is not surprising that their clinical toxicities largely

overlap. The most prominent side-effects of both drugs include nephrotoxicity, neurotoxicity, impaired glucose metabolism, hypertension, and gastrointestinal disturbances. The incidence and degree of nephrotoxicity was similar among patients taking CsA and tacrolimus both in the US multicenter liver transplant trial and in the European trial[26,27]. Transient renal dysfunction immediately following transplantation is frequently observed with intravenous administration of tacrolimus or CsA. However, the degree of renal dysfunction has been minimized with a continuous 24-hour infusion, instead of bolus infusion, of tacrolimus, and concomitant administration[39,40] of low-dose prostaglandin E_1. Hyperkalemia is more common with tacrolimus than with CsA, and is reported to be independent of renal function[41,42]. However, it is easily managed with fludrocortisone (Florinef®),

Neurologic toxicity, which manifests as tremor, insomnia, headache, dizziness and photophobia, is somewhat more prominent in patients taking tacrolimus than in those on CsA[41–43]. In most cases these symptoms are dose-dependent and reversible. Severe neurologic toxicity, including aphasia, coma, and seizures, has been reported but is uncommon[43,44]. The incidence of new-onset diabetes mellitus and hypertension is similar with tacrolimus and CsA[41,42]. Hirsutism, coarsening of the facial features, and gingival hyperplasia, known side-effects of CsA, are notably absent with tacrolimus.

INITIAL CLINICAL TRIALS

Tacrolimus as a 'rescue' agent

In its first clinical trial, tacrolimus was used as a 'rescue' agent in liver transplant patients experiencing rejection refractory to conventional immunosuppression. This initial pilot study at the University of Pittsburgh included 11 liver transplant recipients[19]. In this trial CsA was gradually tapered off while tacrolimus was administered at full dose. Rejection was reversed in seven patients, while three had persistent rejection requiring retransplantation. Serious nephrotoxicity was frequently observed. As a result, in subsequent rescue patients, CsA was discontinued a minimum of 24 hours before tacrolimus was administered.

Later reports of patients rescued from refractory rejection under conventional CsA-based immunosuppression (125 patients with refractory rejection in the US multicenter FK506 liver transplant study group) showed a success rate of 70% at 3 months after treatment, with graft and patient survival at 1 year of 50% and 72%, respectively[45].

Tacrolimus as a primary immunosuppressant

Encouraged by the initial 'rescue' trial, a prospective single-center study of tacrolimus as a primary immunosuppressive agent for commonly transplanted organs (kidney, liver, pancreas, and heart) was launched at the University of Pittsburgh[19–23]. Tacrolimus was found to be an effective primary immunosuppressive agent. Early reports of hepatic transplantation showed 1-year patient and graft survival to be significantly higher than in historical controls. Steroid requirements and the incidence of clinical rejection were also substantially lower in the tacrolimus-treated group.

These initial results were confirmed by subsequent prospective, randomized, multicenter trials both in the USA and in Europe[26,27]. In the US trial, which consisted of 529 liver transplant recipients from 12 centers, primary endpoints were patient and graft survival at 1 year, while secondary endpoints included the incidence of acute rejection, corticosteroid-resistant rejection, and refractory rejection. There was no difference in patient or graft survival between the two groups. However, the incidence of acute rejection (68% tacrolimus vs 76% CsA, $p<0.002$), corticosteroid-resistant rejection (19% tacrolimus vs 36% CsA, $p<0.001$), and refractory rejection (3% tacrolimus vs 15% CsA, $p<0.001$) were all significantly less common in the tacrolimus group.

Similarly, the European trial, which enrolled 545 liver recipients from 19 centers, found no difference in patient or graft survival at 1 year, but did observe a significant reduction in acute, refractory acute, and chronic rejection episodes in the tacrolimus-treated group. In both trials the cumulative dose of corticosteroids administered for both prophylaxis and treatment of rejection was significantly lower in the tacrolimus-treated patients than in the CsA-treated group.

TACROLIMUS IN CARDIAC TRANSPLANTATION

Tacrolimus as a primary immunosuppressant

Tacrolimus has undergone clinical testing at the University of Pittsburgh on the thoracic transplantation service since October 1989. The initial and intermediate-term results have been reported elsewhere[22,23,46–48]. Between 1 January 1989 and 31 December 1994, 243 heart transplant recipients were enrolled in a non-randomized fashion to receive either tacrolimus- or CsA-based immunosuppression. Excluded from this analysis were patients undergoing retransplantation, those receiving multiple-organ transplants, and those dying within 7 days of transplantation from primary graft failure.

There were a total of 121 patients in the tacrolimus group and 122 in the CsA group. The CsA group included two subsets: (a) 72 patients who received CsA plus lympholytic induction (CsA + LI), with either OKT3 (11 patients) or rabbit anti-thymocyte globulin (ATG, 61 patients); and (b) 50 patients who received CsA alone, based on a modified triple-drug regimen described by Bolman and associates[49].

The tacrolimus regimen included 15 mg/kg of methylprednisolone given intraoperatively, with 5 mg/kg of methylprednisolone given in three divided doses on postoperative day 1, followed by 0.3 mg/kg per day in a single dose on subsequent days. Conversion to prednisone was accomplished upon resumption of oral intake. Gradual weaning from steroids was initiated 3 months after transplantation. Early in the trial, tacrolimus was given at a dose of 0.15 mg/kg per day, in two divided intravenous doses within 6–12 hours of transplantation. Due to a high incidence of renal dysfunction, dosing was decreased to 0.01–0.05 mg/kg per day as a continuous infusion, with conversion to oral tacrolimus, at 0.2–0.3 mg/kg per day in two divided doses, upon return of gastrointestinal function. Whole blood (IMx) target levels were maintained at 5–30 ng/ml in the first 2 months after transplantation. The level was subsequently reduced to 5–15 ng/ml, depending upon the patient's renal function and rejection pattern. Azathioprine (2 mg/kg per day) was added if

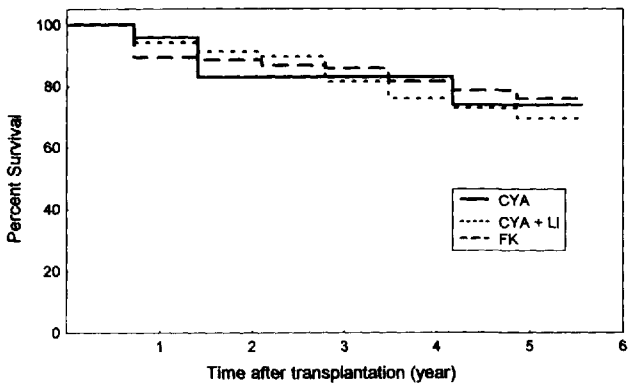

Figure 2 Actuarial survival following heart transplantation according to different immunosuppressive protocols. CYA = cyclosporin alone; CYA + LI = cyclosporin plus lympholytic induction; FK = tacrolimus

serum creatinine exceeded 2.0 mg/dl (to allow a reduction in the tacrolimus dosage), or if there was persistent rejection.

Acute rejection in both groups was treated initially with pulse steroids, while OKT3 was reserved for steroid-resistant rejection.

The tacrolimus and CsA groups were similar with the exception that the mean age in the tacrolimus group was lower than in the CsA group (34.2 ± 22.3 vs 47.8 ± 14.5 years; $p<0.05$). The mean duration of follow-up was longer in the tacrolimus group (3.2 ± 1.3 vs 2.3 ± 1.8 years; $p<0.01$).

One-year and 5-year actuarial patient survival rates were not significantly different (CsA alone: 91% and 74%; CsA + lympholytic induction {CsA + LI}: 93% and 69%; and tacrolimus: 90% and 76%) (Figure 2).

Actuarial freedom from rejection at 3 months for the CsA-alone group was 22%, significantly lower ($p<0.01$) than that of the CsA + LI (53%) and tacrolimus groups (47%) (Figure 3). The rate of rejection (episodes per 100 patient-days) in the tacrolimus group was significantly lower ($p<0.05$) than that of the CsA-alone or CsA + LI groups. Steroid boluses per 100 patient-days were lower in the tacrolimus group than in the CsA-alone ($p<0.01$) and the CsA + LI ($p<0.05$) groups (Table 2).

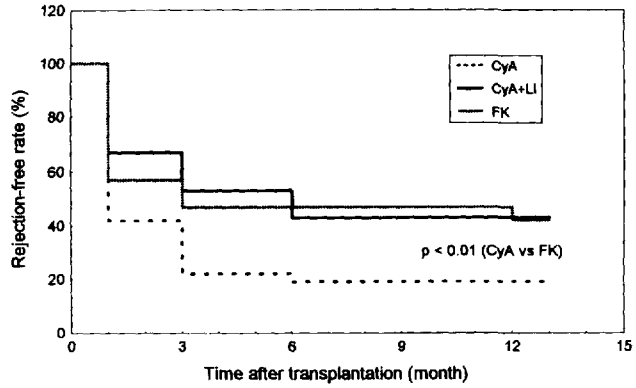

Figure 3 Actuarial freedom from rejection following heart transplantation according to different immunosuppressive regimens. CyA = cyclosporin alone; CyA + LI = cyclosporin plus lympholytic induction; FK = tacrolimus

Table 2 Rejection, and requirement for steroid boluses and lympholytic treatments, under different immunosuppressive protocols

	Immunosuppression		
	Tacrolimus	*CsA*	*CsA + LI*
Rejection-free rate at 30 days	47%	22%*	53%
Episodes of rejection†	0.09	0.26*	0.13**
Steroid bolus†	0.09	0.20*	0.11**
Lympholytic treatment†	0.009	0.06*	0.02

LI: lympholytic induction.
† per 100 patient-days.
* $p<0.01$ compared to tacrolimus group; ** $p<0.05$ compared to tacrolimus group

Thirteen patients in the CsA-alone and five patients in the CsA + LI groups experienced intractable rejection (refractory to at least one course of pulse steroids and one course of lympholytic treatment); all were successfully treated by conversion to tacrolimus. Only two patients in the tacrolimus group developed intractable rejection: one was rescued with methotrexate, while the other required total lymphoid irradiation.

Allograft coronary arteriopathy, defined as luminal irregularity or stenosis seen on coronary angiogram or the presence of diffuse coronary artery disease at autopsy, was evaluated in 183 patients. Actuarial freedom from allograft coronary disease at 4 years was 82% for the tacrolimus group (103 patients) and 73% for the CsA group (80 patients) (p = n.s.).

At last follow-up or at the time of death, 40 of 83 adult tacrolimus patients (48%) were free of steroids as compared to 16 of 95 adult CsA patients (17%) ($p<0.01$). Of those still on steroids, the average daily dose of prednisone was 5.8 ± 2.6 mg/day in the tacrolimus group and 8.0 ± 4.5 mg/day in the CsA group ($p<0.01$). The impact of tacrolimus was most dramatic in the pediatric group; 76% of the 42 pediatric recipients treated with tacrolimus were free of steroids. Of the 10 pediatric patients initially on CsA, eight required conversion to tacrolimus because they could not be weaned off steroids; all were eventually free of steroids.

Mean serum creatinine 1 year post-transplant was significantly higher in the tacrolimus (2.1 ± 0.5) group than in the CsA group (1.8 ± 0.5) ($p<0.001$). This difference disappeared by the second year. We believe that this early difference reflects a learning curve for the use of tacrolimus. New-onset hypertension was more prevalent in the adult CsA patients (84%) than in the tacrolimus patients (47%) ($p<0.01$).

Twenty-five adults and one pediatric patient (21%) who were on tacrolimus had persistent hyperkalemia ($K^+>5.0$ mEq/l) that required treatment. This was easily controlled with a low dose of fludrocortisone (0.2 mg/day). No patient died of hyperkalemia in this trial.

The prevalence of new-onset insulin-dependent diabetes mellitus was the same in both tacrolimus (26%) and CsA (22%) adult patients. One of 50 pediatric patients on tacrolimus (both as a primary agent, and after conversion from CsA) developed new-onset insulin-dependent diabetes mellitus.

Two pediatric patients in the tacrolimus group developed post-transplant lymphoproliferative disease (PTLD) at 4 and 5 months after transplantation; one resolved with reduction in immunosuppression and the other died of disseminated PTLD. Another pedi-

atric patient, who was initially on CsA, died of disseminated PTLD after conversion to tacrolimus. Of the adult patients, one in the tacrolimus group died of brain lymphoma; four in the CsA group died of other malignancies, comprising osteosarcoma (one), recurrent cardiac rhabdomyosarcoma (one), lung carcinoma (one), and testicular embryonal cell carcinoma (one).

Other side-effects of tacrolimus, which have been reported elsewhere[47] and include extremity paresthesias, akinetic mutism, myalgia, and tremor, were infrequent and transient. Notably absent in the tacrolimus group were gingival hyperplasia, hirsutism, and coarsening of facial features. No patients in the tacrolimus group had severe and persistent adverse events that required discontinuation of the drug.

In summary, although survival after cardiac transplantation with tacrolimus immunosuppression is similar to that with CsA immunosuppression, tacrolimus is associated with a lower rate of rejection. In addition, use of tacrolimus permits both lower doses of steroids, and more success in weaning from steroids. The incidence of new-onset hypertension is less in tacrolimus-treated patients. Finally, intractable rejection is more common in CsA-treated patients and shows an excellent response to conversion to tacrolimus.

Tacrolimus as a 'rescue' agent

We have evaluated tacrolimus as a 'rescue' agent for refractory rejection in 26 patients after an average of 435 days under cyclosporin-based immunosuppression. Refractory rejection was defined as persistent rejection of grade 3A or higher after at least one course of pulse steroids and one course of lympholytic therapy. In all patients, CsA was discontinued 1–2 days prior to initiation of tacrolimus. In 24 patients, tacrolimus successfully reversed the rejection within 2 weeks. One patient required an additional course of pulse steroids, while another needed both pulse steroids and OKT3 (after conversion to tacrolimus to reverse the rejection).

In view of the heavy immunosuppression received by this subset of patients prior to tacrolimus therapy (many had received multiple courses of pulse steroids and lympholytic treatment), it is not surprising that four had major infections, while another four developed PTLD. We believe that tacrolimus has an important role as a rescue agent for cardiac transplant recipients experiencing intractable rejection under CsA-based regimens. However, conversion to tacrolimus should be accomplished prior to heavy doses of adjunct immunosuppressive therapy to minimize the risk of PTLD and major infection.

TACROLIMUS IN LUNG TRANSPLANTATION

Encouraged by the results in heart transplantation we initiated a randomized trial comparing tacrolimus- and CsA-based immunosuppression for lung transplantation[40,50]. Between October 1991 and May 1994, 133 single and bilateral lung transplant recipients were randomized to receive either tacrolimus (66 patients) or CsA (67 patients). At the time of graft revascularization all patients were given an intravenous loading dose of azathioprine (4 mg/kg) and a 500 mg bolus of methylprednisolone. Postoperatively, azathioprine was continued at 2 mg/kg per day, with adjustment made to keep the white blood cell count greater than 5000 cells/mm^3.

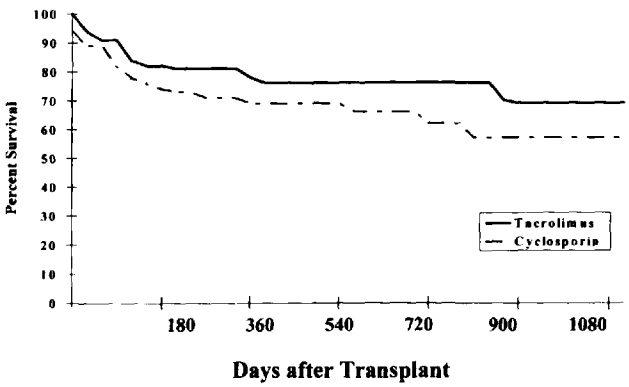

Figure 4 Actuarial survival following lung transplantation under tacrolimus and cyclosporin

Tacrolimus and CsA were administered intravenously at 6–8 hours after transplantation. Tacrolimus was administered at a dose of 0.025 mg/kg per day as a continuous infusion, and the dose was titrated to achieve whole blood levels of 10–20 ng/ml (serum levels of 0.5–2.0 ng/ml). Conversion to oral tacrolimus at a dose of 0.30 mg/kg per day in two divided doses was accomplished upon return of gastrointestinal function. Similarly, CsA was given initially at 2.5 mg/kg per day as a continuous intravenous infusion, and then by oral administration to maintain serum levels of 750–1000 ng/ml.

Overall survival in the groups was similar with respect to 1- and 2-year survival rates (83% and 76% for tacrolimus, and 71% and 66% for CsA) (Figure 4). The predominant causes of mortality in the perioperative period were ischemic lung injury followed by sepsis. Infection (primarily pneumonia) was the leading cause of death in the first year, while respiratory failure from obliterative bronchiolitis, with or without pneumonia, was responsible for six of nine late deaths.

The incidence of acute rejection (number of episodes per 100 patient-days) was lower in the tacrolimus group (0.85 $\pm$ 0.72) than in the CsA group (1.09 $\pm$ 0.72), although the difference did not reach statistical significance ($p = 0.07$).

Patients who survived 60 days or longer after transplantation, and therefore were considered at risk for developing obliterative bronchiolitis (OB), were analyzed separately. Of the 60 patients who received tacrolimus and were at risk, 13 developed OB (21.7%). In contrast, 19 patients (35.8%) treated with CsA developed OB ($p = 0.03$) (Figure 5). These intermediate-term results suggest that tacrolimus might reduce the incidence of OB.

Thirteen patients initially treated with CsA were switched to tacrolimus in the postoperative period. Indications for the conversion included persistent acute rejection in nine patients, with one case each of persistent chronic rejection (OB), acute nephrotoxicity, severe headaches with anorexia, and inability to obtain an adequate CsA level. In contrast, only two recipients initially treated with tacrolimus were converted to CsA – one for anorexia, and one for inability to maintain an adequate tacrolimus level. Six of the nine patients with persistent rejection resolved their rejection after conversion to tacrolimus.

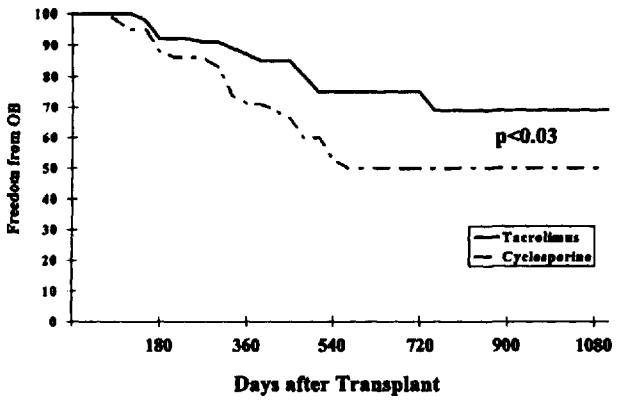

Figure 5 Freedom from obliterative bronchiolitis (OB) in lung transplant recipients treated with tacrolimus versus cyclosporin

At 1 year following transplantation the extent of renal impairment was similar in the two groups with serum creatinine levels averaging 1.95 ± 0.72 mg/ml for patients receiving tacrolimus and 1.68 ± 0.6 mg/ml for CsA recipients. The frequency of new-onset hypertension was similar in the two groups.

Tacrolimus was associated with a reduction in the incidence of obliterative bronchiolitis, a histologic manifestation of chronic rejection. We hope that with further follow-up this reduction in acute and chronic rejection will translate into improved survival.

COMMENT

Tacrolimus is proving an important immunosuppressant for heart and lung transplantation. While tacrolimus and CsA share a similar mechanism of action, clinical experience has demonstrated several distinct advantages of a tacrolimus-based immunosuppression protocol. These include a lower incidence of acute rejection in cardiac transplantation, and a trend toward less acute rejection and a lower incidence of obliterative bronchiolitis in lung recipients. Tacrolimus is highly successful in rescuing recipients of heart and lung transplants who have refractory rejection under CsA-based immunosuppresion. Notably absent in the tacrolimus group are the side-effects of hirsutism, gingival hyperplasia, and facial 'brutalization'. The lack of these cosmetic effects, as well as the reduced requirement for steroids, in the tacrolimus group have made tacrolimus a preferred drug for heart and lung transplant recipients at the University of Pittsburgh, particularly in the pediatric group.

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11
Infection in Patients Undergoing Thoracic Organ Transplantation: Epidemiology, Pathogenesis, and Clinical Management

S.J. THALER AND R.H. RUBIN

INTRODUCTION

Over the past several decades, remarkable progress has been made in the management of patients with advanced cardiac and lung disease – medications such as angiotensin-converting enzyme inhibitors for the management of heart failure, implantable defibrillators for the prevention of cardiac sudden death, such new antibiotics as the fluoroquinolones and imipenem in the treatment of pulmonary infection, and exercise rehabilitation programs have all increased in the quality of life for these patients. However, all of these measures represent disease palliation rather than curative therapy and, in recent years, it has become apparent that the best chance for rehabilitation for patients with advanced cardiac and pulmonary disease (as it is for patients with advanced liver and renal disease) occurs with transplantation[1].

In the United States at the present time the 1-year allograft and patient survival rates following cardiac transplantation are 81% and 82%, respectively; following single lung allografts, 67% patient and graft survival; and following heart–lung allografts 53%. Even more remarkable is that the 5-year survival rate for cardiac allografts now approaches 70%[2]. Reflecting these successes, there are now approximately 1500 cardiac transplants, 500 lung transplants, and 200 heart–lung transplants performed in the United States each year, with approximately 80% of the world's cardiac transplants performed since 1985 and 90% of the lung transplants since 1988[1,2]. Transplantation of the thoracic organs as the treatment for end-stage disease of the heart and lungs has evolved over the past decade from an interesting experiment in human immunobiology to the most practical means of rehabilitating a growing patient population.

Despite this progress, three major hurdles must be overcome in every patient if transplantation is to be successful: the availability of a suitable donor organ in a timely fashion; host immunologic attack of the allograft in acute and chronic rejection processes; and the occurrence of life-threatening infection.

The first two of these are covered in other sections of this monograph; it is the purpose of this chapter to delineate the epidemiology, pathogenesis, and clinical management of infection in patients undergoing thoracic organ transplantation. The importance of these issues is illustrated by the following statistics: more than two-thirds of cardiac allograft recipients have at least one infectious episode in the first year post-transplant, with an even greater number of infectious events occurring in lung allograft recipients[3]. It is also important to emphasize that infection and rejection are intimately linked by the requirement for immunosuppressive therapy. An important axiom of organ transplantation is that any intervention that decreases the risk of infection will permit the more aggressive treatment of rejection, thus salvaging more allografts; conversely, any intervention that decreases the risk of rejection will allow the use of lesser amounts of immunosuppressive therapy, thus decreasing the risk of infection, and resulting in the salvage of more lives. *The therapeutic prescription, then, for the transplant recipient has two components: an antirejection program and an antimicrobial program to make it safe*[1].

RISK OF INFECTION IN THE ORGAN TRANSPLANT RECIPIENT

The risk of infection in the organ transplant recipient is largely determined by the interaction between the nature of the *epidemiologic exposures* the patient encounters and the patient's *net state of immunosuppression*. Table 1 delineates the epidemiologic

Table 1 Epidemiologic exposures of particular importance in the thoracic organ transplant recipient

A.	*Within the community*
	1. The geographically restricted systemic mycoses (histoplasmosis, coccidioidomycosis, and blastomycosis)
	2. Mycobacterial infection
	3. *Strongyloides stercoralis*
	4. Respiratory viruses (influenza, parainfluenza, respiratory syncytial virus, etc.)
B.	*Within the hospital*
	1. *Aspergillus* species
	2. *Legionella* species
	3. *Pseudomonas aeruginosa* and other Gram-negative bacilli

exposures of importance. These can be divided into two general categories: those occurring in the community and those that occur within the hospital environment. Within the community the issues are reactivation of such long-dormant processes as tuberculosis, one of the geographically restricted systemic mycoses (blastomycosis, coccidioidomycosis, and histoplasmosis), and strongyloidiasis; and acute, rapidly progressive infection with one of these or, more commonly, such acute respiratory viruses as influenza, respiratory syncytial virus, and others that may be circulating in the community. The general pattern for all these infections is systemic dissemination and/or a high rate of bacterial superinfection. For the clinician it is clear that a careful epidemiologic history of both recent and remote exposures is important in evaluating in infectious disease episode[1].

Within the hospital environment, such infections as invasive aspergillosis, legionellosis, and Gram-negative infection can be even more important problems, with an opportunity for nosocomial epidemics being constantly present. Two general epidemiologic patterns are observed with these nosocomial outbreaks: *domiciliary* spread, in which exposure occurs on the ward where the patient is housed, due to contaminated air or potable water, with identification of the outbreak being relatively easy because of clustering of cases in time and space; and *non-domiciliary* spread, in which exposure occurs as the patient travels to the radiology suite, the catheterization laboratory, or operating room for an essential procedure. This latter form of epidemic is far more difficult to identify because of the lack of clustering, but is far more common than the domiciliary outbreak. The leading clue to the presence of such a problem is the occurrence of an opportunistic infection at a point in time when the net state of immunosuppression would not normally allow it to occur (see below)[1].

The net state of immunosuppression is a complex and evolving state which is composed of a number of factors (Table 2). Of particular importance are the direct and indirect effects of the immunosuppressive regimen. It is clear that the prime determinant of infection risk is the dose, duration, and temporal sequence in which immunosuppressive drugs are deployed. These not only inhibit multiple limbs of the host defense system (particularly the microbial-specific, MHC-specific T-cell response which is so important in the control of herpes group viruses, fungi, mycobacteria, and other organisms), but also cause the reactivation and amplification of a group of immunomodulating viruses. It is now apparent that such viruses as the herpes group viruses, hepatitis B and C viruses, and the human immunodeficiency virus contribute significantly to the net state of immunosuppression. It is also apparent that a key determinant of the effects of these viruses is the nature of the immunosuppressive program being administered. The most critical of these interactions is the effect of immunosuppressive therapy on cytomegalovirus (CMV), the single most important infection in transplantation. The antilymphocyte antibodies, both the polyclonal antithymocyte globulins and the monoclonal OKT3, have potent effects in reactivating latent virus (in contrast to such drugs as cyclosporin, FK506, rapamycin, and corticosteroids). Once active, replicating virus is present, then these latter drugs have a marked amplifying effect on the virus due to their potent suppressive effects on the key cytotoxic T-cell response[1,4,5].

Table 2 Factors contributing to the net state of immunosuppression in the thoracic organ transplant recipient

1. Dose, duration, and temporal sequence of immunosuppressive therapy
2. Presence of another immunosuppressing condition
3. Neutropenia
4. Presence of indwelling foreign bodies or other factors that compromise the first line of defense against infection: the mucocutaneous surfaces of the body
5. Protein–calorie malnutrition
6. Such metabolic factors as uremia and hyperglycemia
7. Infection with one or more of the immunomodulating viruses (CMV, EBV, HBV, HCV, and HIV)

In addition to these complex interactions it is important for the clinician to recognize that other, more controllable factors contribute to the risk of infections. The vascular access devices, drainage tubes, catheters and other equipment such as pacing wires and stents that are essential for management in the early post-transplant period are important risk factors for infection. Extended intubation greatly increases the risk of pneumonia and sinusitis, and any extended period in the intensive-care unit markedly increases the risk of antimicrobial-resistant infection. Patients with difficulty in the first 2 weeks post-transplant also receive more than their share of antibiotics, which significantly increases their subsequent risk of antimicrobial-resistant infection, antibiotic-associated diarrhea, and loss of appetite. Malnutrition is extremely common in these individuals, with one measure of this, a low serum albumin, being linked to increased in-hospital mortality – usually due to infection[1].

TIMETABLE OF INFECTION IN THE ORGAN TRANSPLANT RECIPIENT

As immunosuppressive programs have become standardized, it has become apparent that there is a temporal pattern of the times at which different infections occur in the post-transplant period; that is, although such infectious disease syndromes as pneumonia can occur at any time point post-transplant, the microbial etiologies are very different at different time points. In the organ transplant patient the 'timetable for infection' can be divided into three periods: the first month post-transplant, the period 1–6 months post-transplant, and the late period, more than 6 months post-transplant (Figure 1)[1].

In the first month post-transplant there are three categories of infection that become clinically manifest[1]: infection that was present in the allograft recipient prior to transplant; infection conveyed with the allograft; and infection related to technical issues involved with the operative and perioperative management. For heart, lung, and heart–lung transplant recipients, many of whom are critically ill prior to the transplant, the most common pretransplant infections that will have a post-transplant impact are pneumonia and vascular access bacteremia, both of which will be exacerbated in the peritransplant period. Indeed, all active infection of the lungs and blood stream should be eradicated prior to transplant. As far as a contaminated allograft is concerned, infection, particularly with *Staphylococcus aureus*, Gram-negative bacilli, and *Candida* species, carries a high probability of seeding of the vascular anastomotic site, with the subsequent development of a mycotic aneurysm and catastrophic disruption of the anastomosis[6–11]. Contamination of the allograft, resulting in this disas-

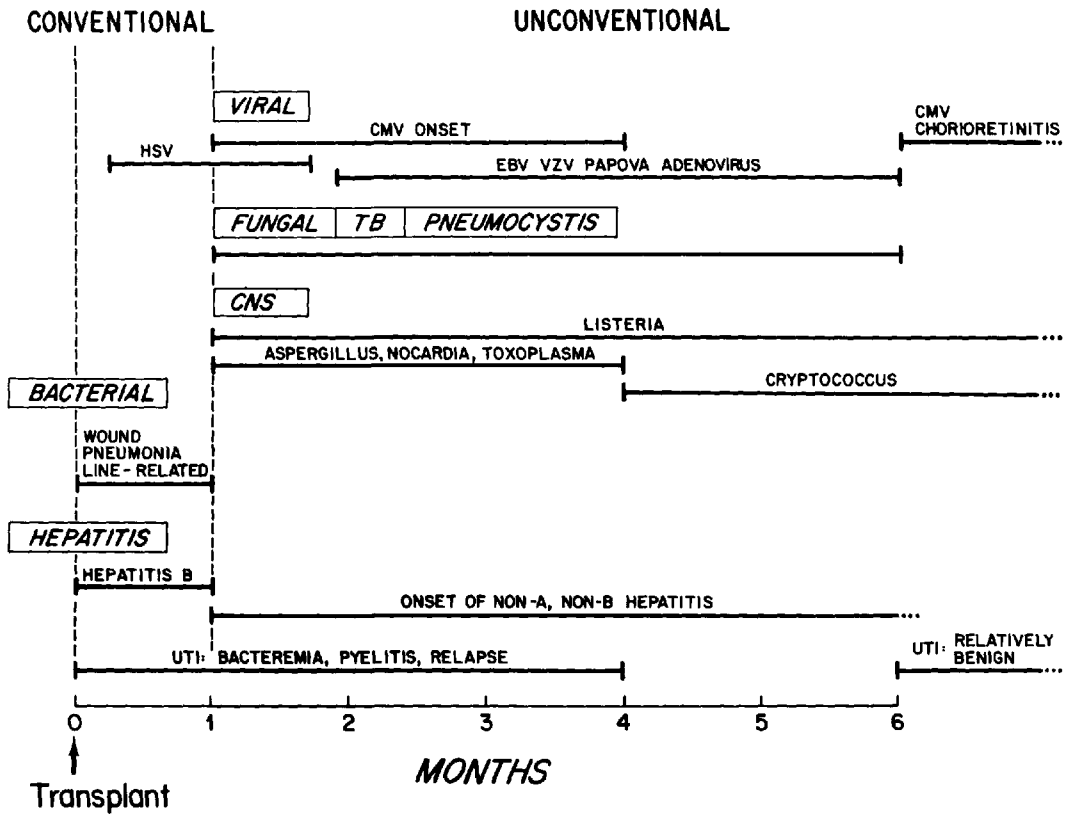

Figure 1 Timetable for the expected occurrence of different infections following thoracic organ transplantation (Modified from ref. 1)

trous sequence of events, may occur as a result of bacteremia or candidemia in the donor, contamination from respiratory flora during the harvesting procedure, or the handling of the organ prior to implantation.

More than 95% of the infections occurring in the first month post-transplant are the same bacterial and candidal infections of the surgical wound, lungs, urinary tract, and vascular access sites that occur in non-transplant patients undergoing comparable surgery, although the impact is greater in the transplant patient[12–15]. The key determinant in the development of these infections is the technical skill with which the operation is performed, the endotracheal tube managed, and the various vascular and drainage catheters assessed. A technical error in these patients is a virtual guarantee of potentially life-threatening infection. Prophylactic antibiotics at the time of the surgery can decrease the incidence of wound infection, but are of less importance than the technical outcome of the surgical operation.

Notable by their absence during this first month 'golden period' are opportunistic infections due to such organisms as *Pneumocystis carinii*, *Aspergillus* species, *Listeria monocytogenes*, etc. The net state of immunosuppression during this first month is normally not great enough for these to occur, *unless an unusually intense epidemiologic exposure has occurred.* There are two important implications of this observation. First, since the daily doses of immunosuppressive drugs are at their highest in this first month post-transplant, it is apparent that the duration of immunosuppression (the 'area under the curve'), the intensity over time, is a more important determinant of the net state of immunosuppression than is the daily dose administered. Second, the occurrence of a single case of opportunistic infection during this time period should be regarded as evidence of an excessive nosocomial hazard, and is usually an early indicator of a potential nosocomial epidemic[1].

During the period 1–6 months post-transplant the infections that occur may be divided into two categories, the second being in part dependent upon the first. By far the most important infections during this time period are those caused by the immunomodulating viruses: cytomegalovirus (CMV), Epstein–Barr virus (EBV), the two hepatitis viruses, B and C (HBV and HCV), and the human immunodeficiency virus (HIV). These not only cause infectious disease syndromes directly during this time period (e.g. CMV is the most common cause of fever in this time period), but contribute significantly to the net state of immunosuppression[16–18]. The combination of sustained immunosuppressive therapy and immunomodulating viral infection now makes it possible for opportunistic infection, particularly due to *Pneumocystis carinii*, *Listeria monocytogenes*, and *Aspergillus* species, to occur in this time period[1].

In the late period, more than 6 months post-transplant, transplant recipients can be divided into three groups in terms of their infectious disease risks: the majority (>75% overall) will have intact graft function without the continuing need for high-dose immunosuppressive therapy. These patients are at minimal risk for opportunistic infection; rather their problems resemble those

of the general community – influenza and other community-acquired respiratory virus infections, pneumococcal pneumonia, and urinary tract infection. In a small fraction of patients (approximately 10%), chronic, active viral infection persists, resulting in the inexorable development of end-organ failure (e.g. the liver from the hepatitis viruses or the retina from CMV), malignancy (e.g. hepatocellular carcinoma as a result of chronic hepatitis or squamous-cell carcinoma as a result of chronic papillomavirus infection, or the acquired immunodeficiency syndrome (AIDS) from HIV. Finally, approximately 15% of patients will have suffered recurrent bouts of acute and chronic rejection, requiring significantly greater amounts of acute and chronic antirejection therapy. Many of these patients will also have chronic immunomodulating virus infection. The end-result is the group of patients with the greatest net state of immunosuppression and the highest risk of opportunistic infection with such organisms as *Pneumocystis carinii*, *Cryptococcus neoformans*, and *Listeria monocytogenes*. Because of this risk, these 'chronic n'er-do-wells' are candidates for preventive therapy with low-dose trimethoprim-sulfamethoxazole and fluconazole on an ongoing basis[1].

Early bacterial or fungal infections following thoracic transplantation

The most common form of early infection following heart transplantation is bacterial pneumonia. Thus, in a recent review of 814 patients from 24 centers, bacterial invasion accounted for 47% of all infections, with pneumonia being the most common form of bacterial infection encountered[14]. Vascular access-related bacteremia, urinary tract infection, and gastrointestinal tract infection (particularly diverticulitis) are also not uncommon. The microbial species responsible for such infections are typical of those causing infection in seriously ill surgical patients: *Pseudomonas aeruginosa*, *Klebsiella* species, *Staphylococcus aureus*, and *Candida* species. Interestingly, wound infections following heart transplantation are relatively uncommon, a testimony to good surgical technique and the efficacy of perioperative antibiotic prophylaxis.

Lung and heart–lung recipients may be considered together, as the infectious disease problems of the latter more closely resemble those of the lung than the cardiac recipient. Once again, bacterial pneumonia is the most common serious early infection, occurring in 35–45% of patients, with Gram-negative bacilli and *S. aureus* being the most important pathogens[19–22]. A number of physiologic reasons explain this high rate of pneumonia: diminished cough reflex, prolonged intubation, abnormal mucociliary clearance, the abnormalities inherent in the bronchial anastomosis (which is particularly susceptible to ischemic injury), abnormal lymphatic drainage, prior colonization with organisms of either donor or recipient origin, as well as the most obvious reason – the lung is the only transplanted organ *in direct communication with the outside world*. Of interest, in a canine animal system, contamination of the donor trachea predicted pneumonia in the recipient[8,23]. The infecting organism did not always correlate with the donor flora; rather, the presence of bacteria was probably an indicator of impaired microbial clearance, setting the stage for overgrowth and pneumonia in the recipient.

A particular problem in the lung transplant patient is pretransplant colonization of the tracheobronchial tree with either *Aspergillus* species or *Pseudomonas cepacia* (both particular issues in patients with cystic fibrosis). In the case of *Aspergillus* colonization, unless eliminated, there is a >50% risk of either invasive pulmonary aspergillosis or a localized necrotizing infection at the bronchial suture line post-transplant[24,25]. Pretransplant and/or post-transplant colonization of the tracheobronchial tree with *P. cepacia* has been associated with a significantly worse prognosis for the successful rehabilitation and/or survival of the lung or heart–lung transplant recipient[26,27].

Mediastinitis and/or deep infection of the chest wound occurs more commonly in lung transplant recipients than in cardiac transplant recipients (13.5% vs 8.0%), reflecting the increased technical challenges associated with lung transplantation[19,28,29]. Although such unusual organisms as *Mycoplasma hominis* and *Legionella* species have been reported in these patients, the great majority of such infections are due to Gram-negative bacilli, *S. aureus*, and *Candida* species[30,31].

Infection due to herpesviruses following thoracic transplantation

All herpesviruses (which include CMV, EBV, herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV) and human herpesvirus-6 (HHV-6) exhibit three characteristics that make them particularly effective pathogens in the transplant recipient: *latency*, *cell association*, and *oncogenicity*[32]. The term 'latency' connotes dormant infection of cells capable of being reactivated; that is, herpesviruses incorporate their genomic DNA into the host chromosomes, with no active viral RNA transcripts produced during the latent state. Once activation occurs, viral RNA is again produced, with viral replication now proceeding. The laboratory marker for the presence of latent herpesvirus infection is the presence of circulating antibody to the particular virus (seropositivity).

In the case of CMV, VZV, and HHV-6, latency is quite stable, requiring particular stimuli (antilymphocyte antibody therapy, an allogeneic reaction, and pregnancy being common stimuli causing reactivation, especially in the case of CMV); in the case of EBV and HSV, although the same stimuli will reactivate virus, spontaneous reactivation or other relatively minor stimuli (e.g. stress, menses, ultraviolet light, etc. – especially in the case of HSV) can result in active viral replication[33,34]. Thus, approximately 25% of transplant patients at any one time have active replicating EBV, with this figure rising to >80% in the face of antilymphocyte antibody therapy of rejection[35]. Whatever the cause of herpesvirus reactivation, cyclosporin, FK506, and high-dose steroid therapy will amplify the level of virus replication due primarily to the interference with the key host defense – a virus-specific, MHC-specific, cytotoxic T-cell response. This is the key host defense against these viruses (although non-specific NK cell activity may also offer some protection), as the highly cell-associated nature of these viruses renders humoral immunity inefficient, and this limb of host defense of prime importance. Finally, all herpesviruses should be considered potentially oncogenic, although clear-cut evidence of this effect has been shown only in the case of EBV and its role in the pathogenesis of B cell lymphoma[36,37].

The impact of CMV on thoracic transplantation

CMV is by far the most important of the herpesviruses in terms of its impact on the outcome of transplantation[38–44]. Not only does it directly cause a variety of infectious disease syndromes (e.g. mononucleosis, pneumonitis, hepatitis, gastrointestinal tract inflammation and ulceration, myocarditis, chorioretinitis, etc.) in the transplant patient, it also has significant indirect effects on the transplant patient. These indirect effects, probably caused by cytokines elaborated in the course of CMV infection, include the following: an increase in the global state of immunosuppression, thus predisposing to opportunistic superinfection; a possible role in the pathogenesis of EBV-associated lymphoproliferative disease; and a probable role in the pathogenesis of both acute allograft injury and such manifestations of chronic allograft injury as accelerated coronary artery atherosclerosis and bronchiolitis obliterans[1].

CMV infection occurs predominantly 1–4 months post-transplant. Although evidence of viral replication (isolation of virus from urine or respiratory secretions, or rise in antibody titer) can be found in >50% of heart, lung, and heart–lung transplant recipients, the best laboratory marker for the presence of *clinically significant disease* is the demonstration of viremia[45–47]. Viremia, as defined by cultural techniques, by an antigenemia assay, or by polymerase chain reaction (PCR), is usually demonstrable 3–8 days prior to the onset of clinical disease, with >60% of these individuals developing symptomatic infection.

There are three general patterns of CMV infection, each having a different potential for causing symptomatic CMV disease[1]:

(1) *Primary* CMV infection, which occurs when a seronegative recipient becomes infected with CMV following the transfer of latently infected cells from a seropositive donor (a D+ R– transplant). More than 90% of the time the source of these latently infected cells is the allograft; however, viable leukocyte-containing blood products from a seropositive donor can also be a source of primary infection. More than 60% of individuals at risk for primary infection will develop symptomatic disease, with lung transplant patients apparently having an even higher attack rate.

(2) *Reactivation* CMV infection, which occurs when a seropositive recipient reactivates his or her endogenous virus from latency (an R+ transplant, with the donor status being irrelevant). Interestingly, although >50% of these patients will reactivate virus, only a minority (an estimated 20%) become ill, unless antilymphocyte antibody therapy is used to treat rejection, in which case the incidence of symptomatic disease rises to >60%.

(3) CMV *superinfection* occurs when an allograft from a seropositive donor is transplanted into a seropositive recipient (a D+ R+ transplant), and the virus that is reactivated is of donor origin (this occurs in approximately 50% of such transplants). It has been suggested that the attack rate for clinical disease is significantly greater for those patients with superinfection with the donor virus as opposed to reactivation with their endogenous viral strain. The known genetic and antigenic heterogeneity of CMV strains in nature makes this a plausible hypothesis, although definitive proof remains to be gathered.

The direct clinical effects of CMV infection are similar in all forms of organ transplantation: fever, often with accompanying leukopenia and thrombocytopenia, pneumonia (the most common of the life-threatening manifestations of CMV infection), hepatitis, and gastrointestinal inflammation and ulceration[48–52]. The major difference among the different forms of organ transplantation is that the organ transplanted is more severely affected than a native organ. Thus, pneumonia is a far more common and severe problem in recipients of lung and heart–lung transplants than in the other forms of transplantation, and myocarditis due to CMV is recognized almost exclusively in heart transplant patients[53,54]. Thus, Ettinger *et al.* found that among 52 lung recipients, the attack rate for pneumonia was 80% for those at risk for primary infection (D+ R–), and 75% among all recipients[43].

The incidence and severity of CMV disease are strongly influenced by two factors: the nature of the immunosuppressive therapy administered, and the past experience of donor and recipient with the virus (as denoted by the presence or absence of seropositivity). Two population groups have been shown to have >60% incidence of symptomatic disease due to CMV infection: D+ R– transplant recipients (primary infection); seropositive (R+) patients treated with antilymphocyte antibody therapy (either the polyclonal antithymocyte globulin or the monoclonal OKT3). Seropositive patients treated with induction antilymphocyte antibody therapy have an increase in CMV disease from approximately 10% to 24%; those treated with an antilymphocyte antibody for rejection have an increase from approximately 20% to >60%[5,55]. Recent evidence suggests that the release of tumor necrosis factor (TNF) is the key factor in these events: TNF acts as a promoter for the immediate early antigen of CMV, activating the virus from latency[56–58]. The increased amount of TNF elaboration in association with antirejection therapy, as opposed to induction therapy with antilymphocyte antibodies, is presumably responsible for the observed difference in CMV disease.

This proposed mechanism also explains two other observations: the occurrence of CMV disease following allograft rejection regardless of the immunosuppressive therapy employed; and the occurrence of CMV following bouts of urosepsis or bacterial pneumonia. In all of these instances TNF elaboration occurs, and CMV is reactivated; whether or not symptomatic CMV occurs, then, depends on the host's ability to control the replicating virus. This, in turn, is determined by the host's responsiveness to the virus, which is a function of the intensity of the immunosuppression being administered (particularly the amount of cyclosporin and FK506 being administered) and the past experience with the virus.

The potency of cyclosporin and FK506 in inhibiting the critical host defense against CMV, the virus-specific cytotoxic T-cell response, is further underlined by another clinical phenomenon: in the pre-cyclosporin era, relapsing CMV disease was essentially unheard of; at present, clinical relapse (defined as the occurrence of symptomatic disease within 6 weeks of completion of a 2–3-week course of intravenous ganciclovir for symptomatic disease which eradicated clinical evidence of CMV infection) occurs in approximately 20% of patients with symptomatic disease[38]. This figure, however, is somewhat deceiving in that the incidence of relapse is only about 10% in the R+ patients, whereas it is >60% for the D+ R– patients, again emphasizing the importance of the host's past experience with the virus.

The most important indirect effects of CMV infection are its contribution to the net state of immunosuppression and its possible role in the pathogenesis of allograft injury. As far as the first of these is concerned, both in the murine model and in humans there is abundant evidence that CMV adds significantly to the net state of immunosuppression, predisposing particularly to those opportunistic infections that are susceptible to cell-mediated immunity: aspergillosis, pneumocystosis, listeriosis, and disseminated candidiasis being notable examples[59–62]. CMV-induced leukopenia is an important marker for profound virus-induced immunosuppression.

Perhaps the most fascinating potential role of CMV is as a causal factor in the chronic vasculopathy of cardiac allograft atherosclerosis and bronchiolitis obliterans in the lung allograft[63,68]. In 1989 the Stanford group published compelling data on 301 cardiac transplant patients, 210 of whom were free of CMV infection[63]. The incidence of acute rejection, graft atherosclerosis, and patient death from atherosclerosis (as well as opportunistic fungal infection) were all greater in the CMV group. Since then a number of investigators have published series suggesting that acute and chronic rejection are more likely during chronic stimulation by CMV infection. The same has been suggested of bronchiolitis obliterans following lung transplantation[69]. Other groups have not found such associations[70]. If CMV is playing a role here, it is unlikely that the damage to the allografts is solely due to either direct viral injury or immunologic cross-reactivity (due to homologies between CMV antigens and certain MHC antigens). Rather, it is our belief that there is a link between allograft injury and CMV, that being the elaboration of cytokines in the course of the infection. These cytokines then modulate the display of MHC antigens on the allograft, as well as the host's response to these antigens[71–73]. Thus, CMV's effects are not the only way to cause those events that lead to serious graft injury; but rather, given the ubiquity of this virus in this patient population, CMV is an important way to enter this final common pathway.

Given the importance of CMV infection, a great deal of effort has been invested in the treatment and prevention of CMV disease[74–82]. Intravenous ganciclovir, at a dose of 5 mg/kg twice daily (with dosage corrections for renal dysfunction) for 2–3 weeks, is the standard of care for treating CMV, with many groups adding anti-CMV immunoglobulin to the regimen in patients seriously ill (as with pneumonia), those with primary infection, and those with relapsing disease. Foscarnet has not been an important therapy in transplant patients, for two reasons: ganciclovir-resistant infection in transplant patients, as opposed to AIDS patients, is essentially unknown; and foscarnet's toxicities for the transplant patient population appear to be significantly greater.

Table 3 delineates the various prophylactic programs that have been tried in patients to prevent CMV disease following thoracic transplantation and their efficacy. At present, although it is clear that the optimal program remains to be defined, the following conclusions appear to be warranted.

(1) One month of intravenous ganciclovir is effective prophylaxis against CMV disease for R+ patients.
(2) The addition of ganciclovir at a dose of 5 mg/kg per day intravenously to regimens that include antilymphocyte antibody therapy provides important added protection to any preventative program (so-called pre-emptive therapy).
(3) The relative values of combined immunoglobulin + antiviral regimens, sequential programs (intravenous ganciclovir followed by oral therapy with either oral ganciclovir or high-dose acyclovir) remain to be established.
(4) New laboratory approaches to diagnosing preclinical viremia (antigenemia assay or PCR) may permit the use of pre-emptive therapy on the basis of presymptomatic diagnosis of systemic infection.

The impact of Epstein–Barr virus on thoracic transplantation

Essentially all adults have experienced infection with the Epstein–Barr virus, with this occurring in developed countries predominantly in adolescence and young adulthood. EBV infects two cell types: primary infection of the epithelial cells of the upper respiratory tract, particularly the oropharynx and parotid duct, followed by secondary B lymphocytes traveling through the oropharyngeal lymphoid tissue[33]. Infection of epithelial cells is lytic, whereas in B cells infection results in transformation and immortalization. Control of this infection, including the immortalized B cells, is primarily via the host's cytotoxic T-cell response. The clinical consequence of these events in the immunocompetent individual is infectious mononucleosis, an illness characterized by fever, cervical adenopathy, pharyngitis, hepatosplenomegaly, and both an absolute and atypical lymphocytosis.

In the transplant patient there is little impediment to ongoing B cell transformation and the proliferation of transformed clones, because of the marked inhibition of the virus-specific cytotoxic T cell response[35]. In EBV seropositive individuals, reactivation of viral replication in the pharynx is quite common, occurring in 20–30% of those being treated with baseline immunosuppression, with this percentage rising to >80% with antilymphocyte antibody therapy. Although usually asymptomatic, EBV may cause a mononucleosis-like syndrome in the seropositive patient that is

Table 3 Current prophylactic programs against herpesviruses for thoracic organ transplant recipients*

Donor serologic status	*Recipient serologic status*	*Current recommendations*
Positive	Negative	4–6 weeks of intravenous ganciclovir, followed by 3 months of oral ganciclovir, ± hyperimmune anti-CMV globulin
Positive or negative	Positive	Intravenous ganciclovir for 2–4 weeks followed by high-dose oral acyclovir for 3–4 months
Negative	Negative	Intravenous acyclovir during anti-lymphocyte antibody therapy (against Epstein–Barr virus)

* Based on currently available information these are the programs we currently utilize. As new data appear, revisions will be made. In addition, intravenous ganciclovir is administered during antilymphocyte antibody therapy (unless donor and recipient are both seronegative for cytomegalovirus, in which case intravenous acyclovir is utilized)

indistinguishable from that caused by CMV. Far more important is the occurrence of EBV-associated post-transplantation lymphoproliferative disease (PTLD), which has been particularly common in lung and heart–lung transplant recipients (an incidence of 5–10%, as compared with an incidence of 1–2% in kidney transplant recipients)[83]. Factors particularly associated with the occurrence of PTLD include primary EBV infection (which primarily occurs in children), intensive immunosuppressive regimens that combine antilymphocyte antibody therapy with cyclosporin or FK506, and preceding symptomatic CMV disease[84–87]. This last may be due to the effects of cytokines elaborated in the course of the CMV infection, or merely due to similar effects of immunosuppressive therapy on these closely related herpesviruses.

Pathologically and clinically, PTLD should be regarded as encompassing a continuum from polyclonal lymphoid hyperplasia to a monoclonal, frankly malignant picture[88]. Symptomatically there is likewise a broad spectrum of disease, with fever, gastrointestinal bleeding, hepatitis, infiltration of the allograft, tonsillitis, bone marrow invasion, and invasion of the central nervous system singly or in combination all being possible. Unlike the situation with lymphoma in non-immunosuppressed patients, PTLD is not infrequently extranodal in presentation.

With evidence that the level of replicating virus present in the pharynx is predictive of the risk of PTLD, and the fact that acyclovir and ganciclovir can turn off EBV replication, it is hoped that the same kind of antiviral programs being developed for CMV prevention may be useful in the prevention of PTLD. Once PTLD develops, it is not yet clear what is optimal management. The role of the antivirals is biologically limited to controlling lytic infection in the pharynx, and has no effect on the episomal form of the virus present in the infected B cells. Thus, antiviral therapy is of only limited efficacy[89–91]. All patients require a significant decrease in immunosuppression, particularly in the use of cyclosporin, FK506, and antilymphocyte antibodies, with an estimated 20% responding to this course of action alone[92]. In patients with localized gastrointestinal tract disease, resection and radiation have been effective. For more widespread disease, survival is poor, although conventional antilymphoma chemotherapy is advocated. Because of the limited success of such programs, experimental therapies with T-cell infusions, anti-B cell antibodies, and α-interferon are currently also being evaluated[93].

The impact of herpes simplex virus on thoracic transplantation

Infections with both HSV-1 and HSV-2 are common in the general population, and approximately 75% of transplant recipients will excrete the virus post-transplant as a result of immunosuppression-induced reactivation[94,95]. About two-thirds of these will have symptomatic mucocutaneous lesions if no antiviral therapy is administered. In addition, a rare patient has acquired primary infection at the time of transplant, resulting in rapid systemic dissemination, multiorgan failure, and death unless early diagnosis and therapy are effected[96–98].

The most common clinical manifestation of HSV infection in transplant recipients is an aggressive form of herpes labialis, with large, ulcerating, hemorrhagic lesions around the mouth, anus, and/or genitalia. This disease process can extend into the tracheobronchial tree or esophagus, most commonly in the presence of an endotracheal tube or a nasogastric tube.

Patients undergoing heart–lung or lung transplantation appear to be at particular risk for pneumonitis due to HSV. In one recent series of 51 heart–lung recipients, nine HSV-seropositive recipients developed severe mucocutaneous disease and another six developed pneumonitis, five of whom died[95]. The high rate of viral excretion, local disease and the risk of pneumonitis all mandate the use of antiviral prophylaxis in lung and heart–lung patients. Fortunately, either acyclovir or ganciclovir is equally effective in this regard, and HSV prevention should be regarded as part of the general antiviral program aimed also at CMV and EBV. In cardiac allograft recipients, because the incidence of severe disease is more unusual, early intervention with acyclovir is an alternative to prophylaxis.

The impact of varicella-zoster virus on thoracic transplantation

Infection with VZV in the setting of transplantation is quite different in the pediatric and adult transplant populations. By the age of 15, >90% of United States residents are seropositive to VZV and no longer at risk for primary disease[99]. Approximately 10% of these individuals will develop dermatomal zoster, with essentially no risk for visceral spread. In contrast, seronegative children and adults with primary infection are at significant risk for disseminated visceral disease, which can include pneumonia, hepatitis, encephalitis, disseminated intravascular coagulation, and other complications[100,101]. Early therapy with high-dose intravenous acyclovir (e.g. 10 mg/kg every 8 h, with dosage adjustment for renal dysfunction) can be life-saving, but prevention is far to be preferred[102]. Zoster immune globulin prophylaxis offers only partial protection to seronegative transplant patients exposed to the virus. For this reason it is recommended that all transplant candidates be serologically screened for VZV, with seronegative individuals being administered the newly licensed varicella vaccine[103].

The impact of human herpesvirus-6 on thoracic transplantation

Both HHV-6 and HHV-7 can be considered 'orphan viruses' in transplantation. Whereas evidence of HHV-6 primary and reactivation infection has been well demonstrated in organ transplant recipients, its clinical impact is unclear[104–107]. HHV-7 has been isolated from the CD4-positive lymphocytes of asymptomatic immunocompetent individuals but, as yet, not from transplant patients[108]. If one were to postulate a role for these agents in the transplant patient, by analogy one would look for the direct production of a mononucleosis-like syndrome and indirect effects similar to those produced by CMV. Clearly, this is an emerging area of transplant infectious disease.

The impact of hepatitis viruses on thoracic transplantation

The prevalence of chronic liver disease in organ transplant recipients is approximately 10%[109]. Although a variety of drugs com-

monly employed in transplant patients (from azathioprine and cyclosporin to antihypertensive and antimicrobial agents) are potentially hepatotoxic, and the herpesviruses CMV and EBV can cause *acute* hepatocellular disease, hepatitis B and C viruses account for >95% of the chronic liver disease in the transplant population. Each of these viruses presents three major management issues: the appropriate approach to a seropositive donor; the suitability of a seropositive patient with end-stage cardiac and/or lung disease for transplantation; and the management of patients with active infection post-transplant.

The efficiency of transmission of HBV with organ transplantation from an infected donor approaches 100%, with a relatively high rate of acute hepatic failure associated with the acquisition of HBV in the peritransplant period[110–113]. Fortunately, with modern screening of blood products, the risk of transmitting HBV with blood transfusion is estimated to be 0.002% per unit tested; since the same techniques are used to screen organ donors, the risk of a seronegative donor transmitting HBV to the recipient should be comparable. Although it is clear that HBsAg-positive donors are to be avoided, it is also important to recognize that HBsAg-negative but HBcAb-positive donors can, on occasion, transmit the virus[113]. Because of this risk, we would only consider using such donors for patients awaiting an organ who are at immediate risk of death without a transplant.

Management of the HBsAg-positive transplant candidate remains controversial[114]. Post-transplant immunosuppression upregulates the level of viral replication and accelerates the rate of progression of the resulting liver disease. Beginning approximately 2 years post-transplant there is an increasing incidence of clinically important chronic liver disease and/or hepatocellular carcinoma. Thus, as reported by Rao *et al.*, in a population of HBsAg-positive renal transplant patients the prevalence of cirrhosis was 42% and the rate of death from liver disease was 54% over a time period of approximately 8 years[115]. Thus, many groups regard HBsAg-positivity as strong relative contraindication to transplantation[115–119].

Hepatitis C is responsible for >80% of the chronic liver disease that occurs in transplant recipients[109]. It is now recognized that there are six genotypes of the virus, with specific geographic clustering: subtype 1a is most prevalent in North America, whereas 1b occurs in North America and western Europe, type 5 in South Africa and type 6 in Hong Kong[120]. Recent studies suggest that these different genotypes have differing levels of viremia, clinical courses, and responses to interferon therapy[121]. The host response to HCV infection is at present incompletely understood: antibody to HCV does not appear for many weeks after the initiation of infection in normal hosts; this delayed response is probably even greater in the immunosuppressed transplant patient. Not only is this important in terms of diagnosis, anti-HCV testing being the most accessible means of assessing infection with the virus, previous infection does not protect against rechallenge against either the same strain or a different strain of the virus. The consequences of HCV infection are starting to become apparent: by 10 years post-transplant approximately 20% of transplant recipients with chronic HCV infection (and virtually all transplant patients with HCV infection will have chronic infection) will have serious consequences of HCV infection[122–124]. Therapy with α-interferon is far less effective in this patient population than in the general population, an approximately 25% initial response rate and a very high rate of relapse[125,126].

HCV infection by itself is not considered a strong contraindication to transplantation at most transplant centers, although careful assessment of the liver is required before proceeding, as post-transplant immunosuppression will clearly amplify the level of replicating virus[127]. Far more controversial is the appropriate approach to donors who might be harboring the virus. It is estimated that 1–5% of potential donors are anti-HCV-positive, with approximately half of these transmitting the virus to the allograft recipient. If PCR assay is performed for HCV RNA in the serum of the potential donor, it is apparent that essentially all viremic individuals transmit the virus with organ donation[128,129]. Thus, the anti-HCV assay has a high false-positive rate in terms of predicting transmission of HCV, as well as a high false-negative rate. Since the PCR assay cannot be performed routinely in the timely fashion needed to assist in evaluating a potential thoracic organ donor, it is unclear how best to optimize the number of organs available, while at the same time decreasing the transmission of HCV. Our own approach is to reserve organs from anti-HCV-positive donors for critically ill patients or for older recipients, feeling that the several years necessary for HCV to become clinically apparent post-transplant offers a trade-off that is acceptable for these two population groups.

The impact of toxoplasmosis on thoracic transplantation

Toxoplasma gondii causes a widespread zoonosis in which infection occurs following ingestion of undercooked lamb or pork contaminated with tissue cyst, vegetables contaminated with infected animal feces (particularly cats), or inhalation of infected cat feces[130]. Following inhalation or ingestion, the tachyzoites or invasive form of the parasite may infect a variety of cell types, with lymphadenopathy, a mononucleosis syndrome, and ocular disease being the most common manifestations in the immunologically normal individual. Although occasional cases of toxoplasmosis, including disseminated disease with a major effect on the central nervous system (both focal lesions and diffuse encephalitis akin to that seen in the AIDS patient), have been reported in organ transplant patients, these are rare in all but cardiac allograft recipients. Particularly important is the instance when the allograft donor is seropositive and the recipient seronegative for *T. gondii*. In these instances there is a >50% incidence of symptomatic toxoplasmosis, with myocarditis and myocardial dysfunction being the most common manifestations of toxoplasmosis in this patient population[131,132]. In addition, dissemination and central nervous system disease may also occur[133,134]. A not-uncommon error in these patients who are at risk for primary toxoplasmosis is to diagnose rejection and treat with increased immunosuppression, as opposed to anti-toxoplasmosis therapy, with this error having disastrous consequences.

All cardiac transplant patients who are seronegative for toxoplasmosis and are receiving an allograft from a seropositive donor should receive anti-toxoplasmosis prophylaxis. Whether or not trimethoprim-sulfamethoxazole is adequate for this purpose is at present unclear, and it is our policy to utilize either pyrimethamine plus a sulfonamide or atovaquone for 4 months in these patients[135,136].

The impact of *Pneumocystis carinii* infection in thoracic transplantation

Pneumocystis carinii infection is a major pathogen in organ transplant recipients. Whereas approximately 10% of renal transplant patients will develop symptomatic *Pneumocystis* pneumonia in the first 6 months post-transplant, the rate is significantly higher in cardiac (38% in one series) and lung (88% in one series) transplant recipients not receiving prophylaxis, although not all of these were symptomatic[137]. Patients with coexistent CMV infection, those receiving higher doses of corticosteroids, and those with chronic rejection are at particularly high risk.

The clinical presentation of *Pneumocystis* pneumonia among thoracic allograft recipients is similar to that of other populations: the subacute onset of fever, chills, dyspnea, non-productive cough, and a peribronchovascular ('interstitial') infiltrate on chest examination, with hypoxemia demonstrable on blood gas measurement. Differential diagnostic considerations include CMV infection, other viruses and, in the lung transplant patient, rejection. Therefore, in patients who have not been on effective *Pneumocystis* prophylaxis, rapid diagnosis, usually via bronchoscopy and bronchoalveolar lavage, is essential.

In addition to the morbidity and mortality associated with *Pneumocystis* pneumonia there is another compelling reason for attempting to prevent this otherwise common infection: treatment of transplant patients receiving antirejection therapy with cyclosporin or FK506 with the necessary dosages of either trimethoprim-sulfamethoxazole or pentamidine (the two first-line drugs for the treatment of *Pneumocystis*) is associated with a high rate of either nephrotoxicity or bone marrow toxicity. This is clearly an infection to be prevented, not treated. Low-dose trimethoprim-sulfamethoxazole (we use one single-strength tablet, containing 80 mg of trimethoprim and 400 mg of sulfamethoxazole, at bedtime for a minimum of 6–12 months post-transplant) provides virtually complete protection against this infection, while providing similar protection against nocardiosis, listeriosis and, perhaps, toxoplasmosis in the patient who is already seropositive for this organism[138–140]. In those patients who cannot tolerate trimethoprim-sulfamethoxazole, prophylaxis with a second-line regimen (e.g. atovaquone, dapsone, or monthly aerosol pentamidine) is obligatory.

The impact of mycobacterial infection on thoracic transplantation

Because of the critical role of T cell immunity and activated macrophages in controlling tuberculosis, it would be expected that, among transplant recipients, active tuberculosis would be an important threat. In fact, although tuberculosis is more common in transplant recipients, with an attack rate of 480 per 100 000 vs 13.1 per 100 000 in the general population, most centers in North America and Europe report that mycobacterial infection accounts for <2% of transplant-associated infections[141]. The rate is, however, significantly higher in populations such as those in developing countries, where the endemic level of tuberculosis in the general population is high. Thus, in India tuberculosis accounts for 10% of transplant-associated infections, with a mortality rate of approximately 25% (11% for pulmonary disease and 37% for disseminated disease)[142].

Most reports of tuberculosis in transplant recipients have concerned kidney transplant recipients. In these reports it appears that both disseminated and joint infection are more common than in the general population[143–145]. Less is known about recipients of thoracic organ transplants. A recent report of three cases of tuberculosis among heart transplant recipients brings the total reported cases to 12 among the approximately 12 000 heart transplants that have been carried out in the world[146]. About half of these reported cases are extrapulmonary or disseminated, with several occurring in conjunction with such other infections as CMV, nocardiosis, and aspergillosis. A small number of cases of tuberculosis have likewise been reported among lung transplant recipients, with the clinical presentation varying from that of a subacute pneumonia or focal pulmonary lesion to an isolated, unexplained pleural effusion[147,148].

Although the majority of patients developing tuberculosis represent instances of reactivation disease, several reports document the transmission of the infection with the allograft. In one report a donor was subsequently found to have tuberculous meningitis; both recipients of kidneys from this donor developed evidence of active tuberculous infection[149]. In another case, fatal miliary tuberculosis developed 11 weeks following heart–lung transplantation[148]. In retrospect a computerized tomographic scan demonstrated calcified lymph nodes, and pathologic examination of the donor lungs revealed an old Ghon complex. Clearly, then, tuberculosis can be conveyed with the allograft, and careful evaluation of prospective donors for this possibility is essential.

A more common issue is the management of individuals known to be tuberculin-positive. Because the bulwarks of antituberculous therapy, isoniazid and rifampin, may cause hepatic toxicity, as well as affect the metabolism of cyclosporin and prednisone, concern has been raised regarding the routine use of antituberculous prophylaxis in transplant patients with positive tuberculin tests. Our approach has been to utilize isoniazid in certain patients when at least one other risk factor for tuberculosis is present. Risk factors of importance include the following: noncaucasian racial background; history of past active disease, particularly if not treated optimally; significant abnormalities on chest X-ray; the presence of protein–calorie malnutrition; or the presence of some other immunosuppressing process[1].

In addition to infection with *M. tuberculosis*, transplant patients have an increased risk for localized and disseminated infection with a variety of atypical mycobacteria. Cutaneous infections, pulmonary disease, septic arthritis, and disseminated disease have all been reported in transplant patients due to these organisms[144,145,150–154]. In 1994 Patel *et al.* presented an extensive review of this topic, noting that 67% of patients presented with soft tissue, bone, or joint infection, and >50% had multiple sites of infection[151]. The clinical presentation was subacute, with active infection being present up to a year before diagnosis. Of the 28% of patients with pulmonary disease the majority were infected with *M. kansasii*; in contrast, those with soft tissue infection were commonly infected with organisms belonging to the *M. chelonae/fortuitum* complex. In addition, other atypical mycobacteria such as *M. avium-intracellulare*, *M. haemophilum*, *M. thermoresistibile*, and *M. scrofulaceum* have been reported. Risks factors for disease with these organisms include, in addition to immunosuppression: tissue injury (particularly to the skin), and exposure to contaminated water (as in aquariums, swimming

pools, freshwater ponds, etc.). Treatment of these infections remains non-standardized, but decrease in immunosuppression and surgical debridement are often necessary. Effective chemotherapy relies heavily on *in vitro* susceptibility testing, although the newer fluoroquinolones and macrolide antibiotics have greatly increased our ability to treat these uncommon infections.

The impact of fungal infection in thoracic transplantation

Fungal infection remains an important problem in thoracic organ transplantation. In the early days of heart transplantation >30% of cardiac allograft recipients developed significant fungal infection; fortunately, this has decreased to 5–21% in recent years[155,156]. In lung recipients, however, it is estimated that 35–45% of patients develop evidence of fungal infection[12,19,155]. The infections that occur can be divided into two major categories: infection with the geographically restricted systemic mycoses (blastomycosis, coccidioidomycosis, and histoplasmosis); and those due to the opportunistic pathogens, particularly *Candida* species, *Aspergillus* species, and *Cryptococcus neoformans*.

With respect to the geographically restricted systemic infections, three general patterns are observed[1]: (a) progressive primary infection following inhalation of the organism, resulting in both significant pulmonary disease and a high incidence of systemic dissemination; (b) reactivation of an old, dormant focus, with a significant risk of secondary dissemination; and (c) reinfection, again with a risk for systemic dissemination, in a previously immune individual, due to the combined effects of immunosuppression and a significant epidemiologic exposure. Thus, for the clinician, knowledge of both recent and remote epidemiologic history is of importance. Common clinical presentations of these infections in the transplant patient include the following: fever of unknown origin, unexplained cough or shortness of breath, or manifestations of metastatic infection (e.g. headaches due to meningeal coccidioidomycosis; mucocutaneous disease due to histoplasmosis; or cutaneous disease due to blastomycosis)[157–160].

Candidal infection accounts for more than three-quarters of the fungal infections in transplant patients. The most common manifestations include mucocutaneous infections, the incidence of which is related to steroid and antibacterial use, as well as adequacy of blood sugar control. Although thrush and vaginitis are bothersome, symptomatic esophagitis is the most important form of mucocutaneous infection. Many of the less invasive infections (such as thrush) respond well to topical therapy with clotrimazole or nystatin, although fluconazole may be used for more rapid control. Esophagitis, however, should be treated with fluconazole or amphotericin B. Candiduria is also common, and is usually treated aggressively, because of fear of dissemination. Again, fluconazole is the cornerstone of therapy, with low-dose intravenous amphotericin plus flucytosine a useful alternative, particularly when infection is due to candidal species resistant to fluconazole (e.g. *C. krusei* and *C. glabrata*). The time-honored method of amphotericin bladder irrigation via an indwelling catheter is less effective than systemic therapy, and carries the additional risk of bacterial superinfection. A particular form of mucosal disease that bears mention is the effects of candidal infection/colonization on the bronchial anastomosis in lung transplant patients[161]. Since this can affect wound healing we advocate aggressive prophylaxis and surveillance for the presence of candidal species in the first weeks following transplantation.

The most important serious infection with *Candida* is blood stream infection, which usually occurs as a consequence of vascular access infection. Candidemia in the transplant recipient carries a >50% risk of visceral dissemination, and even a single positive blood culture necessitates a course of systemic chemotherapy in addition to removal of the vascular access line[156]. The optimal treatment regimen is currently unknown. Pending the development of more data, our approach is the following: in the acutely ill patient, therapy is initiated with amphotericin until control of the process is achieved, at which time the patient is switched to fluconazole. In less acutely ill patients, therapy is initiated with fluconazole as long as the candidal species isolated is not thought to be resistant. The overall duration of therapy is determined by the patient's clinical presentation and response to therapy. In general, particularly when treatment can be accomplished with oral fluconazole, our preference is to treat for 1–2 weeks beyond the point at which the patient has been rendered free of all clinical evidence of the infection.

Aspergillosis, particularly that acquired within the hospital, remains a significant concern in all organ transplant recipients. Approximately 90% of the cases of aspergillosis observed involve the lungs, with the nasal sinuses accounting for most of the remainder (an occasional patient will have inoculation of *Aspergillus* spores directly into a surgical wound or vascular access site, resulting in invasive cutaneous aspergillosis)[162–168]. Wherever the primary site of infection, the cardinal signs and symptoms of aspergillosis in this patient population are due to the vasculotropic nature of this infection: tissue infarction, hemorrhage, and metastasis. Thus, clinical presentation can represent the primary site of involvement (i.e. the lungs or sinuses) or a site of metastasis. As far as the latter is concerned, the most common example of this is the patient with a focal neurologic deficit or seizures due to invasive aspergillosis metastatic from a relatively asymptomatic pulmonary focus[169,170]. In lung transplant patients a necrotizing lesion at the site of the bronchial anastomosis has been a particular problem unique to this population[24].

Aspergillus fumigatus and *A. flavus* account for >90% of these infections, with the other *Aspergillus* species accounting for the remainder. Once the respiratory tract is colonized with *Aspergillus*, more than two-thirds of patients will develop invasive disease, thus justifying an aggressive approach to this infection in this patient population[171–173]. *Aspergillus* species, in the non-immunosuppressed host, have a particular propensity for colonizing structurally and functionally abnormal respiratory tracts (e.g. in cystic fibrosis patients, those with bronchiectasis, and those with chronic sinusitis). Since many of these individuals are awaiting lung transplantation, it is important to demonstrate whether or not such colonization is present, and to attempt to eradicate this infection prior to transplantation. Unfortunately, the options for therapy against *Aspergillus* are limited. Itraconazole, presumably because of its erratic bioavailability, has not proven reliable as primary therapy in transplant patients, and can safely be used only to eradicate carriage in the pretransplant situation where therapeutic failure can still be corrected, or as 'wrap-up' therapy following an extended course of amphotericin. Otherwise,

at present, extended courses of amphotericin remain the standard of care. Because of the toxicities associated with such therapy, the emphasis should be on prevention, particularly on eliminating potential nosocomial hazards.

Cryptococcus neoformans occurs predominantly >6 months post-transplant, with a particularly high attack rate in the patient who has had multiple bouts of acute and chronic rejection, requiring higher than normal amounts of immunosuppressive therapy (the 'chronic n'er-do-wells'). *C. neoformans* also has a pulmonary portal of entry, often asymptomatic, occasionally presenting as a subacute pneumonia or isolated pulmonary nodule discovered on chest X-ray. Far more common, however, is the patient who presents with evidence of metastatic infection, with the skin or the central nervous system being the sites most commonly involved[155,156,174–176]. In >20% of transplant patients with cryptococcosis there is evidence of a skin lesion as the first sign of systemic infection for many weeks before other disease manifestations appear (this is also true for such other infections as nocardiosis and the other fungal infections). Thus, unexplained skin lesions merit biopsy for pathologic assessment and culture.

Other than biopsy, the cornerstone of diagnosis is testing for cryptococcal antigen in blood and cerebrospinal fluid. In addition, blood cultures performed by the lysis centrifugation technique ('Dupont isolators') also have a high yield. The cryptococcal antigen test gives a good measurement of organism load, and serial determinations provide an excellent guide to therapy[177,178]. Therapy of cryptococcosis is similar to that of candidal infection: fluconazole therapy for those subacutely ill and initial amphotericin therapy, followed by fluconazole, for those more seriously ill. Duration of therapy is prolonged and variable, determined by the patient's response, both clinically and in terms of antigen clearance.

PRINCIPLES OF ANTIMICROBIAL USE IN THORACIC TRANSPLANTATION

There are three underlying themes in the prescription of antimicrobial agents for the transplant patient: (a) antimicrobial therapies must be considered in the context of the immunosuppressive therapy being administered, both in terms of the manifold opportunities for drug interaction and in terms of the linkage of antimicrobial programs to the intensity of immunosuppression being prescribed; (b) prevention of infection rather than therapy is the overriding aim; and (c) when therapy of established infection is required, early diagnosis and initiation of therapy, as well as an adequately sustained duration of therapy, form the basis for success[1].

The advent of cyclosporin-based immunosuppression, and now the availability of FK506, have been a major advance in transplantation, responsible for much of the success observed with modern thoracic organ transplantation. At the same time, because of drug interactions, they have greatly complicated the use of antimicrobial drugs. Although clearly established in the case of cyclosporin, much of the following information is likely to be also true of FK506. In the case of cyclosporin there are three ways in which antimicrobial agents can interact in a clinically important way, with two of these being related to the key step in cyclosporin metabolism, which occurs via hepatic cytochrome P450 enzymatic metabolism[1]:

(1) Certain antimicrobial agents, most notably rifampin and, perhaps, isoniazid, up-regulate cyclosporin metabolism, thus decreasing cyclosporin blood levels, potentially leading to underimmunosuppression and allograft rejection.
(2) Certain antimicrobial agents, most notably the azole antifungal agents (ketoconazole > itraconazole ≫ fluconazole) and the macrolide antibiotics (erythromycin, azithromycin, and clarithromycin) down-regulate cyclosporin metabolism, resulting in elevated cyclosporin levels, potentially leading to toxicity or overimmunosuppression.
(3) Many antimicrobial agents, most notably amphotericin, the aminoglycosides, high-dose trimethoprim-sulfamethoxazole, high-dose fluoroquinolones and, perhaps, vancomycin, have a synergistic effect with cyclosporin in terms of producing nephrotoxicity.

Whereas the first two of these can be managed by adjustment in the cyclosporin dose, based on blood level measurements, the last is idiosyncratic. The implication of these observations is that, when antimicrobial therapy is needed, there is a particular emphasis on the use of β-lactams, fluconazole, and low-dose fluoroquinolones (e.g. no more than 500 mg of ciprofloxacin per day), and there is even further emphasis on the prevention of infection. Finally, a good rule of thumb is that if unexplained renal dysfunction occurs in a cyclosporin (and, presumably, an FK506) recipient, a drug interaction should be suspected, with antimicrobial agents being prime suspects.

There are three modes in which antimicrobial agents can be utilized in transplant recipients[1]:

(1) *Therapeutic*, in which curative antibiotics are administered to patients with clinically manifest infection.
(2) *Prophylactic*, in which antibiotics are administered to an entire population to prevent infection prior to an event. For prophylaxis to be appropriate, the infection needs to be important enough and common enough in the population to justify that step, and the antimicrobial therapy in question has to be non-toxic enough to make it worthwhile. Successful prophylactic strategies in the transplant patient include both low-dose trimethoprim-sulfamethoxazole (which is well tolerated, as opposed to treatment doses for *Pneumocystis*, which are poorly tolerated), which effectively eliminates pneumocystosis, nocardiosis, and listeriosis, and peritransplant surgical wound prophylaxis.
(3) *Pre-emptive*, in which antimicrobial agents are administered prior to the onset of clinical disease to a subpopulation identified on the basis of a clinical epidemiologic characteristic or a laboratory marker. For such a strategy to be justified, such a marker has to be highly discriminating, and effective 'emergency intervention' strategies must be available. Examples of successful pre-emptive strategies include the prescription of intravenous ganciclovir during antilymphocyte antibody therapy to prevent symptomatic CMV disease in CMV-seropositive individuals, or the prescription of anti-*Aspergillus* therapy to patients colonized with *Aspergillus* species. In both these instances, without such pre-emptive therapy the incidence of life-threatening infection is >60% for these subgroups and the antimicrobial intervention provides significant protection which is superior to more generally prescribed prophylactic programs.

COMMENT

Considerable strides have been made in the technical, immunosuppressive, and infectious disease management of patients undergoing thoracic organ transplantation. The next decade should see further improvement as new immunosuppressive therapies, as well as new antimicrobial strategies, become available. However, the principles that have become established over the past decades will remain the cornerstones of patient management. The most important of these are the following:

(1) Technically impeccable surgery and management of both the donor and the recipient are now, and will remain, the cornerstones of successful transplantation. A technical mishap is invariably associated with life-threatening infection.
(2) The occurrence of infection is determined by the interaction of the patient's net state of immunosuppression and the epidemiologic exposures encountered. These are translated into a timetable according to which different infections occur at different time points post-transplant.
(3) The therapeutic prescription for the transplant patient consists of an immunosuppressive strategy to prevent and treat rejection, and an antimicrobial strategy to make it safe. Thus, antimicrobial strategies (both drug and environmental protection) are closely linked to the intensity and duration of the immunosuppressive program that is prescribed.
(4) Prevention of infection is the goal.

With further advances in each of these areas it is expected that the opportunities for successful rehabilitation of patients with end-stage heart and/or lung disease via transplantation will only increase over the next decade.

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12
Malignant Neoplasia in the Immunocompromised Patient

I. PENN

INTRODUCTION

The burgeoning numbers of patients who undergo cardiac or pulmonary transplantation make it increasingly important that cardiologists, pulmonologists and cardiothoracic surgeons be familiar with the complications of these treatments. One of these is an increased risk for the development of certain types of cancer. Much of this chapter is based on experience obtained from renal transplantation, but whenever possible observations made in cardiac, cardiopulmonary or pulmonary graft recipients (henceforth referred to as cardiothoracic [CT] recipients) will be stressed. The report is based on data collected by the Cincinnati Transplant Tumor Registry (CTTR) up till May 1995.

The chapter will consider three categories of malignancies: (a) transplanted cancers; (b) pre-existing tumors that were present before transplantation; and (c) *de novo* malignancies that developed in the recipient after transplantation.

TRANSPLANTED MALIGNANCIES

When assessing a potential cadaver donor for harvesting the heart or lungs, it is obligatory to determine whether or not he or she has cancer, or has been treated for it recently, as there is a danger of transmitting tumor cells with the transplanted organ(s)[1-3]. In normal individuals such foreign cells would be rejected promptly by the recipient. However, the immunosuppressive therapy, used to prevent graft rejection, impairs the host's immune defenses and prevents destruction of cancer cells which may grow, invade adjacent structures, and even metastasize.

The CTTR has data on 248 recipients of organs from donors who had malignancies at the time of donation, or had been treated for them within 10 years of transplantation or, in the case of the several living donors, who presented with neoplasms up to 18 months after donation. Allograft recipients included 227 renal, 10 hepatic, seven cardiac, two pancreatic, one cardiopulmonary and one pulmonary. In eight instances small tumors were removed from renal allografts immediately prior to transplantation. If we include these cases 103 recipients (42%) received organs that contained cancers. In 39 instances the tumor involved the allograft only, while in another six there was invasion of adjacent structures, and 58 recipients had distant metastases.

The most common tumors that caused metastases were malignant melanoma (28%), carcinoma of the kidney (19%), carcinoma of the bronchus (17%), choriocarcinoma (16%) and primary brain tumors (9%). Overall, 40 recipients died of cancer. However, 16 renal allograft patients had complete remissions of all transmitted neoplasms, usually following allograft nephrectomy, and cessation of immunosuppressive therapy, which presumably permitted host immunity to recover and reject residual tumor. These measures were supplemented in five patients by chemotherapy, immunotherapy (interferon; interleukin-2) or radiotherapy. Two other patients are currently alive with tumor after undergoing allograft nephrectomy and discontinuation of immunosuppressive therapy.

Seven heart, one heart–lung and one lung patients received organs from donors with cancer. Four recipients (three heart, one lung) died of metastases of carcinoma of the bronchus, medulloblastoma, malignant melanoma, and nephroblastoma respectively. The heart–lung recipient died of rejection 1 month after transplantation. At autopsy a metastasis of choriocarcinoma was found in one lung. Four cardiac recipients are alive from 0.5 to 57 (average 29) months after transplantation without evidence of malignancy, having received organs from donors with choriocarcinoma, adenocarcinoma of the uterine cervix, adenocarcinoma of the kidney, and carcinoma of the prostate, respectively.

While it is possible to remove part or all of a lung allograft that contains a metastasis, excision of a cardiac allograft and cessation of immunosuppressive therapy are not feasible unless an implantable artificial heart can be used, either as a permanent replacement or for a period of several months until all evidence of malignancy has disappeared and a new cardiac allograft can be inserted. None of these procedures has yet been attempted to treat transmitted malignancies in CT recipients.

It is therefore imperative to avoid using donors with tumors, except those with low-grade carcinomas of the skin, or with primary brain malignancies, which rarely spread outside the central nervous system[1-3]. However, it is important to have histologic proof that the cancers actually arose in the brain, as, in

several instances, the cause of the donor's death was misdiagnosed as intracranial hemorrhage, primary brain tumor, or multiple brain abscesses, when, in fact, the donors died of metastases mostly from choriocarcinoma, bronchial carcinoma, malignant melanoma, or renal carcinoma. We should also avoid using donors with primary brain tumors who have been treated with ventriculoperitoneal or ventriculovenous shunts, or who have had extensive craniotomies, radiotherapy or chemotherapy, as these open pathways for tumor dissemination[1-3].

PRE-EXISTING CANCERS

If a neoplasm in the potential recipient was treated before transplantation, it is possible that the immunosuppressive therapy may impair the ability of the host's immune defenses to control any residual cancer cells[4,5].

In a study of 939 pre-existing malignancies that occurred in 913 renal transplant recipients there was a 22% recurrence rate after transplantation[4,5]. Thirteen percent of the recurrences occurred despite removal of the primary malignancies 5 or more years beforehand. It was recommended that with the exception of patients with: (a) incidentally discovered renal malignancies, (b) *in situ* carcinomas of various organs, (c) focal neoplasms (a small microscopic focus) in organs such as the prostate or uterus, (d) low-grade bladder cancers and (e) basal cell carcinomas of the skin, transplantation should be delayed for at least 24 months after treatment of the tumors.

During this time renal transplant patients can be kept alive by dialysis. As regards potential cardiac transplant recipients, current experience with the artificial heart makes it extremely unlikely that they can be kept alive during this long waiting period. Because of the hopeless prognosis of potential CT recipients who do not receive transplantation, it is probably advisable to expeditiously proceed with transplantation as soon as donor organs become available, except in individuals with active major cancers or who have short life expectancies because of their neoplasms.

Pre-existing tumors (total 160) were present in 146 heart, eight lung and two combined heart–lung recipients who were treated from 504 months before to 12 months after transplantation (average 86 months before transplantation). Persistence or recurrence of cancer occurred in 30 patients (19%), a similar percentage to that observed in renal transplant recipients.

A favorable feature in many heart transplant recipients is that they had been successfully treated for a lymphoma (including Hodgkin's disease) or a sarcoma more than 5 years previously, and were apparently cured of their malignancies. Adriamycin-induced cardiotoxicity was a common indication for cardiac transplantation in these patients.

Of the 30 patients with persistent or recurrent tumors, 12 had tumors treated at or after transplantation, five were treated 24 months or less before transplantation, nine were treated more than 24 months before transplantation, and the time of treatment was not stated in four cases. The most common tumors that persisted or recurred were carcinomas of the lung (seven), non-melanoma skin cancers (five), lymphomas (four), carcinomas of the bladder (three) and carcinomas of the pancreas (two). In addition, two patients had primary tumors of the heart (malignant synovioma and angiosarcoma, respectively) removed at the time of transplantation and both, not surprisingly, had recurrences.

DE NOVO TUMORS

The CTTR has data on 8191 patients who developed 8724 cancers *de novo* after transplantation[6-8]. Of these, 772 received heart, and 29 combined heart–lung, and 29 lung allografts. There were also 6821 kidney transplant recipients and 540 recipients of other extrarenal organs (liver, pancreas, bone marrow, upper abdominal cluster organs and small bowel).

Data from several large renal transplant centers show an overall incidence of cancer ranging from 1% to 16%, with an average of 6%[6-8]. In a series of 182 cardiac transplant recipients who underwent 199 transplantations, the incidence was 10%[9]. Lanza *et al.*[10] compared the incidence of malignancies in cardiac and renal allograft recipients. They found a 2-fold greater increase of all neoplasms in cardiac patients, with nearly a 6-fold increased incidence of visceral tumors ($p<0.02$). They attributed the increase to the more intense immunosuppressive therapy used in these patients.

The incidence of cancer increases with the length of follow-up after transplantation. The actuarial probability of developing malignancy among patients who received cardiac transplants during childhood was 7% at 1 and 2 years, 12% at 3 years, and 15% at 4 and 5 years[11]. An Australasian study of 6596 recipients of cadaver renal transplants showed that the percentage probability of developing cancer 24 years after transplantation was 66% for skin neoplasms, 27% for non-skin malignancies and 72% for any type of tumor[12]. These exceptional figures must be interpreted with caution as most malignancies were skin cancers (which are very common in Australia) and the number of 24-year survivors was small. Nevertheless, they stress the necessity to follow transplant patients indefinitely.

Age of recipients

The malignancies affected a relatively young group of persons whose average age at the time of transplantation was 42 years (range 3 months to 80 years). Forty-three percent were under 40 years of age at the time of transplantation.

Sex

Males made up two-thirds and females one-third of the patients with cancer, roughly the same proportions as those undergoing renal transplantation.

Time of appearance of the neoplasms

In the general population there is a latent period of 5–20 years or even more, between exposure to many carcinogens and the development of malignancies. However, in transplant patients tumors appeared after a much shorter interval, at an average of 61, median 46 (range 0.25–313) months[6-8].

Some cancers appeared at fairly distinct intervals after transplantation[6-8]. Kaposi's sarcoma (KS) presented the earliest at an average of 21, median 12 (range 1–225.5) months after transplantation. Lymphomas appeared at an average of 36, median 12.5 (range 0.25–305.5) months after transplantation. Skin cancers appeared an average of 75, median 60 (range 1–313) months post-

transplantation. Carcinomas of the vulva and perineum appeared at the longest interval after transplantation at an average of 112, median 109 (range 1.5–285.5) months.

Types of tumors

The neoplasms that are frequently observed in the general population (carcinomas of the lung, breast, prostate, colon and invasive uterine cervical carcinomas) showed no increase or even a decrease[6–8]. Only two varieties of cancer often seen in the general population were encountered in significant numbers in transplant patients. Excluding lip neoplasms the percentage of non-melanoma skin cancers (31%) was similar to that observed in the general population (37% of all tumors), but the incidence of squamous cell carcinomas (SCC) was markedly increased (see below). The only other relatively common tumor in the general population, seen also in transplant patients, was *in situ* carcinoma of the uterine cervix, which comprised 3% of malignancies in both groups.

If non-melanoma skin cancers and *in situ* carcinomas of the cervix were excluded, as they are from most cancer statistics, we then observed a variety of malignancies in transplant patients that were uncommon in the general population: lymphomas 24% vs 6%; lip cancers 7% vs 0.2%; KS 6% vs a negligible incidence; carcinomas of the kidney 5% vs 2%; carcinomas of the vulva and perineum 3.5% vs 0.5%, hepatobiliary tumors 2.4% vs 1.5%; and sarcomas (excluding KS) 1.7% vs 0.5%[6–8].

These findings are in keeping with several epidemiologic studies that showed that when transplant patients were compared with suitable age-matched controls there was a 4–7-fold increase in skin cancer in people living in areas of low sunshine exposure, but a 21-fold increase in persons exposed to abundant sunshine[6–8,13]; a 29-fold increase in lip cancers[14]; a 28–49-fold increase in non-Hodgkin's lymphoma (NHL)[15,16]; a 400–500-fold increase in KS[17]; a 14-fold increase in carcinoma *in situ* of the uterine cervix[18]; and a 100-fold increase in carcinomas of the vulva and anus[14].

Cancers of the skin and lips

Overall, these were the most common and comprised 3178 of the 8724 tumors (36%)[6–8]. The incidence of skin cancers increased with the length of follow-up after transplantation, as shown by an Australasian study of 6596 recipients of cadaveric renal transplants who experienced a linear increase in the incidence of skin cancer reaching 66% at 24 years post-transplantation[12]. Similarly, a Dutch study showed a 10% incidence of non-melanoma skin cancers in renal transplant recipients at 10 years post-transplantation, which rose to 40% after 20 years[19].

CT recipients differed from the renal recipients in this series in that skin cancers were relatively uncommon, making up only 239 of 859 (28%) of all tumors compared with 39% in renal recipients. In part this may be related to length of follow-up, which was relatively short in many CT recipients.

The skin cancers of transplant patients differed in several respects from those seen in the general population[6–8,13,19]. Whereas basal cell carcinomas (BCC) outnumber squamous cell carcinomas (SCC) in the general population in a ratio of 5:1, the reverse occurs in CTTR patients, in whom SCC outnumber BCC by 1.8 to 1. In the population at large, non-melanoma skin cancers occur mostly in individuals in their 60s and 70s, whereas the average age of transplant patients is 30 years younger[6–8,20]. Multiple skin cancers occur in 42% of patients in the CTTR. This incidence is remarkably high, and is comparable to that seen only in people in the general population who live in areas of abundant sunshine[6–8]. Several patients each had more than 100 skin cancers. Malignant melanomas comprise 5.2% of skin cancers in the CTTR, in contrast with an incidence of 2.7% in the general population of the United States. This finding is consistent with several epidemiologic studies showing a 3.8–5-fold higher incidence of malignant melanoma in transplant patients compared with age-matched controls[13,15,16].

Most skin cancers in the CTTR were of low-grade malignancy but a significant percentage of SCC behaved much more aggressively than their counterparts in the general population[6–8,13]. Lymph node metastases occurred in 5.8% of patients. Seventy-five percent were from SCC and only 17% from melanomas. Another 8% were from Merkel's cell tumors, and <1% were from BCC. In addition, 5.1% of patients died of skin cancer, with 60% of deaths being from SCC and only 33% from melanomas. Five percent were from Merkel's cell tumors and 2% from BCC. These findings are consistent with a more than 10-fold increase in mortality from SCC of the skin observed in Australian renal transplant recipients[15]. The behavior of skin cancer in transplant patients is markedly different from that seen in the general population, in whom they cause only 1–2% of all cancer deaths, the great majority of which are from malignant melanoma. Most patients with SCC who developed lymph node metastases, or who died of their cancers, had skin, rather than lip, lesions.

In the overall series of patients with complications of their skin cancers, nine were cardiac allograft recipients. Eight developed lymph nodes metastases, six from SCC (skin four, lip two) and two from melanomas. Six patients died of their skin cancers, five from SCC (skin three, lip two) and one from malignant melanoma. Of the deaths, five occurred in patients who had had nodal metastases (SCC four, melanoma one).

Lymphomas

In several respects lymphomas were strikingly different from those seen in the general population[6–8]. The majority (94%) were NHL, whereas Hodgkin's disease and plasmacytoma/myeloma comprised less than 3% and 4% of lymphomas, respectively, compared with 11% and 18% respectively in the general population. Morphologically, most NHL were large-cell lymphomas. Of tumors studied by modern immunological techniques, 87% arose from B lymphocytes, 13% were T-cell lymphomas, and less than 1% arose from null cells. Whereas extranodal involvement was reported in 24–48% of NHL patients in the community at large, it occurred in 69% of NHL in transplant patients.

Surprisingly, one of the most common extranodal sites was the central nervous system (CNS), which was involved in 22% of cases[6–8,21]. Ten percent involved the CNS and other organs, a figure similar to the 8–12% of CNS involvement by systemic NHL in the general population[21]. However, most lesions in transplant patients involved the brain parenchyma, whereas in the general population most occurred in the leptomeninges,

perivascular spaces and nerve roots[21]. The other 12% were limited to the CNS, in contrast with a 1–2% incidence of primary CNS involvement by NHL in the general population[21]. In both groups the brain parenchyma was usually involved, and meningeal involvement occurred infrequently[21]. In the CTTR patients, brain lesions were frequently multicentric in distribution. In the CTTR series, spinal cord lesions were very unusual. In this regard the lesions resembled CNS lymphomas in the general population, in whom the spinal cord was affected in only 0–5% in different series[6–8].

A possible explanation for the frequency of brain involvement is that this organ has poor immunologic reactions, so that lymphoma cells that arise in it, or are carried there from other sites, grow more readily in this relatively immunologically privileged site than in other viscera[6–8].

A CNS lymphoma should be suspected whenever a transplant patient presents with neurologic symptoms[6–8]. A thorough workup is necessary to exclude more common causes of these symptoms in such a patient, including hypertensive encephalopathy, meningitis, brain abscess, or intracranial bleeding. Studies may include electroencephalography, brain scan, computerized axial tomography, examination of the cerebrospinal fluid, cerebral angiography, and magnetic resonance imaging.

Epstein – Barr virus (EBV) DNA has been isolated from many B cell lesions in transplant recipients[22,23]. In immunosuppressed individuals it is believed to cause a spectrum of lesions ranging from benign polyclonal B cell hyperplasias on the one hand to frank monoclonal B cell lymphomas on the other[22,23]. We do not know the cause(s) of T-cell lymphomas in transplant patients.

Of 339 CT patients with lymphomas, four had Hodgkin's disease and 11 had plasmacytoma or multiple myeloma. The remaining 324 patients had NHL, of whom 54 had no treatment (the tumor was an autopsy finding in 38 recipients), and in 14 instances treatment data were not provided. Of the 256 treated patients, 102 (40%) had complete remissions (Table 1). These occurred in 70 patients (69%) with disease localized to a single organ or site and 32 patients (31%) with widespread disease. Subsequently, 26 patients who had complete remission died of other causes. At present, of the 324 patients, both treated and untreated, 76 are alive and well, 43 are undergoing treatment, 115 died of their lymphomas, and 90 died of other causes. The results obtained with CT recipients mirror those obtained with all patients with lymphomas in the CTTR.

Table 1 Treatments used in cardiothoracic transplant patients with complete remissions*

Treatment	*No. of patients*	
	Multimodality therapy†	*Single-modality therapy*‡
Decrease or cessation of immunosuppression	43	30
Acyclovir or ganciclovir	20	1
Excision	16	8
Chemotherapy	16	7
Radiotherapy	13	6
Interferon	3	3
Monoclonal anti-B-cell antibodies	1	–

* Treatment given to one patient is not recorded.
† Used in 46 patients.
‡ Used in 55 patients.

Kaposi's sarcoma (KS)

The fact that KS composed 6% of tumors in the CTTR study stands in stark comparison with its incidence in the general population of the United States of only 0.02–0.07% of all tumors (before the AIDS epidemic started)[6,8,24]. Among CT recipients in the CTTR, KS composed 2% of all malignancies.

In the CTTR, patients with KS (356) exceeded those with two common cancers, carcinomas of the colon and rectum (305) and carcinomas of the breast (273), a most remarkable finding[6,8,24]. Apart from individuals with AIDS, in which KS is enormously increased[11], there is probably no other series in which the numbers of KS patients exceed those with either of these two common cancers, except possibly in tropical Africa where KS is common and colon cancer is infrequent.

KS affected males to females in a 3:1 ratio, substantially less than the 9:1–15:1 ratio seen with KS in the general population[6–8,24]. KS was most common in organ transplant recipients who were Arab (25%), black (16%), Italian (15%), Jewish (9%) or Greek (7%). Sixty percent had non-visceral disease confined to skin, or oropharyngeal mucosa, and 40% had visceral disease with involvement of internal organs, most commonly the gastrointestinal tract, lungs and lymph nodes[6–8,24].

After treatment, complete remissions occurred in 113 of the 213 patients (53%) with non-visceral disease. Whereas 78 remissions followed various treatments including alteration of immunosuppression, surgery, radiotherapy, chemotherapy or administration of α-interferon, 35 (31%) occurred when the *only* treatment was a drastic reduction of immunosuppressive therapy. In patients with visceral disease only, 38 of the 143 patients (27%) had complete remissions after treatment. However, 23 of the remissions (60%) occurred after reduction of immunosuppressive therapy only.

Of the 19 CT recipients with KS, 10 had visceral disease and nine non-visceral involvement. In the former group, six patients died of KS, two from other causes, and only two are alive, with one in complete remission of KS. In contrast, in the nine patients with non-visceral disease, three died of unrelated causes and six are alive, with four patients in complete remission of KS.

Carcinomas of the uterine cervix

Carcinomas of the cervix occurred in 300 of the 2744 women in this series (11%). At least 72% were *in situ* lesions[6–8,18]. Thus far, seven have affected CT recipients. It is advisable that all postadolescent female patients have regular pelvic examinations and cervical smears to detect such lesions, and also carcinomas of the vulva and perineum[6–8,18].

Carcinomas of the vulva and perineum

Carcinomas of the vulva, perineum, scrotum, penis, perianal skin and anus occurred in 220 patients: 159 women and 61 men[6–8,25].

The patients were surprisingly young when compared with individuals with similar lesions in the general population, who are mostly in their 60s and 70s. The average age of the women at the time of transplantation was 30 (range 15–59) years, and of the men 35 (range 18–62) years. One-third of patients had *in situ* lesions. In women there was sometimes a 'field effect', with involvement by cancer of the vulva, vagina, uterine cervix, and anus in varying combinations[6–8,25]. In approximately one-third of patients a possible viral etiology of these malignancies is suggested by a preceding history of condyloma acuminatum caused by human papillomavirus[25,26].

Renal carcinomas

There were 301 patients with carcinomas of the kidney, of which 262 involved the patients' own kidney(s) and 28 involved the allograft; in 11 the location was not stated[6–8,27]. These figures excluded 259 patients who had lymphomatous involvement of the native or allograft kidneys and three patients who had renal sarcomas (two involving the allograft and one a native kidney).

In various studies in the general population, 5–10% of renal tumors are carcinomas of the pelvis[27], whereas in the CTTR series these accounted for 14% of tumors. The increase is almost certainly due to the high incidence of analgesic nephropathy among renal allograft recipients, which occurred in 8% of CTTR patients with carcinomas of their native kidneys[27]. This disorder is known to cause carcinomas in various parts of the urinary tract. This is borne out in the CTTR series, in which 68% of patients with analgesia-related renal carcinomas had similar cancers elsewhere in the urinary tract[27].

Another predisposing cause of tumors in renal transplant recipients is acquired cystic disease (ACD) of the native kidneys. This occurs in 30–95% of patients receiving long-term hemodialysis, and is complicated by renal adenocarcinoma, which is increased 30–40-fold over its incidence in the general population[27,28]. With a successfully functioning transplant the ACD tends to regress, and presumably the risk of developing renal cell carcinoma also recedes.

There is no ready explanation for the 16 renal carcinomas seen in CT recipients in the CTTR.

Hepatobiliary tumors

Most cases in the CTTR were hepatomas[6–8,29]. A substantial number of patients had a preceding history of hepatitis B[29] and, less frequently, of hepatitis C infection.

Sarcomas (excluding KS)

Most involved the soft tissues or visceral organs, whereas cartilage or bone involvement was uncommon[6–8]. The major types were fibrous histiocytoma (20 patients), leiomyosarcoma (15), fibrosarcoma (12), rhabdomyosarcoma (nine), hemangiosarcoma (eight), mesothelioma (six) and liposarcoma (five).

Other cancers

In some studies an increased incidence of other malignancies was noted. For example, the Australia and New Zealand Dialysis and Transplant Registry reported a 289-fold increased incidence of endocrine cancers, 5.6-fold increase of leukemia, 2.5-fold increase in digestive organ cancers, 2-fold increase in respiratory system tumors, and 4.6-fold increase of miscellaneous cancers[12].

Biological behavior of post-transplant neoplasms

Malignancies that occur in organ allograft recipients frequently demonstrate a more aggressive nature than do similar neoplasms in patients who have not undergone transplantation[30].

Cancers in renal versus CT recipients

Marked differences in incidence were observed when 7318 cancers that occurred in 6821 renal transplant recipients were compared with 859 neoplasms that occurred in 830 heart CT recipients (Table 2).

The most significant finding was a disproportionately high incidence of lymphomas in cardiac compared with renal patients. They composed 39% versus 12% of all malignancies[6–8]. The difference was even more marked in pediatric compared with adult cardiac allograft recipients. In 49 pediatric patients with post-

Table 2 Cancer in renal vs cardiothoracic recipients

Type of cancer	*No. of tumors in renal recipients**	*No. of tumors in CT recipients†*
Cancers of skin and lip	2850	239
Lymphomas	844	339
Carcinomas of the lung	406	68
Kaposi's sarcoma	324	19
Carcinomas of uterine cervix	288	7
Carcinomas of colon and rectum	267	18
Carcinomas of the kidney	281	16
Carcinomas of breast	250	9
Carcinomas of vulva, perineum, penis or scrotum	209	6
Head and neck carcinomas (excluding thyroid, parathyroid and eye)	207	18
Carcinomas of urinary bladder	186	7
Metastatic carcinomas (primary site unknown)	180	8
Leukemias	153	10
Hepatobiliary carcinomas	134	9
Carcinomas of thyroid gland	99	6
Carcinomas of prostate	106	28
Sarcomas (excluding Kaposi's)	88	10
Carcinomas of stomach	92	5
Carcinomas of testis	65	4
Carcinomas of ovary	59	1
Carcinomas of pancreas	56	8
Miscellaneous cancers	174	24
Total	7318	859

* More than one type of tumor affecting different organs occurred in 464 patients, of whom 31 each had three separate types of cancers and one patient had four.
† One cardiac transplant recipient had three separate types of neoplasm, and 26 others had two each. One pulmonary recipient had two tumors.

transplant malignancies, lymphomas composed 41 (84%) of the tumors compared with 298 of 810 neoplasms (37%) in adults. A similar high incidence of lymphomas was also seen in recipients of other extrarenal organs, in whom they composed 302 of 547 neoplasms (55%). The high incidence of lymphomas in CT recipients is confirmed by a study of 131 lymphomas from the University of Pittsburgh, which showed a 1% incidence in renal transplant recipients, a 2.3% incidence in cardiac allograft recipients, and a 3.8% incidence in recipients of combined heart and lung transplants[31].

In contrast, the renal patients in the CTTR exceeded the cardiac and related recipients in the incidence of skin cancers (39% vs 28%), carcinomas of the cervix (4% vs 0.8%) and carcinomas of the vulva and perineum (2.9% vs 0.7%). Somewhat similar findings have been reported by other workers[10].

Several factors probably account for these differences. In CT and other extrarenal organ recipients intense immunosuppressive therapy is frequently necessary to save lives by reversing rejection, whereas with severe rejection of kidney allografts physicians are likely to discontinue immunosuppression and return patients to dialysis therapy[6–8]. A complication of intense immunosuppressive therapy is a disproportionate increase in the incidence of malignancies that occur in the early months after transplantation, namely NHL.

This viewpoint is supported by a study of 75 survivors of heart and heart–lung transplantation who were treated with cyclosporine, prednisone and, in some instances, antithymocyte globulin. Lymphomas occurred in six patients. Measured quantitative parameters of immunosuppression during the first 3 months after transplantation (mean cyclosporine level, total antithymocyte globulin dosage, number of days of T cell suppression and mean cyclosporine level during T cell suppression) were higher in patients with lymphomas than in those who did not develop tumors[32].

Many renal transplants were performed in the era when immunosuppressive therapy consisted of azathioprine and prednisone (supplemented in some patients by ALG or ATG). In contrast, most CT transplants have been performed in the era of polypharmacy, usually involving triple therapy (azathioprine, prednisone and cyclosporine) sometimes supplemented by ALG or ATG or monoclonal agents such as OKT3. We are still learning how to use these drugs in varying combinations and doses. A problem is that some patients are being very heavily immunosuppressed, either inadvertently or intentionally to treat rejection, and the price for this is, again, an increased incidence of NHL[6–8].

A third factor is the length of follow-up after transplantation[6–8]. Many renal transplant recipients have been followed for a decade or even two decades or more, whereas most CT recipients were treated in the past 8–10 years. As neoplasms such as skin cancers, carcinomas of the cervix, and carcinomas of the vulva and perineum occur late after transplantation this may explain the discrepancy in the incidence of these tumors in renal versus CT recipients. The relatively small number of CT patients compared with the large number of renal transplant recipients may also have contributed to differences in the incidence of the various cancers.

Treatment of post-transplant malignancies

In dealing with patients with premalignant skin lesions or early skin tumors a useful treatment is a 6-week course of topical 5-fluorouracil cream applied twice daily[6–8]. This will eliminate many premalignant lesions and even superficial carcinomas. This treatment is rapidly being superseded by topical use of 0.05% tretinoin cream (a retinoid). This is efficacious in treating warts and keratoses in organ allograft recipients and, perhaps, may inhibit cutaneous carcinogenesis[33]. Treatment of skin cancers includes surgical excision, cryosurgery, chemosurgery or radiotherapy[6–8].

In situ carcinomas of the uterine cervix respond well to simple hysterectomy, cervical conization, or cryotherapy[6 8]. *In situ* carcinomas or small neoplasms of the vulva and perineum are treated by local excision. Large lesions require extensive operations such as total vulvectomy and inguinal node dissection, or abdominoperineal resection[25]. Other cancers are treated by standard surgical, radiotherapeutic or chemotherapeutic modalities.

The antiviral agents acyclovir or ganciclovir may be used to treat EBV-related NHL[6 8,22,23,31]. Alpha-interferon has been used to treat some patients with KS or NHL[6 8]. However, it may stimulate the immune system and cause rejection, and this has occurred in occasional patients[34].

In addition, we must consider reduction or cessation of immunosuppressive therapy[6–8,22,31]. The value of reducing immunosuppressive therapy is borne out by experience with certain neoplasms[6–8,22,31]. As mentioned above, several malignancies that were inadvertently transplanted from organ donors underwent partial or complete regression following cessation of immunosuppressive therapy[1–3]. Similarly, a significant percentage of KS and NHL (see above) regressed partially or completely after reduction of immunosuppressive therapy[6–8]. Disappointingly, regression of epithelial tumors following such treatment has been a rare event[6–8]. Nevertheless we may decrease the level of immunosuppressive therapy in patients with highly malignant, or extensive, or advanced cancers, in the hope that the immune system may recover and aid in their destruction. However, such treatment carries the risk of allograft rejection with return of renal allograft recipients to dialysis therapy, but CT recipients may die of this complication.

In individuals needing cytotoxic therapy for widespread cancers we must remember that most agents depress the bone marrow[6–8]. It is therefore prudent to stop or reduce azathioprine dosage during such treatment, to avoid severe bone marrow toxicity. As most cytotoxic drugs have immunosuppressive side-effects, satisfactory allograft function may persist for prolonged periods. Treatment with prednisone may be continued, as it is an important component of many cancer chemotherapy protocols.

As lymphomas are the single most common group of tumors in CT recipients, special mention is made of their management. In suitable patients with localized disease, excision or radiotherapy may suffice. In other patients with localized lesions, or in those with widespread disease, immunosuppressive therapy should be reduced, or even stopped (except for a small maintenance dose of prednisone) in patients who are seriously ill. As most lesions are associated with EBV infection the patients should be given acyclovir. If no improvement occurs, α-interferon should be administered. Failure to cause complete regression is an indication for using chemotherapy. As the patients are already heavily immunosuppressed, these agents should be used with caution as some patients have died of overwhelming infections following their use. It may be wise to reduce dosage of the drugs to 67–75% of that used

in non-immunosuppressed patients, and to restrict the number of courses given if the patient responds satisfactorily. Monoclonal anti-B cell antibodies have been used successfully to treat some B cell lymphomas but, apparently, these are no longer being manufactured.

Etiology of *de novo* malignancies

The causes of cancers in organ transplant recipients are not known, but a complex interplay of multiple factors is probably responsible[6–8,14,22,23,26,31]. A major role can be assumed for impaired immunity. Oncogenic viruses are also important, and may account for the short 'latent period' between transplantation and the development of many tumors. Other factors that may play a role include possible carcinogenic effects of some immunosuppressive agents; possible synergism of these agents with environmental carcinogens such as smoking, sunlight, radiation, food additives, etc.; the administration of other potentially carcinogenic drugs to some renal transplant recipients (such as diphenylhydantoin or isoniazid); and genetic susceptibility or resistance to certain types of neoplasia.

COMMENT

Although cancer is a complication of transplantation, one must stress that the great majority of organ transplant recipients do not develop this problem. Transplantation of malignancies can nearly always be avoided by careful selection of donors. CT transplantation should be considered in patients who were previously treated for cancers, except for those who have a short life expectancy from their tumors. Many patients who develop *de novo* cancers after transplantation have readily treatable malignancies of the skin, uterine cervix, or vulva and perineum. However, with the limited experience gained thus far, CT recipients appear to be more prone to develop potentially life-threatening malignancies, particularly lymphomas, related to the more intense immunosuppressive therapy that they require compared with renal transplant recipients.

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Long-term Management and Late Complications of the Thoracic Organ Transplant Recipient

D.P. NELSON, N.K. IMES AND D.K.C. COOPER

INTRODUCTION

The care of thoracic transplant patients centers on the use of immunosuppression to balance rejection (Chapters 8–10) against infection (Chapter 11), and on the management of these two major complications when the balance achieved is uneven. Primary care delivery to these patients is accomplished in the context of the unique toxicities and drug interactions of the immunosuppressive agents used (Chapters 8 and 10). Although some management issues directly relate to surgically altered anatomy, most are the consequence of the drugs that permit the coexistence of self with non-self.

ARRHYTHMIAS

Bradycardia

Bradycardia occurs within the first 2 weeks postoperatively in about 20–40% of heart transplant (HTx) patients, but only 5–15% of heart recipients will require a permanent pacemaker[1–6]. Approximately 60% of bradycardias are related to sinus node dysfunction and 40% to AV block[5]. Infrequently, bradycardia may be related to atrial fibrillation with a slow ventricular response[4].

Bradycardia may be caused by rejection, pretransplant amiodarone administration, ischemic-preservation injury, and surgical injury to the sinus node, conduction system or sinoatrial blood supply. Although not highly sensitive or specific for rejection, the correlation of bradycardia with rejection is great enough that cardiac biopsy should be considered when it occurs. Late bradycardias (beyond 5 months post-transplant) have been reported in about 1.5% of patients, and half of these were associated with cardiac rejection[4].

Oral administration of slow-release theophylline has been used to reduce the incidence of pacemaker placement[7], but not everyone has enjoyed success with this approach. Oral terbutaline has been used in at least one case to convert a hemodynamically significant junctional rhythm to sinus rhythm[8].

Atrial fibrillation or flutter

These occur in about 25–50% of cardiac recipients and, although relatively insensitive for rejection, are moderately specific and their identification should generally result in cardiac biopsy[9,10]. In one study almost 80% of atrial flutter episodes were associated with rejection, but only about half of these were moderate or severe[9]. Histologic resolution of rejection following immunosuppressive therapy sometimes fails to convert the patient to normal sinus rhythm, but atrial overdrive has been successfully used for conversion in this setting[9].

Although originally it was not contemplated that digoxin would have a significant beneficial effect on slowing or converting atrial fibrillation or flutter, it is generally found to be helpful. Calcium channel blocking agents and quinidine may also need to be employed.

Ventricular arrhythmias

These are common early after HTx, but are not generally predictive of rejection[10].

Atrial dysrhythmias

Atrial dysrhythmias can develop following lung transplantation (LTx), as after any major pulmonary operative procedure, but can usually be controlled adequately with digoxin or calcium channel blocking agents.

LATE SURGICAL COMPLICATIONS

Pericardial effusion

Although pericardial effusions are common and usually benign sequelae to cardiac surgery, little has been published regarding their occurrence following HTx. Effusions usually occur within the first 3 postoperative months. Large effusions are relatively uncommon, although tamponade should always be considered in the

differential diagnosis of early postoperative hypotension, independent of effusion size. Both acute and chronic rejection have been associated with the development of pericardial effusions, but small effusions that do not increase are usually insignificant[11,12].

Persistent inguinal lymphocele

If the femoral vessels have been used for access for cardiopulmonary bypass, then a persistent lymphatic leak may result in the inguinal–femoral region. Although such lymphatic leaks are uncommon following routine heart surgery, they appear to be significantly more frequent following HTx. This is almost certainly related to the effect of corticosteroid therapy on wound healing. Lymph collects beneath the groin incision, forming a fluctuant, non-inflamed mass.

Although it can be repeatedly drained by syringe and needle, a permanent solution almost always requires a further operative procedure. Goldstein *et al.*[13] have described a rectus femoris flap repair, which combines ligation of obvious lymphatic fistulae with a muscle transposition to increase vascularity and to fill the dead space created by the lymphocele. We have preferred a technique in which the sartorius muscle rather than the rectus femoris is immobilized and utilized (E. Dalton, unpublished). Rotating the sartorius muscle into the defect is efficacious, is technically easier, and leads to no apparent loss of muscular function or strength in the lower extremity. The technique has been elegantly described by Mathes and Nahia[14].

COMPLICATIONS OF ENDOMYOCARDIAL BIOPSY

Cardiac perforation occurs in less than 1% of endomyocardial biopsies performed on HTx recipients[15]. The biopsy complication rate of 3% reported by one group using the jugular vein approach was predominantly a result of venous puncture (e.g. carotid puncture and bleeding)[16]. The elimination of 'major' complications has been reported by using the femoral vein approach, utilizing a catheter designed specifically for this purpose[17]. Ultrasonographically guided biopsies (in place of fluoroscopy) slightly improved safety and sampling in one study[15], but few operators have experience using this approach, despite its additional advantages of mobility (bedside biopsy) and elimination or radiation exposure. The selection of biopsy approach should depend on the patient's anatomy and level of hydration, and the personal preference of the physician performing the procedure.

Coronary-artery-to-right-ventricle fistulae have been angiographically documented in about 5–15% of HTx recipients and are thought to result from cardiac biopsies[18–21]. They are, however, generally clinically insignificant due to the small shunt volume.

Although the tricuspid valve may be damaged by the bioptome during the biopsy procedure, the incidence of tricuspid regurgitation does not appear to correlate with the number of cardiac biopsies performed[22].

Atrioventricular valve regurgitation

Tricuspid regurgitation is commonly seen immediately post-HTx. At about 5 months post-transplant, mild or moderate tricuspid regurgitation is seen in about 25% and 12% of recipients, respectively[23]. Mild mitral regurgitation occurs in about 20% of patients by the fifth postoperative month[23]. The incidence of tricuspid regurgitation increases to about 40% by 1 year post-HTx and to about 60% at 2 years[22]. Post-HTx tricuspid regurgitation has shown an association with the presence of pre- and postoperative pulmonary hypertension[24]. These valvular regurgitant problems, however, are usually hemodynamically insignificant[22].

COMPLICATIONS OF TRANSBRONCHIAL LUNG BIOPSY

Patients who receive lung or heart–lung allografts will probably undergo multiple bronchoscopies and transbronchial lung biopsies. Serious complications are unusual, but abnormalities of gas exchange are common during the procedure[25–28]. Transient hypoxemia, hypercapnia and hypotension during bronchoscopy can generally be satisfactorily tolerated by the patient, and the use of agents such as flumazenil and naloxone to reverse the effects of conscious sedation further add to patient safety.

Performance of transbronchial biopsies is associated with a greater risk of serious complication than is routine bronchoscopy, including: (a) perforation of the lung with subsequent pneumothorax, (b) excessive bleeding, and (c) dissemination of infection.

In early series, pneumothorax was reported to occur in as many as 5.5% of transbronchial lung biopsies[26,28]. However, pneumothorax did not occur in a series of 200 consecutive procedures reported by Trulock *et al.* in the evaluation of lung transplant recipients[29]. Post-bronchoscopy chest radiography or fluoroscopy to check for the presence of a pneumothorax is mandatory, since these patients may tolerate even a relatively small pneumothorax poorly, and may require chest tube placement to allow lung expansion.

Excessive bleeding is uncommon during transbronchial lung biopsy, but may be life-threatening. Patients with severe pulmonary impairment clearly tolerate bleeding particularly poorly. Emergency intubation and possibly balloon tamponade of a bleeding lung segment may be necessary to control post-biopsy hemorrhage[28,30]. When large numbers of biopsies are taken during a single examination, the incidence of bleeding appears to increase. Scott *et al.*[31] reported that 27 of 219 patients had 100 ml of bleeding or more when an average of 17.3 transbronchial biopsies were obtained. Preoperative screening for coagulopathy should be carried out in all patients who are to undergo transbronchial lung biopsy, and should include protime, partial thromboplastin time, platelet count, and any other studies suggested by preoperative evaluation.

The incidence of dissemination of infection within a transplanted lung is difficult to assess. Trulock *et al.*[29] reported a 2% incidence of pneumonia in their series of transplant patients undergoing transbronchial biopsies. Our policy has been to give broad-spectrum antimicrobial therapy to patients who have suspected infection, or who demonstrate grossly purulent secretions in the airways. More specific therapy is prescribed once the culture and sensitivities of the bronchoscopy specimens are reported.

NEPHROTOXICITY

The most important factor in the post-transplant development of nephrotoxicity in thoracic transplant patients is cyclosporin (CsA)

therapy. The pathophysiology is characterized by intrarenal afferent arteriolar vasoconstriction mediated by altered vascular responsiveness to vasodilators and vasoconstrictors, and perturbations in the balance between these vasoactive mediators, favoring constriction. These vasoactive mediators may be of endothelial, platelet, or other origin, and include endothelin, thromboxane A_2, nitric oxide, serotonin and prostaglandin. Renal tubular injury may also contribute to renal insufficiency[32–39]. Acute nephrotoxicity is dose-related and reversible, but chronic nephrotoxicity appears to be neither.

Chronic maintenance CsA therapy results in a decline in renal function that has been characterized as biphasic, with the initial decline occurring more rapidly over the first 6–24 months post-transplant, and a subsequent decline occurring more slowly thereafter[40–42]. Although reduction of CsA target levels beyond 18 months post-transplant has been correlated with increased rejection without improvement in renal function in heart recipients[43], it seems possible that the reduction of CsA administration which conventionally occurs in most programs during the first postoperative year is contributory to the stabilization of renal function beyond 6 months post-transplant. The observation that late renal function stabilizes, and that further reductions in CsA administration may result in rejection, but not improved renal function, has also been reported in renal transplant patients[44,45].

In HTx recipients, however, impaired cardiac function from acute rejection should always be excluded as a cause of decreasing renal function.

In 1990 the Pittsburgh group reported a 1.5% incidence of end-stage renal disease by 18 months post-transplant in their heart transplant population[40], results which are comparable to our own experience. The 6% incidence of renal failure reported in LTx patients[41] correlates well with the early experience following HTx nearly a decade earlier[39]. It is reasonable to expect that the renal failure rate in LTx patients will decline with experience, as it did with HTx.

Early postoperative CsA nephrotoxicity can be ameliorated in the patient with (or at risk for) acute renal failure by reducing or eliminating CsA dosing and employing antithymocyte globulin or OKT3 therapy instead, particularly during the first 14 postoperative days. However, this approach has its potential complications. (Polyclonal or monoclonal antithymocyte globulin use beyond the first 7–14 days has been associated with an increased incidence of post-transplant lymphoproliferative disease and xenosensitization, often resulting in graft loss from vascular rejection.) In our experience, if renal function compatible with maintenance CsA administration has not been achieved with a CsA-sparing protocol by 7–10 days post-transplant, such improvement often does not subsequently occur.

Less impaired renal function has been seen in renal transplant patients receiving omega-3 fatty acid therapy[46], but this benefit may result from reduced rejection, rather than from the effects of suppressed eicosanoid synthesis on CsA-induced nephrotoxicity. Cilastatin, which currently is available only in combination with imipenem, has been reported to reduce CsA nephrotoxicity during the first postoperative week[47], but experience is limited. The mechanism of protection has yet to be defined, but may be related to impaired tubular cell uptake of CsA. The early reports of reduced CsA nephrotoxicity with the use of prostaglandin E-series agents[48] have unfortunately not been supported by subsequent studies[49,50].

Pentoxifylline has been shown to improve renal function in bone marrow transplant recipients receiving amphotericin and CsA[51], and in animal studies has been shown to reduce CsA nephrotoxicity[52]. Possible mechanisms may include impairment of TNF m-RNA transcription, and reduced release or action of interleukin-2. In our own experience we have not found pentoxifylline to be helpful. The continuous intravenous infusion of CsA, particularly during the early post-transplant period, may offer some protection from nephrotoxicity by the elimination of peak CsA levels. Although this approach is not well established in the literature, our experience with it has been modestly favorable.

Because CsA nephrotoxicity results in the hyperexcretion of creatinine, serum creatinine level and creatinine clearance may overestimate the true glomerular filtration rate[53]. Several other tests of renal function, which are more accurate than creatinine clearance in CsA-dependent patients, have been identified and include the inulin and ^{99}mTc-DTPA tests, and urographic contrast media clearance methods[54,55]. While these methodologies are important in studying the comparative effects of various interventions on renal function, we have not found them practical or cost-effective in the routine clinical management of thoracic transplant recipients.

SYSTEMIC HYPERTENSION

Essential hypertension commonly predates transplantation, particularly in HTx candidates. The fluid-retaining properties associated with steroid administration further contribute to this problem post-transplant, but the major potentiator of post-transplant hypertension is the use of CsA. The incidence of hypertension in extrarenal organ transplant recipients approaches 90%[56–58].

As described in the discussion of CsA-induced nephrotoxicity, preglomerular vasoconstriction occurs at the level of the afferent arteriole through a variety of proposed mechanisms, and results in increased salt and water reabsorption by the proximal tubule. These latter effects, combined with the enhanced peripheral and renal sympathetic activity elicited by CsA, are thought to play an important role in the pathogenesis of CsA-associated hypertension[59].

Weight control, sodium restriction, and exercise play an important role in the management of hypertension in thoracic transplant recipients, just as they do in the general population. Renal magnesium loss (a consequence of CsA administration) may further potentiate hypertension. Normalization of serum magnesium levels with magnesium supplementation should, theoretically, contribute to improved blood pressure control, although to our knowledge no correlation between serum magnesium level and blood pressure has been reported.

Protocols for the treatment of post-transplant hypertension vary considerably from center to center. Our first therapeutic agent of choice is a calcium channel blocker. These agents inhibit afferent arteriolar vasoconstriction. Dihydropteridine calcium antagonists, other than nicardipine (isradipine, nifedipine, nitrendipine, and fleodine) do not interfere with CsA metabolism, but diltiazem and verapamil both interfere with the cytochrome P450 enzyme system metabolism of CsA, resulting in higher CsA levels. This latter effect has been intentionally used to reduce CsA dosages and thus defray costs[60], but may also result in elevation of the

serum creatinine. Diltiazem and verapamil may also have negative inotropic and, in the case of verapamil, chronotropic effects which may be important in some patients. For these reasons (impaired CsA metabolism and negative inotropic effect), we prefer using the dihydropteridine calcium blockers.

Angiotensin-converting-enzyme (ACE) inhibitors vasodilate the efferent glomerular arteriole, and in conditions of preglomerular vasoconstriction may result in a decline in glomerular filtration rate. At least one study has shown a significant improvement in renal function when hypertensive heart transplant recipients were converted from ACE inhibitors to calcium channel blockers[61]. ACE inhibitors have the additional disadvantage of causing hyperkalemia (adding to the CsA-associated hyperkalemia that not infrequently occurs), and sometimes cause a troublesome cough.

Diuretic administration for the control of peripheral edema has the added advantage of being adjunct therapy for hypertension. In our experience, thiazides have generally been less effective than loop diuretics. Overzealous diuretic use, however, will lead to intravascular depletion with resulting impaired renal function. Caution is therefore recommended when using these agents. If there is no edema or other evidence of fluid overload, diuretics should probably not be added as the second therapeutic agent for hypertension that is refractory to calcium channel blocker therapy.

We have found that the use of beta-receptor antagonists in HTx recipients commonly results in fatigue and decreased exercise tolerance. Others have noted a marked reduction in renal blood flow associated with their use[62]. These effects reduce the suitability of beta-receptor antagonists for the management of hypertension in thoracic organ transplant patients. Furthermore, their negative inotropic effect is unwelcome, particularly in patients with heart transplants.

In patients whose hypertension is refractory to calcium channel blockers with or without diuretics, and when a second hypertensive agent is required (which is not uncommon), we usually add a central alpha-receptor stimulator, such as clonidine, or a peripheral alpha-receptor antagonist, such as prazosin. HTx recipients (whose heart is vagotomized) may experience significant orthostatic hypotension with sympatholytics, particularly peripheral alpha-antagonists. We have, nonetheless, enjoyed some success with long-acting transdermal clonidine patches in those patients who demonstrate poor compliance with oral medications.

A loss of the usual nocturnal decline in blood pressure has been demonstrated in HTx recipients[63], and early-morning hypertension is commonly observed in patients who may remain relatively normotensive throughout the remainder of the day (following their morning antihypertensive drug therapy). Without the employment of nocturnal continuous blood pressure monitoring it is difficult to assess the duration or severity of hypertension in such patients. Our approach has been to employ evening antihypertensive dosing when an individual's blood pressure is clearly trending up by bedtime, and/or the early-morning blood pressure consistently demonstrates marked elevation (e.g. diastolic >105 mmHg). The role of ambulatory blood pressure monitoring in solid organ recipients is poorly defined, but when normative data with such monitoring are better established, such monitoring may facilitate improved management of nocturnal, early-morning, or activity-associated hypertension.

Supine hypertension with orthostatic hypotension is sometimes encountered in HTx recipients. We have attempted to manage this state with small (30 mg) doses of diltiazem, which may be effective because of the increase in intravascular volume associated with this therapy. Effective treatment of the supine (or sitting) hypertension is sometimes complicated by an increase in the orthostatic hypotension, although this fortunately generally resolves with time.

GASTROINTESTINAL COMPLICATIONS

It has been reported that approximately one-third to one-half of HTx recipients experience gastrointestinal (GI) complications, and one-third to one-half of these (or about 10–20% of the total) require some form of abdominal surgery[64-67]. Upper GI disease, such as esophagitis, gastritis, duodenitis, and peptic ulceration, is more frequent than lower GI disease, which includes diverticulitis, various types of hernia, perirectal abscess, anal fissure, and thrombosed hemorrhoids[68,69]. Cholelithiasis is common in HTx recipients, but there exists significant variability in the reported incidence of cholecystitis in these patients. Pancreatitis also occurs with variable frequency, but today is generally reported in <5% of patients (see below).

Reports of the GI complications associated with LTx are too limited to-date for meaningful comparison with the HTx experience, but similarities between these populations are anticipated. Procedure-specific surgical complications (e.g. omentopexy-associated morbidity in LTx patients) may, of course, occur.

Most GI problems resulting in the need for hospitalization or surgery develop during the first 3 postoperative months[66,70], which suggests that high-dose immunosuppression may play a role in their pathogenesis.

Pathogenesis

Pharmacologic immunosuppression, which is almost always more intense during the first 90 days post-transplant, potentiates infectious GI complications, such as CMV-associated enteropathies and herpes simplex virus (HSV) or candidal upper GI disease (Chapters 32 and 57). Immunosuppressive agents also have direct GI toxicities.

CsA and azathioprine can both be hepatotoxic, CsA is known to be lithogenic, OKT3 commonly causes diarrhea, and steroids may promote upper GI ulceration and possibly diverticular perforation. Steroid-sparing immunosuppressive protocols have been reported to offer some protection from significant GI complication. Merrell *et al.*'s study in Utah[70], where aggressive steroid tapering took place, reported that all HTx patients experiencing major abdominal complications were on steroids; no steroid-free patient developed such morbidity. Furthermore, one-third of the major abdominal complications were directly associated with a course of steroid pulse therapy. Of the newer agents, tacrolimus (FK506) may cause motility disturbances, and mycophenolate mofetil may cause nausea and exacerbate acid-peptic conditions.

Hepatotoxicity

Hepatotoxicity is common before and after thoracic organ transplantation. Passive congestion of the liver often predates trans-

plant, and pre-existing injury from alcohol abuse may also be present. Postoperative liver disease may result from drugs (e.g. CsA, azathioprine, or antilipemic agents), infection (usually viral, particularly CMV or hepatitis viruses) or cholecystitis. Azathioprine is perhaps the most common agent to cause disturbances of liver function, but these are generally mild and respond to reduced dosage or discontinuation of the drug.

Upper GI disease

Upper GI disease is usually secondary to sterile acid-peptic disease or infection by CMV, *H. pylori*, *C. albicans*, or HSV. Surgical complications, such as injury to the vagus nerve (resulting in delayed gastric emptying) or diaphragmatic hernia (associated with the use of omental wrap in LTx[71]), may contribute to symptomatology.

CMV disease involving the esophagus, stomach or duodenum may present with fever, dysphagia and/or epigastric pain, or even hemorrhage and/or perforation. Oropharyngeal CMV involvement is uncommon[72–74]. Nausea and bloating may result from CMV-induced impairment of gastric emptying[75]. The diagnosis is facilitated by the endoscopic appearance of lesions and biopsy demonstration of inclusion bodies. CMV is frequently present in the absence of symptoms. In one study of renal transplant recipients[76], almost half of the gastric and duodenal biopsies showed CMV inclusions even though these patients were asymptomatic. Diagnosis by shell vial assay or culture of biopsies is strongly supportive, but contamination by ingestion of salivary-shed virus is possible.

Intravenous ganciclovir therapy for 2–3 weeks is usually effective, although relapse is not uncommon. Ganciclovir resistance may emerge in the future, particularly with the anticipated increased use of the drug with the recent introduction of oral ganciclovir.

In severe infection, or when heavy immunosuppression is being utilized or is anticipated (e.g. during a rejection episode), the addition of intravenous immunoglobulin therapy, particularly of hyperimmune CMV globulin, is probably beneficial.

Helicobacter pylori infection is considered by some to be the most common cause of peptic ulcer in the general population[77], but its role is still not yet well defined in transplant recipients. Endoscopic sampling of 33 randomly selected renal recipients found a 40% incidence of *H. pylori* in the gastric antrum[78]; all patients positive for *H. pylori* were symptomatic. Given the known propensity of immunosuppressed patients for lymphoma (post-transplant lymphoproliferative disease), the recent report of a possible association between *H. pylori* infection and gastric B-cell lymphoma[79] warrants attentive management of *H. pylori* infections. *H. pylori* is diagnosed by the CLO test (*Campylobacter*-like organism, Delta West Ltd) on biopsied gastric mucosa. Various treatment regimens for this infection have been successfully employed. A 2-week course of a combination of bismuth, tetracycline, ampicillin, metronidazole (Flagyl), and an H-2 blocker has proved successful at our own center. Ampicillin is omitted in patients allergic to penicillin.

Although *esophageal candidiasis* and *herpes simplex esophagitis* are common sequelae of immunosuppression, the incidence of these problems has markedly declined in heart and lung recipients with the widespread use of nystatin and acyclovir prophylaxis. Nonetheless, a recent report suggests that intense antacid treatment with omeprazole (Prilosec) may result in esophageal candidiasis[80]. Both fungal and herpetic infection can occasionally develop during aggressive treatment for rejection despite prophylactic regimens or when prophylaxis has been discontinued. The choice of treatment of these infections (generally a choice between fluconazole and amphotericin for *Candida* and intravenous and oral acyclovir for herpes) is based on a variety of factors such as the patient's overall condition, the elapsed time since transplant, renal function, site and severity of the disease, actual or anticipated immunosuppressive therapy, and venous access.

Cholelithiasis

Approximately one-third of cardiac recipients have been shown to have cholelithiasis[81]. Between 6% and 60% of those cardiac recipients known to have asymptomatic cholelithiasis pretransplant will become symptomatic some time post-transplant[81,82]. Seventeen percent of those without gallstones pretransplant will develop them post-transplant[82].

Our current therapeutic approach is to consider pretransplant candidates with *symptomatic* gallbladder disease for pretransplant elective laparoscopic cholecystectomy, weighing the risks of their cardiopulmonary condition against the significant risk of emergency abdominal surgery in the post-transplant setting. Patients with *asymptomatic* stones identified pre- or post-transplant do not undergo laparoscopic cholecystectomy until the development of symptoms.

Oral therapy with ursodeoxycholic acid may dissolve non-calcified stones, but the mobilization of such stones following size reduction may result in common bile duct obstruction. The use of this agent has also been reported on at least one occasion to markedly increase the CsA level in the blood secondary to enhanced absorption[83]. We have used ursodeoxycholic acid in several patients for drug-associated cholestasis with only occasional, modest success, but with no adverse effects.

Pancreatitis

Clinically evident pancreatitis is reported to occur in 2–18% of HTx recipients[65,67,70,84]. The incidence and severity of disease appear to vary between centers. Subclinical pancreatitis may be fairly common. Aziz *et al.*[68] reported a 6% incidence of pancreatitis identified at autopsy in heart and heart–lung recipients (without clinical evidence of pancreatitis) dying of other causes. Fernandez and Rosenberg[85], however, found autopsy evidence of clinically unsuspected pancreatitis in 35% of kidney transplant recipients. Possible etiologic factors include cholelithiasis, hyperlipidemia, CMV infection, drug toxicity (CsA, azathioprine, cyclophosphamide, steroids, sulfonamides, tetracycline, furosemide, ethacrynic acid, phenformin, and procainamide)[86], and recent cardiovascular surgery.

The relationship between cardiopulmonary bypass (CPB) and pancreatitis has been thought in part to be perfusion related, but Fernandez-Del Castillo *et al.*[87] demonstrated the perioperative administration of calcium chloride (in dosages exceeding

800 mg/m^2 of body surface area) to be an independent predictor of pancreatic cellular injury in patients undergoing CPB. The comparable incidence of pancreatitis in heart and kidney transplant recipients suggests that CPB may play a relatively small etiologic role[85,88–90].

The diagnosis, prognostic indicators and treatment of pancreatitis are well described in a recent review[91]. Contributing factors (including drugs) should be eliminated or ameliorated, if possible. The temporary conversion of oral CsA administration to a continuous intravenous infusion may improve the pancreatitis, presumably by the elimination of high peak CsA levels.

Colonic perforation

There is scant material in the literature focusing specifically on colon perforation in heart or LTx recipients. Colon perforation has been reported in <2.5% of HTx recipients[70]. Despite its overall low incidence, it did represent 12% of major 'abdominal complication' described in one study[70].

This subject has, however, received more attention in the renal transplant literature[91–94], which has reported a possible decline in the incidence of colonic perforation in the CsA era, thought to be related to a reduction in steroid use. The postulate is that steroids cause lymphoid atrophy, gut wall thinning, decreased mucosal epithelial replacement, and decreased fibroblast reparative activity. Furthermore, when a perforation occurs, an impaired inflammatory response prevents abscess formation.

Risk factors for colon perforation identified by Church *et al.*'s review of the literature[92] include renal failure, ischemia, fecal impaction, and steroid therapy. A small percentage of perforations reported in the renal transplant literature were secondary to Ogilvie's syndrome (non-obstructive colonic dilatation), but by far the majority were related to perforated diverticula. The etiology of Ogilvie's syndrome is unknown, although it tends to occur in critically ill and/or uremic patients; it commonly responds to colonoscopic decompression. Diverticular perforation usually occurs in the left hemicolon, whereas Ogilvie's syndrome is usually on the right.

The diagnosis of *diverticulitis* may be confounded by maintenance steroid administration. Vague complaints of generalized abdominal, deep pelvic or lower quadrant (especially left) or even flank pain should arouse suspicion of diverticulitis, pyelonephritis, nephrolithiasis, or adnexal disease. Radiation of discomfort to genitalia or inner thighs may also occur in any of these conditions. Coexisting diarrhea suggests a colonic etiology. Physical signs may be unimpressive, and fever and leukocytosis may be absent or, if present, unhelpful in narrowing the differential diagnosis. CMV colitis and other enteric pathogens (e.g. *Clostridium difficile*, *Bacillus cereus*, *Salmonella*, etc.) must be excluded in patients with diarrhea. Computerized tomography of the abdomen with infusion may be helpful in making the diagnosis.

Free perforation into the peritoneum from any etiology is a surgical emergency. Empiric perioperative antibiotic selection should be based on a variety of considerations, including the local incidence of *Bacillus fragilis* resistance, a history of penicillin allergy, which would also exclude imipenem–cilastatin (Primaxin) use, current renal function, and recent antimicrobial use. The results of intraperitoneal cultures should be aggressively sought, and special attention given to the identification of enterococcal or *Candida* growth. Serial postoperative abdominal computerized tomography scans are commonly obtained to exclude the development of a phlegmon, which would require surgical drainage.

Diverticulitis without perforation should be treated by hospitalization and intravenous antibiotics, but consideration should be given to elective hemicolectomy, particularly with recurrent disease.

Emergency surgery for GI complications

Emergent abdominal surgery has been associated with a 71% mortality rate in one study, while elective procedures in the same study resulted in no mortality[65]. Most emergent surgeries are carried out for upper and lower GI perforations, and are infrequently performed for GI bleeding. Surgery for acute cholecystitis was problematic in some programs several years ago, but has become less so in recent years, probably because of the advent of laparoscopic cholecystectomy, which has also enabled elective surgery to be performed with low morbidity before such complications develop.

The presence or absence of free perforation may be extremely difficult to confirm without surgery, especially in patients on maintenance steroids, yet it is important not to delay surgery unduly. The Pittsburgh group's experience[94] demonstrated the timing of surgery for colonic perforation to be critical. If surgery was performed within 24 hours of perforation, the patient survival rate was 86%. If surgery was delayed for more than 24 hours following perforation, survival was only 25%.

HYPERLIPIDEMIA

Hyperlipidemia is a common complication of thoracic organ transplantation, and both steroid and CsA use have been shown to contribute to this problem. Although one study failed to show an association between pre- and post-transplant hyperlipidemia[95], another found post-transplant hyperlipidemia to be associated with pretransplant coronary artery disease[96]. Hyperlipidemia usually peaks by 3–6 months post-transplant[95,97]. Although both CsA and steroids have been reported to elevate both total and LDL cholesterol, triglyceride elevation has been inconsistently ascribed to either agent[98,99]. Cumulative prednisone dose has been reported to be a stronger independent predictor of elevated total and LDL cholesterol in HTx recipients than cumulative CsA dose[98].

Although graft vasculopathy in HTx recipients is thought to be primarily immunologic in origin, elevations in cholesterol and triglycerides have each independently been associated with transplant vasculopathy in a few reports. The consequences of immunologic vascular injury may be potentiated in a hyperlipidemic milieu. Treatment of hyperlipidemia must therefore balance the undefined role of hyperlipidemia in graft vasculopathy (combined with the extracardiac vascular risks of hyperlipidemia) against the known toxicities of pharmacologic intervention.

Antilipemic agents other than the 3-hydroxy-3-methylglutaryl coenzyme A (HGM-CoA) reductase inhibitors have risk/benefit characteristics which may limit their utility in thoracic organ

transplant recipients. Bile-acid-binding resins, such as cholestyramine, may interfere with CsA absorption and elevate triglycerides[99]. Nicotinic acid is hepatotoxic (as are CsA and azathioprine) and may contribute to the glucose intolerance already associated with steroid use. Time-released nicotinic acid reduces the skin-flushing experienced with the drug's use, but increases the risk of hepatotoxicity[100]. Gemfibrozil, a fibric acid derivative, is more useful than HMG-CoA reductase inhibitors in the treatment of hypertriglyceridemia, but is less effective in the treatment of elevated total or LDL cholesterol[101]. Fibric acid derivatives have been associated with cholelithiasis[99], which is of concern because of the increased incidence of cholecystitis in HTx patients. Because fibric acid derivatives and their metabolites are eliminated in the urine, their use in patients with CsA nephrotoxicity is limited.

HMG-CoA reductase inhibitors, such as lovastatin, simvastatin, and pravastatin, are probably comparably effective antilipemic agents, and are our agents of choice for hypercholesterolemia[101-104]. When these drugs are used in combination with other antilipemic agents (nicotinic acid or gemfibrozil), or used in high doses (lovastatin >20 mg daily or equivalent), there is a marked increase in risk of myositis, which may result in rhabdomyolysis[105,106].

Myositis in this population may result from CsA-induced changes in antilipemic drug metabolism, which has been shown to result in increased lovastatin enzyme inhibitor levels[102]. A commercial manufacturer of simvastatin (Merck) has recommended not exceeding a daily dose of 10 mg in patients taking CsA.

Kobashigawa *et al.*[103] have shown that pravastatin is not only effective and safe in lowering cholesterol levels after HTx but would also appear to have some immunosuppressive effect. When begun early (within 2 weeks) after HTx, at an initial dose of 20 mg/day (being increased to 40 mg/day if tolerated), it has been shown to be associated with less frequent cardiac rejection accompanied by hemodynamic compromise, improved patient survival, and a lower incidence of graft vasculopathy as determined by angiography and at autopsy. The exact mechanism whereby these beneficial effects are produced remains uncertain, but the cytotoxicity of natural killer cells was demonstrated to be lower in patients receiving pravastatin than in control patients.

OSTEOPOROSIS

The reported incidence of vertebral compression fractures in HTx recipients ranges from 1% to 44%, with most studies reporting an incidence >25%[107-111]. The program reporting the lowest fracture incidence[107] treated 38% of its patients with calcitonin, and the primary HTx physician at the time was a practicing endocrinologist who was particularly attentive to hormonal therapy (52% of the men received testosterone and 92% of the women received estrogen). The vertebral fracture incidence after HTx is comparable to that reported after liver transplantation (29%)[112]. Comparison with renal transplant populations is complicated by the occurrence of renal osteodystrophy in renal transplant patients.

Although some have reported a lower mean bone (mineral) density in those with fractures than those without, bone density has proven to be an unreliable predictor of fracture in heart and kidney transplant patients[107-111,113]. Patients with low bone densities are commonly free of fractures, and vertebral compression fractures may occur in those with normal vertebral bone densities. Nonetheless, despite its limitations, we believe that bone densitometry may be useful in these patients. No mineral biochemical predictors of fracture have been identified[110].

The pathogenesis of osteoporosis in thoracic organ transplant patients is primarily related to: (a) pre-existing osteoporosis antedating transplant and (b) the use of immunosuppressive agents post-transplant. The occurrence of osteoporosis in HTx candidates has been reported variably to be both common[107] and uncommon[110]. It is reasonable to presume that some thoracic transplant candidates have osteoporosis of multifactorial origin (hypogonadism, loop diuretics, inactivity, cachexia, cigarette smoking, anticoagulants, or steroid administration).

Steroid administration has direct effects on bone resorption and formation, and causes changes in: (a) calcium absorption from the gut, (b) gonadal secretion, and (c) vitamin D metabolism[114]. Steroids affect trabecular bone more than cortical bone, and vertebral bodies and ribs are the bones that most commonly suffer atraumatic fracture in steroid-dependent patients[114,115]. Although CsA can also adversely affect trabecular bone, the co-administration of CsA and steroids may be mutually protective because of the high and low bone turnover rates that result from CsA and steroid administration, respectively[116]. A recent study of bone and mineral metabolism in renal transplant recipients concluded that CsA did not significantly contribute to osteoporosis[117]. Tacrolimus, rapamycin, mycophenolate mofetil, azathioprine, and methotrexate have all shown effects on bone metabolism, which have not yet been exhaustively studied[116].

Although the occurrence of osteopenia in HTx recipients has been shown to correlate with the cumulative dose of steroids[110], the incidence of fractures has been shown to correlate with the number of rejection episodes rather than the cumulative steroid dose[109]. High-dose steroid administration may cause rapid bone loss resulting in fractures within weeks to months[118] and, because very rapid bone loss may result in micro-architectural abnormalities[119], it seems possible that high-dose steroids may result in fracture-prone architectural changes unappreciated by bone densitometry. Osteopenia has been reported to develop most rapidly in the first 6–12 post-transplant months[108,120].

Dual-energy X-ray absorptiometry (DEXa) appears to be the bone densitometry method of choice, and is preferable to either single or dual photon absorptiometry (SPA or DPA). Quantitative computerized tomography (QCT) is more expensive, is less precise, has greater radiation exposure, and is more time-consuming. However, it offers a truer measurement of cancellous density through three-dimensional imaging, and may avoid the posterior spine and aortic calcifications[119].

The treatment and prophylaxis of transplant-associated osteoporosis includes treatment of hypogonadism[107], avoidance of excessive thyroid replacement[121], and minimizing steroid administration. Although steroid elimination may expose the patient to the unopposed bone effects of CsA, the net effect of steroid reduction is almost certainly favorable to bone metabolism. Hormonal replacement is indicated in the oophorectomized or postmenopausal woman, and testosterone is probably beneficial in older men. Treatment of osteoporosis identified pretransplant seems prudent.

Treatment/prophylaxis with calcium supplementation, vitamin D administration, cyclic etidronate therapy, and calcitonin are common approaches. Although milk-alkali syndrome may result from calcium carbonate administration to cardiac recipients (especially those with renal insufficiency), it is the least expensive method of calcium supplementation if used with discretion[122]. Calcitonin has been successfully used to reduce bone loss in HTx patients, but is expensive[107]. Cyclic etidronate therapy has proven successful in other steroid-dependent populations[123], and a new biphosphonate, alendronate, appears particularly promising with continuous (non-cyclic) administration[124]. Experience with etidronate in patient with creatinine clearances <60 ml/min is limited, and calcitonin should be considered in these patients (S. Epstein, personal communication). Calcitonin and etidronate therapies have not been compared in the solid organ transplant population. It is unclear from existing publications whether the inclusion of 25-hydroxylated vitamin D with other therapeutic modalities is essential. The use of 1,25-hydroxylated vitamin D may be necessary in patients with advanced renal insufficiency, but caution should be exercised in use of this agent because of the risks of hypercalcinemia. Deflazacort has been reported to have less adverse effect on bone than prednisone[125], but experience with this agent in thoracic transplant recipients is still limited.

ASEPTIC BONE NECROSIS

Aseptic bone necrosis is known to occur in association with steroid administration, although its correlation with cumulative steroid dose has been poor. It has been reported to occur in 2.7–10% of HTx patients[109–126,127], but long-term survivors may demonstrate an even higher prevalence. Eighteen percent of 5-year survivors had required bilateral total hip or knee arthroplasties in one program[126]. The incidence in kidney transplant patients has varied from 3% to 41%[128,129] and has been reported in 10% of allogeneic bone marrow transplant recipients[130]. The mean time to diagnosis following HTx is reported to be 44 months[127], but the onset of hip pain has been reported to occur at a mean of 29 months post-transplant[126]. It develops earlier post-transplant in renal (12–22 months) and bone marrow (median of 12 months) transplant patients[130–133].

The incidence in patients with LTx is not yet certain, but is likely to be relatively high in view of the fact that many LTx recipients have received steroid therapy for treatment of their pre-transplant pulmonary condition.

Although a review of steroid-associated osteonecrosis found a correlation with mean daily steroid dose[134], this has been inconsistently demonstrated in individual studies. An association between rejection and osteonecrosis has been reported following heart[109] and kidney transplantation[129,132].

In those afflicted, a mean of 3.3 joints per patient were reported to be involved in one study, which also reported that 64% of these patients had hip involvement, 61% knee, 29% ankle, 21% shoulder, and 7% elbow involvement[130]. Half of the afflicted patients had bilateral hip involvement and half had bilateral knee involvement[130]. Clinical presentation is usually with pain, which may initially be episodic. Although disease is commonly bilateral, clinical presentation is often asymmetric. MRI is more sensitive than conventional radiologic imaging for the diagnosis of aseptic necrosis.

Non-surgical treatment, consisting of analgesics and reduced weight-bearing, is palliative; surgical therapy is frequently required[126,130].

HYPERURICEMIA AND GOUT

Hyperuricemia occurs in about 70–80% of HTx recipients[135]. Six percent of these patients have experienced gout pretransplant and 4–8% experience gout attacks post-transplant[135]. A history of pre-transplant gout is an unreliable predictor of post-transplant gout severity. Acute gout attacks occur at a mean of 17 months post-transplant[135]. Almost half of the patients with definite post-transplant gout have been reported to progress to polyarticular arthritis and/or tophaceous gout within a mean of 31 months[135]. Serious life-threatening joint infection may occasionally complicate gout in transplant recipients[136]. Although hyperuricemia does not correlate with CsA levels or renal insufficiency in HTx recipients[135] (comparable to the experience in renal transplantation at the same institution[137]), hyperuricemia is thought to result from CsA-induced reduction in renal urate clearance[138]. Hyperuricemia resulting from tacrolimus (FK506) administration is also thought to result from a change in renal urate excretion[139]. Diurectics (both loop and thiazide) may exaggerate hyperuricemia, and gout attacks may be precipitated by alcohol ingestion, binge eating, infection, or surgical stress.

Treatment and prophylaxis of gout in solid organ recipients is complicated because of drug toxicities and interactions. Treatment of the acute attack can be accomplished with colchicine, although both hepatic and renal insufficiency increase the risk of myelosuppressive and neuromyopathic toxicity with colchicine. Indomethacin may be effective, but may be nephrotoxic, myelosuppressive, and ulcerogenic. We have experienced modest success with surprisingly low doses of indomethacin (e.g. 25 mg p.o. every 8 hours for three doses and then every 12 hours for two doses). The use of maintenance prednisone may facilitate the success of this lower-than-usual indomethacin dosing. Care should be taken not to manipulate uric acid levels until the acute attack is resolved.

Allopurinol can be used to treat hyperuricemia, but its interference with azathioprine metabolism will result in severe myelosuppression if the azathioprine dose is not empirically reduced in concert with allopurinol administration. It has been recommended that the dose of azathioprine be reduced by 50–75% before initiation of low-dose allopurinol[136]. The risks of allopurinol administration may outweigh the benefits, and it is our general policy not to use allopurinol in our thoracic transplant recipients. The myelosuppressive effects of cyclophosphamide may also be increased by co-administration of allopurinol. Mycophenolate mofetil is not reported to interact with allopurinol, and may be an appropriate alternative to azathioprine in the patient with poorly controlled gout.

Probenecid uricosuric therapy for hyperuricemia is limited by the drug's relative ineffectiveness at glomerular filtration rates <30–50 ml/min[138]. If hyperuricemia is treated with allopurinol or probenecid, maintenance colchicine at 0.5 mg p.o. twice daily should probably be administered until the uric acid level is within normal limits, to prevent precipitation of an acute attack. Uncontrolled acute attacks very infrequently require an intra-articular steroid injection. Maintenance prophylaxis with

colchicine (0.5 mg p.o. twice daily), with careful monitoring for toxicity, should be considered in the patient with frequent exacerbations. A purine-rich diet should be avoided.

CHANGES IN SEXUAL ACTIVITY AND FUNCTION

There are a few data available on the incidence of impotence following heart or lung transplantation. In renal transplant series, however, impotence has been reported in 22–43% of male recipients[140]. Male potency is a complicated process, and the etiology of any impotency may be multifactorial.

Hypogonadotrophic hypogonadism occurs commonly immediately following organ transplantation or other open-heart surgery. We have documented reductions in serum testosterone from normal levels to levels at the lowest measurable limits in as few as 4 days following HTx. The duration of this hypogonadism appears to vary with the clinical status of the patient. For those who suffer repeated illnesses the hypogonadism will likely persist. Others who regain their vigor quickly may have relatively few weeks of hypogonadism. Older patients have a more prolonged period of hypogonadism. Impotence may last for only a few days or become permanent.

We now frequently provide testosterone supplementation therapy following cardiac or pulmonary transplantation, mostly because low levels may be an important contributing factor in the rapid loss of trabecular bone which occurs in many patients. Unfortunately, impotence may still occur, particularly in those with prolonged or frequent illness (such as from CMV infection) or psychological factors.

Erectile impotence, without loss of libido, may be reported by patients with extensive (diabetic or non-diabetic) atherosclerotic vascular disease or peripheral neuropathy. Relative impotence may occur during the period of weaning from a prolonged course of testosterone replacement therapy. Antihypertensive agents may also be a cause of impotence, but this can frequently be avoided by careful selection of the agent.

In contrast, post-transplant improved cardiac output or respiratory function may occasionally restore lost potency, and an increased sexual appetite may follow the increased capability and improved feeling of well-being.

DIABETES MELLITUS

We have not considered uncomplicated diabetes to be a contraindication to transplantation, though we have not accepted the patient with significant diabetic complications, such as microvascular disease or neurotrophic foot ulcers. Some degree of impairment of glucose tolerance is seen in virtually all patients at some point in the transplantation process. It is easy to overlook the presence of hyperglycemia in transplant patients since it is usually asymptomatic. In the post-transplant period, therefore, each patient must be willing to monitor his/her glycemia at home, keep records, and adjust therapy as prescribed. Certain patients will establish themselves as having a greater tendency towards hyperglycemia, and these patients should be monitored closely. Periodic monitoring of the glycosylated hemoglobin will help in identifying prolonged unacceptable hyperglycemia.

In its simplest form, hyperglycemia is seen in the first few days following transplantation during high-dose methylprednisolone therapy. It is seen again during the treatment of an acute rejection episode with methylprednisolone pulse therapy. In these circumstances the hyperglycemia usually lasts only a few days. Without treatment, plasma glucose levels >16.7 mmol/l (300 mg/dl) are commonly encountered. In the unproven belief that it is beneficial to maintain a near-normal metabolic milieu, we routinely monitor and treat this hyperglycemia with the goal of maintaining glucose levels closer to 5.6 mmol/l (100 mg/dl), and certainly below 8.3 mmol/l (150 mg/dl). Granulocyte chemotaxis may be impaired at higher plasma glucose levels.

Some patients will present for transplantation with familial type II diabetes. The degree to which they display this diabetes will depend on several factors, which include: (a) relatively body masses of muscle and adipose, (b) diet, and (c) activity. Their diabetes can often be controlled with proper diet and activity. These persons are profoundly affected by pharmacological doses of corticosteroids. Until the corticosteroid dose is reduced to physiological levels, or eliminated entirely, treatment for diabetes will be required. After reduction of corticosteroid dosage, those who follow the prescribed diet and activity instructions rarely require pharmacological treatment of their diabetes. Many patients, however, are unable or unwilling to follow such instructions, and require prolonged treatment, usually with oral agents.

At our own center we have transplanted very few patients with uncomplicated type I diabetes. They have done well, but have required large doses of insulin (<300 units daily) during the periods when they were taking corticosteroids in high or moderately high doses.

Treatment of diabetes in the transplant patient is, as in other diabetics, largely empirical. Home blood glucose monitoring is essential, and patients are encouraged to follow a program of diet and exercise. Those patients with relatively normal fasting blood glucose levels, but with postprandial hyperglycemia, are given glipizide, which rarely produces fasting hypoglycemia. Those with fasting hyperglycemia are treated with glyburide. When these agents alone prove insufficient, regular (soluble) insulin is added before some or all meals. Some patients also require intermediate-acting insulin. As the patients' overall health improves, and corticosteroid dosages are lower or discontinued, the insulin may be phased out, and the diabetes controlled with oral agents alone.

Good diabetic control is strongly encouraged. Our goal is to achieve preprandial glucose levels of 3.9–5.6 mmol/l (70–100 mg/dl) and 2 or 3 hours postprandial glucose levels of <8.3 mmol/l (150 mg/dl). Not all patients are sufficiently disciplined to achieve these goals. In long-term follow-up we monitor the glycosylated hemoglobin, the goal being to achieve levels of 7% or less. Levels of >8% are considered unacceptable. A glycosylated hemoglobin level of 9% would correspond approximately to a glucose level of 8.3 mmol/l (150 mg/dl).

GROWTH RETARDATION AND DELAYED ONSET OF PUBERTY

Before the introduction of CsA, because of the complication of growth impairment, many renal, liver and cardiac transplantation units were reluctant to accept children as potential recipients.

Growth retardation in children under the age of 12 or 13 could lead to such gross dwarfism that severe psychological problems ensued. The proportion of children who demonstrated normal growth following organ transplantation varied considerably from 13% to over 60% from one series to another[141,142].

Fortunately, it is now possible to immunosuppress some children without the need for steroid therapy (Chapters 9 and 38). In those that require steroids there is some evidence that the growth retardation effect is minimized by administering a double dosage of steroids on alternate days with no therapy on intervening days[143,146]. The availability of growth hormone preparations may now help prevent this complication.

OCULAR COMPLICATIONS

The eyes can be involved in several post-transplant complications, including CMV retinitis, diabetes mellitus, and temporary changes in visual acuity, almost certainly steroid-induced.

Cataracts can be induced by prolonged high-dose steroid therapy, but have become less common since the introduction of CsA. The typical lens opacity following long-term use of corticosteroid either systemically or topically is a posterior subcapsular opacity[145]. The high doses administered during rejection episodes produce subcortical crystalline opacities which usually affect the posterior pole of the lens. Low-dose corticosteroids sometimes provoke the development of cataracts, especially with prolonged use over at least 12–18 months. There remains controversy as to whether cataract formation is dose dependent[145,146].

BENIGN SKIN LESIONS

In addition to a high incidence of skin cancers (Chapter 12), patients on long-term immunosuppressive therapy not infrequently develop multiple benign skin lesions, which may require no treatment. Warts and keratoacanthomas are not uncommon. Warts are thought to result from reactivation of latent viruses rather than from primary infection[147,148]. It is important, however, to differentiate benign lesions from malignant tumors, such as squamous and basal cell carcinomas, which are the most common malignancies in immunocompromised patients (Chapter 12). All suspicious lesions should be biopsied or excised.

OTHER COMPLICATIONS

The list of other complications of long-term immunosuppressive therapy, especially if corticosteroid therapy, is long (see Chapter 8, Table 3). Clinical features of fluid and salt retention can usually be controlled by diuretic therapy. Increased appetite and weight gain are common and may lead to obesity if not controlled by a strict diet (Chapter 16). Changes in menstrual cycle, acne, night sweats, myopathy, joint pains, and spontaneous petechial hemorrhages in the skin are also common side-effects of steroid therapy.

Patients with widespread atheroma are, of course, at risk from progression of this disease. Reconstructive surgery to peripheral vessels, especially in the legs, or even amputation for painful ischemic ulceration, has been necessary in some patients with progressive peripheral arteriopathy.

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14
Psychiatric Aspects

E.S. NASH AND D.K.C. COOPER

INTRODUCTION

Cardiac transplantation emerged in 1967 as a dramatic new way of saving the life of a dying cardiac patient[1]. It was not so much the radical nature of the procedure that triggered the reactions, but the removal and replacement of an organ that is seen by some solely as a physiological pump, but by others as the symbolic seat of love and loyalty[2,3].

Psychiatric experience in the fields of both heart and lung transplantation is indebted to contributions from two allied areas: firstly, the care of patients undergoing closed and open heart surgery, and secondly, experience obtained from involvement in renal and liver transplantation programs. The psychiatric implications of closed and open heart surgery have been extensively documented[4–6]. Of particular relevance was Kimball's identification of nuclear patterns of emotional reaction in patients who were assessed preoperatively; these patterns were found to have predictive value. Of the four groups that he identified, namely (a) the adjusted, (b) the symbiotic, (c) the depressed, and (d) those denying anxiety, it was the members of the latter two who caused concern. Depressed patients had a high postoperative mortality rate, while those who denied anxiety had a high incidence of postoperative psychiatric complications.

The second area, psychiatric experience obtained from involvement in organ transplantation since the first kidney transplant was performed in 1950, has centered around the issues of (a) selection criteria, (b) organ incorporation versus rejection, (c) postoperative psychosis, (d) the complications of immunosuppressive medications, (e) compliance, and (f) rehabilitation.

Psychiatric experience in heart transplantation over the past 25 years (and in lung transplantation for the past decade) has been similar; recipients require psychosocial screening, develop early and late postoperative emotional or behavioral disturbances and have to incorporate a new life-giving organ[7–11]. Thoracic organ transplant recipients have to face not only the physical challenges of organ rejection and systemic infections, but also the psychological challenges of reorganizing family relationships and of reentering employment. It is in all these areas that psychiatric insights have helped transplant teams. Each of these aspects will be considered in greater detail.

The importance of the role of the psychiatrist is well illustrated by the early observation in Cape Town that no fewer than 26% of deaths or loss of cardiac allograft function were related, in some part, to non-compliance on the part of the patient[12], thereby drawing attention to the psychological problems such patients display.

PATIENT SELECTION

Patient selection is important because basic issues of social policy, the limits of medical responsibility, and major ethical and legal considerations are encapsulated in the decision to choose or reject patients[13].

In the early years of cardiac transplantation, mental deficiency and overt psychosis were the only psychiatric grounds used to justify exclusion from a program[14]. With improved survival, and the subsequent expansion of transplantation, resulting in an inadequate number of donor organs, the selection of suitable recipients has become an important issue[15]. The situation with candidates for lung transplantation is similar. Many centers now have a committee of medical personnel who take medical, psychological and social criteria into account (Chapters 5 and 15). A preliminary screening inevitably occurs prior to the actual referral, which reflects to some extent the responses of the referring physician to the patient's personality and to the character of his or her family. Self-referrals for organ transplantation must be viewed with some caution, since this may be indicative of exhibitionistic traits in patients seeking publicity. (The self-referral of donors, however, requires immediate psychiatric intervention, since this suggests suicide intent.)

Many groups today carry out an initial screen using a self-report questionnaire, e.g. the Millon Behavioral Health Inventory (MBHI), or symptom checklist[16] that is completed by the patient. Although this will give helpful information regarding the patient's present psychological status, it may not necessarily predict post-transplant medical problems[16]. Whenever the questionnaire, or clinical history, suggests significant past or current psychological concerns, the patient should be referred for

further psychiatric assessment. This will include an in-depth interview that reviews the patient's personal development, family background and history of psychiatric illness, as well as his or her attitudes to current illness, disability, death, and transplantation.

During this interview it is of particular value to assess the patient's ego strengths, such as his or her capacity to: (a) understand the information offered, to think about problems in a rational way, to communicate needs, and be motivated to regain health; (b) face reality, and not resort to excessive denial or fantasy; (c) maintain a stable mood, without wide emotional swings from elation to the depths of despair; (d) control basic impulses, such as anger, greed, sexuality and self-abuse; (e) use mature ego defenses in everyday life and under conditions of stress; (f) perform age-appropriate life tasks, e.g. education, employment and social responsibilities; (g) make mutually rewarding relationships and use social support; and finally (h) define his/her values, abide by these, and use social resources that reinforce these. These ego strengths are based on those described by Beard[17] and Bellak *et al.*[18].

Dreams are useful cues to reveal unspoken concerns. It is also important to examine current mental functioning using a standardized method[19], which should include inquiry about depression, suicidal ideas and memory impairment, as well as assessment of intelligence, insight, and capacity to make sound judgements, with special relevance to the giving of informed consent. Formal psychological testing may be of assistance[20,21], but critically ill patients are frequently not able to carry out such tests. Where there are language barriers, competent and empathic interpreters, who are familiar with the transplant program, are invaluable.

Evidence of organic impairment may be found in patients with poor cerebral perfusion or chronic hypoxia or hypercarbia, but if this is associated with neurological deficits the impairment is likely to be permanent, and due to brain damage. Evidence of a disturbed personality, indicated by alcohol and drug dependence, an erratic work record, unstable interpersonal relations, and antisocial behavior, have been added in most programs to the original exclusion criteria of mental deficiency and overt psychosis[13]. Affective disorders, anxiety, or problems with adjustment must also be investigated, but are not necessarily contraindications to acceptance for transplantation[15,22–25]. The quality of the patient's psychosocial support is of great importance, and must also be taken into account (Chapter 15). This is particularly so in the occasional patient who has been fortunate in never before having to make a major adjustment in his or her life; such patients may find the adjustments forced on them by the need for thoracic organ transplantation particularly difficult, complicating their preparation for operation and their convalescence.

However, Kuhn *et al.*[26] found that pretransplant psychiatric diagnosis frequently reflected the patient's emotional reaction to current illness and, with the exception of behavioral management problems, did not correlate with subsequent emotional responses. Indeed, most psychiatric distress after evaluation appeared to be related to the protocol (e.g. the waiting period, endomyocardial biopsy, etc.) rather than to any pre-existing psychopathology.

In patients being assessed for cardiac transplantation, the nature of the heart disease in itself may be a cue to the patient's personality and his/her customary ways of adapting to stress. Since some candidates have a history of myocardial infarction (complicating coronary artery disease), they may well show features of the type A behavior pattern described by Friedman and Rosenman[27]. Such individuals characteristically show ambitiousness, striving, overcommitment to work, time urgency and impatience[28]. They strive to be 'good' patients postoperatively and are highly motivated to survive. Another identifiable group comprises those with cardiomyopathy linked to significant alcohol abuse, which has been associated with shorter survival. Careful thought has to be given before a transplant is offered to patients with a history of unpredictability, which may include unstable work and relationship patterns.

While patients with long-standing heart or lung disease, particularly those who have tolerated previous operative intervention, are likely to handle the stress of the transplantation procedure satisfactorily, they may have difficulty in relinquishing a well-established sick role; rehabilitation then becomes a major exercise. Factors that are predictive of a favorable outcome include a patient's ability to discuss the possibility of his or her own death, as well as having a clear use for the time gained by longer survival, reinforced by sound social support[29].

Olbrisch and Levenson[30] carried out a survey of heart transplant programs with regard to the psychosocial evaluation process. There was considerable disagreement among programs as to when a patient was not suitable on psychosocial grounds. Wide discrepancies in the criteria used, and in the rate of patients refused on psychosocial grounds, were discovered. More than 70% of all programs excluded patients for transplantation on the grounds of dementia, active schizophrenia, current suicidal ideation, history of multiple suicide attempts, severe mental retardation, current heavy alcohol use, and current use of addictive drugs. Lack of consensus was found for some exclusion criteria (cigarette smoking, obesity, non-compliance, recent alcohol or drug abuse, criminality, personality disorder, mild mental retardation, controlled schizophrenia, and affective disorder). The proportion of patients rejected for transplantation on psychosocial grounds ranged from 0% to 37%, with an average of 5.6% in the USA and 2.5% in non-USA programs.

WAITING FOR A TRANSPLANT

Once a patient has been accepted for a program, he or she has to await a donor heart[31,32]. Patients referred from distant places may have to live temporarily near the hospital, and are often supported by only one family member. This alien environment can be stressful, especially if the patient has left a well-established support structure and has to wait many weeks or even months[33]. Initial optimism may give way to anxiety, despair, and depression. Individuals who use acting-out, impulsive behavior have difficulty tolerating this phase; exclusion from the program, even at this late stage, should be considered.

POST-TRANSPLANT COMPLICATIONS

There is now a considerable literature on the psychiatric complications of heart transplantation[9,10,34–39] and some early experience after lung transplantation[40].

Immediate postoperative period

In the early days of cardiac transplantation, many patients became psychologically disturbed during the initial postoperative period, just as they did in the early days of heart surgery[6]. These changes were attributed to the convergence of such factors as altered cerebral circulation, prolonged anesthesia, overstimulation by the monitoring systems, the sensory deprivation of immobility, and the unfamiliar bland environment peopled by masked strangers.

The acute psychosis that can occur in this phase bears the features of both 'organic' and 'functional' disturbance; symptoms include reduced level of consciousness, hallucinations, paranoid delusions, disorientation from time and place, and mood disturbance such as depression or undue euphoria. Unconscious anxiety and fantasies about the transplanted heart may be voiced in this context[41]. In addition, there is some evidence that emotional factors can influence the immunologic balance of the body, thereby affecting the organ acceptance or rejection[42].

More recent reports from heart transplant centers indicate that there is now little disturbance in the early postoperative period, which is possibly indicative of improved patient preparation prior to the procedure[21]. However, postoperative organic mental syndromes were reported in a high percentage of the early lung transplant patients[40]; episodes of dementia were found particularly in patients who had undergone cardiopulmonary bypass, had high levels of cyclosporin, or who had to relocate to await surgery. Although many patients are persistently euphoric at having survived the procedure, and are delighted with their increased vital capacity and physical strength, this state of well-being can be threatened by episodes of organ rejection or infection that may trigger depression and anxiety. If hospitalization is prolonged, boredom and social isolation may also take an emotional toll. The steroids required to combat organ rejection pose an additional hazard since they are known to produce depression and even psychosis[43].

Mild symptoms respond to a psychotherapeutic interview and program modification; more severe disturbances such as regressed behavior, irritability, paranoid fears, and suicidal feelings and ideas may emerge and require treatment with neuroleptic medication such as chlorpromazine or thioridazine. Such drugs, however, have a relatively long half-life, and should be used with caution in debilitated patients; a butyrophenone, such as haloperidol, or a thioxanthine, may be preferable in such cases. Risperidol, a new antipsychotic, has a different side-effect profile from the neuroleptics in that it does not carry significant risk of extrapyramidal side-effects. Antidepressants such as the tricyclic or tetracyclic agents should also be administered with caution[44,45], again as they are long-acting, may share a metabolic pathway for degradation with cyclosporin, and may have an arrhythmogenic effect. Shorter-acting non-tricyclics, such as trazadone and maprotiline, offer an alternative. In more recent years, several newer antidepressants have become available, with relatively fewer side-effects. These include fluoxetine, paroxetine and sertraline, which are selective serotonin reuptake inhibitors. Bupropinon, a unicyclic, is also available.

Regressed behavior, which is often triggered by medical complications, responds to empathic and firm handling by staff. As dependency on the staff lessens, it becomes necessary for the patient to practice autonomy in taking control of some aspects of treatment. Some patients are anxious about leaving the security of the hospital and require gradual weaning from the intensive-care unit and, later, the ward environment. The spouse and/or other close family members also need reassurance and instruction. The psychiatrist, clinical psychologist, or psychiatric social worker can help the surgical team understand the anxieties that inevitably occur during and after any hospitalization, particularly if the hospital stay has been for organ transplantation[46]. It is also very important to remember that disturbed behavior, confusion, and headaches may have a neurological cause, such as an intracranial bacterial, viral, or fungal infection, which occurs more frequently in the immunologically compromised host. Epileptic seizures, local pain, or paralysis are indications for immediate neurological investigation to rule out organic pathology, including infection. Neoplasia may account for late neurological/psychiatric disturbance[47].

REHABILITATION

The successful transplant recipient has survived a unique life experience. During the past 50 years, cardiac resuscitation and cardiac surgery have introduced a new dimension of human experience: the patients both 'die' and are 'reborn' or 'resurrected'[48]. Some survivors of cardiac arrest admit to thoughts of resurrection and fantasies of rebirth[38,49]. Organ transplantation brings a new lease of life with the promise of improved health and renewed physical vigor. To quote Paul Coffey, an early British heart transplant survivor: 'Following the transplant, and being given a second chance of life, one has time to think about what really matters'[50]. The early risk of rejecting the transplanted heart or of other post-transplant complications, however, may initially inhibit long-term planning.

Integration of the new organ into the body image is effected in various ways. The organ has to be 'taken in' and become part of a healthy body representation. The heart is seen as a pump to the mechanically-minded patient, devoid of emotional significance, which can be replaced if worn out. At the other extreme, however, a few recipients unmistakably identify with the sex and personality of the donor. Reactions to the organ influence compliance with medication. Complete integration of the organ into the body image involves dealing at some level with feelings of guilt and indebtedness for having received an organ at the cost of another life[41,51].

Mai[52] has observed that, in 18 of the 20 heart transplant subjects that he studied, significant denial with respect to the graft, the donor or both, was present in the 30–90-day period following surgery. Bunzel *et al.*[53] also investigated how heart transplant patients cope with the fact that their own heart has been replaced by a donor organ from an unknown dead donor who was the target of disease, accident or even suicide. Three groups of patients were identified: (a) the complete deniers, who denied thinking about the donor; (b) the partial deniers, who were aware of avoiding thinking about the donor; and (c) those who coped, who accepted the death of the donor as reality and also reported having more or less close connections with the donor. Eighty-two percent of the patients interviewed accepted the donor heart immediately as their own, whereas the remaining 18% avoided talking and thinking about the graft and donor.

Recipients of donor organs, particularly the heart, have to deal with fantasies about the donor. Louis Washkansky, the first heart transplant patient, received a heart from a female donor. He enquired, 'Do you think ... that I might develop busts (breasts) like a woman? ... or become chicken-hearted?'[54] Other male recipients receiving hearts from female donors have also had this type of reaction, though, with the increasing frequency of the operation, such fantasies are becoming rare.

At almost every center the initial heart or lung transplants gained considerable media attention. The early transplant patients' 'fame', even if only local, nourished the rehabilitation process and ensured a 'survivor mission' reaction which was useful in gaining their co-operation. For subsequent survivors the rehabilitation process has been a less dramatic affair, with mundane issues such as housing, employment, and restored marital relationships to be faced (Chapter 15). As thoracic organ transplantation has become more commonplace, however, the patient has had a greater exposure to the experiences of fellow-recipients, and such interchange and sharing can be of great emotional support. Many centers have initiated support groups to enable patients to share their experiences and concerns.

While euphoria and improved self-esteem are found in many patients following operation, the steroid facies, which still occurs in a few, may make the recipient feel self-conscious. In some patients there are difficulties in concentration, emotional lability, and irritability, while, in others, features of mild organic brain impairment can be found[55]. Occasionally, recipients may show a morbid interest in their surgical 'twins' who have received organs from the same donor (e.g. kidneys), and identify closely with them.

Follow-up has to be carefully planned. On the one hand, transfer to physicians or surgeons remote from the transplant center, who have had little experience with transplantation programs, engenders insecurity and negative transference reactions. This may jeopardize compliance with dietary instructions and medication – and hence survival. On the other hand, follow-up by the patient's own doctor lessens the overidentification with fellow transplant patients and grief at their death.

The return to the family means there has to be a reorganization of the family system[20,56]. Wives often feel insecure in adapting to a more active spouse, though marital relations may be jeopardized by inadequate sexual function, to which several causative factors may contribute[57,58]. Although libido remains strong in post-heart-transplant male patients, erectile rigidity and orgasmic ability, already often impaired by pretransplant debility, decline further after the transplant[58]. Marital and family counseling is sometimes required.

Tabler and Frierson[59] documented sexual dysfunction in a number of patients following heart transplantation, such dysfunction including impotence, ejaculation problems, altered libido, and avoidance of sexual opportunities. Contributing factors to these sexual difficulties were fear of death during coitus, effects of medication on interest and ability to function, body image concerns, depression, uncertainty about the sexuality of the donor, and altered roles and responsibilities within the family. They recommended that members of transplant teams should: (a) obtain a routine sexual history during the evaluation of candidates; (b) be more aware of such sexual concerns; (c) aggressively treat clinical depression; (d) establish peer support groups for spouses of transplant patients; (e) provide didactic material on sexual issues after transplant; (f) adjust medications when sexual problems arise; and (g) address their own level of comfort in discussing sex-related topics with transplant patients.

TEAMWORK

Human organ transplantation is notable for its intensive interdisciplinary team work. It is a collaborative enterprise that requires the skills of a large number of health-care professionals. A key individual is the liaison nurse or coordinator, who mediates between the team caring for the donor, who have to abandon all life-saving procedures, and the transplantation teams, eager to receive organs in good condition. He/she has an important role in sustaining the donor's family in their grief and obtaining consent for organ usage. Here, too, psychiatric skills play a major role. Newcomers to the service may find that the unique demands of the program tax their professional equilibrium, especially if they have unrealistic expectations of their roles. Psychiatric assistance may be necessary for such individuals if team support is not enough. Frequent team meetings are valuable to allow members to express their feelings and ensure good communication and support.

COMMENT

Thoracic organ transplantation raises the cosmic issues of death, resurrection and immortality that transcend the mundane daily concerns of lymphocyte counts and blood cultures. Programs of this kind demand the best that an academic or a research center has to offer, and stretch the imagination in seeking how to extend productive lives. Psychiatrists and social workers have played a useful role in transplantation programs by assisting with selection, and helping transplant teams handle the more complicated behavioral, emotional and family problems which regularly accompany major procedures that aim to prolong life. Donor families may also need support in coming to terms with their grief and even anger. These families occasionally seek to find out the identity of the organ recipients, since the survival of the recipients gives their loss some meaning.

In addition, organ transplantation has opened up important ethical issues of selection, the finality of life, the definition of death, informed consent, and priorities in the allocation of scarce lifesaving resources[60–62]. The giving and receiving of a body organ – a gift of enormous value – is the most significant aspect of human organ transplantation. It is not a private transaction between the donor and recipient, but rather takes place within a network of personal relationships that includes families, the medical teams, and society.

Mauss[63] has described the gift relationship as a series of implied obligations – to give, to receive, and to repay. In the context of organ transplantation it is life that is given up, received, and renewed. Thus, donor, recipient, and kin can become bound to one another emotionally and morally in ways that can be fettering as well as self-transcending. Organ transplantation has brought issues of gift exchange and social solidarity to the fore, and has shown how technical advances tend to outstrip contemporary psychological and social organization. Thoracic organ trans-

plantation highlights the value medical science places on individual human life, and the progress that is possible through the application of science with compassion.

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15
Medico-social Aspects

W. D. PARIS, S.E. THOMPSON, N.J. BRAWNER, M.L. PENIDO, M.J. BRIGHT AND C.M. ROBERTSON

INTRODUCTION

With the introduction of cyclosporin a new era in transplantation began. Improved immunosuppression was partly responsible for the exponential growth of heart transplant programs from 1984 to 1987[1]. The first successful lung transplant was performed by the Toronto Lung Transplant Group in 1983 and, though less dramatic, there has been a steady increase in the number of lung transplant programs since that time[2]. More importantly, the increased frequency of organ transplantation has been accompanied by improved long-term survival. At the five heart and lung transplant programs represented by the authors of this chapter we found combined 1- and 5-year survival rates for heart (n=654) transplantation to be 89% and 75%, and for lung (n=196) transplantation to be 67% and 38%, respectively.

Survival statistics alone, however, do not reflect the challenges patients undergoing transplantation must face. The enduring focus of attention for patients and families will be the 'process' they undergo, rather than the surgery itself[2].

Medical social work services have historically been available to patients and their families to help meet the challenges of transplantation. As a member of the transplant health-care team the social worker assists in the selection, monitoring and rehabilitation of transplant recipients. The patients and their families are assisted in their adjustment to the psychological, emotional, social, and financial impact of transplantation. For the social worker the challenge is to continue to be a liaison between the transplant team, the patient and the family, while developing greater skills in assessing and following prospective candidates.

Bright *et al.*[2], Christopherson[3], and others[4] have identified a fairly predictable sequence of adjustment stages for early heart and lung transplant patients which include: (a) assessment, (b) awaiting donor, (c) immediate post-surgical, (d) recovery (first infection/rejection), (e) hospital discharge, and (f) early convalescence. In this chapter some of these stages will be combined and reference will be made to the social work services provided for heart and lung transplant recipients during the selection, perioperative, and rehabilitation phases.

ASSESSMENT PHASE

Psychosocial

The psychosocial assessment is the most important contact that a social worker has with a prospective candidate and his/her family. It should include an overall picture of the patient's personality and attitudes, mental stability and level of functioning, and current social matrix. In particular, a detailed history should be obtained of current or previous substance abuse, medical noncompliance, and psychiatric problems. Support system strengths and weaknesses, patient and family attitudes about transplantation, and the motivation and potential for post-transplant rehabilitation (physical, psychological and vocational) need to be fully evaluated. For lung transplant candidates their participation in a required post-assessment pretransplant pulmonary rehabilitation program provides an excellent opportunity to observe and further evaluate their behavior. The willingness with which they accept this requirement may in itself provide insight into their character, and into their potential post-transplant behavior.

The importance of the psychosocial assessment in determining proper patient selection has been well documented. Historically, patients have been denied transplantation on the basis of a history of (a) poor medical compliance, (b) mental illness, or (c) current or recent evidence of alcoholism or drug abuse[5]. Recently, the validity of some of the assumptions that have been made in relation to psychosocial factors and transplantation outcomes has been questioned.

Renal transplant recipients with a prior history of heroin abuse did well when compared with other recipients[6]. Preoperative depression and psychiatric distress were found to have little predictive value in regard to mortality and morbidity after heart transplantation[7,8]. Heart transplant recipients selected with liberal psychosocial criteria, and exposed to aggressive psychosocial intervention, had medical results at 1 year similar to those of patients selected with less liberal criteria[9].

At many centers the policy with regard to alcohol or drug abuse is to defer patients with a current, continuing problem. However, patients are accepted once they have maintained 6 months sobriety or drug-free behavior. Following this policy at the Oklahoma

Transplantation Institute, no patient has resumed alcohol or drug consumption post-transplant, although this group has been found to be significantly more likely to develop compliance or psychological problems which were associated with increased morbidity (but nor mortality) after the first post-transplant year[10].

Patient non-compliance, while sometimes difficult to identify or quantify, especially pretransplant, is an important issue that must be explored. Non-compliance has been documented to be more common in younger patients (<40 years), those single or divorced (presumably lacking family support), those with a lower level of education (less than high-school diploma), and those with no career skills[11]. Some believe that non-compliance may indicate depression, intellectual deficit, cognitive impairment, or ambivalence about surgery and/or survival[12], while others report that non-compliance in regard to taking medication is mainly associated with financial restrictions[13].

Any history of failing to follow medical advice should be noted. If there are social reasons for this behavior, attention and effort must be focused on identifying the underlying issues and helping the patient to develop an effective plan of corrective action. When there is a psychological or intellectual basis for this non-compliant behavior, its potential for treatment must be assessed.

The pretransplant psychosocial evaluation must also include, when applicable, an exploration of the possibility of the patient's return to work post-transplantation, and any need for training or education. It is reasonable to expect that the majority of thoracic organ transplant recipients will be able to resume a fully functioning lifestyle. According to a 1987 UNOS (United Network for Organ Sharing) public opinion survey on heart transplantation, 72% of respondents agreed that a patient's ability to return to work or other regular activities was second in importance only to patient survival (with which 83% of respondents agreed)[14]. It is at this early stage that the expectations of the transplant team with regard to the patient's return of work post-transplant should be clearly stated. The center's policy regarding the support (or misuse) of post-transplant disability claims should be emphasized to the patient[15]. Studies have shown that a clearly stated policy which expects employment (coupled with social work intervention) results in 12–15% of patients securing new employment[16,17].

On occasion, patients and/or family members experience some degree of disbelief and even anger when informed of the diagnosis of end-stage heart or lung disease and the need for transplantation. At times this may result in a reluctance to be totally factual during the assessment, because of the underlying fear of being denied candidacy. Potential recipients who have limited expectations of potential benefit, or view the transplant as merely a delay in the dying process, must be screened particularly carefully. Some lung transplant candidates (e.g. those with cystic fibrosis) may have difficulty in anticipating or imagining any meaningful post-transplant improvement because, due to the early age of onset of the disease and prolonged length of illness, they have no prior experience and little expectation of good health.

Financial

Before admission or transfer to the transplant service the social worker coordinates an assessment of the patient's financial history. In countries such as the USA, with a health-care system based largely on the patient's ability to pay, finances are a major issue. Ideally, a prospective candidate will have insurance which will cover 80–100% of medical expenses. In the absence of private or state insurance the patient may well have to rely on his or her own personal resources or local fund-raising efforts. When problems or potential problems are identified, the social worker may provide counseling services or refer the family to appropriate community resources. It is important to remember that insurance that covers pharmacy costs is as important as hospital and physician benefits because of the expensive, and long-term, post-transplant immunosuppressive regimen.

PERIOPERATIVE PHASE

The perioperative phase refers to the time from selection through early convalescence, and is characterized by contradictory emotions for the patient and family. Although patients may express relief about being accepted as a candidate, guilt and anxiety often occur once they realize that their return to health is dependent on another's death and the bereaved family's willingness to donate the organs. Alternatively, the patient may secretly wish for another's death. In some cases the patient will make known his or her idea of a suitable donor. Time spent awaiting a donor produces a severe strain on even the best of relationships within the family, or among friends. The social worker should be available to provide reassurance and emotional support and, when needed, to facilitate the ventilation of feelings[2]. Communication with patients who have undergone transplantation is encouraged, and has proven especially helpful to candidates.

The immediate post-surgical period is, if all goes well, a time of euphoria and relatively little stress which requires only minimal social work involvement. At some point most recipients will experience a real or suspected infection and/or rejection episode. It is because of this and the other side-effects of immunosuppressive medication that a patient soon realizes that he or she may have traded one set of symptoms or problems for another. This is perhaps particularly true for the lung recipient, due to the increased risk of infection and the difficulty of diagnosing rejection after lung transplantation.

In general, recovery and the preparation for hospital discharge may be a time of incongruous feelings (i.e. frustration and depression about functional limitations and ongoing weakness, etc., accompanied by excitement about leaving the hospital). At discharge a patient is expected to once again assume responsibility for his/her own physical and emotional care, which may result in conflicts with family members who have been fulfilling a 'caretaker' role during the patient's period of ill-health. Early convalescence is a furthering of this process, where a variety of family conflicts are likely to develop[18]. The amount of social work involvement at this time is dependent entirely upon the needs and wishes of the patient and family.

Though successful discharge may mean resolution of many of the medical factors that precipitated admission to the hospital, what lies ahead may be uncertain. As a result, discharge is often an anxious (fearful) time for both patient and family, and they may react with confusion, shock, or denial[19]. Any early unrealistic expectations about transplantation and its influence on social relationships are likely to be evident at this time.

The social worker should be available to work with the patient and family for any post-hospital counseling and/or referral needs. With improved medical results, and better pretransplant screening and counseling, discharge planning is a less important role for the transplant social worker than it once was.

REHABILITATION PHASE

Depending upon improved physical capacity, a transplant recipient should be restored to a level of general health comparable with that of the general population[20]. For both heart and lung transplant recipients aggressive cardiopulmonary rehabilitation efforts begin once the patient is medically stabilized post-transplant[21,22]. Successful long-term rehabilitation can be realistically claimed if a patient returns to (a) competitive employment, (b) school, (c) homemaking duties, or (d) active retirement.

Psychosocial problems identified at pretransplant assessment are likely to continue in some form, and will serve as a hindrance to a patient's rehabilitation potential. Therefore, the social work role during the rehabilitation phase should be clinically focused and directed towards reducing psychosocial problems and increasing the likelihood of returning to an active lifestyle.

Only recently have psychosocial factors and their relationship to transplantation outcomes been subjected to critical evaluation[6–9]. Patients with a history of substance abuse within 6 months of acceptance for transplantation, even if currently abstinent, should be monitored closely because of the risk of recurrence. Patients abstaining for longer than 6 months pre-acceptance are at risk of exhibiting previously undiagnosed psychological or compliance problems[10]. Psychological and non-compliance problems that existed pretransplant, whatever their form, are almost certain to recur post-transplant. Medication non-compliance may occur even in the previously compliant patient as the result of financial restrictions[13].

Since it has not been conclusively shown that the presence of any post-transplant psychosocial problem will increase mortality, social work intervention should be based upon the assumption that attempting to resolve these issues will improve the quality of life for heart and lung transplant recipients, but not necessarily increase longevity.

The rate at which transplant patients are gainfully employed is not synonymous with the medical results of transplantation. A recent multicenter study found 81% of heart transplant recipients were physically able to work, but only 45% were doing so[16]. There are no comparable data available as yet for lung transplant patients.

Obviously there are many barriers to employment post-transplantation. The factors typically associated with continued unemployment are: (a) potential loss of health insurance or disability income if the patient becomes employed, (b) less than a high-school diploma, and (c) lengthy pre- and post-transplant disability. However, heart transplant centers that refuse to support unsubstantiated (or poorly substantiated) disability claims post-transplant have been successful both in encouraging patients to return to their former jobs (if available) and in stimulating an additional 12–15% of patients to secure new employment[16,17].

A difficult and often-ignored aspect of a patient's rehabilitation is the patient's perception of continued disability. The discrepancy between the physician's assessment and the patient's perception of work ability is problematic, and as yet unresolved. Whether this is a real or imagined incapacity on the part of the patient is uncertain. Neither vocational rehabilitation nor psychotherapy has shown much success in altering patient perceptions, although this does not imply that they should be ignored.

Vocational programs offer the support necessary to remove educational barriers, and counseling can help to reduce the stress and anxiety associated with change. However, for patients to achieve full rehabilitation potential consistent with their medical improvement will require meaningful social reform and, in the USA, full implementation of the Americans with Disabilities Act.

COMMENT

The complexity of the issues confronting transplant patients and their families requires both sensitivity and creative intervention strategies. The multifaceted nature of the social workers' knowledge and role allows them to function in a clinical capacity and provide supportive, educational and/or counseling services and, when needed, referral for community services. It is incumbent, however, on social workers that they document their clinical experience and research knowledge. This will require them to refine outcome measurement tools and report the effectiveness of their intervention through publication. Solely reporting transplant patient or family needs is inadequate in an environment driven by the outcome mandates of insurance carriers, restructured organizations, and/or government regulations. Certainly, transplant social workers have already contributed towards these goals, but there remains much to be accomplished.

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16
Nutrition and Diet

M. KANOSKI

INTRODUCTION

Organ transplantation has become a therapeutic option for hundreds of victims of cardiac and pulmonary failure each year. Since the first human heart transplant was performed by Christiaan Barnard in Cape Town, in December 1967, medical centers have been performing transplant procedures worldwide. Heart and lung transplants have made survival and rehabilitation possible for individuals with end-stage cardiac and pulmonary failure who would otherwise have died or have remained severely disabled. However, the number of patients waiting for transplantation increases each year, and many wait weeks or months before they receive a transplant. During this waiting period the patients' nutritional status can be maintained, or possibly improved, though repletion of the nutritional stores of a patient with end-stage organ disease may not be possible until a functioning organ is received.

The nutritional care of transplant patients is unique to the preexisting cardiac and pulmonary disease and postoperative therapy. Frequent reviews of the dietary requirements of the patient are essential, as they may differ markedly with changing clinical status. The nutritional status of transplant candidates and recipients significantly affects wound healing, surgical complications, length of hospitalization, quality of life, and mortality[1].

Blackburn *et al.*[2] noted a 53% incidence of significant malnutrition in patients with cardiac disease who were usually in New York Heart Association (NYHA) class III or IV. Poindexter's report on the assessment of 54 patients who underwent heart transplantation demonstrated that 25 exhibited compromised nutritional status preoperatively[3]. Advanced cardiac failure may result in cardiac cachexia, evidenced by multiple organ insufficiency (from hypoxia), muscle and adipose tissue loss, hypoalbuminemia, malabsorption, nausea, vomiting, and anorexia. The risk of mortality and postoperative complications increases with each of these factors[4].

Patients with advanced pulmonary disease usually present with weight loss. Possible mechanisms for this weight loss include impaired gastrointestinal function, altered dietary intake as a result of early satiety, the presence of hypermetabolism, increased resting energy expenditure, and increased work of breathing[5,6].

The degree of malnutrition in patients with respiratory disease usually correlates with deterioration in lung function. Depletion of the somatic protein stores is common in patients with chronic obstructive emphysematous pulmonary disease (COPD). Weight loss in COPD patients (who begin with normal weight) causes deterioration in respiratory and skeletal muscle strength. Weight loss in COPD patients who are initially overweight, however, usually improves respiratory muscle function and decreases arterial $PaCO_2$[5,7,8].

Patients with cystic fibrosis (CF) have difficulty absorbing fat, and require pancreatic enzyme replacement. Undernutrition is caused by unfavorable energy balance and there appears to be a direct correlation between the degree of undernutrition and the severity of pulmonary disease. Three major factors have been shown to lead to undernutrition: (a) reduced dietary intake caused by various factors, (b) malabsorption, and (c) increased energy expenditure. Girardet's research stressed that the resting energy expenditure (REE) in CF patients is higher than normal, even when pulmonary function is normal, and that it increases approximately 150% with deteriorating lung disease[9].

The above observations, combined with the known deleterious complications of immunosuppressive drugs and the stress of major surgery, support the need for nutritional intervention prior to transplantation, to improve the probability of a successful outcome. This chapter will discuss the primary areas of concern, and recommendations for nutritional therapy.

NUTRITIONAL ASSESSMENT OF THE TRANSPLANT CANDIDATE

At a time when candidates for transplantation are more numerous than donors, long-term pretransplant nutritional care becomes increasingly difficult, especially for those who require support by mechanical ventilation, a mechanical assist device, or an artificial heart. In order to treat nutritional abnormalities and malnutrition, such patients must first be identified. Accurate initial and follow-up assessments by the dietitian will identify those patients at risk in each phase of transplant care.

With transplant candidates presenting in a wide variety of nutritional status generally deteriorating while awaiting transplantation, routine pretransplant follow-up is essential to allow early intervention. Patients with better nutritional status at the time of transplant have a shorter length of hospital stay, and lower hospital costs[10].

The patient with slowly progressive disease (>6 months) may seem to have adjusted metabolically. The severe malnutrition that may accompany chronic heart failure has been termed cardiac cachexia. The condition may exist for many months or years. Factors contributing to the pathogenesis of this syndrome are: (a) dietary, (b) metabolic abnormalities, and (c) excessive loss of nutrients[11].

In cardiac patients, weight loss often appears minimal, but is usually masked by the presence of fluid overload[12]. Anthropometric measurements commonly detect a reduced lean muscle mass with varying degrees of adipose (calorie) reserves. Poor appetite, nausea and vomiting may reduce food intake at a time when all physical activities, including breathing, are placing greater nutritional demands on the patient. Abel *et al.* established that early malnutrition adversely affects cardiac function[13,14]. In turn, impaired cardiac function from nutritional deficit further interferes with food intake, which creates a downward spiral of deterioration until parenteral nutritional support is initiated[15].

Signs of a hypermetabolic state may include weakness, muscle wasting (in particular in the temporal region), severe weight loss, increased temperature, and hypoalbuminemia. The cardiovascular system may exhibit decreased cardiac output, increased blood pressure, and increased heart rate. Altered respiratory function may include increased respiratory rate, presence of rales, and dyspnea. Gastrointestinal aberrations may include decreased gastric motility, early satiety, anorexia, steatorrhea, hepatomegaly, hepatic congestion, and ascites. Renal function may be impaired, and result in increased nitrogen loss and rises in blood urea nitrogen and creatinine levels.

Pretransplant nutritional assessment should include biochemical and anthropometric measurements, history of weight loss, and diet history. The appropriate biochemical and anthropometric measurements are well documented in the literature[3]. Nutritional assessment includes recording of age, sex, height, and weight, and estimations of serum albumin and/or prealbumin and 24-hour urinary urea nitrogen and creatinine, when appropriate[2] (Table 1).

A thorough diet history, with estimated caloric intake levels, should be taken, to detect potential deficiencies, and may suggest methods to improve oral intake. The history should include usual home diet, utilization of any special food supplements, chewing and swallowing ability, and smell and taste perception. In addition, limitations on the procurement of food products, meal preparation, and eating arrangements should be evaluated. An attempt should be made to estimate dry weight (e.g. the weight 6 months prior to the onset of illness) and used to determine the patient's percentage weight change over a period of time. Current weight should be compared with desirable weight standards. The estimated desirable weight is then used to determine basal energy expenditure (BEE)[16]. When available, indirect calorimetry can be measured by a metabolic chart, to determine resting energy expenditure (REE).

A standardized, easily performed, cost-effective assessment tool is important to the success or failure of nutritional intervention. The subjective global assessment (SGA) of nutritional status (Table 2) is a clinical technique that estimates nutritional status on the basis of medical history, which includes current weight and weight history, dietary intake compared with usual pattern, gas-

Table 1 Nutritional assessment of the transplant patient

Height (cm) ___ Age: ___ Sex: M/F ___

Weight (kg) Actual: ___ Usual weight: ___ Weight 6 weeks ago: ___ Weight 6 months ago: ___

___ % change in ___ weeks (based on Blackburn*)

Albumin ___ Prealbumin ___ Total protein ___ Lymphocytes % ___

Na ___ Potassium ___ Chloride ___ CO_2 ___

Glucose ___ A1C ___

Hgb ___ Hct ___ WBC ___ BUN ___ Creatinine ___

24-hour UUN ___ 24-hour creatinine ___

Nitrogen balance study ___

Metabolic chart study ___

Diet history ___

Blackburn's evaluation of weight change[16]

Time	*Significant weight loss (%)*	*Severe weight loss (%)*
1 week	1–2	>2
1 month	5	>5
3 months	7.5	>7.5
6 months	10	>10

* Values charted are for percentage weight change.
Percentage weight change = (usual weight − actual weight) × 100 (usual weight).

Table 2 Patient-generated subjective global assessment of nutritional status

A. *History*

1. *Weight change*
   I weigh about ___ pounds
   I am about ___ feet ___ inches tall
   A year ago I weighed about ___ pounds
   Six months ago I weighed about ___ pounds
   During the past 2 weeks my weight has ___ decreased ___ increased ___ not changed

2. *Food intake*
   I would rate my food intake during the past month (compared to my normal) as
   ___ no change ___ changed
   (a) ___ more than usual
   (b) ___ less than usual
   (c) ___ much less than usual
   ___ only solids
   ___ only liquids
   (d) ___ very little of anything

3. *Problems with eating*
   Over the past 2 weeks I have had the following problems that have kept me from eating (check all that apply)
   ___ no problems, just did not feel like eating
   ___ no appetite, just did not feel like eating
   ___ nausea
   ___ vomiting
   ___ diarrhea
   ___ constipation
   ___ mouth sores
   ___ dry mouth
   ___ pain
   ___ things taste funny or have no taste
   ___ smells bother me
   ___ other _____

4. *Functional capacity*
   Over the past 2–4 weeks I would rate my activity as generally
   ___ 0 = normal, no limitations
   ___ 1 = not my normal self, but able to be up and about with fairly normal activity
   ___ 2 = not feeling up to most things, but in bed less than half the day
   ___ 3 = able to do little activity, and spend most of the day in bed or chair
   ___ 4 = pretty much bedridden (rarely out of bed)

The remainder of the form will be filled in by your doctor, nurse or therapist. Thank you.

5. *Disease and its relation to nutritional requirements*
   Primary diagnosis _____
   Metabolic demand ___ no stress ___ low stress ___ moderate stress ___ high stress

B. *Physical examination*
(For each trait, specify: 0 = normal, 1 = mild, 2 = moderate, 3 = severe)
___ loss of subcutaneous fat (triceps, chest)
___ muscle wasting (quadriceps, deltoid)
___ ankle edema ___ sacral edema ___ ascites

C. *Subjective global assessment (SGA) rating*
(Select one)
___ A = well nourished
___ B = moderately malnourished
___ C = severely malnourished

trointestinal symptoms >2 weeks duration, functional status, and metabolic demands, as well as physical examination of muscle, fat and fluid status. Based on these features a patient is categorized as: (a) well nourished, (b) having moderate (or suspected) malnutrition, or (c) severely malnourished. The original SGA has been validated in surgical patients and transplant patients[17].

Caloric requirement

The total estimated non-protein calorie requirements are calculated for maintenance and stress (e.g. surgery, sepsis) (Table 3). In the patient taking oral nutrition, requirements vary from 20% to 50% above BEE values[1,7]. These additional requirements should be assessed on an individual basis. An extremely stressed patient receiving enteral or parenteral support may need up to 75% above BEE[7].

In severely cachetic patients, repletion of fat and muscle stores may not be achieved for several months. Normalization of serum albumin can require several weeks. Extended periods of therapy during the preoperative stage are impractical due to the uncertainty of organ availability and the severity of end-stage disease.

Table 3 Estimation of nutritional needs

Basal energy expenditure (BEE) at ___ weight = ___ calories
Male = 66 + (13.7 × dry weight in kg) + (5 × height in cm) − (6.8 × age) =
Female = 655 + (9.6 × dry weight in kg) + (1.7 × height in cm) − (4.7 × age) =

Cachetic patients have an impaired tolerance to oral intake, and enteral (nasogastric tube feeding) and parenteral (intravenous) hyperalimentation support may also be poorly tolerated. Such refeeding must be cautiously approached, to avoid the refeeding syndrome, in which malabsorption and distressing diarrhea can occur. Support should ideally be initiated before the patient is severely malnourished, to allow gradual increases in caloric and fluid intake[12]. Supplying the optimal nutritional support requires constant reassessment and monitoring. Overfeeding a patient can stress the heart, liver and kidney, thus eliminating the benefits of such support[18].

Although BEE provides an effective estimate of average energy needs, the actual metabolic requirements of the individual patient may be difficult to assess. Resting energy expenditure (REE), a form of indirect calorimetry, is useful for determination of energy requirements and appropriate substrate utilization[19]. Indirect calorimetry is used to measure actual oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). The ratio of CO_2 produced and O_2 consumed results in specific respiratory quotient (RQ) values. The specific RQ values are as follows: Carbohydrate 1.0 (maximum carbohydrate utilization is calculated[17] at 5 mg/kg per hour; protein 0.8; fat 0.7; mixed substrates 0.85.

The RQ reflects the oxidation of a mixed fuel of fat, carbohydrate, and protein. An RQ >1.0 is thought to represent fat synthesis. With higher RQ values, CO_2 production is increased and can result in increased respiratory demands, causing increased work of breathing for a compromised pulmonary patient. This would be appropriate if the exact energy requirement of a non-ambulatory patient were needed[16].

Protein requirement

The protein requirement of any individual patient is based on laboratory and anthropometric findings (Table 4). Needs range from 1.2 to 1.5 g of protein per kilogram of appropriate dry body weight[1]. Protein requirements may increase to levels of 1.5–2.0 g of protein per kilogram in the severely depleted patient – for example, the patient supported by an artificial heart. In the depleted patient, renal failure should be treated by dialysis rather than by protein restriction.

Table 4 Nutrient needs

	Calorie	*Protein*
Lung		
Maintenance	1.0–1.2 × BEE	1.0–1.5 g protein/kg dry weight
Repletion	25–35 kcal/kg dry weight	1.5–2.0 g protein/kg dry weight
	(Promote weight gain with CF patients)	
Heart		
Maintenance	1.2–1.5 × BEE	1.2–1.5 g protein/kg dry weight
Repletion	25–35 kcal/kg dry weight	1.5–1.75 g protein/kg dry weight

NUTRITIONAL SUPPORT PRETRANSPLANT

The obese patient

The obese transplant candidate (>20% above desirable body weight due to adipose tissue) is also at nutritional risk. Protein status, eating habits, and family eating patterns are often poor. Detailed histories of diet, of nutritional status of members of the family, and of change in weight are helpful. If time allows, the patient should work with a dietitian and be guided toward gradual weight loss (0.5–2 kg/week) while maintaining adequate protein status. The patient should not be instructed to decrease weight without professional guidance and monitoring, as this often leads to severe protein and nutritional deficits.

The patient in the early stage of cardiac failure

The goal of nutritional support is to provide adequate calories, protein and other nutrients without overfeeding the patient. The New York Heart Association class II or III patient may show no problems with anorexia or weight loss. Education regarding nutritional requirements, suggesting a high-protein, high-calorie, moderate sodium intake, may be sufficient treatment at this stage (Table 5).

The patient in advanced cardiac failure

As cardiac function decreases, more intense support is necessary. Intake may vary daily, depending on gastrointestinal function and overall well-being. Cellular hypoxia, secondary to increasing cardiac failure, gradually leads to multisystem failure. Malabsorption occurs as the viscera become engorged with fluid, and the body is unable to utilize nutrients provided enterally[17]. Gastrointestinal motility may decrease, resulting in constipation and/or diarrhea. Reduced renal blood supply results in conservation of nutrients and water by the kidneys[1]. Free water, supplied in the form of nutritional support and medications, is retained unless diuretics are used. Diuretics deplete mineral and vitamin levels, requiring careful monitoring and supplementation[20].

Sodium restriction is commonly advised, yet should not compromise adequate nutritional intake, particularly protein intake. A high-protein diet can contain as little as 87–176 mEq (2–4 g) of sodium. Small frequent meals, snacks, and high-protein shakes are recommended to increase total protein intake as much as possible.

Calorie and protein counts and nitrogen balance studies can accurately indicate actual daily intake. If oral intake is consistently at or below the calculated BEE, additional support will clearly be necessary. Alternate routes of nutritional support, such as enteral or parenteral hyperalimentation, are often required. While enteral support is preferred, tolerance is generally poor. Peripheral venous hyperalimentation can be beneficial in supplementing oral intake in hospitalized patients. Central venous hyperalimentation is occasionally necessary, and should not be postponed until serial laboratory studies indicate severe nutritional deficits.

Table 5 Constituents of recommended diet*

The calorie level is calculated on an individual basis to achieve and maintain desirable body weight and should consider an activity factor for exercise if feasible.

The total number of calories should be made up of:
- *Carbohydrate*: 45–55%
  - 33% from simple sugars in fruits
  - 67% from complex carbohydrate high fiber (3–4 g/day)
  - Limited concentrated sweets/sugar/alcohol
- *Protein*: 25–30%
- *Fat*: 18–25%
  - 33% or less of calories from saturated
  - 33% monounsaturated
  - 33% polyunsaturated

The daily diet should not contain more than approximately 200 mg cholesterol and 176–220 mEq sodium (4–5 g)

*Recommendations listed are modified American Dietetic Association and American Heart Association guidelines. The modifications listed were deemed necessary after experience with transplant patients[1].

The patient with cardiac cachexia

The patient with rapidly deteriorating cardiac function may develop cardiac cachexia while awaiting transplantation[2]. Nutritional treatment often includes a form of hyperalimentation. Although cachexia may be a compensatory mechanism to reduce oxygen consumption and decrease cardiac demands, at least minimal energy and nutrient needs should be supplied to maintain the patient's immune status and survival[7]. The absolute quantities and relative proportions of carbohydrate, protein and fat desirable to maintain minimal nutritional support and reduce cardiac stress remain highly debated, and require further detailed investigation.

The patient on a mechanical assist device or artificial heart

The artificial-heart patient has postoperative metabolic stress in addition to pre-existing malnutrition. Increased cardiac output and nutrient distribution to atrophied tissues stimulates the anabolic process. Energy and protein requirements will exceed those before implantation of the device. If the patient had been taking adequate nutrition prior to implantation, his or her nutritional needs may not increase so significantly. A high-protein, high-calorie diet, with vitamin and mineral supplementation (and frequent 'snacks') is appropriate.

Oral intake may require supplementation in the form of enteral or parenteral hyperalimentation. The gastroenterologist, dietitian, and nutrition support team should monitor tolerance and intake closely. Weekly calorie counts and nitrogen balance studies should be checked to assess adequacy of nutritional support.

The patient with respiratory disease

In patients with respiratory problems, respiratory muscles, as well as other somatic muscles, are catabolized to meet energy needs when nutrition is not adequate. With a reduction in weight and body mass, respiratory muscle mass decreases. When this occurs, a decrease is observed in the availability of substrates that are used for production of energy and cellular growth. Severe hypoalbuminemia may be a contributing factor to pulmonary edema[20], with a reduction in oncotic pressure and fluid shifts into the interstitial space. The primary goal is to minimize CO_2 production while providing adequate nutritional needs, and avoidance of excess kilocalories, which could prove detrimental to pulmonary function and contribute to hypercapnia in some patients[8].

NUTRITIONAL SUPPORT IMMEDIATELY POST-TRANSPLANT

Immediate postoperative care should be focused on establishing hemodynamic stability, hydration status, and respiratory function, and on monitoring complications related to the surgery. The goal of post-transplant nutritional support is to provide adequate nutrients to promote anabolism and prevent infection. Well-nourished patients able to begin a diet within 3–4 days after surgery usually do not need nutritional support. Others who are unable to begin a diet within this time-frame, or who were malnourished pretransplant, may benefit from nutritional support. Nasogastric tube feeding is the preferred method of nutritional support. Total parenteral nutritional support is indicated when a patient is unable to tolerate tube feedings.

Post-transplant patients require increased amounts of nutrient for wound healing at a time when renal and hepatic function are frequently impaired, appetite may be poor, and side-effects of medications may result in gastrointestinal distress[4]. Postoperatively, the patient's diet is advanced to solid foods as tolerated, the goal being to provide a high intake of calories[16] (BEE $\times$ 1.5 = 1.75) and protein (1.2–1.5 g/kg dry body weight). For cachetic patients an injury factor of 1.2 for calories and 1.0–1.2 g of protein/kg dry body weight would be more appropriate. Hyperalimentation may be continued to support a previously malnourished patient. Small frequent snacks between meals, including commercial liquid nutritional supplements, are continued, but may be modified to conform to new dietary restrictions.

Biochemical values to be monitored include serum electrolytes, glucose, BUN, creatinine, albumin, and/or prealbumin. If BUN and creatinine rise, protein may need to be restricted. Intake is monitored by frequent calorie nutrient counts and nutritional assessments.

The patient's nutritional status and intake ability dictate the extent to which dietary restrictions can be implemented during the first week post-transplant. The restriction on sodium intake is usually relaxed to 176–220 mEq (4–5 g) per day, and sugar intake, especially in the form of concentrated sweets, is limited, to

minimize the effects of corticosteroids on glucose metabolism. Cholesterol and saturated fat restrictions are also implemented.

The protective isolation precautions used by some institutions may eliminate fresh fruit and vegetables from the patient's diet. Although aerobic Gram-negative bacilli are commonly found on such foods, they are no longer considered dangerous unless the patient is neutropenic[1,21]. Allowing fresh fruit and vegetables in the diet often improves intake, and has not been associated with increased infection rates[22].

LONG-TERM NUTRITIONAL CARE

Long-term nutritional care is best provided by a dietitian in conjunction with follow-up appointments with the cardiologist/pulmonologist or surgeon. The patient should be encouraged to record all food intake over a 3-day period at designated intervals: (a) immediately after discharge from the hospital, (b) after 6 weeks, and (c) 6 months later. A careful review of such intake is helpful in determining compliance, and the necessity for further dietary education and guidance. Changes in the diet may cause confusion, and therefore should be explained. Patient education is critical for long-term compliance with any modification in daily dietary habits.

Readmission or extended hospitalization is not uncommon in patients with organ transplants, due to episodes of infection or rejection. Nutritional assessment, monitoring, and support are recommended with any readmission. In the case of sepsis and high metabolic needs, a high-protein, high-calorie diet with supplements may be necessary to maintain adequate intake. Antibiotic therapy and poor oral intake add to nutritional risks.

Drug-related nutritional problems

The long-term nutritional care of the transplant patient must take into consideration drug-induced metabolic changes and the side-effects of medications. Because of more effective immunosuppressive drug regimens, the success of transplantation is increasing and recipients are living longer. Long-term post-transplant complications are therefore emerging. Obesity, hypertension, hyperlipidemia, diabetes mellitus, and osteoporosis are common complications. Since these complications affect survival, a treatment program in the form of diet and exercise should be included.

Corticosteroids affect carbohydrate, protein and fat metabolism, as well as adipose distribution in the body. Changes in carbohydrate metabolism can result in steroid-induced diabetes mellitus. Appetite and craving for sweets are also stimulated, contributing to obesity and abnormal blood sugar levels. Protein catabolism results in muscle wasting, thinning of the skin, dissolution of vertical bone matrix, and poor wound healing[23]. The influence of steroids on fat tissue results in loss of subcutaneous fat from extremities and excessive deposition in the trunk areas[23]. Excessive fat in the trunk has been associated with an increased risk of atherosclerotic disease[24].

Prednisone's antagonistic effects on vitamin D lead to changes in the body's calcium balance, contributing to osteoporosis, and its mineralocorticoid effect is associated with hypertension[22]. Zinc depletion, contributing to delayed wound healing, is another possible long-term effect[23]. These side-effects are reduced when low-dose prednisone therapy is administered, in conjunction with cyclosporin, though the incidence of hypertension may increase. Nutritional support aimed at reducing these effects of corticosteroids is a primary goal of post-transplant dietary care.

Cyclosporine therapy may be associated with hypertension, renal and hepatic dysfunction, and an increased risk of viral infection[25]. The intake of foods with high protein quality (e.g. meat and milk) and restricted sodium is recommended to counter these side-effects.

Azathioprine therapy may be associated with hematologic changes, gastrointestinal problems, hepatitis, or pancreatitis. Dietary modifications play an important part in the care of patients with gastrointestinal problems, and should be based on individual symptoms.

Tacrolimus (known as FK506 during the research phase of its investigation) is one of the newest immunosuppressive agents available for patient use (Chapter 10). Its use may be associated with hyperkalemia, hyperglycemia and hypermagnesemia. In addition, loss of appetite, along with nausea and/or vomiting, has been observed in some patients. Dietary modification should be based on individual symptoms and tolerance, but when gastrointestinal symptoms are severe, patients may benefit from taking liquid supplements (2 ounces every 2 waking hours).

Recommended diet for the heart or lung transplant recipient

Total caloric intake, as well as the types of carbohydrate, protein and fat taken, are controlled to minimize the short- and long-term nutritional complications that may be associated with immunosuppressive therapy. Calorie intake should be adjusted to achieve and maintain ideal body weight, and may in turn prevent complications of hypertension and carbohydrate intolerance. A low-cholesterol, low-saturated-fat diet with limited sugar and no concentrated sweets, and a moderate restriction of sodium of approximately 2–4 g per day is recommended. Dietary sodium is primarily restricted to reduce the sodium and fluid retention induced by steroid therapy. Potassium restriction may be necessary due to cyclosporin therapy. Cholesterol restriction is recommended on the basis that it may reduce the high incidence of atherosclerotic disease seen in patients with transplants (Chapters 13 and 35). Intake of polyunsaturated fat is increased to achieve at least a balance with saturated fat.

The pretransplant nutritional status of the patient will be an important factor in the strictness with which this diet can be applied in the early post-transplant period, though the long-term goal includes all of the restrictions listed. Specific recommendations for this special diet are listed in Table 5.

Vitamin and mineral supplementation may be necessary if dietary compliance is strictly followed. For example, if the patient avoids all red meats in order to reduce cholesterol and saturated fat, the intake of iron should be monitored carefully and iron supplements given if necessary. Detailed adjustments for food preferences should be made for each patient. Adequate amounts of extra lean (>95% fat-free), high-quality protein sources are encouraged to offset muscle wasting. A high intake of skim or 1% fat milk is beneficial as a source of protein, calcium and vitamins A and D.

However milk intake should be limited if renal failure results in hyperkalemia.

If the patient is underweight, it is important to increase body weight so that there are adequate fat and protein reserves for periods of infection or rejection. Conversely, once desirable body weight is attained, it is equally critical to prevent excessive weight gain, since obesity can lead to increases in blood cholesterol, triglyceride and glucose levels and in blood pressure, all of which are risk factors for coronary disease.

Whenever possible, nutritional counseling should include both the patient and his/her family members. Emotional support can enhance compliance with drug and nutritional therapy. This is important, as non-compliance with the drug regimen and other instructions has been documented as a major contributory factor to mortality in some recipients[26].

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17
A Commentary on Quality of Life after Thoracic Organ Transplantation

M.M. EDGAR

INTRODUCTION

Survival of a patient and graft after transplantation was once thought to be the measure of a successful transplant. Today quality of life (QOL) issues are regarded as just as important as, if not more important than, simply the survival of the patient. Mai[1] suggests that 'Quality of life, probably more than any other aspect of a transplant program, is the acid test of the success and efficacy of the operation.'

At the time of writing, one of my childhood idols, the former baseball star Mickey Mantle, has just had a liver transplant. Organ transplantation has become commonplace at most major medical centers across North America, Europe and Australasia. The facts appear incontrovertible that organ transplantation does increase the recipient's lifespan. Unfortunately, in today's climate of bureaucracy and health economics, most medical decisions are as much a product of the business administrators as they are of the physicians. In other words the cost–benefit of any therapy is becoming increasingly analyzed and questioned.

Examples of this growing awareness of the cost of health care are numerous throughout the Western world, particularly in the USA.

'U.S. health care is the biggest industry in world history. In 1995, the United States is expected to spend an estimated $1 trillion on health care. The United States represents about 5% of the world's population; however, according to estimates by the World Bank and the World Health Organization, more than 40% of world health care expenditures occur in the United States' (World Bank, 1993, quoted by Kaplan[2].

'The system costs too much, and the accelerating costs have the potential to ruin the entire economy (affordability problem). Despite these high costs, there are significant numbers of people who have too little or no health insurance (access problem), and the United States is unable to demonstrate that its high expenditures on health care result in better outcomes for patients (accountability problem)'[2].

In February 1994 the state of Oregon began a plan that excluded payment for medical treatments for certain conditions where there was considered little or no evidence that the therapy improved the patient's longevity or improved his or her QOL. End-stage cancer and AIDS were included as disease processes for which there is no treatment that is known to be effective. Therefore, it behooves each and every health-care provider to be able to document to the fullest extent possible that the treatments offered (including organ transplantation) will increase longevity and/or will improve the patient's QOL. Increasing attention is therefore being paid to assessing QOL.

WHAT IS MEANT BY 'QUALITY OF LIFE'

In 1902 it was suggested to the readers of Mark Twain that 'the value of life must be discerned from the individual's perspective'[3]. Is there, therefore, a satisfactory method of measuring such QOL when it is such a subjective observation? The answer remains uncertain, but many conscientious attempts have been made to do so. It may clearly be preferable if we know the patient before the transplant, and his or her own preconceived expectations and notions about transplantation, and what he or she is hoping to accomplish post-transplant. Only then will we be in a position to know if indeed those expectations have been achieved.

There is a growing body of evidence that pretransplant expectations have a profound bearing on estimates of QOL post-transplant. In an article by Leedham, *et al.*[4], preoperative positive expectations were demonstrated to affect physical health after transplantation. Many explanations for this finding are possible, but it suggests that helping the patient pretransplant to become more optimistic about the outcome of transplantation could be helpful, and could indeed affect the outcome positively. The old adage that 'you get what you expect' may be very true.

Molzahn[5], in her review of the literature pertaining to QOL after organ transplantation, commented: 'It is generally known that these procedures increase the length of life, but less is known about the quality of that life.' She drew attention to several issues that remained to be addressed, including 'definitions of quality of life, dimensions of quality of life, expectations of quality of life and clinical assessment of quality of life'. Four years later these same issues remain in the forefront of QOL discussions.

'The major problem with QOL research is that nearly every researcher has defined the term differently'[5]. 'The first issue pertains to defining QOL. Although thousands of publications have been written on the topic, the definition of QOL is not at all clear. Most researchers define QOL in terms of happiness or satisfaction with life. However, the terms "happiness" and "satisfaction" differ from each other, with happiness generally referring to a transient feeling of pleasure, and satisfaction referring to an assessment of a life situation.

'Quality of life is a complex concept: there is no agreed definition nor is there a universally used measuring instrument' (McDowell and Newell, 1987 quoted by Mai[1]. Quality of life may, therefore, be exactly what you think it is; nothing more and nothing less. It may be just like 'beauty' – in the 'eye of the beholder'. We all appreciate there are certain things that cannot be measured by any method except personal choice.

My bias for a philosophical definition of QOL is best expressed by Kaplan[2] in his presidential address to the Division of Health Psychology of the American Psychological Association. It is entitled *The Ziggy Theorem: toward an outcomes-focused health psychology*. In the cartoon, Ziggy confronts a wise man and asks him, 'Tell me, old wise one, what is the meaning of Life?' The wise man replies, 'Ah, yes ... the meaning of life. Life, my boy, is doin' stuff!' The shocked Ziggy responds, 'Life is doing stuff? That's it?' The wise man then reflects, 'As opposed to death, which is *not* doin' stuff.' Ziggy reacts, 'It's a more elementary theory than I expected, but one you can't argue with!'

Over the past 20 years Kaplan has developed a 'general health policy model'. 'The model separates aspects of health status into distinct components. These are life expectancy (mortality), functioning and symptoms (morbidity), preference for observed functional states (utility), and duration of stay in health states (prognosis).'

'A model of health outcomes necessarily includes a component for mortality. Death is an important outcome that must be included in any comprehensive conceptualization of health. In Ziggy's terms, death is the most extreme and most permanent state of "not doin' stuff". Thus, death serves as an anchor against which to evaluate levels of wellness'[2].

'Researchers generally agree upon three important properties of quality of life: it is a multifactorial, subjective, and temporal concept'[3].

Schipper *et al.*[6] suggested four areas that contribute to QOL: '(i) physical and occupational function, (ii) psychologic state, (iii) social interaction, and (iv) somatic sensation'[3]. Bunzel *et al.*[7], however, considered nine areas that contributed to life quality: 'physical, emotional, mental, vocational, and sexual status, financial situation, leisure activities, partnership, and overall life quality.'

In fact, 100 life areas have been identified to constitute quality of life[8]. However, it appears an insurmountable task to measure each area. That researchers often attempt to sum up the results of a QOL assessment as a single score on a single scale illustrates an effort to simplify a complex subject that probably defies such simplification. Nevertheless, if we are to assess the results of our therapeutic procedures, we must make an effort to come up with some relatively simple scale.

METHODS OF MEASURING QUALITY OF LIFE

Numerous questionnaires have been designed in an effort to glean information from which QOL can be evaluated in patients who have undergone various procedures, not confined to organ transplantation. Appendix A reproduces one such questionnaire developed by Lim *et al.*[9]. This provides a good and rapid assessment of QOL in post-myocardial-infarction patients and, with appropriate word changes, could be used for patients with other clinical conditions, including those after organ transplantation.

Many such 'good' methods have been designed to assess quality of life (Appendix B). By 'good' it is meant to indicate that the method gives us information we believe to be true, but which otherwise we would not be able to present in an objective format. Many of the questionnaires or 'instruments' suggest substantial *reliability, but we must be cautious in interpreting the results obtained.* Whether you choose to take showers or baths *might* be a reliable indicator of the presence of schizophrenia. Unfortunately, it might equally be related to whether or not you have a shower. Thus, most instruments report significant reliability, but may not be measuring what the investigators think they are. One needs to be reminded of the teaching that an IQ is what an IQ test measures – nothing more and nothing less! Perhaps the same can be said for QOL.

RESULTS OF QOL STUDIES IN THORACIC ORGAN TRANSPLANT PATIENTS

Numerous QOL studies have been carried out on: (a) patients awaiting transplantation of hearts and other organs[3,10–13], (b) patients with heart transplants (all of which studies reported improved QOL for virtually all patients whose surgical outcome was successful)[4,7,14–21], and (c) patients, both adults and children, who received heart–lung transplants[19,22,23]. Certain authors have concentrated attention on pediatric recipients[19,22]. There appear to be no such studies as yet on patients who have undergone lung transplantation.

Dracup and colleagues[12], in a study of QOL in patients with advanced heart failure, concluded: 'These findings support the inclusion of quality of life as an outcome measure in any evaluation of treatment efficacy and suggest the interventions to improve the quality of life of patients with advanced heart failure need to be targeted at reducing depression and hostility and increasing daily activity levels.'

Levenson and Olbrisch[24] compared psychosocial evaluations of organ transplant candidates in terms of process, criteria and outcomes. In summary, cardiac programs, when compared with renal and hepatic programs, were the most 'stringent' in criteria for acceptance and in rates of refusal. Muirhead *et al.*[13] observed that 'patients awaiting heart transplantation, although dissatisfied with quality of life, maintain positive psychological and social adjustment.'

Wray and colleagues[25] concluded that 'early postoperative findings indicate an improvement in quality of life after heart or heart–lung transplantation but longer-term follow-up is now necessary.'

Jones *et al.*[26] reported longitudinal results on QOL and psychological adjustment after heart transplantation, and concluded that there was 'no evidence of mood disorder and a high level of well-

being in this sample of cardiac transplant recipients up to four years after transplantation.'

In 1990 Rosenblum *et al.*[15] followed 200 heart transplant recipients for as long as 10 years after heart transplantation and monitored their QOL. 'The most commonly reported complaints were generalized weakness (54%), fatigue (42%), and low back pain (37%) ... analysis of the individual items of the Sickness Impact Profile (SIP) revealed highly prevalent dysfunctional behaviors such as decreased sexual activity, decreased housework, abnormal sleep patterns, and decreased endurance.' Rosenblum suggested a program of physical and occupational therapy 'such as endurance training, body care and movement, and home activities/housework'. Interventions in these symptom constellations might prove beneficial in circumventing these QOL issues at a later date.

Bunzel *et al.*[7], however, in a QOL study on heart transplant patients, 'found an absolute increase in quality of life'.

Riether's group[27] measured outcomes in liver and heart transplant patients, and concluded that 'both groups showed significant improvements after transplant in neurocognitive functioning, depressive symptoms, and quality of life'.

In contrast, however, Baumann *et al.*[19] demonstrated that although 'life improved for the majority post-transplantation, recipients continue to experience work problems, financial burdens, family role changes, life-style changes, and side effects associated with long-term drug treatment'.

COMMENT

Hypothesis, theory, reliability, and validity of instruments (questionnaires) all contribute to an assessment of QOL, and indeed confirm that it is to some extent a measurable entity. However, Lofton[28] advises the transplant patient to ask questions to help clarify what it might be like to have a surgically successful transplant yet still feel 'I think I am merely dying a bit slower now.' He emphasizes how an organ transplant changes the patient's whole perspective on life. 'I hate it when people say "At least you're alive". I find myself more understanding of people who take their own life when they suffer serious medical problems.' Lofton delivers an insightful message to anyone involved in organ transplantation, and his article is recommended reading for all involved in the care of transplant patients. From time to time we should put aside the science and research, and take the time to listen to a real patient. His is a sobering message.

An old principle of treatment and research might be applicable to QOL researchers. This is the KISS principle, i.e. Keep It Simple, Stupid! Not infrequently it appears to the writer that lawyers, physicians, researchers, and, indeed, most educated individuals complicate subjects unnecessarily. In 25 years of working with people it never ceases to amaze me what we try to find out without actually asking the direct question. How do you really know what a patient wants from life and expects from a transplant without asking? It may be sufficient simply to ask the patient whether he or she would undergo the procedure (i.e. the organ transplant) again. As Bunzel *et al.*[7] say: 'When one understands life quality as a subjective experience ... the only sensible source of information is the patient.'

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APPENDIX A: EXAMPLE OF A QOL QUESTIONNAIRE

An adaptation of a questionnaire directed towards an assessment of QOL after acute myocardial infarction utilized by Oldridge *et al.* and adapted by Lim *et al.*[9] In the interest of brevity, and in order to give the reader a general outline of the salient points, aspects relating to time periods have been omitted. For example, in the original questionnaire item 1 in full reads 'In general, how much of the time *during the last two weeks* have you felt frustrated, impatient or angry?'

Responses to all questions were to be differentiated as: (1) None of the time, (2) A little of the time, (3) Some of the time, (4) A good bit of the time, (5) Most of the time, (6) Almost all of the time, (7) All of the time.

Questions

1. How much of the time have you felt frustrated, impatient or angry?
2. How often have you felt worthless or inadequate?
3. How much of the time did you feel very confident and sure that you could deal with your heart problem?
4. How much of the time did you feel discouraged or down in the dumps?
5. How much of the time did you feel relaxed and free of tension?
6. How often have you felt worn out or low in energy?
7. How happy, satisfied or pleased have you been with your personal life?
8. How often have you felt restless or as if you were having difficulty trying to calm down?
9. How much shortness of breath have you experienced while doing your day-to-day physical activities?
10. How often have you felt tearful or like crying?
11. How often have you felt as though you were more dependent than you were before your heart trouble?
12. How often have you felt unable to do your usual social activities or social activities with your family?
13. How often have you felt as if others no longer have the same confidence in you as they did before you had the heart problem?
14. How often have you experienced chest pain while doing your day-to-day activities?
15. How often have you felt your heart problem limited or interfered with sexual intercourse?
16. How often have you felt unsure of yourself or lacking in self-confidence?
17. How often have you been bothered by aching or tired legs?
18. How much have you been limited in doing sports or exercise as a result of your heart problem?
19. How often have you felt apprehensive or frightened?
20. How often have you felt dizzy or light-headed?
21. How much have you been restricted or limited as a result of your heart problem?
22. How often have you felt unsure as to how much exercise or physical activity you should be doing?
23. How often have you felt as if your family is being overprotective toward you?
24. How often have you felt as if you were a burden on others?
25. How much of the time have you felt that you could manage chest pain if and when you had any?

APPENDIX B: QOL INSTRUMENTS OR METHODS

This is not designed to be a comprehensive list of QOL instruments or methods. For a further listing of psychological testing instruments and reliability and validity information on each, the reader is referred to *Buros Mental Measurements Yearbooks* or *Tests In Print* (both published by the Buros Institute of Mental Measurements, University of Nebraska, Lincoln, Nebraska).

Health Concerns Questionnaire (Spoth & Dush)
General Health Questionnaire (Goldberg)
The Nottingham Health Profile
Sickness Impact Profile
Quality of Life After Acute Myocardial Infarction (Oldridge)
Perceived Quality of Life Scale (Patrick *et al.*)
Health Behavior Scale (Miller *et al.*)
Profile of Mood States (McNair *et al.*)
Cardiac Health Knowledge Scale (Hanvik)
Hospital Anxiety and Depression Scale (Zigmond)
Coronary Prognostic Index (Norris)
UCLA-Social Support Inventory (Dunkel)
Specific Activity Scale (Goldman)
The Heart Patient's Psychological Questionnaire (Jan Van Dixhoorn)
State–Trait Anxiety Inventory (Speilberger)
Social Readjustment Scale (Holmes and Rahe)
The Psychosocial Adjustment to Illness Scale (Derogatis)
Karnofsky Performance Status Scale (Karnofsky)
Life Stressors and Social Resources Inventory
Coping Responses Inventory (Moos)
Strait–Trait Anger Expression Inventory (Speilberger)
Psychosocial Pain Inventory (Heaton *et al.*)
Coopersmith Self-Esteem Inventory (Coopersmith)
Coping Resources Inventory (Hammer and Marting)
Problem Solving Inventory (Heppner)
Millon Behavioral Health Inventory (Millon)
Brief Symptoms Inventory (Derogatis)

18
Experimental Development and Early Clinical Experience

D.K.C. COOPER

INTRODUCTION

Clinical heart transplantation was made possible by the considerable experimental work carried out earlier this century, which embraced mainly the technical, physiological and immunological aspects of the procedure. This chapter endeavours to review briefly the evolution and results of experimental surgical techniques utilized by cardiac transplant research workers; a comprehensive review appears elsewhere[1].

Experimental work on cardiac transplantation evolved through several overlapping phases. In the earliest experiments animals were given a second, often parasitic, heart which enabled certain physiological, pharmacological and pathological studies to be made. Initially the neck was chosen as the locus, though the abdomen and inguinal regions were occasionally used. The subsequent evolution of surgical techniques permitted the insertion of the donor heart into the chest as an auxiliary pump in circuit with the recipient organ. With the advent of hypothermia and the pump–oxygenator, total excision and replacement of the recipient heart became more feasible. Finally, after technical and physiological problems had been studied and minimized, efforts were made to combat the immune response with immunosuppressive agents.

TRANSPLANTATION OF AN ACCESSORY HEART

The first reported attempts at experimental heart transplantation were by Carrel (Figure 1) and Guthrie in 1905[2,3]. The principal technique they used is inadequately described as 'anastomosing the cut ends of the jugular vein and the carotid artery to the aorta, the pulmonary artery, one of the vena cava and a pulmonary vein. Although contractions of the donor atria appeared immediately, effective contractions of the ventricles did not begin for approximately 1 hour. The experiment was interrupted after a further 2 hours when coagulation occurred in the cavities of the heart.

The crucial factor of donor coronary perfusion (viviperfusion) was simplified in 1933 when Mann and his colleagues developed a technique of cervical transplantation[4] (Figure 2). Numerous investigators have subsequently used modifications of the Mann technique to study problems of heart transplantation and the response of the denervated heart to pharmacological agents and physiological stresses[1]. One such modification remains a standard model in many laboratories, including our own, for experimental animal studies on acute rejection and immunosuppression (Figure 3).

Figure 1 Alexis Carrel was awarded the Nobel Prize for Physiology and Medicine in 1912, primarily for his work on the anastomosis of blood vessels

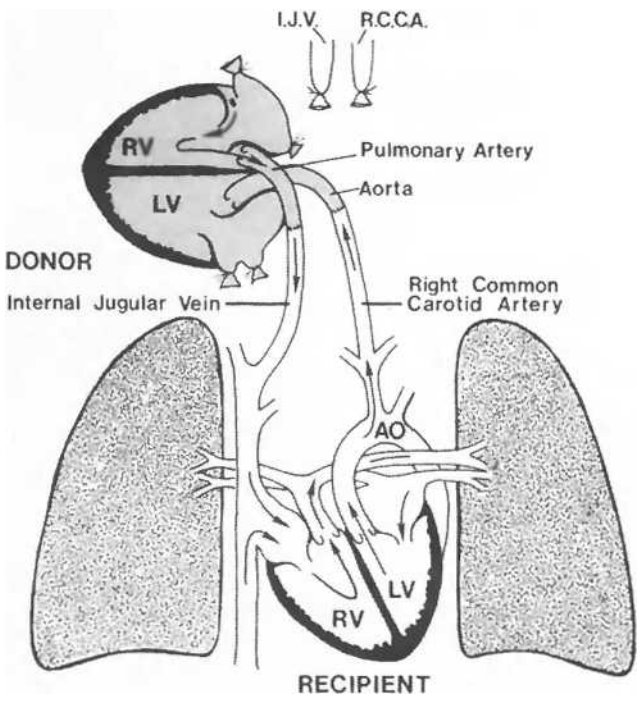

Figure 2 Technique of experimental heterotopic heart transplantation in the neck (Mann *et al.* 1933)[4], I.J.V. = internal jugular vein; R.C.C.A. = right common carotid artery

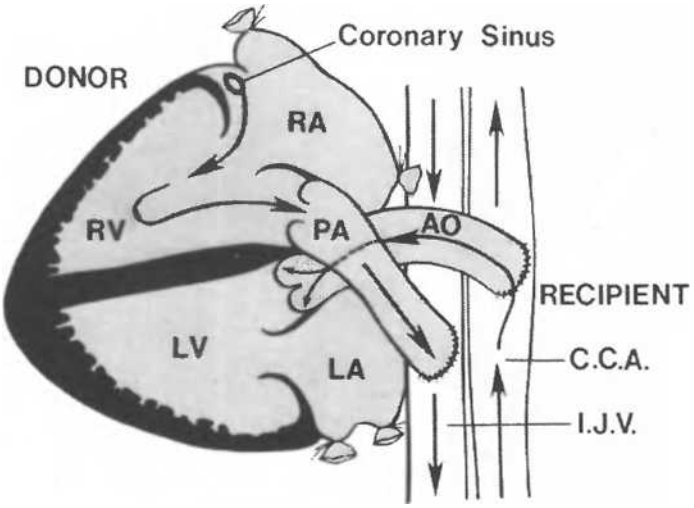

Figure 3 Modification of the Mann experimental cervical heterotopic heart transplantation technique[4] as used in our own laboratory

From their results, Mann and his co-investigators concluded that a functioning cardiac allograft was no less 'resistant' than a renal allograft, the graft failing to survive due to the same 'biologic factor' which also prevented survival of other homo (allo) transplanted tissues and organs. Such a transplanted heart, however, proved a valuable test object for the investigation of various physiological problems. For example, the effect of the intravenous administration of thyroxine to the host animal was investigated; the denervated donor heart was demonstrated to be more sensitive to the accelerating influence of the drug since central nervous system influence was inhibitory.

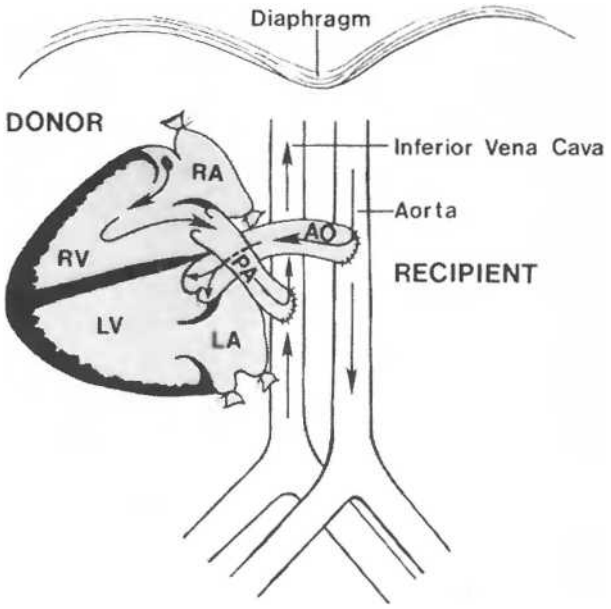

Figure 4 Technique of experimental heterotopic heart transplantation in the abdomen (Abbott *et al.* 1964)[5]

In more recent years, techniques for transplanting the auxiliary donor heart into the abdomen of the recipient have been described, principally for the study of the immune response and its modification by therapeutic agents (Figure 4); using microsurgical techniques it remains an important experimental model in rats[5,6].

THE TRANSPLANTED HEART AS AN AUXILIARY INTRATHORACIC PUMP

In 1946, Demikhov (Chapter 66, Figure 1) began extensive studies on transplantation of the heart into the thorax. These involved the addition of a second heart (occasionally with an attached lobe of a lung) as an auxiliary pump, as well as orthotopic transplantation of the heart with and without both lungs[7]. The ambitious nature of Demikhov's attempts can be appreciated best when it is remembered that supportive techniques, such as hypothermia and cardiopulmonary bypass, had not yet been developed.

In all, Demikhov described 24 variants of his technique to place an additional heart within the thorax, performing 250 operations on dogs utilizing most of the major vessels within the chest cavity. Few animals survived more than a few days, most of the early deaths being associated with technical problems. The best results with regard to functional activity of the transplanted heart and preservation of its structure were obtained after operations using the technique illustrated in Figure 5. Physiologically the transplanted heart was distinguished by the comparative constancy of its rhythm and by its greater resistance to the action of toxic doses of various cardiac glycosides. The physiological and pharmacological responses of the denervated, transplanted heart are discussed more fully in Chapter 27.

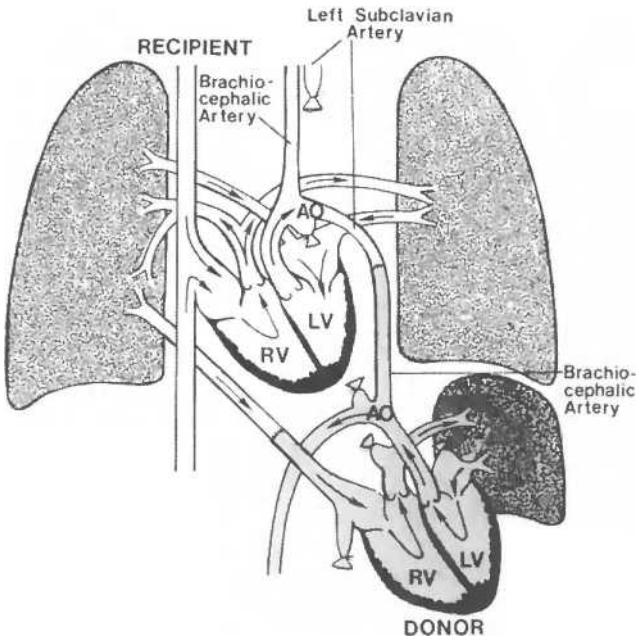

Figure 5 Technique of insertion of the heterotopic heart in the chest as an auxiliary pump (Demikhov 1962)[7]

In 1964, Reemtsma[8] described a method of inserting an additional intrathoracic heart as an auxiliary pump, which was similar in principle to that later developed and used clinically in the heterotopic heart transplant program in Cape Town[9,10]. The donor inferior vena cava was anastomosed to the recipient's right atrial appendage, followed by anastomosis of the two left atrial appendages, and end-to-side anastomoses of the two pulmonary arteries and aortae. Function as an auxiliary pump was maintained for a maximum period of 72 hours.

One year later, Sen and his colleagues described a further technique in which the transplanted heart supported only the systemic circulation of the recipient[11]. This auxiliary heart functioned in one animal for 48 hours, when it was surgically excised and the animal supported solely by its own heart once again, thus demonstrating the heterotopic heart transplant as a temporary left ventricular assist device.

ORTHOTOPIC TRANSPLANTATION OF THE HEART

On 25 December 1951 – a date which surely tells us a great deal about this surgeon – Demikhov made the first recorded attempt to replace the heart alone[7]. Without the availability of hypothermia or pump–oxygenator support, the technique was necessarily complicated. The procedure consisted of end-to-side anastomoses between the corresponding thoracic aortae, superior and inferior venae cavae, and pulmonary arteries. The two inferior pulmonary veins of the donor were joined together and connected to the recipient's left atrial appendage. After these anastomoses the ascending part of the recipient's thoracic aorta and pulmonary artery were ligated, and the recipient's left atrium was indrawn at its border with the ventricle by means of a purse-string suture. The entire segment of the recipient's heart thus excluded from the circulation was then excised.

Demikhov performed this procedure on 22 occasions, and in 1955 was successful in obtaining good cardiac function in two cases for periods of just over 11 and 15 hours respectively. In all but one case, death resulted from technical problems. These were amongst the first reported experiments, however, where animals survived for a few hours solely on the activity of a transplanted heart.

ADVENT OF SUPPORTIVE TECHNIQUES

With the advent of methods of supporting the recipient during the operative procedure, workers in this field became more ambitious. Significant early attempts were made by Neptune and his colleagues, using hypothermia[12], by Webb and Howard[13,14] and by Goldberg and co-workers[15,16], both using mechanical pump–oxygenator support.

In 1959, Cass and Brock reported six attempts at autotransplantation and homotransplantation using a modification of Goldberg's technique, where both atria were left intact in the recipient, thus simplifying the procedure[17]. Anastomoses of the

Figure 6 Richard Lower, who worked with Norman Shumway at Stanford Medical School over many years, and performed much of the early experimental work on heart transplantation. He subsequently set up the heart transplant program at the Medical College of Virginia

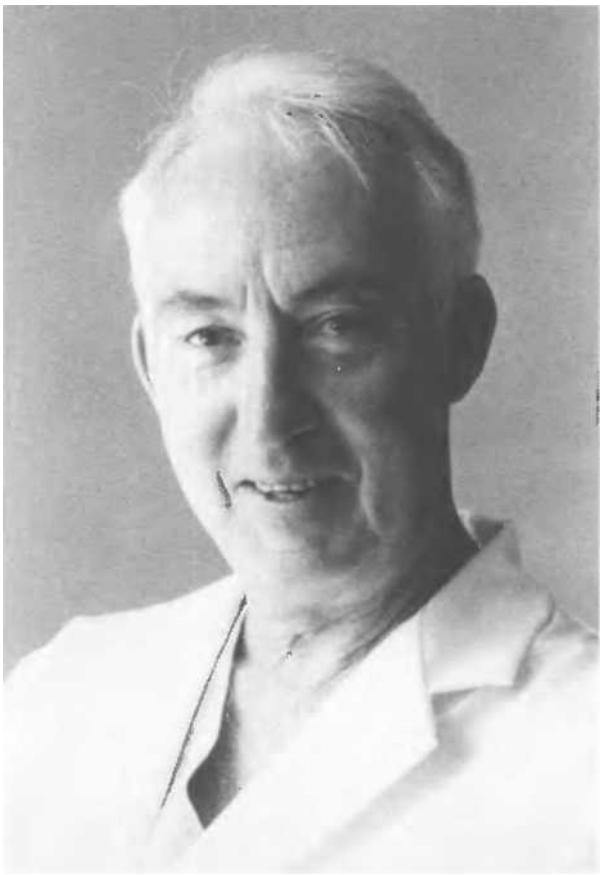

Figure 7 Norman Shumway, one of the major pioneers of heart transplantation. Much of the experimental work that led to the initiation of clinical heart transplantation was carried out in Shumway's laboratory at Stanford Medical School

atria, aorta and pulmonary artery were now all that were required. This procedure was described independently 1 year later at Stanford Medical School by Lower (Figure 6) and Shumway (Figure 7), who obtained the first consistently successful results[18]. With further modifications made by Barnard[19], the technique is now used in the clinical operation of orthotopic heart transplantation, and is described in detail in Chapter 24.

It was, therefore, not until 1960 that the major experimental advance was made, when Lower and Shumway reported that five out of eight consecutive dogs undergoing transplantation had lived for 6–21 days[18,20]. During convalescence the dogs ate and exercised normally, the pulse rate was variable and increased moderately with exercise, and only a few hours before death the ECG remained virtually normal, showing no evidence of arrhythmia or conduction defects. After death, microscopic examination of sections of myocardium demonstrated severe myocarditis, with massive round-cell infiltration, patchy necrosis, interstitial hemorrhage, and edema. The authors concluded that in all likelihood the graft would have continued to function for the normal life span of the animal if the immunologic mechanisms of the host had been suppressed.

These investigators and their colleagues subsequently studied autotransplantation[21,22] and allotransplantation[23–25] of the heart, achieving long-term survival, and contributing extensively to our knowledge of this subject[26–28]. In the experimental animal the transplanted heart was found to have the capacity to increase cardiac output under a variety of physiological stresses; a normal cardiac output was demonstrated 1 year after allotransplantation and $5\frac{1}{2}$ years after autotransplantation; evidence of autonomic reinnervation of the heart after autotransplantation was obtained.

Beginning in 1962, Willman and his colleagues produced the first of several papers on the subject of myocardial structure and function following autotransplantation of the heart[29], including autotransplantation in the primate[30].

USE OF PROFOUND HYPOTHERMIA

Orthotopic transplantation performed in puppies under profound hypothermia, rather than with a pump–oxygenator, was described by Kondo and his colleagues in 1965[31]. Total body cooling by iced water immersion of both recipient (to 16–17°C rectally) and donor (to 27–29°C) was carried out, allowing complete circulatory arrest of the recipient for the 45-minute operation. Heart massage was begun immediately after the anastomoses were completed. After being warmed to 26–28°C by body immersion and flushing of the chest cavity with warm saline, the heart was electrically defibrillated, and rewarming continued until the temperature returned to normal. One animal remained alive and well 112 days after operation.

PROLONGATION OF GRAFT SURVIVAL BY IMMUNOSUPPRESSION

When many of the technical and physiological problems had been overcome, investigators turned their attention to the problem of combating the immune response by chemotherapy. Reemtsma and his colleagues used Mann's cervical transplantation technique to study the effect of methotrexate, a folic acid antagonist[32]. In a control series of untreated dogs, cardiac activity continued for a maximum of only 10 days, whereas in the methotrexate-treated group maximal survival extended to 27 days. When the recipient was given azathioprine, a drug introduced as an immunosuppressive agent by Calne (Figure 8), the maximal survival was 32 days[8]. Blumenstock and his colleagues, using methotrexate in dogs with orthotopic transplants, obtained five dogs that survived from 12 to 42 days[33].

The Stanford group reported its experience with a combination of steroids (hydrocortisone or methylprednisolone) and azathioprine or mercaptopurine, achieving a mean survival of 17 days, in comparison to 7 days in control dogs[25]. Six further dogs were given immunosuppressant drugs only during threatened rejection, as demonstrated by diminution of the R-wave voltage in all leads of the ECG, which had previously been found to accompany immune rejection of the cardiac allograft; five of the six dogs survived for over a month, and three for over 3 months.

CARDIAC TRANSPLANTATION IN MAN – FIRST ATTEMPT USING A XENOGRAFT

By the mid-1960s a considerable fund of knowledge had been acquired. The increasing success of experimental cardiac transplan-

Figure 8 Sir Roy Calne, of Cambridge University, who was responsible for the introduction of azathioprine as an immunosuppressive agent in transplantation. Later, with David White, he carried out much of the experimental work on cyclosporine, and was the first to use this drug in a clinical transplant program

Figure 9 James Hardy, who, in 1963, led the team that performed the world's first single lung transplant and, in the following year, the first heart transplant. In this latter operation he used a chimpanzee as donor

tation led Hardy (Figure 9) and his colleagues at the University of Mississippi to consider heart transplantation in man. This group had considerable experience of cardiac and lung transplantation in animals, and had carried out the first lung transplant in man[34] (Chapter 43).

In 1964 they reported their attempt to transplant the heart of a large chimpanzee into the chest of a 68-year-old man with hypertensive cardiovascular disease, widespread atheroma, and evidence of previous myocardial infarction[35]. Before operation the patient deteriorated suddenly and passed into terminal shock. He was taken to the operating room, and supported by a pump–oxygenator just as effective heart action ceased. As no human donor was available, and as some of the members of the group had been impressed by the early results of kidney xenografts from chimpanzees to man reported by Reemtsma *et al.*[36], the heart of a 96 lb (43.6 kg) chimpanzee was used for orthotopic transplantation. After defibrillation the donor heart beat regularly and forcefully, but it soon became apparent that the rather small heart would not be able to support the circulation unless its rate were increased. The heart was paced at 100 per minute to maintain a systolic blood pressure of 60–90 mmHg. About 1 hour after the removal of the bypass catheters, however, the heart was judged incapable of accepting a large venous return without intermittent decompression by manual massage. Further support was abandoned.

THE FIRST HUMAN-TO-HUMAN HEART TRANSPLANT

After nearly 4 years and much further experimental work another attempt was reported. Barnard (Figure 10) and his colleagues performed the first human-to-human heart transplantation on a 57-year-old man with ischemic heart disease at Groote Schuur Hospital in Cape Town on the night of the 2–3 December 1967[37]. The operative procedure was successful, and the patient's orthotopically transplanted heart functioned satisfactorily throughout the early postoperative course. Immunosuppression was with azathioprine and corticosteroids. Like many patients who followed, however, he developed pneumonia, and died on the 18th postoperative day. At autopsy his transplanted heart showed features of mild to moderate acute rejection (Figure 11)[38].

The first patient who could realistically be acclaimed as a 'long-term' survivor was operated on in Cape Town 1 month later. This 60-year-old patient lived an active and full life for over $1\frac{1}{2}$ years, until he died from the hitherto undescribed complication of chronic rejection[39].

Figure 10 Christiaan Barnard, who led the team that performed the first human-to-human heart transplant at Groote Schuur Hospital in Cape Town in December 1967

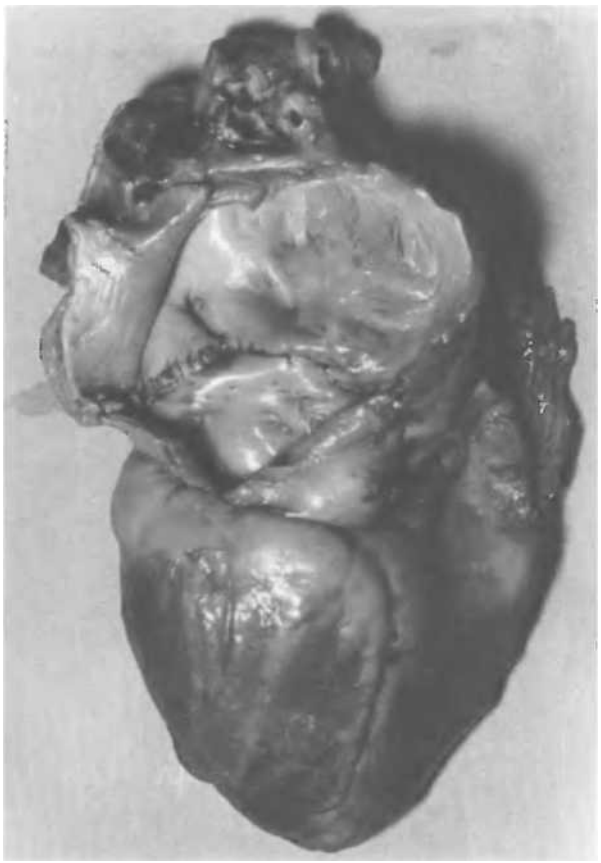

Figure 11 Post-mortem appearance of the donor heart from the first human-to-human heart transplant. The atrial suture line can clearly be seen. Histopathologic features of mild to moderate acute rejection were present on microscopic examination

EARLY CLINICAL PROGRESS

The initial enthusiasm for heart transplantation waned as the problems of acute rejection and infection became apparent to those who had embarked upon a transplant program without a full understanding of the complications which might be involved. Four centers, those of Stanford University[40] and the Medical College of Virginia[41] in the USA, Hôpital La Pitié in Paris[42], and Groote Schuur Hospital in Cape Town[43], continued with planned programs of heart transplantation.

With improved patient selection based on experience, and improved postoperative care, in particular with regard to the administration of immunosuppressive drugs and the prevention, diagnosis and treatment of infectious complications, the results in these centers slowly improved. The introduction of the technique of percutaneous transvenous endomyocardial biopsy to diagnose acute rejection, by Caves and his colleagues in 1973[44,45], contributed much to the successful management of patients with cardiac allografts, allowing timely increases in immunosuppression or, of equal importance, the avoidance of over-immunosuppression.

The introduction of the operation of heterotopic heart transplantation by Barnard and Losman in 1975[9] added a further surgical technique, with some advantages and some disadvantages over orthotopic transplantation, which could be used by those treating patients with terminal myocardial disease.

During the late 1970s cardiac transplantation came to be accepted as a definitive form of therapy rather than as a clinical research program. As a result, in the late 1970s and early 1980s several other groups in North America and Europe initiated clinical heart transplant programs.

INTRODUCTION OF CYCLOSPORINE

Following the discovery of the immunosuppressive effects of cyclosporin A by Borel (Figure 12) in 1976[46], and extensive experimental studies at several centers, notably Cambridge in the United Kingdom[47] and Stanford in the USA[48], cyclosporin was introduced into a clinical cardiac transplantation program in 1980[49]. Until this time, immunosuppression had been achieved with a combination of azathioprine[50], corticosteroids[51], and anti-lymphocyte globulin[40], which was probably first used in cardiac transplantation in 1968 (Barnard, C.N., personal communication).

Prolonged survival of heart and heart–lung transplants in non-human primates was achieved using immunosuppressive regimens in which cyclosporin played a major role[48]. Based on these studies, the initial clinical programs suffered from incorporating

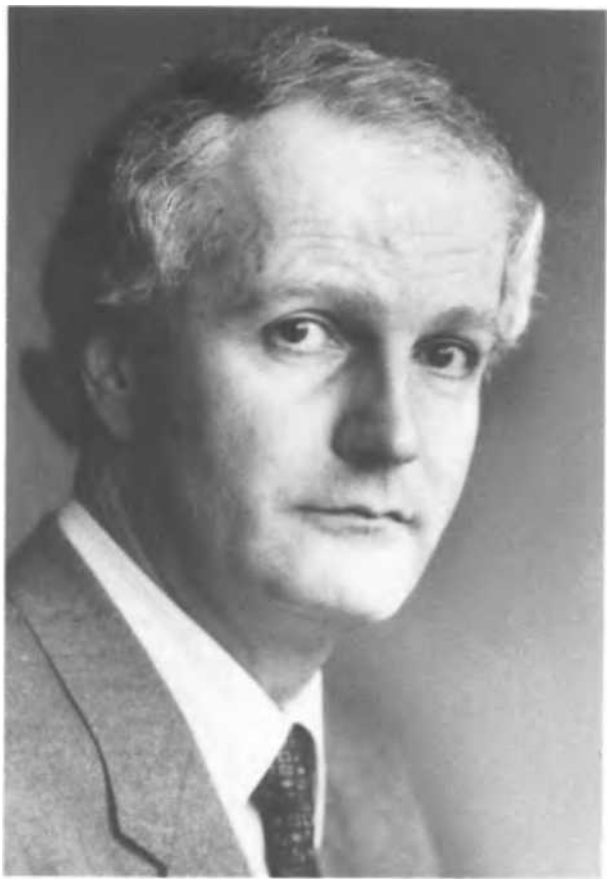

Figure 12 Jean Borel, of Sandoz Ltd, Basel, Switzerland, who discovered the immunosuppressive properties of the drug, cyclosporine A

an excessively high dose of cyclosporin in the pre- and early post-transplant period, and many patients suffered morbidity or even death from complications of cyclosporin therapy, in particular renal failure.

With experience, however, the dose of cyclosporin was gradually reduced, and the potential complications minimized.

The subsequent good results obtained with a combination of a lower dose of cyclosporin with azathioprine and low-dose corticosteroids, reserving antilymphocyte globulin and, more recently, the monoclonal antibody OKT3[52] primarily as treatment for rejection episodes, has encouraged many centers world-wide to embark on heart transplantation programs.

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19
Selection and Management of the Potential Candidate for Cardiac Transplantation

L.W. STEVENSON

INTRODUCTION

The potential benefits of transplantation were already recognized in 1968, as reflected in the statement from the Bethesda conference chaired by Francis Moore[1]: 'Cardiac transplantation, still in an early stage of development, shows promise for the future treatment of many people with severe heart disease.' At that time there were 20 survivors of 50 heart transplant procedures. Since then, cardiac transplantation has evolved from an experimental to an accepted clinical procedure, endorsed by Medicare in 1986 as 'best therapy' for end-stage heart failure. The current survival rate is 80–85% at 1 year and 60–70% at 5 years[2]. There have been over 30 000 heart transplants performed in the world, involving over 250 heart transplant centers.

When transplantation was an experiment, patients were selected from those facing imminent death. The indications were obvious, and the contraindications could be liberally defined by the investigators. Improving results led to consideration of candidates for whom the immediate need for transplant was less urgent, but the longer waiting times required earlier anticipation of that need. At the same time, continuing refinement of immunosuppression diminished the immediate negative impact of many conditions such as diabetes and older age, which were initially criteria for exclusion due to associated higher risks of post-transplant complications. These changes have widened the channels into an ever-expanding pool of potential candidates (Figure 1). It is currently estimated that up to 40 000 people each year in the United States would potentially benefit from cardiac transplantation, an estimate surprisingly consonant with the 10 000–40 000 estimated in 1968. The original estimate of potential donor heart availability at that time, however, was 45 000 yearly in the United States, compared to the 2000–2500 actually achieved yearly for the past 5 years. Interestingly, their original estimate of cost was $50 000 in 1968 dollars[1], which is only

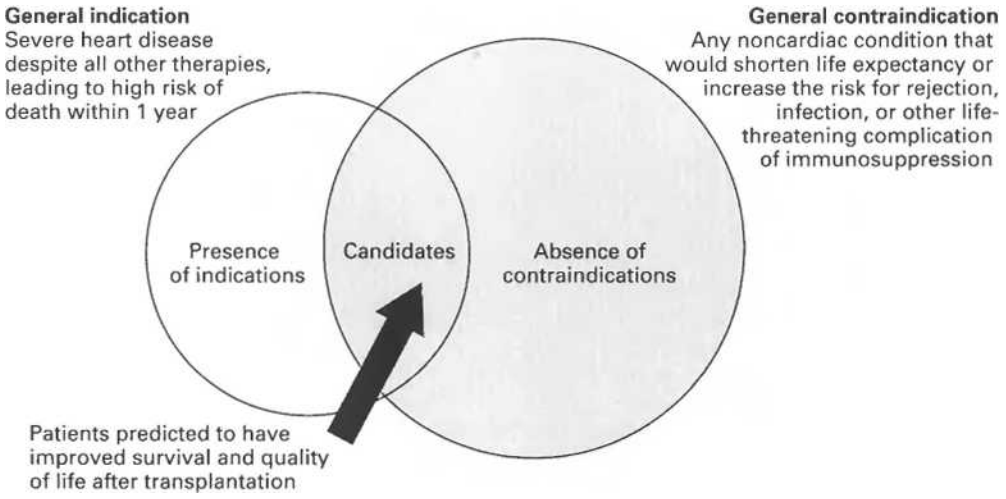

Figure 1 Intersecting circles demonstrate the principle of selection for cardiac transplantation of candidates who demonstrate indications without serious contraindications. As results of transplantation have improved, the indications broaden and the contraindications become less strict

slightly lower than the absolute figure currently negotiated for some contracts in 1995 dollars.

As cardiac transplantation has evolved, other medical and surgical alternatives to transplantation have also developed. Heart transplantation now represents only one facet of the therapies which should be offered by centers dedicated to the heart failure population. A left ventricular ejection fraction ≤25% no longer means that a new heart must be substituted in order for a patient to survive with a good quality of life. Surgery for reversible ischemia, distorted ventricular geometry, and valvular disease is successful in some patients despite poor left ventricular function and symptoms of heart failure[3-5]. Medical therapy has had dramatic impact on the symptoms of heart failure, with less but still significant impact on survival, challenging previous assumptions of when left ventricular dysfunction becomes 'end-stage'[6-8].

APPROACH TO THE PATIENT REFERRED FOR CARDIAC TRANSPLANTATION

The most common diagnosis in adults referred for transplantation is dilated heart failure, due in almost equal proportion to coronary artery disease and non-ischemic dilated cardiomyopathy. Primary restrictive cardiomoyopathy, primary valvular disease, and congenital heart disease account for slightly fewer than 10% of all candidates[2], with rare cases of cardiac trauma or tumor. The general approach to the identification of indications and contraindications is applicable regardless of etiology (Table 1), but most of the specific considerations below focus on advanced heart failure with low left ventricular ejection fraction.

Table 1 Approach to the potential candidate for heart transplantation

Address potentially reversible components of heart failure
Tailor medical therapy to relieve congestion
Evaluate functional capacity
Assess risks of deterioration or sudden death
Identify indications for transplant
Exclude contraindications to transplantation
Determine candidacy for transplantation: now, when needed, or conditional
Maintain and re-evaluate

Factors which are potentially reversible

All patients should undergo extensive investigation to identify the primary cause and any potentially reversible factors contributing to decompensation (Table 2). Occasionally a systemic cause of disease is identified which will preclude cardiac transplantation due to expected effects on other organs after transplantation. A low left ventricular ejection fraction may in some cases reflect major areas of hibernating or stunned myocardium, which may demonstrate improved function after revascularization with coronary artery bypass grafting or catheter-based interventional procedures[4,5]. Angina is frequently absent, and thallium redistribution after reinjection or prolonged delay is not always present. Areas of glucose uptake in regions with decreased flow may identify viability otherwise not evident. A history of multiple reoperations or chronic diabetes mellitus may predict worse outcome. The quality of distal vessels appears critical for success, particularly in this population.

Table 2 Potentially reversible factors in heart transplantation

Intrinsic factors
Recent-onset cardiomyopathy
Extensive myocardial ischemia with potential for revascularization
Secondary viral infection superimposed on primary disease
Major alcohol consumption
Tachycardias
Metabolic factors: thyroid disease, electrolyte disturbances, obesity
Anemia or other high-output state
Factors of therapy
Ineffective drug regimen:
ineffective doses or combinations of vasodilators
inadequate diuresis
Non-compliance:
with drug regimen
with salt and fluid restriction
Concomitant drug therapy causing:
increased fluid retention
depressed contractility

Recent practice surveys from metropolitan transplant centers suggest that no more than 3–10% of potential transplant candidates with coronary artery disease may be appropriate revascularization candidates[9]. There is even less information available regarding valve replacement in patients with severely reduced ejection fractions, although it is generally considered indicated in any patient with significant aortic stenosis, and recent experience suggests that mitral valve reconstruction may be feasible and helpful in some patients[3]. Vigorous searching for surgically reversible conditions is warranted, however, due to the implications both for the patient whose own heart may improve without transplantation and for another patient who may receive the donor heart which is spared.

Considering the limitation of donor hearts, an operative risk for an alternative procedure which is higher than for the same procedure in a patient with good ventricular function is not itself an indication for cardiac transplantation. Those who survive often demonstrate gradual improvement after 'salvage surgery', or at least stabilize sufficiently to undergo elective transplantation after discharge. Reliance upon 'transplant back-up' for post-cardiotomy shock, however, may be dangerous, as outcomes are uncertain for such patients. Patients requiring mechanical assistance to bridge from post-cardiotomy shock have been reported to have poorer outcome than patients receiving a primary bridge[10,11], although those surviving to transplantation have subsequent survival comparable to elective transplantation.

Recent cardiomyopathy, defined as less than 6 months of symptoms in the absence of major coronary artery or primary valvular disease, may improve spontaneously in up to 50% of patients, whether or not associated with a recent viral infection or with myocarditis on endomyocardial biopsy. When the clinical severity of symptoms leads to referral for transplantation, major improvement defined as ≥0.15% increase in left ventricular ejection fraction occurred in 27% of patients in one series[12], most often in those with the least elevation in filling pressures and least mitral regurgitation at the time of referral. For patients with this defined improvement, subsequent prognosis is excellent, although exercise capacity may remain somewhat impaired by diastolic dysfunction[13]. Recent-onset cardiomyopathy which does not improve, however, confers worse short-term prognosis than for

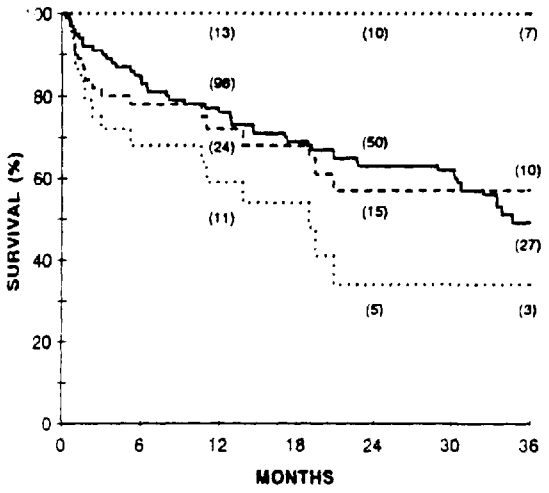

Figure 2 Survival for 297 patients with primary dilated cardiomyopathy referred for cardiac transplantation. The survival with chronic cardiomyopathy was not different from the total survival of all patients with recent-onset cardiomyopathy, but this recent-onset group could be divided into: (a) those with ≥15% ejection fraction improvement to ≥30% and (b) those with no improvement, for whom survival was significantly worse ($p = 0.0009$)[12]

patients with more chronic disease (Figure 2), particularly in patients under 33 years[12]. Occasionally, young patients present with a fulminant picture of acute cardiac and other organ failure, usually in association with a viral syndrome, from which the chance of complete recovery may exceed 50%, although high-dose catecholamine support, and occasionally mechanical ventricular support, may be necessary for 5–10 days. The incidence of this syndrome varies from year to year and is usually highest in the winter months.

Cardiomyopathy presenting within the last trimester of pregnancy or initial post-partum months may have a slightly higher chance of improvement than cardiomyopathy of other etiologies[14]. Symptoms often improve remarkably after assisted diuresis post-partum of the excess volume of water accumulated during pregnancy. Heart failure presenting earlier in pregnancy often reflects exacerbation of previous conditions.

Patients with known heart failure due to cardiomyopathy or coronary artery disease often demonstrate prolonged deterioration after respiratory and viral syndromes, perhaps as a result of the negative inotropic effects of cytokines, the accompanying tachycardia, or increased metabolic demands. Many patients are first referred for transplantation within weeks after such an episode. Restoration of fluid balance and adjustment of vasodilator therapy frequently allows recovery to previous levels of compensation within the next few months.

Approximately 10% of cardiomyopathy in the United States has been attributed to heavy alcohol consumption, although the incidence may be underestimated[15]. Consumption of two drinks daily, which is common in the general population, may be sufficient to worsen heart failure of other primary causes. Occasional dramatic improvement in the left ventricular ejection fractions of patients with old myocardial infarctions is sometimes explained later by the patient's retrospective admission of heavy alcohol consumption prior to referral. Complete abstinence from alcohol should be mandated for at least 3–6 months prior to transplantation candidacy, both to demonstrate the irreversibility of decompensation and to ensure the patient's ability to avoid excessive alcohol consumption after transplantation, although modest consumption is then acceptable.

Tachycardia is increasingly recognized as a primary cause of cardiomyopathy in both adults and children[16]. Supraventricular tachycardias and relatively slow ventricular tachycardias may not be initially recognized. Atrial fibrillation, present in approximately 20% of patients referred for cardiac transplantation, is frequently associated with excessive ventricular rates during exertion. Conversion to sinus rhythm usually leads to clinical improvement, but has also frequently been associated with major improvements in left ventricular ejection fraction[17]. Amiodarone is the safest and most effective antiarrhythmic agent in this population, of whom more than half may still be in sinus rhythm a year after cardioversion on amiodarone[18]. Atrioventricular node ablation and pacemaker implantation may be considered when atrial fibrillation is refractory and the rate cannot be well controlled.

Obesity has been implicated as a primary cause of cardiomyopathy. Weight loss is achievable and frequently easier during heart failure, even though activity is curtailed. Weight loss itself allows more effective distribution of limited cardiac output but, in addition, is frequently associated with significant improvement in left ventricular function[19], such that cardiac transplantation need not be considered. This should be emphasized to all potential transplant candidates. A pattern of weight maintenance is also critical to avoid morbid weight gain after transplantation, which limits rehabilitation, contributes to osteoporotic complications, and has been associated with transplant vasculopathy.

Tailored therapy prior to transplantation

At the time of serious consideration for transplantation, most patients have a left ventricular ejection fraction <25% (Table 3) (although this is not necessary for acceptance, see below), and symptoms of heart failure which limit daily life. These symptoms are dominated by elevated intracardiac filling pressures which on the left side cause orthopnea, paroxysmal nocturnal dyspnea

Table 3 Profile of 265 patients discharged after referral with class IV symptoms and ejection fraction ≤25%

Ejection fraction (%)	18 ± 5
CHF duration (months)	33 ± 34
Left ventricular end-diastolic dimension (mm)	75 ± 10
Mitral regurgitation (0–3)	2.0 ± 0.8
Tricuspid regurgitation (0–3)	1.7 ± 0.9
Serum sodium (mEq/l)	134 ± 5

Hemodynamics	*Initial*	*On revised therapy*
Right atrial pressure	13 ± 7	7 ± 4
Systolic blood pressure	106 ± 14	96 ± 13
Pulmonary wedge pressure	27 ± 9	17 ± 16
Systemic arterial pressure	85 ± 11	76 ± 10
Cardiac index ($l\ min^{-1}\ m^{-2}$)	1.9 ± 0.6	2.5 ± 0.5
Heart rate (beats/min)	94 ± 17	91 ± 15

(PND), and immediate dyspnea on light exertion (IDLE). (In contrast, dyspnea occurring only after several minutes of moderate exertion is more often due to the failure to increase cardiac output to levels adequate for aerobic metabolism during increased demand.) Elevated right-sided cardiac filling pressures cause the symptoms of systemic venous congestion, which can be manifest as gastrointestinal discomfort, anorexia, early satiety, ascites, and peripheral edema. Most patients have a history of recent hospitalizations during which intravenous diuretics, often in conjunction with a brief course of an intravenous inotropic agent, such as dobutamine or milrinone, have caused only a temporary clinical improvement, following which the congestion rapidly recurs.

The majority of patients at the time of referral are already receiving standard 'triple therapy', which includes digoxin, diuretics, and angiotensin-converting-enzyme inhibitors, which have in some cases been reduced or stopped due to hypotension. Although effective doses of vasodilators have been established in trials of mild–moderate heart failure[6,7], the use of different doses or different combinations frequently improves clinical status in patients with more severe heart failure[8,20]. For all potential candidates, transplant evaluation provides a vital opportunity to redesign the medical regimen, which is of central concern regardless of whether or not the patient is ultimately found to be a candidate for transplantation.

Therapy for severely symptomatic patients is dominated by the need to reduce congestive symptoms, and thus the filling pressures which cause those symptoms. The first challenge is to recognize the excess volume present in most of these patients[21]. Although many patients have 3–5 liters of excess fluid at the time of evaluation, the lungs are usually clear of rales in chronic heart failure, and peripheral edema and/or ascites occur in fewer than 30% of these patients. Orthopnea and jugular venous distension are the most reliable clinical indicators of volume overload, and almost always indicate the need for further therapy.

Previous therapy to relieve congestion has often been hampered by concern that therapy to decrease volume status will further depress cardiac output. This misconception is often strengthened by small rises in creatinine and blood urea nitrogen during diuresis, which is more often a direct result of reflex responses to decreased atrial distension than an indication of falling cardiac output. The majority of patients with chronically dilated heart failure[22] will achieve their highest cardiac outputs with pulmonary capillary wedge pressures in the range of 12–15 mmHg. Forward stroke volume often increases by 30–50%, due largely to forward redistribution of mitral regurgitant flow[23].

Resting hemodynamic compensation is maintained on standard doses of diuretics and vasodilators despite low left ventricular ejection fractions in most patients with left ventricular dysfunction, who have not been shown to benefit from hemodynamic monitoring to achieve more precise goals when already clinically compensated. Cardiac transplantation is rarely indicated in such patients except for other indications such as refractory angina or arrhythmias. Adjustment of vasodilators or diuretics can be guided by clinical assessment in some patients with mild hemodynamic abnormalities. When severe symptoms persist after empiric therapy, however, further intervention can frequently still restore compensation (Table 4)[20].

In the Bethesda conference on cardiac transplantation, the summary of general recommendations specifies that functional status should not be assessed until patients have undergone aggressive therapy with combinations of vasodilator and diuretic therapies[24]. 'Therapy should be adjusted until clinical congestion has been resolved or until further therapy has been repeatedly limited by severe hypotension (generally systolic blood pressure <80 mmHg) or marked azotemia. Patients should not be considered to have *refractory* hemodynamic decompensation until therapy with intravenous followed by oral vasodilators and diuretic agents has been pursued using continuous hemodynamic monitoring to approach hemodynamic goals.'

Hemodynamic monitoring allows the coupled optimization of both volume status and vascular resistances using simultaneous diuretic and vasodilator therapy, which can rarely otherwise be achieved safely and completely once decompensation is severe (Table 5). Hemodynamic status is often easiest to optimize initially during titration of intravenous vasodilators, such as nitroprusside. Intravenous inotropic agents, such as dobutamine, have also been used, but are less predictive of ultimately successful maintenance on oral regimens because the inotropic component cannot currently be duplicated with available oral drugs. Use of

Table 4 Suggested indications for invasive monitoring of hemodynamics during therapy of congestion

Congestion with concomitant hypoperfusion suggested by:
Mental obtundation
Pulse pressure < 25%
Cool extremities
Declining renal function
Hemodynamic intolerance to ACEI
(likely when systolic blood pressure < 90 mmHg or serum sodium < 133 mEq/l)
Congestion in the presence of:
Active ischemia
Symptomatic ventricular arrhythmias
Suspected active pulmonary disease
Impaired baseline renal function
Congestion persisting or recurring despite all of:
ACEI as tolerated
Combination high-dose diuretics
Sodium and water restriction
Serious consideration of heart transplantation for symptoms of heart failure

ACEI = angiotensin-converting-enzyme inhibitors

Table 5 Tailored therapy for advanced heart failure

1.	Measurement of baseline hemodynamics
2.	Intravenous nitroprusside and diuretics tailored to hemodynamic goals
	PCW ≤ 15 mmHg
	SVR ≤ 1200 dyne s cm $^{-5}$
	RA ≤ 8 mmHg
	SBP ≥ 80 mmHg
3.	Definition of optimal hemodynamics by 24–48 h
4.	Titration of high-dose oral vasodilators as nitroprusside weaned
	Combinations of: captopril, isosorbide dinitrate, hydralazine as needed as alternative or addition
5.	Monitored ambulation and diuretic adjustment for 24–48 h
6.	Maintain digoxin levels 1.0–2.0 ng/dl, if no contraindication
7.	Detailed patient education
8.	Flexible outpatient diuretic regimen including PRN metolazone
9.	Progressive walking program
10.	Vigilant follow-up

longer-acting inotropic agents is occasionally necessary for prolonged intravenous support, but the long half-life complicates monitored weaning onto oral agents. In addition to restoring clinical stability, reduction of left ventricular filling pressures over several days often demonstrates reversibility of pulmonary hypertension which during acute therapy appeared fixed.

The oral regimens established by tailored therapy often consist of relatively high doses of angiotensin-converting-enzyme inhibitors. Some data suggest that the best survival may be obtained in this population when angiotensin-converting-enzyme inhibitors are combined with oral nitrates[8]. Patients with the most severe decompensation, as indicated by very low serum sodiums and/or inability to tolerate sufficient doses of angiotensin-converting-enzyme inhibitors to optimize loading conditions, often derive sustained benefit from the combination of hydralazine and oral nitrate therapy[8].

Tailoring of therapy for hemodynamic goals in class IV heart failure often leads to dramatic improvement in hemodynamics and clinical status (Table 3). Prolonged maintenance of hemodynamic goals has been associated also with measured reductions in atrial sizes and mitral and tricuspid regurgitation, and with improvement in peak oxygen consumption (Table 6)[25–27]. In combination with patient education, progressive exercise, and meticulous ongoing care by an experienced heart failure team, this approach has been shown to reduce the rehospitalization rate by over 75%[27]. The impact of this care extends not only to the patient who can postpone transplantation, but also to the patient who can await transplantation in greater comfort and a more favorable condition for surgery, and perhaps most importantly, to the larger majority of patients for whom transplantation is not an option.

Table 6 Outcome of tailored therapy in patients referred for cardiac transplantation

	Pre-referral	*Post-referral*
NYHA Class	3.3	2.4*
Orthopnea (0–4 scale)	3	0.2*
Jugular venous distension (0–4 scale)	3	0.5*
Edema (0–4 scale)	1	0.1*
Atrial overload		
Left atrial volume (cc)	100	65*
Right atrial volume (cc)	85	52*
Mitral regurgitant units	33	13*
Tricuspid regurgitant units	36	18*
Peak Vo_2 (ml kg^{-1} min^{-1})	11	15*
Hospital/6 months	2.0	0.2*

*$p < 0.05$ compared to baseline
Adapted from refs 26 and 27

Adjunctive outpatient therapies for heart failure

On the foundation of tailored therapy, other therapies may offer additional benefit in selected patients (Table 7). The use of adrenergic blocking agents has been shown to improve ejection fraction and clinical status in some patients with heart failure, but their benefit in decompensated heart failure has not been demonstrated[28,29]. The limited experience in advanced heart failure involves patients who were free of apparent volume overload or congestive symptoms when the drug was cautiously initiated in very low doses[30]. While patients frequently experience some fatigue during initiation of these drugs, administration should usually be stopped if accompanied by fluid retention unresponsive to diuretics. Withdrawal should be considered, although it is controversial, in patients presenting with severe decompensation while receiving these agents. Amiodarone has been associated with similar increases in ejection fraction, possibly related to similar decreases in heart rate[31]. Unlike other antiarrhythmic agents studied in heart failure, amiodarone does not appear to increase mortality[32,33]; in fact, several lines of evidence suggest that amiodarone may actually improve survival in advanced heart failure. This effect appears to be independent of the degree of baseline arrhythmia and to result in decreased heart failure endpoints, as well as sudden death[32].

Multiple non-glycosidic oral inotropic agents have been investigated in heart failure populations, often with deleterious effects at the doses used. There is not yet any consistent evidence that any such agents decrease mortality in the population for whom cardiac transplantation would otherwise be considered. Intermittent or continuous ambulatory infusions of dobutamine have appeared beneficial in some patients, but sustained benefit has not been proven.

Table 7 Outpatient therapies for potential heart transplant candidates

Routine use	*Selected use*	*Detrimental*	*Under clinical investigation*
Angiotensin-converting-enzyme inhibitors	Anticoagulation	Amrinone, milrinone	Carvedilol
Digoxin	Hydralazine	Flosequinan	Vesnarinone
Diuretics	Amiodarone	Prostacyclin infusion	Home dobutamine
Nitrates	β-blockers	Diltiazem, nifedipine	Pimobendan
Potassium	Magnesium	Type I anti-arrhythmic agents	Amlodipine
Exercise	AICD	Non-steroidal anti-inflammatory agents	Ibopamine
	Nocturnal oxygen		AII rec antagonist Coenzyme Q10 L-Carnitine Ultrafiltration Nocturnal CPAP (continuous positive airway pressure)

INDICATIONS FOR CARDIAC TRANSPLANTATION

The goal of cardiac transplantation is to maximize the benefit derived from each donor heart transplanted (Figure 3). Benefit is a function of both quality and length of life, with different relative values assigned by different patients. If the goal were instead to maximize overall survival after transplantation, the optimal recipient would be a healthy young athlete, who would himself derive negative benefit from the procedure. For the patient who remains critical in an intensive-care unit despite consideration of all other medical and surgical options, the expected benefit of transplanta-

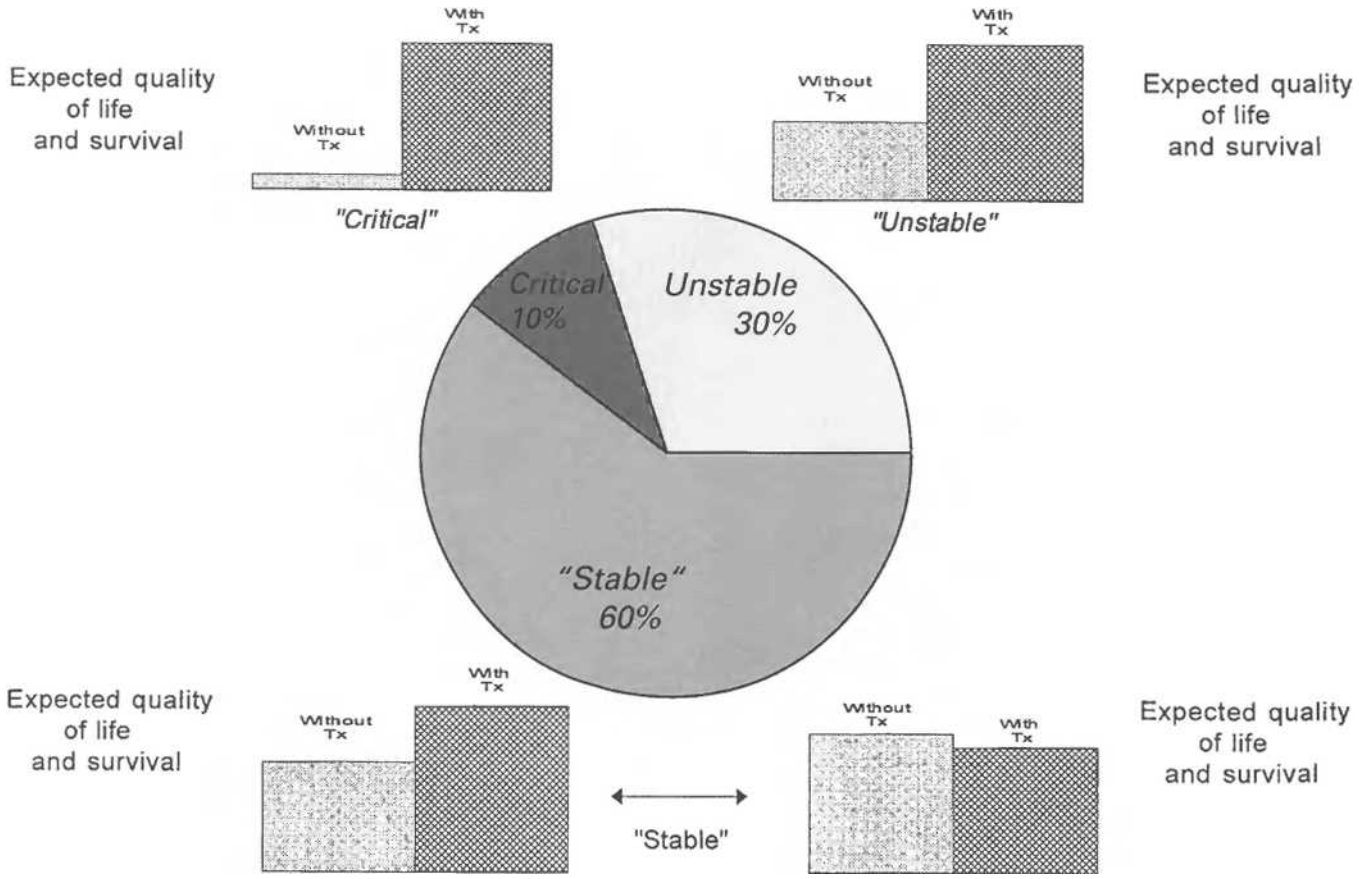

Figure 3 Expected benefit from transplantation according to clinical status achieved after therapy tailored for advanced heart failure. The relative proportions of the pie reflect the population referred to a major metropolitan transplant center, and would be expected to vary at other programs. Eligible patients remaining hospitalized in critical condition can expect major benefit in both quality and length of life. Patients remaining unstable, whether in or out of the hospital (see definition of stability, Table 13) can expect significant benefit. More refined analyses of functional and survival benefits are required to identify appropriate candidates from the population of patients who appear 'stable'

tion for both function and survival is obvious. For the patient who remains unstable, in or out of the hospital, with recurrent symptoms of congestion, the benefit is also obvious.

A major challenge of selection is the identification of the ambulatory patient at home who has sufficient clinical limitation or sufficient risk of deterioration and death to warrant the risks and limitations of cardiac transplantation. Many of the adverse prognostic factors validated in large heart failure trials are consistently present in the patients considered for cardiac transplantation. Factors proposed more specifically in severe heart failure relate to cardiac and hemodynamic parameters, the substrate for arrhythmias, and the systemic cardiovascular and neuroendocrine integration[34–40].

Table 8 Peak exercise oxygen consumption and expected benefit from transplantation

Peak Vo_2 with heart failure	*Expected after transplant*	*Estimated 1-year survival with heart failure*	*Estimated 1-year survival after transplant*	*Decision regarding transplant*
< 10	< 14–18	< 50–60%	≤ 80–90%	Transplant (if eligible)
10–14	14–18	60–75%	80–90%	Toward transplant
14–18	14–18	70–85%	80–90%	Away from transplant
> 18	> 14–18	80–95%	≥ 80–90%	No transplant (unless other indications)

Adapted from refs 39, 43, 45 and 47

Symptoms of heart failure

The presence of class IV symptoms of heart failure was originally considered to indicate 'end-stage' heart failure, and thus represented the major indication for transplantation. Since the early days of transplantation, however, medical therapy has evolved such that even patients with class IV symptoms can often improve to regain good quality of life. Although survival remains limited, it has also improved (Figure 4). While the extended prognosis of advanced heart failure remains worse than that of transplantation, the limitations both of donor supply and of lifespan after transplantation require that indications for transplantation be based on the expected increment in 1–2-year prognosis, with frequent

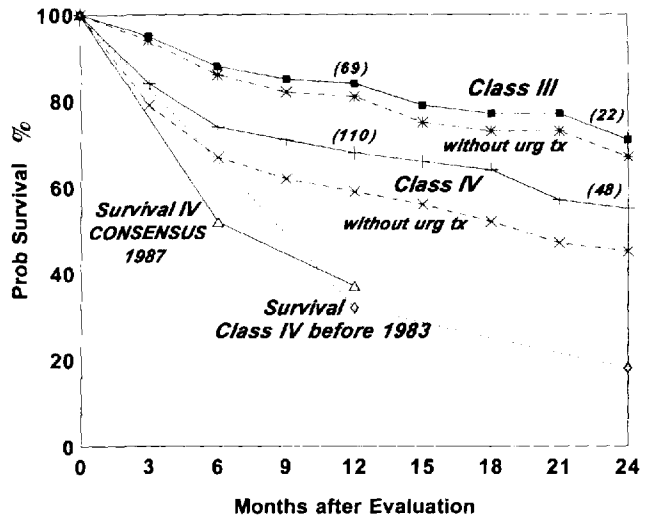

Figure 4 Overall survival and survival without urgent transplantation for 404 patients presenting with left ventricular ejection fraction ≤25% and New York Heart Association class III (n = 139) or class IV symptoms (n = 265). Recent survival of class IV patients is compared to those described by Wilson *et al.* in 1983[34] and the CONSENSUS trial in 1987[86]

reassessment. Considering the 70–80% 2-year survival after transplantation at major centers, it has been suggested that cardiac transplant candidates should have a predicted 2-year survival of ≤50% without transplant[41].

Left ventricular ejection fraction

Left ventricular ejection fraction below 20–25% has also been suggested to confer an unacceptable risk of mortality[42]. While this is certainly true when a population covering the spectrum from mild to severe disease is included, the prognostic value of left ventricular ejection fraction once it is below 25–30% is less clear.

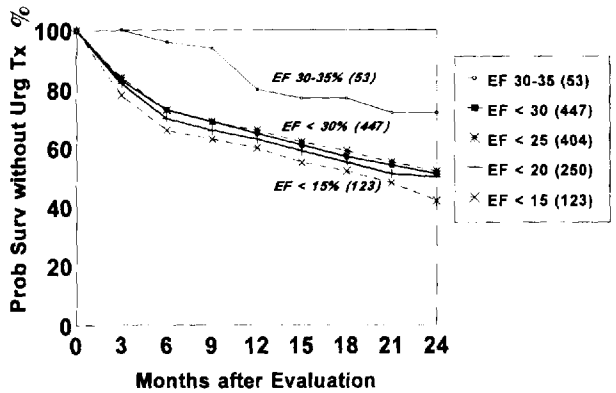

Figure 5 Relationship of left ventricular ejection fraction to actuarial survival without urgent transplantation (urg tx) in 500 patients presenting with New York Heart Association class III or IV symptoms 1988–1993 in one center. Left ventricular ejection fraction over 30% was associated with better survival but, once below 30%, progressively lower ejection fraction did not portend worse survival

If only those patients with class III or IV symptoms are considered, the left ventricular ejection fraction is not very helpful once it is lower (Figure 5)[43]. Interestingly, potential transplant candidates with massive left ventricular dilatation have a significantly worse prognosis than those with moderate dilatation, even when etiology of disease and degree of hemodynamic compromise are comparable[44]. Even for presentation with class IV symptoms and left ventricular ejection fraction ≤20%, prognosis after discharge on tailored medical therapy is not uniformly dismal, 45% surviving without death or urgent transplant[43]. (When comparing the outcome of other therapies to transplantation, it is important to consider the patients who are saved by 'urgent' transplantation as failures of alternative medical therapy, who would presumably have died had they not been hospitalized and supported until transplantation.)

Peak oxygen consumption

Measurement of peak oxygen consumption during exercise provides an index of overall cardiovascular reserve that is useful both to quantitate functional limitation and to estimate prognosis (Table 8). In mild–moderate heart failure of the Veterans Administration Heart Failure trials, a peak oxygen consumption <14.5 ml kg^{-1} min^{-1} predicted worse survival whether left ventricular ejection fraction was above or below 28%[45]. The experiences of Szlachic and Likoff in other populations confirmed the measurement of peak oxygen as an independent prognostic guide[46,47]. Mancini *et al.*[48] provided the initial validation of peak oxygen consumption as a criterion for transplant candidacy from her analysis of 114 potential transplant candidates, suggesting 14 ml kg^{-1} min^{-1}. Other experience has identified values between 10 and 14 ml kg^{-1} min^{-1} (Figure 6A)[49,50].

Some differences between programs may reflect varying practices of excluding patients with obvious resting symptoms. In addition, bicycle exercise yields peak oxygen consumption values slightly lower than treadmill exercise. Synthesis of the currently available information suggests that patients who are unable to perform exercise, or who can achieve peak oxygen consumption of <10–12 ml kg^{-1} min^{-1}, have the worst prognosis. The importance of indexing to predicted values remains controversial (Figure 6B). Patients with peak oxygen consumption over 16–18 ml kg^{-1} min^{-1} have 2-year survival similar to that of cardiac transplantation, in the absence of other confounding factors such as active ischemia or rapid deterioration (Table 8).

Many patients are unwilling to accept the burdens and risks of immunosuppression unless a major improvement in functional capacity is anticipated in addition to the survival benefit. For some patients with stable heart failure by clinical criteria[51,52], quality of life may not be significantly improved after transplantation[53]. Despite a left ventricular ejection fraction usually within normal limits, exercise capacity after transplantation is limited by multiple cardiac and systemic factors. Peak oxygen consumption, and other measures of exercise capacity such as the 6-minute walk distance, are often similar between patients with stable heart failure and cardiac transplant recipients, in the range of 50–70% of values predicted on the basis of age, size, and gender[52]. The perception of prolonged fatigue after exertion is less easy to quantify, but appears less common after transplantation.

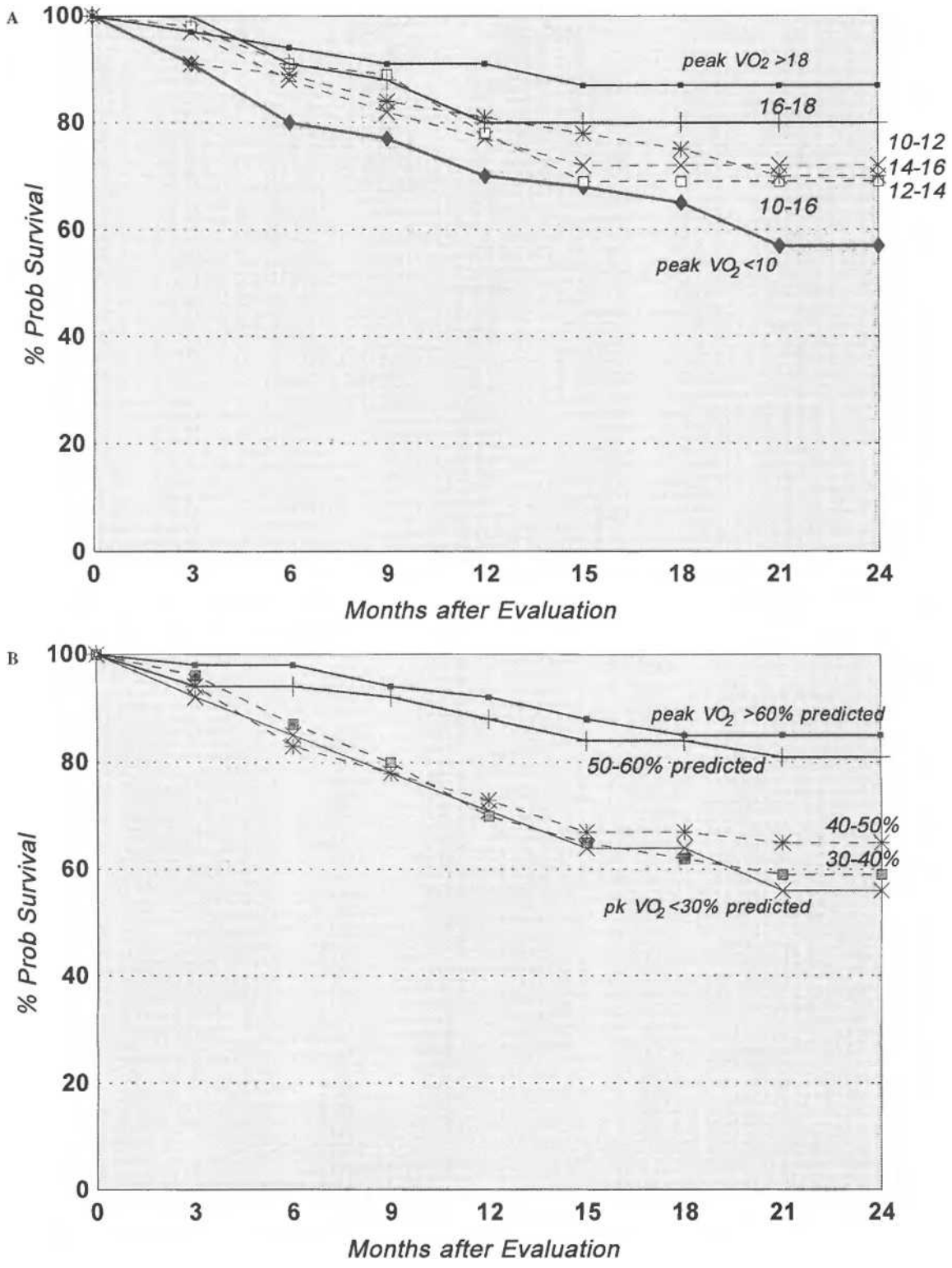

Figure 6 Actuarial curves of survival without hospitalization for urgent transplantation, analyzed for 320 patients undergoing cardiopulmonary exercise testing during the initial evaluation. **A**: Analysis according to peak oxygen consumption achieved <10 (n = 73), 10–12 (n = 67), 12–14 (n = 62), 14–16 (n = 46), 16–18 (n = 37) and over 18 ml kg^{-1} min^{-1} (n = 35). The test was performed with a bicycle ergometer, after a 6-min warm-up at 20 W, followed by a 5–10 min rest period and symptom-limited testing with a ramping interval of 15 W/min. **B**: Analysis according to percentage of predicted peak oxygen consumption which was actually achieved, demonstrating threshold value of 50% with little additional discrimination. (Adapted from ref. 87)

The current guidelines for cardiac transplantation focus on peak oxygen consumption as the basis for predicting improvements in survival and functional capacity after transplantation[24] (Table 9). While considerable debate surrounds the issue of whether to adjust for age- and gender-predicted maximal values, the threshold of peak oxygen consumption below which transplantation is indicated is generally adjusted upward for younger candidates and downward for older candidates. Functional capacity and prognosis should ideally be assessed after the impact of a revised medical regimen can be appreciated[51,52]. In practice, however, functional capacity and prognosis are usually assessed at the conclusion of a hospitalization for transplant evaluation, and interpreted in the light of improvement expected from changes in the medical regimen.

A patient referred, for example, after months of repeated hospitalizations for congestive symptoms might have a peak oxygen consumption of 11 ml kg^{-1} min^{-1} after evaluation, but the effective diuresis of 10 kg of fluid and enhanced vasodilator regimen might allow further symptomatic improvement and peripheral muscle reconditioning due to relief of exertional dyspnea. On the other hand, the same result would be an indication for listing of a patient referred on a stable regimen of angiotensin-converting-enzyme inhibitors, diuretics, and digoxin with an initial pulmonary capillary wedge pressure of 14 mmHg, who is unlikely to improve significantly with any changes in medical therapy.

Restrictive cardiomyopathy

A severely reduced left ventricular ejection fraction is neither necessary nor sufficient indication for transplantation (Table 9). Although the majority of patients referred have ejection fractions below 25%, patients may also have severe symptoms of congestion due to restrictive disease in which the ventricle is minimally dilated and the ejection fraction is 30–45%. Such patients may have severe difficulty maintaining fluid balance even with meticulous salt and fluid restriction. Amyloidosis needs to be excluded in such patients, even in the absence of a characteristic echocardiographic appearance. When restrictive disease has progressed slowly over many years, liver function should be carefully assessed, because these patients may be among the few to develop true irreversible 'cardiac cirrhosis'.

Hypertrophic cardiomyopathy

Cardiac transplantation is rarely indicated for hypertrophic cardiomyopathy when still in the hypercontractile stage. Diuretics and agents that decrease contractility can generally control congestive symptoms. Dual chamber pacing, myomectomy, and mitral valve replacement should be considered. In the minority of patients who progress to 'burned-out' cardiomyopathy, congestive symptoms and exercise intolerance may become severe, with only modest reduction of contractility to ejection fractions in the range of 30–40%, due to concomitant impairment in compliance. The natural history of these patients has not been well established, but their clinical limitation suggests that quality of life and outcome may be sufficiently compromised to warrant cardiac transplantation.

Other indications

Transplantation is occasionally indicated for reasons other than heart failure. Intractable angina may be an indication when multiple revascularization procedures have failed and no further attempts at surgical or catheter-based intervention are feasible. The left ventricular ejection fraction is usually below 30% in such patients, because those with better left ventricular function are generally candidates for some revascularization procedure. Transplantation is occasionally performed in patients disabled by recurrent discharges from automatic implantable defibrillators despite all attempts at catheter ablation and chemical control. Unusual trauma or isolated intracardiac tumors are rare indications for transplantation.

CONTRAINDICATIONS TO CARDIAC TRANSPLANTATION

Evaluation for transplantation includes a careful search for any non-cardiac condition that limits life expectancy or increases the risk of complications from the procedure, particularly from immunosuppression[24,54] (Table 10). Although this component of evaluation might logically take place after a patient has demonstrated indications for transplantation, in practice it is often more efficient to perform it simultaneously. Furthermore, in patients

Table 9 Selection criteria for benefits from transplantation

I. *Accepted indications for transplantation*
   1. Maximal Vo_2 < 10 ml kg^{-1} min^{-1} with achievement of anaerobic metabolism
   2. Severe ischemia consistently limiting routine activity not amenable to bypass surgery or angioplasty
   3. Recurrent symptomatic ventricular arrhythmias refractory to all accepted therapeutic modalities

II. *Probable indications for cardiac transplantation*
   1. Maximal Vo_2 < 14 ml kg^{-1} min^{-1} and major limitation of the patient's daily activities
   2. Recurrent unstable ischemia not amenable to bypass surgery or angioplasty
   3. Instability of fluid balance/renal function not due to patient non-compliance with regimen of weight monitoring, flexible use of diuretic drugs and salt restriction

III. *Inadequate indications for transplantation*
   1. Ejection fraction ≤ 20%
   2. History functional class III or IV symptoms of heart failure
   3. Previous ventricular arrhythmias
   4. Maximal Vo_2 > 15 ml kg^{-1} min^{-1} without other indications

Adapted from ref. 24

Table 10 Contraindications for cardiac transplantation

General eligibility
Absence of any non-cardiac condition that would itself shorten life expectancy or increase the risk of death from rejection or from complications of immunosuppression, particularly infection
Specific contraindications
Approximate age limit of 60–65 years (various programs)
Active infection
Active ulcer disease
Severe diabetes mellitus with end-organ damage
Severe peripheral vascular disease
Pulmonary function (FEV_1, FVC) < 60%* or history of chronic bronchitis
Creatinine clearance < 40–50 ml/min*
Bilirubin > 2.5 mg/dl, transaminases > 2 × normal*
Pulmonary artery systolic pressure > 60 mmHg*
Mean transpulmonary gradient >15 mmHg*
High risk of life-threatening non-compliance
Inability to make strong commitment to transplantation
Cognitive impairment severe enough to limit comprehension of medical regimen
Psychiatric instability severe enough to jeopardize incentive for adherence to medical regimen
History of recurring alcohol or drug abuse
Failure of establish stable address or telephone number
Previous demonstration of repeated non-compliance with medication or follow-up

* May need to provide optimal hemodynamics with nitroprusside and/or dobutamine for 72 h to determine reversibility of organ dysfunction caused by heart failure

who initially appear too well for transplantation, but may deteriorate, transplantation can be performed more expeditiously when eligibility has already been established.

The appropriate candidate for cardiac transplantation is sick enough to need a new heart, but sufficiently well in terms of overall condition and non-cardiac organ function to expect a good result. Age limits are controversial and usually expressed in relative rather than absolute terms. Highly selected older patients have good 1-year survival, but large series demonstrate decreased longer survival in older patients[55–57]. The older candidates are usually evaluated very carefully for evidence of diseases which commonly cause comorbidity in this age group.

Active systemic disease

Considerations regarding the etiology of disease are important to exclude patients with active systemic disease such as lupus erythematosus, rheumatoid arthritis, or scleroderma, which could cause disease after transplantation. In most programs, amyloidosis is a contraindication due to the tendency for systemic progression and recurrence in the allograft[58]. Chagas' disease may reactivate after cardiac transplantation, but is a common disease in South America, where suppressive therapy has been successfully used after transplantation[59].

Considerable emotional debate may develop regarding patients with chronic conditions with the potential to deteriorate after transplantation, as some patients at high risk will nonetheless do well after transplantation. The severe shortage of donor hearts curtails the systematic validation of each apparent contraindication. As described by Copeland, selection must therefore reflect 'a combination of empirically derived contraindications with limited natural history and considerable common sense'[60].

Diabetes mellitus is no longer an absolute contraindication for transplantation, although, in the early days, patients were excluded for abnormal glucose tolerance tests. Increasing severity of disease in terms of duration and insulin doses renders candidacy less likely. Initiation and augmentation of immunosuppression render glucose control very difficult, and hyperglycemia predisposes to infection. Patients with diabetes are evaluated carefully for evidence of other organ damage such as proteinuria and nephropathy, peripheral neuropathy, retinopathy, and small-vessel peripheral vascular disease, which are generally grounds for exclusion. Adult survivors of juvenile-onset diabetes are generally excluded for one or more of the above conditions.

Psychosocial factors

Failure to adhere to a rigorous regimen of medications, biopsies, and clinic visits remains a major factor in rejection and mortality for all organ transplant recipients[61,62]. Heavy psychological and financial burdens of chronic heart failure followed by transplantation, combined with labile mood changes during glucocorticoid augmentation, can precipitate lethal episodes of overt suicidal behavior or, more commonly, passive attempts to commit suicide through withdrawal of immunosuppression. Considerable debate surrounds the importance of various psychiatric and psychological conditions. Similarly, the importance of family support varies from patient to patient. Relative weaknesses in one area may be compensated by other strengths. The multiple factors relating to the patients and their support systems may best be combined into a profile from which the chances for long-term compliance can be assessed (Table 10). One of the many reasons that effective transplantation programs include integrated heart failure programs is the opportunity for reassessment of patients with a non-compliance history, who might later demonstrate sufficient compliance on complicated medical therapy to warrant acceptance[63].

Previous malignant disease

The incidence of malignancy is increased in organ transplant recipients and other patients on chronic immunosuppression[64], presumably due to impaired policing of potentially oncogenic

viruses and malignant clones, particularly of lymphomas, which may occur up to 40 times more frequently in transplant recipients. Transplantation is generally not performed within 3–5 years of neoplasms other than superficial skin lesions. A history of tumors with a predilection for recurrence, such as breast cancer and renal cell cancer, requires vigorous screening for recurrent disease. There is a growing population, however, of patients with successful transplantation late after successful chemotherapy with adriamycin-containing regimens for lymphoma, particularly Hodgkin's lymphoma.

Irreversible pulmonary hypertension

Multiple criteria for selection of recipients are profoundly affected by hemodynamic compromise, which may need to be addressed before candidacy can be confirmed (Table 10). Demonstration of sufficiently low pulmonary vascular resistance may require several days of vigorous reduction of left-sided filling pressures with vasodilators and diuretics, occasionally requiring support with inotrope-dilators also. Early pulmonary hypertension presents a heavy burden to the donor right ventricle, even if pulmonary pressures later decrease. Acute right heart failure continues to be a major factor in early postoperative morbidity.

Pulmonary hypertension is generally evaluated not by one number alone, but by a combination of calculations, including pulmonary vascular resistance, which should generally be reducible to below 240–300 dyne-s-cm^{-5}, pulmonary artery systolic pressure which should be reducible to levels below 50–60 mmHg, and transpulmonary gradient. The gradient, calculated as the mean pulmonary artery pressure minus the pulmonary capillary wedge pressure, usually shows least change during pharmacologic therapy[65] and should be below 12–15 mmHg. Although evaluation in some centers includes acute titration of intravenous nitroprusside to systemic blood pressure tolerance[66], reversibility of pulmonary hypertension in patients with pulmonary capillary wedge pressures chronically above 25 mmHg may be easier to demonstrate after sustained reductions in filling pressures over several days. The average patient with symptoms at rest, or with minimal exertion, has chronically elevated ventricular filling pressures and some reversible elevation in pulmonary pressures (Table 11). A brief trial of prostaglandin E1 may occasionally help to demonstrate reversibility after other modalities and assist in planning of postoperative hemodynamic management. Nitric oxide appears to be a potent pulmonary vasodilator, but its use should be tempered with caution, as it frequently leads to elevation in left-sided filling pressures, most likely due to increased right-sided cardiac output to the failing left ventricle. Heterotopic transplantation ('piggy-back' of the new heart on the old) has at times been employed for irreversible pulmonary hypertension, but this procedure has been associated with a 1-month mortality of 25% compared to 10%, and is now rare[67,68].

Impaired pulmonary function

Pulmonary function testing should be postponed until after hemodynamic optimization in patients with obvious resting congestion. Both obstructive and restrictive patterns may be observed with pulmonary congestion[69]. Maintained reduction of filling pressures and volume status, often for several days, allows optimal performance. General thresholds for acceptibility have been 50–70% of predicted forced vital capacity and forced expired volume. Cessation of smoking is generally required by most programs for at least 3 months, both to reduce perioperative pulmonary complications and to decrease the chance of postoperative smoking, which may increase the risk of early graft coronary artery disease[70]. Compliance with smoking cessation may be assessed with unscheduled urinary nicotine levels. Regardless of pulmonary function test results, a history of chronic sputum production and 'smoker's cough' is sometimes considered a contraindication due to risks of pulmonary infection during immunosuppression. No organized data have been collected on post-transplant outcome for patients with mild intrinsic asthma, which has generally not been considered a complication unless it has required intensive chronic therapy or multiple hospitalizations.

Hepatic dysfunction

Hepatic function is also optimized by vigorous diuresis and vasodilator therapy to reduce right-sided filling pressures and tricuspid regurgitation. This is important not only to establish transplant candidacy, but to minimize coagulopathy which may become profound after cardiopulmonary bypass during transplantation. All patterns of abnormal liver function have been observed with 'passive congestion'. Depressed cardiac output is much less important for hepatic function, except when circulatory collapse

Table 11 Preoperative reversibility of pulmonary hypertension during tailored therapy* prior to transplantation in 100 patients later receiving transplantation

	Initial PVR > 240 dyne-s-cm^{-5}	*Initial PAS > 50 mmHg*	*Initial TPG > 15 mmHg*
No	59% (9%) †	35% (6%) †	86% (7%) †
Yes	41% (5%)	65% (8%)	14% (7%)
If yes, reversible*	25% (11%)	41% (3%)	8% (17%)
Not reversible	16% (0%)	24% (10%)	6% (0%)

Numbers in parentheses indicate 30-day mortality after transplantation.‡

PAS = pulmonary artery systolic pressure; PVR = pulmonary vascular resistance; TPG = transpulmonary gradient (mean pulmonary artery pressure minus pulmonary capillary wedge pressure)

* Reversibility determined after 72 h of therapy tailored to reduce pulmonary capillary wedge to 15 mmHg, followed occasionally by a trial of prostaglandin E_1, if necessary.

† Reproducibility of this post-transplant survival may depend in part on the vigor with which pulmonary congestion is prevented preoperatively, the preservation and age of the donor heart, and early postoperative hemodynamic management.

leads to 'shock liver', when elevations of transaminases into the thousands may occur. This pattern should be allowed to recover during support with either devices or drugs prior to transplantation to avoid postoperative hepatic failure.

Renal dysfunction

Unlike pulmonary and hepatic function, renal function is more dependent on adequate cardiac output. In fact, even when cardiac output is adequate, renal function may decline temporarily after brisk diuresis of chronically congested patients, perhaps due to sudden decompression of distended atria and resultant reflex increase in renal vasoconstriction[71], and perhaps compounded by decreased atrial natriuretic peptide secretion[72]. Several days of inotropic infusions may be required to optimize function in some cases. Creatinine clearance of at least 50 ml/min is preferred, but lower rates may occasionally be accepted if clearly the result of acute decompensation, with normal renal size on ultrasound and absence of proteinuria. Disproportionate elevation of blood urea nitrogen is common. Patients with creatinine over 2 mg/ml, blood urea nitrogen over 50 mg/dl, or preoperative dependence on inotropic infusions, are at particularly high risk for early postoperative renal dysfunction, which may in some cases be decreased by the use of antithymocyte globulins rather than cyclosporin in the immediate postoperative period.

The critically ill patient

Evaluation presents a particular challenge when performed in a candidate seen first in critical condition. When the patient's major organ and cerebral function are acutely compromised, decisions regarding medical risk and patient commitment are based on experienced guesswork and emotional bias. Peripheral vascular disease is often under-appreciated, while renal and hepatic dysfunction believed (or hoped) to be reversible may become major impediments to postoperative recovery. A common ordeal is the decision regarding a young patient with a previous history of noncompliance or substance abuse for whom there is no time to confirm a commitment to reform. Some patients in critical condition must be refused transplantation, with the cost of immediate disappointment preventing the tragedy of protracted postoperative misery prior to death, and the tragedy of the premature end of a donor heart. Transplantation for otherwise doomed patients, however, is often the most rewarding, with the infinite relative increment in both quality and length of life (Figure 3). Increasing availability of mechanical support may allow many such patients to achieve stabilization and rehabilitation before transplant, following which the chance of favorable post-transplant outcome may be highest.

Documented risk factors

Collaboration between transplant programs is now yielding increasing information regarding the likelihood of good post-transplant outcomes. Of the two major multicenter experiences, the International Society for Heart and Lung Transplantation Registry has established older age, left ventricular ejection fraction <11%, mechanical support while waiting, and female gender as risk factors for death after transplantation[2]. It should be noted, however, that some risk factors for post-transplant death also identify high risk without transplantation. The Cardiac Transplant Research Database provided the first multivariate analysis of death, demonstrating older age, elevated serum creatinine, low cardiac output, and mechanical ventilation prior to transplantation to be associated with worse survival, while female gender was associated with more rejection but equivalent survival[73]. A separate study of program attributes found the most important program factor in patient survival to be the previous experience of the transplant cardiologist, with strong contribution from the transplant nurse coordinator[74].

CANDIDATES ON THE WAITING LIST: MANAGEMENT AND RE-EVALUATION

The average waiting period for candidates has increased from 6 weeks for all candidates in 1984 to over 6 months on average. Patients waiting at home frequently do not undergo cardiac transplantation for over a year after listing, particularly if they have blood group O. During the prolonged waiting time, outpatients require careful management and re-evaluation for both deterioration and improvement. As recommended by the Consensus conference on transplantation, waiting candidates should be seen at least monthly by the heart failure/transplant cardiologist at the center where the transplant will be performed[9]. Assessment of clinical stability by history, particularly evidence of congestion, examination of postural vital signs and jugular venous pressure, and laboratory monitoring of electrolytes, renal and hepatic function, and anticoagulation are critical to ensure that the candidates are in optimal condition for transplantation. More frequent visits with the primary physician are often necessary.

Medical management for transplant candidates is dominated by the same principles developed to decrease the need for transplantation and provide alternative hope to ineligible patients. Maintenance of low filling pressures not only serves to minimize congestive pulmonary and abdominal symptoms and improve nutrition, but also reduces the risk of postoperative pulmonary hypertension, prolonged intubation, coagulopathy, and hepatic dysfunction during the postoperative course. Patients should be compliant with a regimen which includes, in most cases, restriction to $\leq$2 g of sodium and $\leq$2 l of fluid daily, and always a daily weight diary which guides patient adjustment of diuretic dosage. The spectrum of medications in this population is shown in Table 7.

Anticoagulation

The issue of anticoagulation for patients with low left ventricular ejection fractions and dilated ventricles remains controversial. It is accepted that patients with an additional risk factor such as atrial fibrillation, history of previous embolic event, or pedunculated thrombus need anticoagulation, with the strongest risk factor being atrial fibrillation, associated with a yearly embolism risk as high as 18% in the presence of heart failure[75]. In 120 transplant candidates without any of these risk factors, the incidence of embolic events during a mean follow-up of 300 days without anticoagulation was 4%[76]. The official National Practice Guidelines

for Heart Failure do not at this time recommend routine anticoagulation for heart failure patients without other risk factors[77]. The decision reflects the estimated balance of risks of embolic events, which can lead to tragic strokes and death, and the risks of hemorrhage, which can rarely lead to intracranial hemorrage or other life-threatening events. The risks of bleeding are low when anticoagulation is monitored closely and doses decreased for amiodarone and impaired hepatic function. Perioperative bleeding is often greater after coumadin therapy despite administration of vitamin K prior to the transplant.

Ventricular dysrhythmia

Non-sustained ventricular tachycardia occurs in 50–80% of patients with heart failure severe enough to warrant evaluation for transplantation[40]. Although sudden death occurs in 15–30% of these patients, its relationship to previous non-sustained ventricular tachycardia remains controversial. The risk of sudden death is increased in heart failure patients with a history of syncope, which is an indication for admission and evaluation. Therapy for asymptomatic non-sustained ventricular tachycardia has generally not been undertaken unless the runs are long and rapid. Type I antiarrhythmic agents appear to increase the risk of sudden death in heart failure patients, and are rarely used except, occasionally, to decrease the frequency of discharges from an implantable cardioverter–defibrillator. Therapy with amiodarone does not worsen and may improve survival in severe heart failure[32], with benefits for ventricular function and heart failure endpoints as well as sudden death. The GESICA trial studied patients with an overall mortality of 55% at 2 years, similar to that of ambulatory transplant candidates with class IV history, and found a 28% decrease of mortality with amiodarone[32]. The differences between this trial and the Veterans Administration trial may reflect in part the different disease severity[33]. Perioperative pulmonary and hemodynamic problems attributed to prolonged amiodarone use have been described in other surgical populations, but have rarely occurred after transplantation[78].

Hospitalization

Candidacy is a dynamic state from which movement is possible, particularly during the lengthening waiting periods (Figure 7). Deterioration to require hospitalization has in the past occurred in up to 30% of candidates during the first 6 months, and may be more frequent with growing adherence to more defined criteria of disease severity before listing[79]. Hospitalization may be indicated to prevent imminent death, or to prevent serious organ system deterioration which could compromise the outcome of transplantation (Table 12). Progressive right heart failure, and worsening renal or hepatic dysfunction, could be indications for hospitalization even if the candidate finds them compatible with life at home. Escalating fluid retention can increase perioperative

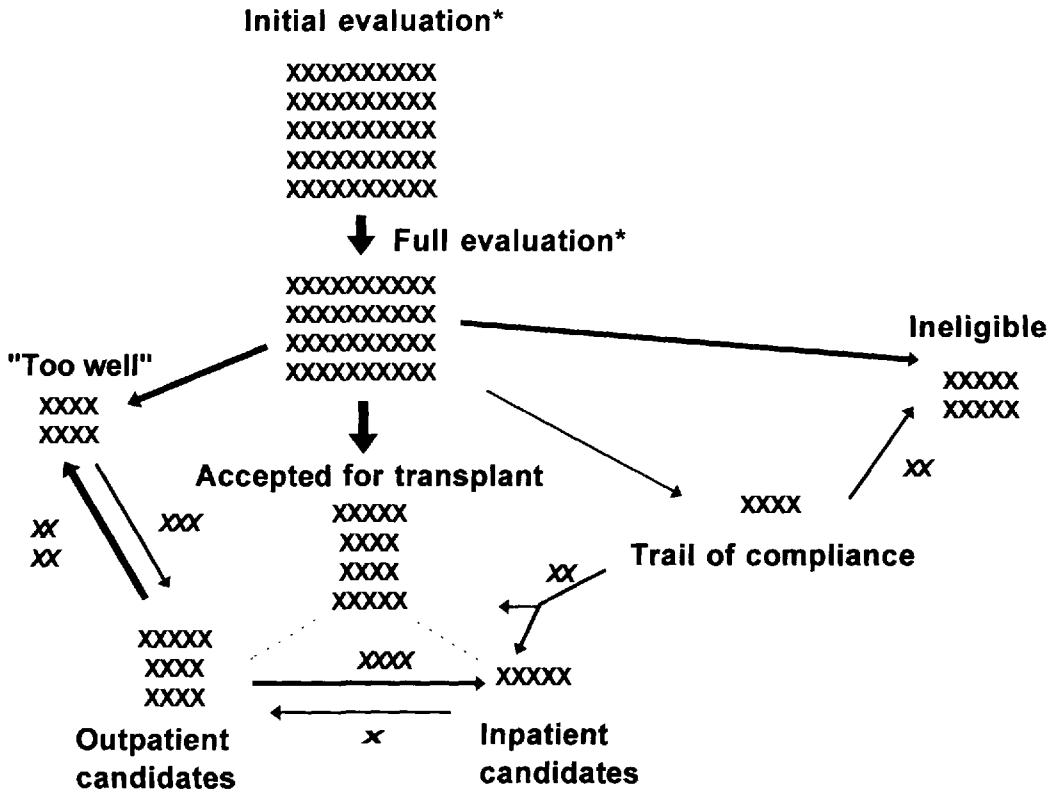

Figure 7 Progress through evaluation and continual re-evaluation for cardiac transplantation, demonstrating the dynamic nature of candidacy. The relative frequencies of outcomes will depend upon the local policies regarding complete and partial evaluation of candidates (*) who appear to be ineligible or 'too well' for transplantation

Table 12 Frequent indications for admission of waiting candidates

General considerations
To prevent death at home
To prevent conditions which jeopardize perioperative outcome
Specific considerations
Unstable angina
Syncope
Frequent Implantable cardioventer defibrillator discharges
Suspected embolic event
Congestion refractory despite good compliance to increased diuretics, which:
(a) renders patients bedridden
(b) causes marked hepatic congestion
(c) may worsen borderline pulmonary hypertension
Systolic blood pressure persistently < 70–75 mmHg
Pulse pressure < 12 mmHg, particularly with cool extremities
Creatinine > 2.0 and rising

pulmonary hypertension, prolong intubation requirements, and worsen coagulopathy, but also seems to be associated anecdotally with increased risk of unexpected death at home, which may in part be related to the difficulties of controlling potassium, both high and low, during fluctuating diuresis and electrolyte replacement. Although patients with the most severe compromise can expect the greatest improvement from transplantation, perioperative condition is a critical determinant of postoperative outcome, and should be optimized.

For the hospitalized patient the escalating approach includes hemodynamic monitoring for 24–48 hours when major changes are made in vasoactive therapy, although prolonged maintenance of indwelling lines should be avoided due to the risks of infection. Low–moderate doses of dobutamine, dopamine, and phosphodiesterase inhibitors, alone or in combination, are frequently adequate to stabilize patients to a level compatible with ambulation with an intravenous pump. The full agonists, epinephrine and norepinephrine, can be useful to increase cardiac output in an emergency setting, but the continued need for these agents should usually trigger consideration of a mechanical assist device.

Mechanical support

The indications for mechanical support continue to evolve. The hemodynamic criteria often suggested for heart failure were actually originally proposed for the very different setting of post-cardiotomy shock needing intra-aortic balloon counterpulsation[80]. These criteria include cardiac index <2.0 l min^{-1} m^{-2}, pulmonary capillary wedge pressure >20 mmHg, systolic blood pressure <90 mmHg and systemic vascular resistance >2100 dyne-s-cm^{-5}, which are typical of many heart failure patients who can be not only stabilized but also discharged after adjustment of medical therapy. The clinical impact of hemodynamic parameters varies greatly, particularly in relation to the duration of compromise, but at the most severe end of the spectrum, mechanical support would generally be indicated for continued inability to maintain a systolic blood pressure >75 mmHg, cardiac index ≥1.5 l min^{-1} m^{-2}, and pulmonary venous saturation >50% on maximal pharmacologic support. (Severely elevated filling pressures, on the other hand, generally indicate the potential for improvement from further adjustment of medical therapy). More subtle trends of declining cardiac index and renal function on maximal therapy are difficult to interpret, but are at least as important as the absolute measured numbers[81].

Patients who require mechanical support in the absence of coronary artery disease may be considered for direct placement of left ventricular assist devices without intervening therapy with an intra-aortic balloon, from which the benefit is controversial in this population. Patients with coronary disease who demonstrate continued dependence on intra-aortic balloon counterpulsation may eventually also be considered for placement of a left ventricular assist device, which allows ambulation and rehabilitation prior to transplantation. Over 300 left ventricular assist devices, both the HeartMate and Novacor models, have been implanted in the United States[82]. Complications include infection, usually through the drive line, bleeding, and thromboemboli from the heart and from the device itself, which may be less common with the HeartMate due to the endothelialization of the titanium surface.

Recent experience with left ventricular assistance as bridging to transplantation has shown approximately 70% survival to transplantation. In an uncontrolled trial of candidates with cardiac indices below 2 l min^{-1} m^{-2} on intravenous inotropic agents and balloon counterpulsation, survival after transplantation was 90% for recipients who had been bridged, compared to 70% for those compromised patients surviving without bridging (Eric Rose, personal communication). The good outcome for bridged patients after transplantation results from better preoperative status, but may also reflect the selection by death of the highest-risk transplant candidates during the period of mechanical support.

Re-evaluation

The long waiting periods also allow demonstration of improvement in some patients able to wait at home. The highest period of risk for outpatients may be the first few months, after which some of the factors which led to deterioration and referral may resolve spontaneously, and the benefits of optimal medical therapy may be realized. The Bethesda Conference and the Consensus Conference on Selection both emphasize the important of periodic re-evaluation of waiting candidates[9,24]. Suggested criteria for re-evaluation include an assessment of clinical stability and demonstration of improved exercise capacity measured by peak oxygen consumption (Table 13). Up to 30% of ambulatory pa-

Table 13 Assessment of clinical stability for re-evaluation of waiting candidates

Clinical criteria
1. Stable fluid balance without orthopnea, elevated jugular venous pressures, or other evidence of congestion on the flexible diuretic regimen
2. Stable blood pressure with systolic at least 80 mmHg
3. Stable serum sodium (usually >133 mEq/l)
4. Stable renal function (blood urea nitrogen usually <60 mg/dl)
5. Absence of symptomatic ventricular arrhythmias
6. Absence of frequent angina
7. Absence of severe drug side-effects
8. Stable or improving activity level without dyspnea during self-care or 1 block exertion.
Exercise criteria (if initial peak oxygen consumption <14 ml kg^{-1} min^{-1})
1. Improvement in peak oxygen consumption of ≥2 ml kg^{-1} min^{-1}
2. Peak oxygen consumption of ≥12 ml kg^{-1} min^{-1}

tients initially listed with initial average peak oxygen consumption <14 ml kg^{-1} min^{-1} (average 11) demonstrated sufficient improvement to leave the list, with a subsequent 2-year survival of 92%[51].

COMMENT

The personal dedication of the early teams for heart transplantation, combined with the advances in surgical techniques and immunosuppression, have established this as the best current therapy for patients with truly end-stage heart disease. Once referred for transplantation with New York Heart Association class IV symptoms and an ejection fraction less than 25%, even when patients can be maintained out of the hospital, survival without urgent transplant is less than 50% at 2 years[43], compared to a 60% chance of surviving 6 years and a 35% chance of surviving 10 years after transplantation with current protocols[2]. Most recipients achieve a good to excellent quality of life, although less than 50% return to full-time employment[83].

As originally projected at the time of the first Bethesda conference on transplantation over 25 years ago, however, the promise of transplantation has not been fulfilled[1]. Despite arduous efforts the donor heart supply is limited to 2000–2500 yearly in the United States, compared to the 40 000–45 000 originally projected in 1968. Over 70% of these hearts are being used for patients waiting in hospitals. It has been said that heart transplantation is currently to heart failure what the lottery is to poverty (attributed to Arnold Kats and others).

Left ventricular assist devices currently offer hope for those hospitalized patients who might otherwise not survive until transplantation. Although now employed only as 'bridges' to transplantation, increasing refinement and experience suggest that permanent ambulatory devices may extend a highway even to patients ineligible for transplantation. At the other end of the spectrum of heart failure, there is now increasing evidence that early intervention can reduce development of heart failure[84,85]. Even after symptoms of heart failure have appeared, new approaches to both medical and surgical therapy may prolong the period of cardiac and clinical compensation[3–5,20]. In particular, recognition of the contributions of mitral regurgitation and left ventricular distortion to the progression of heart failure has stimulated the development of new surgical approaches to cardiac remodelling.

A beneficial side-effect of cardiac transplantation has been the increased medical and public focus on the problem of heart failure, which affects over 3 million patients in the United States, almost 1 million of whom have class III–IV heart failure. The magnitude of the miseries and costs of this problem warrant increasing focus on innovation and collaboration at all levels of research and clinical care.

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20
The Problem of Pulmonary Hypertension in the Potential Cardiac Transplant Recipient

J.M. CHEN AND R.E. MICHLER

INTRODUCTION

Despite advances in perioperative management, right-sided circulatory failure (RSCF) remains a significant source of morbidity and mortality early after cardiac transplantation. Perioperative RSCF, while clearly multifactorial in nature, has traditionally been associated with pretransplant pulmonary hypertension, as suggested by elevated pulmonary hemodynamic indices revealed on pretransplant cardiac catheterization. While severely elevated values of such indices represent a contraindication to cardiac transplantation at many centers worldwide, there currently exist no standard guidelines regarding the validity and importance of these preoperative abnormalities for postoperative outcome. The purpose of this chapter is to review the problem of pulmonary hypertension and the potential cardiac transplant recipient – its diagnosis, management and manipulation, and long-term clinical significance.

Severe preoperative pulmonary hypertension is thought to result in post-transplant RSCF due to a marked increase in afterload presented acutely to the donor right ventricle. Since Griepp and colleagues first reported a relationship in 1971 between elevated preoperative pulmonary vascular resistance and the risk of death from acute right ventricular failure post-transplant, numerous studies have confirmed this association[1]. Indeed, preoperative pulmonary hypertension has not only been associated with overall post-transplant morbidity from acute right heart failure and all-cause perioperative mortality, but also has been associated with other sources of postoperative morbidity, including post-transplant infections and arrhythmias[2-12].

Despite this, substantial controversy has persisted regarding the power of individual hemodynamic indices to predict perioperative mortality (and RSCF) accurately. Transplant physicians have traditionally placed an emphasis on calculated estimates of pulmonary hypertension (pulmonary vascular resistance (PVR), pulmonary vascular resistance index (PVRI) and transpulmonary gradient (TPG)) in the preoperative evaluation of cardiac transplant candidates in an effort to assess the right ventricular afterload that will be presented to the transplanted heart[2-10]. The equations from which these indices are derived are outlined in Figure 1.

As shown, a substantial degree of co-dependence exists among these variables; PVR and PVRI differ by a factor of body surface area, and PVR and TPG differ by a factor of cardiac output. Addonizio and co-workers have favored PVRI on the basis that it represents the PVR 'indexed' to body surface area, thus theoretically eliminating bias due to body size and weight (especially important in the pediatric population)[7]. Proponents of the transpulmonary gradient have suggested that PVR (and thus PVRI) may prove unreliable due to inherent inaccuracies in the method of thermodilution, particularly at low cardiac outputs[6].

Pulmonary vascular resistance (PVR, Wood Units (WU))

$$PVR = \frac{(PA\ mean - PCW)}{CO}$$

Pulmonary vascular resistance index (PVRI, Wood Units · m^2)

$$PVRI = \frac{(PA\ mean - PCW)}{CI} = PVR \cdot BSA$$

Transpulmonary gradient (TPG, mm Hg.)

$$TPG = PA\ mean - PCW$$

Figure 1 Equations for the calculation of pulmonary hemodynamic indices

Table 1 Compilation of the literature regarding individual preoperative pulmonary hemodynamic indices as predictors of postoperative mortality

			Endpoint			
Reference	*n*	*Index studied*	*RSCF*	*30-day mortality*	*1-year mortality*	*Mortality*
Addonizio *et al.* (1987)*	73	PVR < 6 WU	7			4
(pediatric)	8	PVR > 6 WU	4			1
	48	PVRI < 6 WU m^2	0			0
	33	PVRI > 6 WU m^2	11			5
Kirklin *et al.* (1988)[5]	132	PVR (continuous)†				54
Kirklin *et al.* (1988)[5]	63	PVR (continuous)††				29
Erickson *et al.* (1990)[3]	104	TPG < vs ≥ 12 (probability)		NS	5% vs 24% (6 months)/5% vs 36% (1 year)‡	
		PVR < vs ≥ 3 (probability)		NS		
		PVRI < vs ≥ 3 (probability)		NS		
Bourge (1991)[4]§	182	PVR < 4		7.4%		
		PVR ≥ 4		19%		
Anguita *et al.* (1991)[9]¶	57	PVR < vs > 5 and/or TPG < vs > 12			38% vs 87%	
Costard-Jackle and Fowler (1992)[17]‖	288	PVR ≤ 2.5 (n = 143)				6.9% (0)
		PVR > 2.5 (n = 145)				17.9% (9)
		TPG ≤ 15 (n = 240)				11.3% (5)
		TPG > 15 (n = 45)				20.0% (4)
		PVRI ≤ 5 (n = 151)				8.6% (2)
		PVRI > 5 (n = 130)				17.7% (7)
Bando *et al.* (1993)[10]** (pediatric)	67	PVR < vs ≥ 4		NS		
		TPG < vs ≥ 15		50%	49%	
Murali *et al.* (1993)[6]‡‡	425	(I) TPG < 15 and PVR < 5 (n = 332)		9.9%		
		(II) TPG < 15 and PVR > 5 (n = 15)		13.3%		
		(III) TPG ≥ 15 and PVR < 5 (n = 46)		21.7%		
		(IV) TPG ≥ 15 and PVR > 5 (n = 32)		25.0%		

* PVRI > 6 WU m^2 correlated with both death and RSCF, $p < 0.001$.
† Where an elevated PVR was determined as a continuous variable for risk analysis; †† where an increased PVR was associated with mortality by multivariable comparison.
‡ 5% vs 24% mortality at 6 months ($p = 0.003$) and 5% vs 35% at 1 year ($p = 0.0005$) for TPG < vs ≥ 12.
§ $p = 0.06$ for PVR < 4 vs ≥ 4.
¶ Where actuarial survival curves at 1 year and 18 months for those with either PVR or TPG less than threshold were compared with those greater than threshold values ($p<0.01$).
‖ For an endpoint of: incidence of 90-day mortality (no. of patients who died from RSCF) – PVR comparison $p < 0.02$; TPG comparison $p < 0.02$; PVRI comparison $p < 0.05$
** For TPG < vs ≥ 15, $p = 0.01$ by univariate analysis, $p = 0.03$ by multivariate analysis; for PVR < vs ≥ 4, p = not significant over entire experience.
‡‡ Group I vs group III, $p < 0.05$; group I vs group IV, $p < 0.05$.
NS = not significant

The transpulmonary gradient, it is argued, is flow-independent and thus may better reflect resistance to flow across the pulmonary bed[6]. In contrast, others have argued that the gradient across the pulmonary bed is itself flow-dependent, and thus requires that total flow (or its estimate as represented by cardiac output) be included in its definition.

Table 1 summarizes reports in the literature that analyze the utility of calculated hemodynamic indices as predictors of both right heart failure and perioperative mortality[2–12]. As shown, the relative acuity of individual indices to predict either 30-day mortality or right heart failure remains unclear. In an effort to compare these indices quantitatively, we recently evaluated data from 476 patients transplanted at the Columbia-Presbyterian Medical Center from 1983 to 1994 using standard historical threshold values to divide patients into cohorts with elevated and non-elevated preoperative profiles[13]. These findings were then correlated with 30-day all-cause mortality.

Quantitative comparison among these cohorts was established with receiver operating characteristic (ROC) curves, traditionally plotted as graphic representations of the relationship between sensitivity and specificity (the true-positive and false-positive rates). The area under these ROC curves (AUC) not only represents the probability that normal and abnormal test results are associated accurately with the presence or absence of a given outcome, but also reflects a means by which the discriminating ability of individual indices may be compared[14–16]. Representative ROC curves and their AUC are depicted in Figure 2. We found no statistical difference in the AUC for PVR, PVRI or TPG in the prediction of 30-day mortality, suggesting that none of the tests was superior in its ability to predict perioperative mortality. The high degree of co-dependence of the variables may explain these findings, and further suggests that different combinations of such elevated indices would likely have similar results.

REVERSIBILITY

Once the diagnosis of preoperative pulmonary hypertension has been confirmed by cardiac catheterization, some have suggested that patients may be subdivided further into those with 'fixed' or 'reactive' components to their pulmonary hypertension, the relative proportions of which may be elucidated using pulmonary vasodilator therapy such as sodium nitroprusside[17,18]. Those patients, it is argued, with a high degree of 'reactivity' to their pulmonary hypertension (i.e. who have a substantial drop in their PVR, PVRI or TPG from an elevated value to a non-elevated value with vasodilator provocation) may have a large 'reversible' component and, as such, may have a perioperative mortality comparable to patients who have no preoperative pulmonary hypertension. Conversely, those patients whose pulmonary hyper-

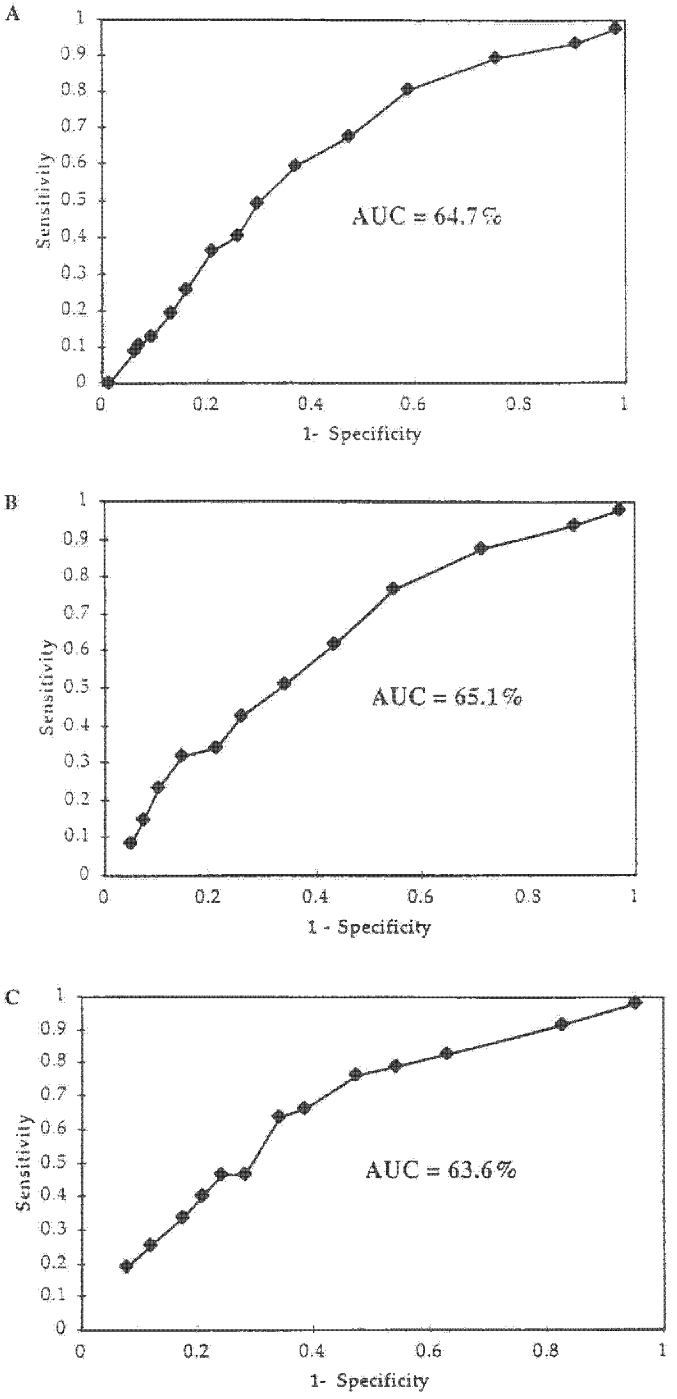

Figure 2 Receiver operating characteristic curves and their areas for (**A**) PVR, (**B**) PVRI and (**C**) TPG as predictors of 30-day all-cause mortality

tension responds less well may have a larger 'fixed' component that may, it is postulated, adversely affect their post-transplant outcome.

The largest study in the literature to date regarding the use of nitroprusside in the evaluation of patients for cardiac transplantation examined 293 patients and revealed four distinct cohorts of patients separated on the basis of risk stratification for 90-day

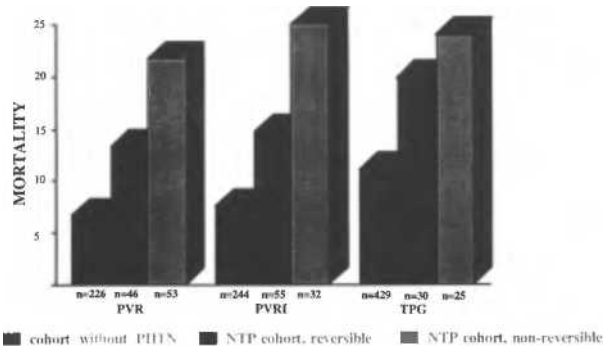

Figure 3 Bar graph representing the effect of preoperative 'reversibility' on 30-day mortality (PHTN = pulmonary hypertension; NTP = nitroprusside

mortality[17]. We examined a similar cohort of 181 patients with elevated preoperative pulmonary hemodynamic indices who under went vasodilator challenge with nitroprusside preoperatively, using 30-day perioperative mortality as the end-point of the study (Figure 3)[13]. The conclusions of these investigations were three-fold. First, those patients with irreversible pulmonary hypertension had a nearly four-fold increase in their incidence of mortality when compared with those without preoperative pulmonary hypertension. These differences were statistically significant. Second, those patients with reversible hypertension appeared to demonstrate a 25% reduction in their incidence of 30-day mortality when compared with those patients who were non-reversible. These differences, while clinically relevant, were not statistically significant. Finally, those patients with reversible hypertension still exhibited a marked increase in the incidence of mortality when compared with patients without preoperative pulmonary hypertension. These differences demonstrated statistical significance for the parameter TPG, and borderline significance for PVR and PVRI, with p-values between 0.05 and 0.1. In sum, these data suggested that while 'reversibility' with nitroprusside therapy was associated with improved overall 30-day mortality, it was not associated with improvement to a level comparable to those patients who did not have preoperative pulmonary hypertension.

VASODILATOR CONDITIONING

Some investigators have suggested that continuous low-dose nitroprusside therapy may help to 'optimize' those patients with a substantial reactive component to their pulmonary hypertension. However, this modality is limited by the adverse systemic effects of nitroprusside, the most pronounced being severe hypotension from peripheral vasodilatation[17–19]. Recent enthusiasm has arisen regarding other pulmonary vasodilators and cardiac inotropes that may have actions specific enough to the pulmonary vasculature to limit their systemic effects[20–29]. Table 2 summarizes the literature regarding these agents.

Adenosine, while potentially superior to nitroprusside (NTP) in its ability to lower TPG, and comparable in its ability to lower PVR, has been shown (a) to have little effect on cardiac output and (b) to increase pulmonary capillary wedge pressure, rendering it a poor modality for long-term use in patients with congestive

Table 2 Compilation of the literature regarding vasoactive agents used in the preoperative pulmonary hemodynamic evaluation of transplant candidates (DBA = dobutamine; NTG = nitroglycerin)

	Agent studied	*No. of patients*	*Response*	*Comment*
Nitroprusside (NTP)				
Kawaguchi *et al.* (1989)[30]	NTP	13	PVR < 4 ($n = 6$)	One RSCF, two biventricular failure in each group
			PVR 4.8 → 2.5	No side-effects noted
Simonsen (1990)[42]	NTP	23		NTP given introperative and post-transplantation also No side-effects noted
Costard-Jackle and Fowler (1992)[17]	NTP	78	PVR ≤ 2.5, SBP ≥ 85 → 3.8% 90-day mortality	
		40	PVR ≤ 2.5, SBP ≤ 85 → 27.5% 90-day mortality	
		32	PVR > 2.5, no effects on SBP → 41% 90-day mortality	
Nitric oxide (NO)				
Semigran *et al.* (1994)[27]	NO	16	PVR: 3.2 → 1.7 ($p < 0.05$) TPG: 11 → 7 ($p < 0.05$)	2 patients had postoperative RSCF 7 patients had significant hypotension
	NTP	16	PVR: 3.3 → 2.1 ($p < 0.05$) TPG: no change	
Adatia *et al.* (1995)[28] (pediatric)	NO–high LAP	6	PVRI: 14.9 → 7.6 ($p < 0.05$; TPG: 45 → 27 ($p < 0.05$)	No noted side-effects of NO, NO most effective in patients with LA hypertension
	NO–low LAP	5	PVRI: 36 → 31 ($p < 0.05$); TPG: 64 → 62	
	Ach-high LAP	3	PVRI: 12.9 → 12.2 (NS); TPG: 43 → 42 (NS)	
	Ach-low LAP	4	PVRI: 33.2 → 32.3 (NS); TPG: 64 → 63 (NS)	
Adenosine				
Haywood *et al.* (1992)[20]	Adenosine	21	↓ PVR 41%, ↓ TPG 35%	No effects on cardiac output, but increase in PCW with adenosine
	NTP	18	↓ PVR 42%, ↓ TPG 9%	MAP < 60 in 21% of NTP patients
Amrinone				
Bolling *et al.* (1988)[21]	Amrinone	27	PVRI: 6 → 2.4 ($p = 0.001$)	Two patients with thrombocytopenia requiring transfusion
			TPG: 19 → 10 ($p = 0.001$)	89% of total 27 were 'responders'
Deeb *et al.* (1989)[22]	Amrinone	22	PVR: 5.8 → 2.5 ($p = 0.0001$)	Two patients with thrombocytopenia requiring transfusion
			TPG: 19 → 11 ($p = 0.0001$)	
	'Conventional' therapy	16	PVR: 5.1 → 3.3 ($p = 0.003$)	
			TPG: 18 → 13 ($p = 0.03$)	
Prostaglandin E1				
Murali *et al.* (1991)[23]	NTG	8	TPG: 16 → 15(NS); PVR: 4.1 → 3.5 ($p < 0.005$)	Hypotension, headache
	NTP	7	TPG: 18 → 16(NS); PVR: 5.4 → 3.6 ($p < 0.01$)	Hypotension
	PGE1	29	TPG: 17 → 13($p < 0.001$); PVR: 4.9 → 3.0 ($p < 0.001$)	Hypotension, headache, flushing, nausea, vomiting
	DBA	11	TPG: 16 → 17; PVR: 3.5 → 1.2 ($p < 0.001$)	Increase in premature ventricular complexes (PVC)
	Enoximone	11	TPG: 18 → 21; PVR: 5.8 → 3.9 ($p < 0.01$)	Nausea, vomiting, increase in PVC
Murali *et al.* (1992)[24]	NTG	9	No change in TPG; PVR 4.3 → 3.7 ($p < 0.01$)	Nine transplanted, no RSCF, but nausea, vomiting, hypotension in eight patients who were non-responders to PGE1
	NTP	12	TPG: 20 → 17 ($p < 0.01$); PVR: 6.2 → 3.9 ($p < 0.01$)	
	PGE1	39	TPG: 18 → 12 ($p < 0.01$); PVR: 5.1 → 2.7 ($p < 0.01$)	
Iberer *et al.* (1993)[25]	PGE1	13	PVR: 5.3 → 2.7 ($p < 0.005$)	Given 6-day course of PGE1, restudied 7 days later; side-effects included edema, joint pain, headache, abdominal pain. Eight transplanted, no RSCF but also given PGE1 post-transplant
Combination therapy				
Zales *et al.* (1993)[29] (pediatric)	DBA	7	Combined drug study:	
	DBA and NTP	9	TPG: 18.5 → 12.0	
	DBA/NTP and/or Amrinone	8	PVRI: 10 → 4	

Ach = acetylcholine, LAP = left atrial pressure, NO = nitric oxide

heart failure[20]. Amrinone has been shown in long-term therapy studies to be more effective in reducing PVR and TPG than conventional triple-drug therapy with digoxin, diuretics and ACE-inhibition, despite a potential risk of thrombocytopenia[21,22].

In short-term therapy preoperatively, prostaglandin E1 (PGE1) has been demonstrated to be more effective than nitroglycerin (NTG) and NTP in reversing pulmonary hypertension, and to achieve postoperative mortality rates comparable to patients without preoperative pulmonary hypertension[23–26]. However, although PGE1 is hypothesized to have a 90% clearance in the lung on a single pass (thus theoretically limiting its systemic effects), it has also been shown to result in significant hypotension, and increased facial flushing and nausea when compared with NTG, NTP dobutamine (DBA) or enoximone[23].

Nitric oxide (NO) has been demonstrated to have substantially fewer systemic effects when compared with other vasoactive agents, and to induce a reduction in hemodynamic indices to a comparable degree to NTP[27,28]. Studies using NO in the pediatric population have described a greater degree of reversibility for those patients with elevated left atrial pressures prior to administration[27,28]. Adatia and colleagues have suggested that preoperative evaluation with NO challenge may further help to establish which pediatric patients would better benefit from heart–lung rather than heart transplantation[28]. Interestingly, evaluation of NO in the adult population, however, has demonstrated an increase in left ventricular filling pressures in patients with severe heart failure, a finding that may potentially limit its long-term preoperative use[27,28].

In addition, Zales and co-workers have also suggested that because of both the poor left ventricular systolic function and elevated diastolic pressures in patients with end-stage heart disease, a more substantial decrease in pulmonary hemodynamic indices may be revealed with additional inotropic support[29]. Their results in pediatric patients with combined DBA, NTP and amrinone therapy, while not compared specifically to vasodilator therapy alone, indicate potential new methods for preoperative evaluation with combination therapy[29].

OUTCOME

The question remains how to utilize these data regarding preoperative hemodynamic indices and reversibility with vasodilator challenge in the pretransplant evaluation. For example, does reversibility truly identify those with fixed elevated pulmonary indices, and should such patients be denied cardiac transplantation, or be referred for heart–lung transplantation? Or, as Kawaguchi has suggested, is perioperative mortality more reflective of graft viability and overall graft ischemic time than reversibility of hemodynamic indices in the potential recipient[30]? In attempting to answer these questions, the natural progression of preoperative pulmonary hypertension both prior to and following cardiac transplantation must be evaluated.

Grant and colleagues have documented that patients with marginally acceptable or slightly elevated pulmonary hemodynamic indices may improve on maximal medical therapy, and thus should be studied serially pretransplant to establish candidacy for transplantation[31]. These findings have also been confirmed by Boffa and co-workers, who similarly noted changes in pulmonary hemodynamic indices over time, the magnitude of which, however, was unpredictable preoperatively[32].

Postoperatively, patients with previously documented pretransplant pulmonary hypertension (PVR > 6) have been noted to resolve their pulmonary hypertension by the first week post-transplant, as evidenced on two-dimensional echocardiogram and right heart catheterization[33]. Bhatia *et al.* evaluated post-transplant hemodynamic changes over time for patients with preoperative pulmonary hypertension, and reported a continual decrease in hemodynamic indices throughout the first year[33]. Eighty percent of such patients demonstrated normalization of a previously elevated PVR when studied at 1 year post-transplant. Interestingly, they further reported right ventricular remodeling in the transplanted heart in response to the increased afterload, as evidenced by the gradual development of right ventricular dilatation and tricuspid regurgitation at 1 year postoperatively despite a resolution in pulmonary hypertension[33].

Similarly, Corcos *et al.* have described a resolution of elevated right-sided circulatory pressures documented at 1 week post-transplant by 6 months post-transplant in most recipients[34]. Von Scheidt and co-workers further evaluated long-term post-transplant hemodynamics in a cohort of 57 patients and found no substantial changes in PVR from 12 to 84 months (no preoperative hemodynamic data given), suggesting a stabilization of hemodynamic indices for those long-term survivors of cardiac transplantation[35]. In a cohort of pediatric recipients, Gajarski *et al.* described a resolution of elevated PVRI by the first post-transplantation biopsy, the absolute value at which time, interestingly enough, was not statistically different from PVRI values achieved during vasodilator challenge preoperatively[12].

SURGICAL ALTERNATIVES

Empirically, the most substantial morbidity associated with RSCF after cardiac transplantation occurs within the first 30 days. Numerous strategies have been attempted in an effort to decrease the perioperative morbidity for this select group of transplant recipients. Donor/recipient size mismatching, with a deliberate oversizing of the donor heart, has been employed based upon the premise that the oversized right ventricle would be more suited to tolerate the increased afterload of the pulmonary hypertensive recipient. In two separate investigations, Costanzo-Nordin *et al.*[36] and Yeoh *et al.*[37] evaluated the deleterious relationship of cardiac allograft size mismatching and post-transplant function. Costanzo-Nordin *et al.* demonstrated a significantly greater risk of death for recipients of oversized donor hearts, independent of preoperative TPG values[36]. Similarly, Yeoh *et al.* described a compromise in long-term cardiopulmonary function (as evidenced by peak exercise oxygen uptake) in those patients with preoperative pulmonary hypertension who were recipients of oversized donor hearts[37].

Another surgical option based on the same principle is heterotopic heart transplantation. In related investigations, Villanueva *et al.*[38] and Kawaguchi *et al.*[30] assessed the utility of heterotopic heart transplantation in patients with severe preoperative pulmonary hypertension. Villanueva and colleagues described a decrease in PVR and TPG for *both* heterotopic transplant study patients and orthotopic transplant control patients: these improvements were maintained for the first year postoperatively[38]. Kawaguchi *et al.* similarly reported a reduction in postoperative PVR for *both*

heterotopic and orthotopic heart transplant recipients[30]. However, heterotopic transplant recipients in this cohort had a significantly increased rate of pulmonary complications and infections, presumably due to the anatomic position of the transplanted heart. In addition, heterotopic recipients were noted to have a higher incidence of biventricular failure and graft ischemia.

These studies indicate that operative strategies to overcome the potential post-transplant RSCF may not provide benefits in perioperative mortality enough to outweigh the procedural morbidity. The natural long-term course of patients with an elevated preoperative pulmonary hemodynamic profile suggests that those patients who survive the perioperative period may undergo a normalization of elevated right-sided pressures due to right ventricular remodeling in response to the increased afterload.

COMMENT

What, then, is the value of an elevated preoperative pulmonary hemodynamic profile, and what is its utility in the overall pretransplant evaluation? Naeije *et al.* have suggested that PVR calculations are insufficient to describe the functional state of the pulmonary circulation in patients with advanced congestive heart failure[39]. This finding has further been confirmed by Martí and co-workers, who have documented that an elevated PVR *per se* may not directly influence the incidence of graft dysfunction[40]. Further, in a multivariable analysis by Morley and Brozena, only right atrial pressure and a calculated 'high risk score' (incorporating parameters of both left- *and* right-sided circulatory function) were significantly associated with 1-year post-transplant mortality, despite a strong univariate association of PVR with 1-year mortality[41].

These findings suggest that, while generally useful as part of the entire pretransplant evaluation, preoperative pulmonary hemodynamic profiles must be viewed with caution. There does not appear to be agreement regarding threshold values for pulmonary hemodynamic indices beyond which cardiac transplantation is absolutely contraindicated; rather, threshold values (based on theoretical estimates of the norm) have previously been arbitrary in definition. Pulmonary hemodynamic indices represent a spectrum of values traditionally thought to have a direct linear relationship with postoperative mortality; the higher the value, it was postulated, the more likely postoperative mortality. Data described herein, however, refute this notion, and instead suggest that the relationship between an elevated preoperative index and postoperative mortality is but an association – direct but not exact, and by no means linear.

For example, can a patient with a preoperative PVR of 15 Wood Units undergo successful transplantation? Can a patient with a preoperative PVR of 2 Wood Units develop RSCF? Anecdotal data would support both of these scenarios. The preoperative pulmonary hemodynamic profile must therefore be evaluated as but one part – albeit important – of the entire pretransplant evaluation. Clearly, those candidates with severely elevated pulmonary hemodynamic indices are at great risk for postoperative complications. However, these risks must be weighed in concert with all other potential physiologic and psychological risks of transplantation.

At our institution no absolute threshold values are employed as inclusion or exclusion criteria for transplantation; this represents a policy that has evolved over our nearly two decades of experience with cardiac transplantation. Those candidates with an elevated preoperative pulmonary hemodynamic profile currently undergo vasodilator challenge with nitroprusside and additionally may receive either repetitive nitroprusside conditioning or long-term dobutamine therapy. It is our hope that, as these and other substantial preoperative risk factors for post-transplant morbidity and mortality are more effectively elucidated, complex algorithms will be developed that help to assess the cumulative risk of transplantation for the individual patient. These, in addition to investigations evaluating both waiting time and postoperative mortality, may then potentially unveil the true problem of pulmonary hypertension for the potential recipient.

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21
Mechanical Circulatory Support Before Heart Transplantation

J.S. SAPIRSTEIN AND W.E. PAE Jr

INTRODUCTION

Cardiac transplantation has become a standard therapy for carefully selected patients with incapacitating end-stage cardiomyopathy. Since the introduction of the procedure, refinements in organ procurement, surgical technique, and immunosuppression have prompted the loosening of recipient inclusion criteria, while at the same time generating better survival. The large numerical discrepancy between organs available for cardiac transplantation and potential recipients is well recognized[1]. Today the number of patients for whom transplantation is clinically indicated – between 10 000 and 40 000 in the USA alone[2] – far exceeds the supply of donor organs. Between 10% and 40% of waiting recipients die before a suitable heart becomes available[3], and donor supply will probably remain the major impediment to broader use of this procedure. Efforts to expand the donor pool in the future are being made[4]; currently many patients can lengthen their status as appropriate allograft candidates with mechanical circulatory assistance.

INDICATIONS AND PATIENT SELECTION

Mechanical circulatory support is indicated when the heart can no longer safely meet the perfusion requirements of the body. Devices such as the intra-aortic balloon pump (IABP) are currently used routinely for relatively short-term support of end-stage cardiomyopathic patients awaiting an allograft. Bridging to transplantation with ventricular assist devices (VAD) and total artificial hearts (TAH) has been successfully performed for over a decade[5–7], and mechanical circulatory support systems specifically designed for chronic bridging are now commercially available. There is a wide spectrum of devices available today to treat a patient with a failing heart. The best support system, though, will always be that device which can provide the best potential results in the simplest possible manner.

Table 1 presents criteria that make an individual an appropriate candidate for mechanical bridging. In general this patient, who otherwise is an acceptable candidate for transplantation, must have deteriorating cardiac function that cannot be supported by more conservative pharmacological techniques. Coexisting processes such as renal failure or sepsis would be contraindications to bridging, to the extent that they would preclude allografting. However, if renal dysfunction is believed to be readily correctable by an improvement in cardiac performance, then mechanical support would be an appropriate intervention. Likewise an infection such as pneumonia should not prevent bridging if the resultant improved perfusion could reasonably be expected to facilitate recovery before transplantation.

Mechanical support is most often required to support the failing left ventricle (LV). Isolated right heart failure in the adult population is rare. When present, right ventricular (RV) failure usually manifests after the initiation of LV mechanical support. Either improved left-sided hemodynamics 'unmask' concomitant right heart dysfunction, or successful decompression of the LV may

Table 1 Criteria for insertion of cardiac assist device

(1)	Cardiac index < 1.8–2.0 l/min per m^2
(2)	Systolic blood pressure < 90 mmHg (or mean arterial < 60 mmHg)
(3)	Left atrial (or pulmonary capillary wedge) and/or right atrial pressure > 20–25 mmHg)
(4)	Urine output < 20 ml/hour (in adults)
(5)	Systemic vascular resistance > 2100 dyne s cm^{-5}
(6)	Trial of volume infusion, maximal pharmacologic (vasopressor and vasodilator) support, and intra-aortic balloon counterpulsation (if vascular access permits) without adequate improvement of ventricular function
(7)	Other signs of poor perfusion (e.g. metabolic acidosis, decreased mentation) may be present
(8)	If the cardiac assist device is intended as a bridge to transplantation, then clearly the patient must be acceptable as a candidate for cardiac transplantation

actually precipitate RV failure[8] as a result of the static and dynamic interactions (cross-talk) that functionally link the ventricles. If RV failure does develop, pharmacological support with inotropic agents and pulmonary vasculature dilators (isoproterenol, prostaglandin E_1, and nitric oxide) can be tried. Persistent dysfunction may then require mechanical support of both ventricles, either with two independently functioning devices or with a TAH.

INTRA-AORTIC BALLOON PUMP

Sarnoff *et al.* proposed in the 1950s that myocardial oxygen consumption is a function of ventricular wall tension[9]. The 'tension–time index' (TTI), the integral of the arterial pressure trace, came to reflect the heart muscle's consumption of O_2. In 1958 Harken suggested that decreasing the TTI would aid the failing heart, and his group described a device that lowered the TTI by withdrawing blood from the arterial tree just prior to ventricular systole and returning it to the circulation during diastole[10]. In 1962 Moulopoulos *et al.* described a catheter-mounted balloon with hemodynamic effects similar to the Harken pump, but without an extracorporeal circuit[11]. The balloon was positioned in the aorta and inflated during ventricular diastole, thereby augmenting diastolic blood flow. Deflation just before cardiac ejection essentially decreased the LV afterload, and thus systolic 'work'.

Kantrowitz *et al.* reported the first clinical use of the intra-aortic balloon pump in 1968 for the treatment of cardiogenic shock secondary to acute myocardial infarction[12]. Reemtsma *et al.* presented the use of IABP counterpulsation before cardiac transplantation in 1978[13]. The development of percutaneous insertion techniques in 1980 enabled the institution of counterpulsation to be much more timely. The efficacy of using this device in a transplantation candidate is well established. Hardesty *et al.*[14] reported that IABP-supported patients' results compared favorably with those obtained in healthier patients who did not require aggressive hemodynamic support. The University of Utah employed the IABP in 9% of 401 heart transplantation procedures[15]. Rosenbaum *et al.*, in their review of 43 patients supported before transplantation with an IABP, concluded that counterpulsation was both safe and effective, even with durations of support exceeding 1 month[16].

Nonetheless, recent series continue to show complication rates for the IABP (all indications) between 12% and 30%[17–20]. Ischemia distal to the catheter's insertion site is the most common complication, occurring in 9–25% of recipients; experience suggests that between 40% and 80% of these patients will require surgical intervention to treat the ischemic event. Other, less common complications include: (a) infection and localized hematoma, (b) pseudoaneurysm formation at the vessel puncture site, (c) vessel perforation with extensive hemorrhage, (d) lymphatic disruption with fistula formation, (e) aortic dissection from catheter passage below the intima, and (f) peripheral nerve damage. The IABP severely restricts patient mobility, thereby limiting the physical rehabilitation of transplantation candidates awaiting a donor heart.

Because of the potential morbidity associated with chronic IABP support, many transplantation centers rely upon this strategy as a 'first-step' mechanical circulatory support. At our own institution 74% of recent ventricular assist device (VAD) recipients awaiting heart transplantation were initially supported by an IABP, while 24% of all transplantations involved IABP support followed by a VAD[21].

VENTRICULAR ASSIST DEVICES

Overview

Over the past 20 years many types of VAD have been designed, tested and, less frequently, marketed. Some devices can support only the left ventricle, whereas others are appropriate for left- and/or right-sided use. The delivered flow may be pulsatile or non-pulsatile in character. We are limiting this discussion to those devices currently available in the United States, and to newer systems in their final stages of development with the highest likelihood of routine clinical use in the near future. The fundamental indication for VAD use is severe, refractory cardiac dysfunction. During the 1960s and 1970s most of the recipients fulfilling this criterion were in cardiogenic shock secondary to acute myocardial infarction. In general, the results of such device applications were poor. More recently the predominant indications for a VAD have overwhelmingly consisted of postcardiotomy cardiogenic shock (PCCS) and bridging to cardiac transplantation[22]. Indeed, our own group's recent use of circulatory support beyond the IABP has been exclusively for bridging to transplantation.

Implantation of a VAD usually requires the use of cardiopulmonary bypass. Major complications of the devices include: (a) perioperative bleeding, (b) infection, (c) thromboembolism, and (d) hemolysis. The risk for bleeding depends in part upon the anticoagulation requirements of the specific device. Excessive bleeding may require transfusion of blood products, predisposing the patient to infection with blood-borne pathogens.

Of particular concern for the bridge-to-transplantation patient is transmission of cytomegalovirus, which can severely complicate post-transplantation management. Exposure to foreign antigens also makes subsequent location of an antigenically acceptable organ more difficult. Similarly, pre-existing infection in a transplantation recipient about to receive induction immunosuppression is very dangerous. Patients requiring mechanical circulatory support are at increased risk for infection for several reasons. At the time of device placement these patients are hemodynamically unstable, a condition that compromises the immune response. The implanted components of the systems serve as a large, potential nidus for infection. Additionally, all current systems employ percutaneous elements that amplify the risk of developing foreign-body infections.

Blood pumps can result in substantial blood trauma. Hemolysis results from the physical trauma to erythrocytes caused by the mechanical pumps; high rates of hemolysis can in turn lead to organ systems' injury and anemia. Stress on the blood and all of the elements suspended within it is directly proportional to the shear rate, the rate of change of the blood's velocity upon moving away from the pump walls. The stress imposed upon erythrocytes can lead to membrane instability and hemolysis with release of free hemoglobin into the bloodstream. If the body's ability to scavenge hemoglobin (predominantly through haptoglobin-binding sites) is overwhelmed, plasma free hemoglobin levels

will rise. Hemoglobin casts form in the renal tubules when plasma free hemoglobin levels approach 40 mg/dl, and acute renal failure can then develop. Hemolysis also contributes to hepatic insufficiency, coagulopathies, and anemia. A relatively high degree of hemolysis has been documented in non-pulsatile pumps[23].

Shear stresses have profound effects on platelets and the clotting cascades. Shearing can elicit changes in the expression and functionality of platelet membrane glycoproteins[24], inducing the binding of von Willebrand factor and fibrinogen. These events are critical to the activation and aggregation of platelets and the formation of platelet thrombi. Shearing also causes platelet fragmentation, exposing more cellular surface upon which catalytic amplification of the clotting cascade can occur[25]. Coupled with the obligatory exposure of blood to various biomaterials, the pumping action of a VAD always carries a risk of thrombus formation and subsequent embolization.

Non-pulsatile VAD

The efficacy of supporting the circulation with non-pulsatile blood flow is clearly demonstrated by the more than 300 000 cardiac procedures performed each year with CPB. There are three general types of non-pulsatile VAD: roller-head pumps, centrifugal pumps, and axial flow pumps. These devices operate in a continuous, 'one-direction' fashion; consequently the pumps are, from a design standpoint, mechanically simple. However, the high-speed nature of non-pulsatile pumps creates particularly high shear stresses. Thrombus formation and relatively high levels of hemolysis, as well as some unique operational constraints mentioned below, have limited non-pulsatile ventricular support to short-term use lasting several days to a week. Non-pulsatile pumps therefore appear not to be the optimal choice for bridging to transplantation.

Roller-head pumps

The majority of CPB machines in use today incorporate roller-head pumps to deliver flow. Roller-heads compress the circuit's flexible tubing, thereby generating flow in a peristaltic fashion. Given the general familiarity with the set-up, it is not surprising that roller-head pumps have been employed outside of the operating room as VAD[26]. The primary advantage of a roller-head VAD is the fact that every institution performing cardiac surgery can conceivably use this relatively inexpensive support strategy. Generally, the inflow cannulation is via the left atrial appendage, with the tubing exiting the left chest tunneled parasternally by way of the second or third intercostal space, or below the xiphoid process. The outflow cannula is brought out through the sternotomy incision or tunneled below the xiphoid[27,28]. Right heart bypass may be instituted alone or in combination with left heart bypass by inflow right atrial cannulation and outflow pulmonary artery cannulation.

These pumps were not designed for protracted use in a single patient, and limitations to extended VAD application arise. Compression of pump tubing creates a jet of blood involving high shear stresses. Prolonged exposure to repetitive compression also leads to fatigue deformation of the circuit's flexible tubing, and thus the physical integrity of the circuit must be carefully monitored. Constant oversight by trained staff is also mandatory, since outflow obstruction can produce pressure overload and catastrophic tubing disruption. Conversely, an inlet cannula obstruction may result in air aspiration from around the cannulation site, leading to air embolization.

Centrifugal pumps

A so-called centrifugal pump draws upon Bernoulli's theorem to generate dynamic pressure from kinetic energy imparted to blood by a spinning chamber. Two centrifugal pumps have been used extensively in the USA. The Bio-Medicus Bio-Pump (Medtronic Bio-Medicus, Inc., Eden Prairie, MN) contains rotating cones within the blood-spinning chamber (Figure 1). The Sarns centrifugal pump (Sarns/3M Health Care, Inc., Ann Arbor, MI), shown in Figure 2, relies upon an impeller mechanism to deliver energy to the blood.

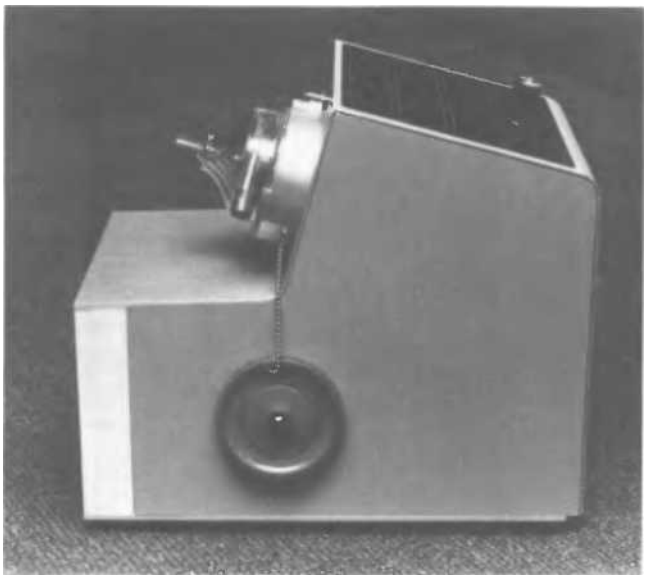

Figure 1 The BioPump console with centrifugal pump head in place

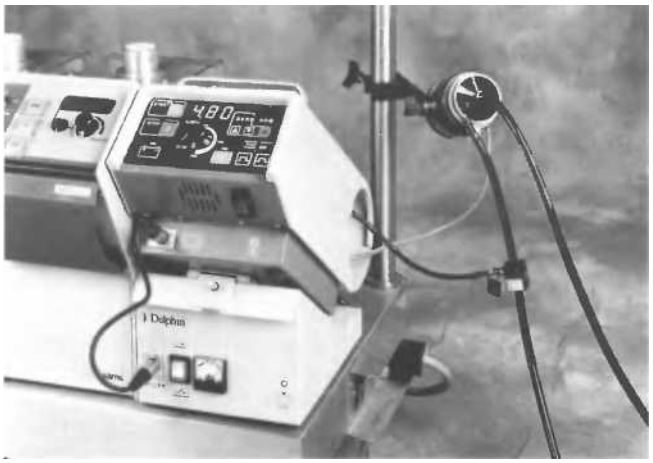

Figure 2 The Sarns centrifugal blood pump. The spinning pump head continuously drains and returns blood with non-pulsatile flow

An axle and bearing support these blood-contacting elements. Reusable consoles spin the pumping chambers through the technique of magnetic coupling. Seals are designed to prevent the leakage of blood onto the axle–bearings assembly. Implantation techniques are similar to those employed with roller-head pumps.

Data from the voluntary registry of mechanical circulatory support show that, between 1983 and 1993, centrifugal pumps were the most commonly applied support devices (other than IABP), making up approximately 50% of all devices used[22]. The vast majority of patients, though, were being treated for PCCS, not for bridging to transplantation. Nearly half of the patients were reported to have had excessive bleeding. This complication is not wholly unexpected given the anticoagulation regimens that are usually used, and reoperation is often necessary[29]. Seal disruption, sometimes precipitating abrupt device failure, does occur in centrifugal pumps, and the development of 'seal-free' pumps is underway[30]. Seal leakage and thrombus formation have restricted the typical duration of support with centrifugal pumps to between 2 and 8 days, and the physical size of a complete system prevents, or at best severely limits, patient mobility. For these reasons the centrifugal pump's role as a definitive bridging device is limited.

Axial flow pumps

Several groups are developing small, implantable axial flow blood pumps. These pumps deliver non-pulsatile blood flow in much the same way that a ship's propeller displaces water[31–33]. Most of the systems are being designed to provide circulatory support lasting up to several months. The projects are all in the *in-vitro* and early animal studies stages, meaning that clinical availability lies many years away. Considerable engineering challenges remain to be solved, most notably the long-term maintenance of pump bearings, and the potentially deleterious effects from long-term non-pulsatile blood flow will need to be thoroughly evaluated. Nonetheless, these compact systems may eventually prove to be the mainstays of long-term circulatory support.

Pulsatile VAD

Pulsatile VAD contain blood-pumping chambers that are completely isolated from their actuating mechanisms. As a result the need for biocompatible seals and bearings is greatly reduced, and the systems are therefore better suited for longer-term periods of pumping. Pulsatile VAD are therefore particularly advantageous when bridging a patient until heart transplantation. The issue of non-physiological blood flow is also less important with pulsatile pumps.

Pierce–Donachy VAD

The Pierce–Donachy VAD (Thoratec Laboratoires Corp., Berkeley, CA) has been used in extensive clinical trials for bridging to transplantation, PCCS, and support of acute myocardial infarction. The VAD (Figure 3) is based upon a seamless, 70 ml polyurethane blood sac which fits within a rigid polycarbonate case. An attached drive console withdraws and injects air into the case, thereby causing the sac to fill or empty. The device lies in a paracorporeal position over the patient's upper abdomen, with the atrial or ventricular apex inlet cannula and the aortic or pulmonary arterial outlet cannula exiting the body through subcutaneous tunnels. The cannulae are fabricated from wire-reinforced polyurethane, and arterial graft material permits a standard end-to-side arterial anastomosis. Tilting-disc prosthetic valves ensure unidirectional blood flow. A magnetic switch in the pump detects a completely filled sac, prompting the initiation of pump ejection with an air pulse. Alternatively, the pump can be run in synchrony with the native heart rhythm via the electrocardiogram signal. Fixed-rate pumping, independent of sac filling or heart beat, is also possible.

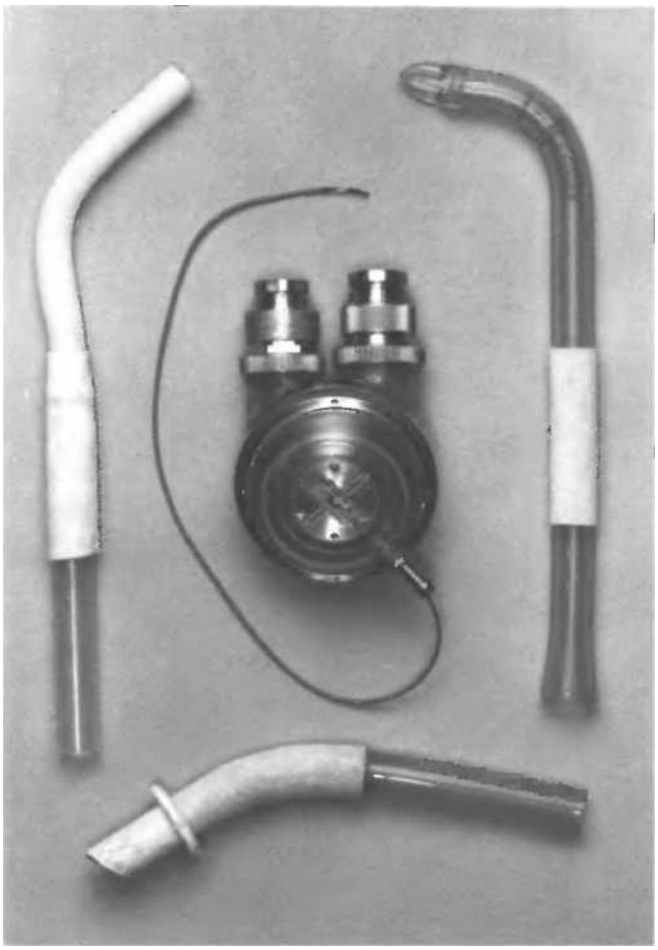

Figure 3 Prototype of the Pierce–Donachy (Thoratec) ventricular assist device

Left, right, or biventricular support is available with this type of device (Figure 4). The implantation generally is made during CPB. In instances where atrial pressures are high, the risk of air embolization is small, and atrial inlet cannulation can be performed without CPB. A median sternotomy provides the optimal exposure for assist pump placement. A patent foramen ovale must be searched out and closed if only a left ventricular assist device (LVAD) is being placed, since decompression of the left heart may lead to significant right-to-left shunting and hypoxemia. We prefer the LV apex as the site of cannulation for VAD inflow, since maximal LV drainage can be obtained, the risk of ventricular thrombus formation is decreased, and more atrial remnant tissue is available for subsequent allografting. In instances where

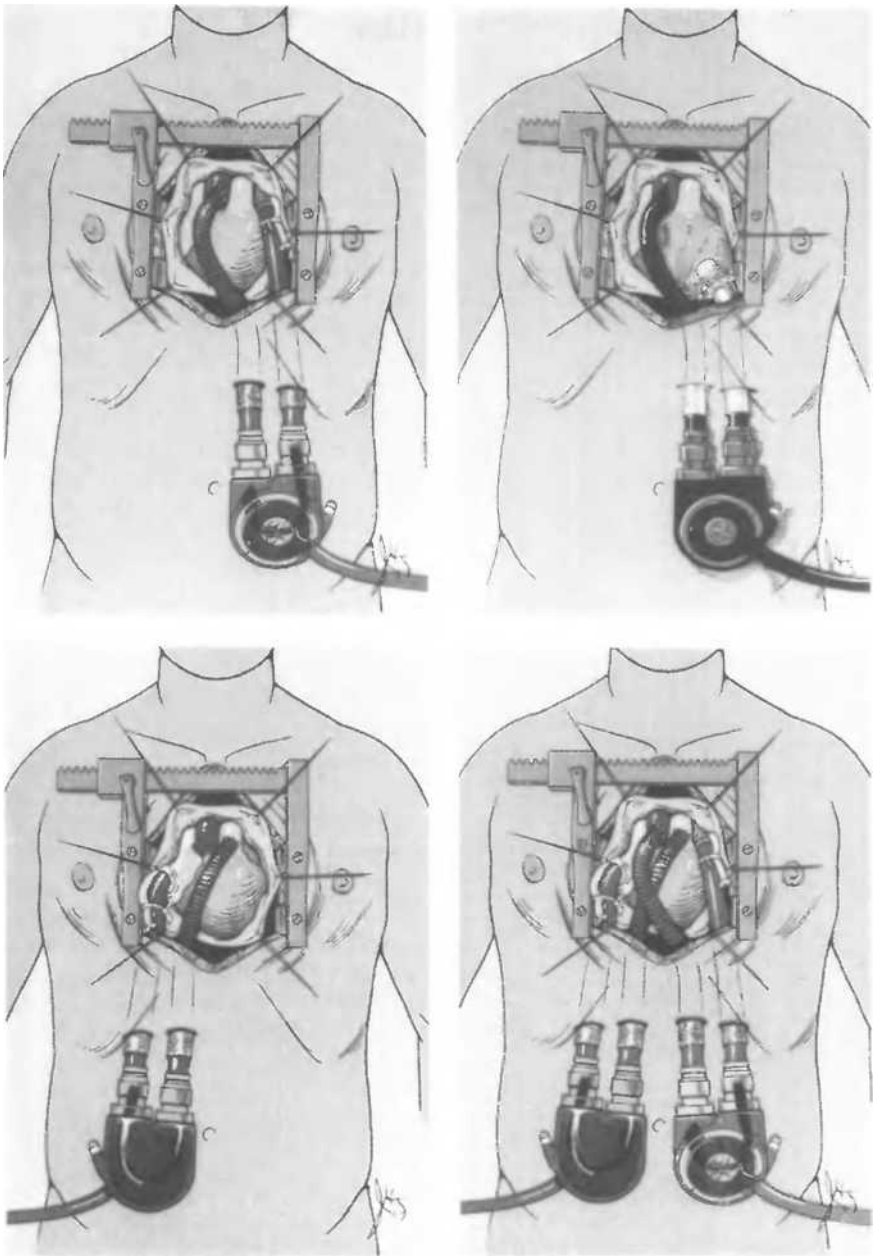

Figure 4 Illustrations of the Pierce–Donachy ventricular assist device in various applications. Clockwise from top left: **A:** left ventricular assistance with atrial inflow cannulation and blood return to the ascending aorta; **B**: left ventricular assistance with ventricular inflow cannulation; **C**: biventricular assistance with left and right atrial cannulation; **D**: right ventricular assistance with right atrial inflow cannulation and blood return to the pulmonary artery. This pump is positioned intraperitoneally, and a percutaneous wire (left) connects with a portable power supply and control unit

adequate exposure of the ventricular apex involves considerable operative risk (e.g. extensive mediastinal scarring from prior coronary artery bypass grafting), left atrial inlet cannulation can be employed with good results. The outlet graft is sutured as low as possible to the ascending aorta in order to allow for its excision during recipient cardiectomy. Pumping is gradually initiated after the pump has been de-aired. A drive-line vacuum pressure can be used to promote faster, more complete pump filling.

At our institution a standard approach is taken to the post-operative management of bridging-to-transplantation patients. Patients return to the intensive-care unit having resumed their perioperative inotropic medications. These agents are weaned as rapidly as RV function will permit. If present, the femorally placed intra-aortic balloon is removed within the first 12 post-operative hours. Patients are extubated according to standard respiratory parameters. The presence of prosthetic, mechanical

valves mandates systemic anticoagulation. Anticoagulation with intravenous heparin followed by oral sodium warfarin begins when mediastinal bleeding has slowed, with the goal of maintaining the prothrombin time 1.5–2 times the control value. Antiplatelet aggregation agents (aspirin and dipyridamole) are added only if signs of embolism develop. Antibiotic prophylaxis includes vancomycin and gentamicin. Appropriate therapy for infections is based upon culture results; if no specific organism can be identified, broad-spectrum, empirical treatment is instituted. Early postoperative mobility leads to a rehabilitation regimen that includes daily exercise with a treadmill and/or stationary bicycle. Duodenal tube feeding or total parenteral nutrition is administered if the dietary intake is inadequate.

TCI Heart-Mate

The Heart-Mate 1000 IP (Thermo Cardiosystems, Inc., Woburn, MA) is indicated solely for left ventricular support as a bridge to transplantation and a system appropriate for chronic, longer-term use is under development (Chapter 78). The blood-contacting surfaces of this pump consist of sintered titanium microspheres and a textured polyurethane diaphragm. These surfaces promote the formation of a biologically active pseudoneointima shortly after exposure to blood[34]. Additionally, porcine xenograft valves are used at the pump's inlet and outlet; therefore the requirement for systemic anticoagulation is greatly reduced or obviated. The diaphragm is bonded to a rigid pusher-plate that moves back and forth with air pulses delivered from an external drive unit; as the pusher-plate moves, blood alternately enters and leaves the pump via attached cannulae. The pump is placed intraperitoneally in the left upper quadrant, with the left ventricular apex inflow and aortic outflow cannulae traversing the patient's diaphragm. A percutaneous airline connects the pump with its drive console. This bridging device was the first to gain approval for commercial use from the United States Food and Drug Administration (FDA).

The Heart-Mate VE LVAD is an electromechanical device based upon the pneumatically driven unit (Figure 5)[35,36]. A diaphragm separates the pump's electric motor from its blood chamber. Compression of the diaphragm causes ejection of blood, and filling of the pump occurs passively. Again, the pump is placed intraperitoneally in the left upper quadrant. The LVAD is externally controlled; permanent, percutaneous wires connect the pump to a wearable controller-battery pack. A permanent percutaneous vent permits the displacement of air from the pump as the LVAD diaphragm moves. This system is already being tested clinically under an Investigational Device Exemption (IDE) for bridging to transplantation[37].

Novacor N100

The Novacor N100 Left Ventricular Assist System (Novacor Division, Baxter Healthcare Corp., Oakland, CA) is also designed specifically for bridging to transplantation. This device, implanted in the preperitoneal space or within the abdomen, consists of a polyurethane blood sac compressed by two opposing pusher-plates (Figure 6). The N100 does not use air pulses to compress the blood chamber. Instead, the pump is actuated by a pulsed solenoid energy converter. Bovine pericardial valves direct the flow of blood. An external controller and power console connects with

Figure 5 The TCI HeartMate VE LVAD. The left ventricular outflow attachment is on the left and the aortic inflow graft is on the right

the pump by means of a percutaneous wire, and a percutaneous vent allows the implanted pusher-plates to oscillate freely[38]. A wearable, electrical control console that allows for improved patient mobility during long-term use is now under clinical investigation[39]. Modifications that make the system appropriate for permanent implantation are also being investigated.

TOTAL ARTIFICIAL HEARTS

A total artificial heart (TAH) consists of orthotopically positioned blood pumps that physically and functionally replace the native left and right ventricles. Akutsu and Kolff achieved the first experimental success with a TAH when they temporarily supported a dog with two pneumatically activated polyvinyl chloride blood pumps[40]. Cooley (Chapter 77, Figure 1) *et al.* then first used a TAH clinically in 1969, as a bridge to transplantation[41]. Currently, the primary indication for placement of a TAH is biventricular failure that cannot be corrected through the use of a left VAD and pharmacological support of the right ventricle. This

Figure 6 Unencapsulated Novacor left ventricular assist device

situation might also be appropriately treated by the implantation of bilateral VAD. In instances where the heart is extremely dilated, though, there may be an advantage in excising the theoretically thrombogenic native ventricles. The TAH is also the device of choice for a small number of patients with left-sided failure whose myocardium is so compromised that LVAD placement would be unsafe or ineffective. This group includes transplantation candidates with ischemic cardiomyopathy who develop ventricular rupture or an irreparable ventricular septal defect with a large shunt.

The decision to use a TAH as opposed to VAD support is not trivial, for the implantation of the more complicated TAH puts the patient at increased risk of perioperative morbidity. The procedure, involving cardiectomy, carries a higher risk of bleeding. If the device were to fail, there is no residual native heart function that might minimally sustain the circulation while corrective measures are taken. Control of a TAH is also intrinsically more difficult than that of a VAD. Typically a VAD maximizes its output in direct proportion to the pump's 'preload', a function of the intravascular volume status. However, the equilibrium blood volumes pumped by the native left and right ventricles are not equal, the discrepancy due in part to a left-to-left shunt of bronchial blood and to different characteristics of the great vessels. A TAH control algorithm must be able to respond to differing left and right volume requirements if adequate perfusion is to be maintained. This response must also include consideration of passive pulmonic perfusion, or left-to-right 'pass-through' flow, that has been identified in TAH[42]. Two TAH, both pneumatically driven, are under clinical investigation in the United States.

CardioWest C-70 TAH

The CardioWest C-70 (CardioWest Technologies, Inc., Tucson, AZ) represents a smaller, modified version of the Jarvik heart (Fig. 7) made famous by DeVries (Chapter 77, Figure 2) *et al.*, who in 1982 electively implanted the device in an individual who was not a transplantation candidate (Chapter 77)[43]. The Jarvik heart, manufactured by Symbion, had been withdrawn from the US market due to manufacturing difficulties, but it continued to have fairly wide use in foreign countries. In the early 1990s CardioWest Technologies assumed responsibility for the TAH, and the result has been renewed clinical evaluation of the 70 ml stroke volume device. The C-70 consists of two pneumatically compressed, polyurethane ventricles placed in the orthotopic position (Figure 8). Percutaneous drive lines connect the pumps to an external drive unit; two mechanical valves in each ventricle assure the correct direction of blood flow[44].

The Penn State Heart

The 'Penn State Heart' (Figure 9), designed and built by our group at the Pennsylvania State University, consists of two valved, pneumatic ventricles; it shares many features with the Pierce–Donachy VAD also developed at Penn State. Our experi-

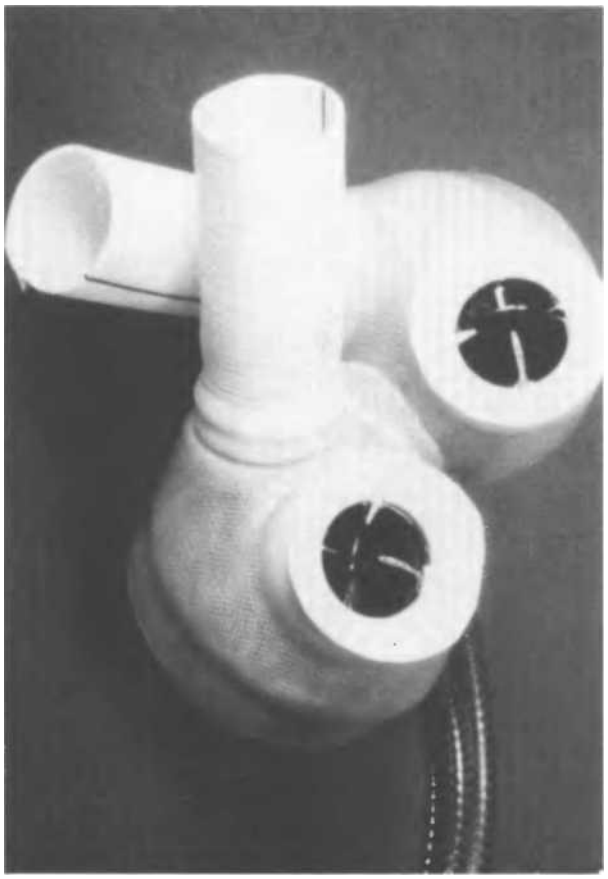

Figure 7 Jarvik-7-100 total artificial heart

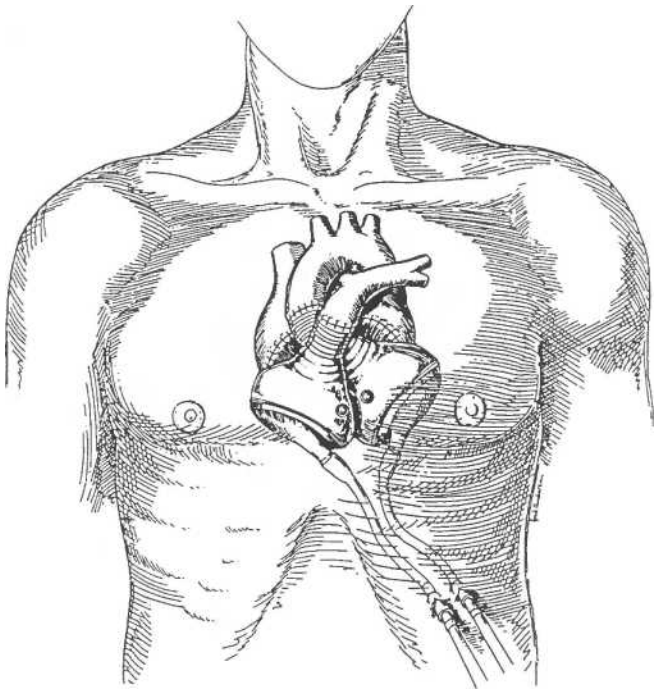

Figure 8 Implantation of the CardioWest C-70 Total Artificial Heart

Figure 9 Implanted blood pumps of the Penn State Heart

ence with clinical use of this system is limited; only five patients have presented to our institution thus far with the need for a temporary TAH. One of these patients was supported for 223 days with the device. The device remains approved by the FDA for clinical application.

RESULTS OF BRIDGING TO TRANSPLANTATION

As experience with bridging techniques has increased, results have improved. A voluntary device Registry shows that, from 1983 to 1993, 584 patients received mechanical circulatory support (excluding IABP) for the specific purpose of bridging to transplantation[22]. Four hundred (69%) of these patients successfully underwent transplantation, with 274 patients (69% of those transplanted) ultimately being discharged from the hospital. Reported results in patients who are bridged now suggest outcomes identical to transplantation alone[6,38,45].

Device-specific results are also very encouraging. Fifty-four percent of all patients implanted with the Thoratec Pierce–Donachy VAD as a bridge to transplantation have survived after receiving an organ[46]. A recent series from our institution had 74% of patients with a bridging Pierce–Donachy left VAD (Sarnes/3M Healthcare, Ann Arbor, MI) successfully supported until transplantation; each of these individuals was eventually discharged from the hospital[21]. Approximately 68% of patients supported with the TCI HeartMate IP during clinical trials subsequently underwent cardiac transplantation (K.A. Dasse, personal communication), and 80% of transplanted patients have ultimately been discharged from the hospital[47]. Since 1984 over 200 patients have received the Novacor Left Ventricular Assist System as part of a multicenter clinical trial (L. Strauss, personal communication), and approximately 60% of these patients went on to cardiac transplantation. Overall, about 50% of the recipients left the hospital after transplantation[48].

The data for the newly investigated CardioWest C-70 TAH are obviously less voluminous, but the results indicate a similar trend. Between January 1993 and May 1996 74 patients had received the device as part of an international clinical trial; 59% of these patients had successfully undergone transplantation and been discharged from the hospital (R.G. Smith, personal communication).

A review of the indications for 476 reported bridging procedures[49] showed that hemodynamic deterioration before transplantation is by far a more common indication (92%) than is acute rejection necessitating retransplantation (8%). Of 40 patients treated with circulatory support during rejection, only 23 (58%) underwent a second transplantation. Eight of the 23 patients (35%) ultimately were discharged from the hospital, with the overall salvage rate being 19%. When the indication was hemodynamic instability and organ unavailability, the best outcome was seen with univentricular support (62% of patients discharged); the rates of subsequent transplantation, as well as hospital discharge, were independent of the type of left ventricular assist device used (i.e. pneumatic, electrically activated, or nonpulsatile). Biventricular paracorporeal support yielded a 45% post-transplantation discharge rate. Only 35% of TAH recipients ultimately left the hospital.

Kaplan–Meier survival estimates for all patients undergoing staged cardiac transplantation at 1 and 2 years after the procedures, inclusive of the 30-day operative mortality, were nearly 65%. This is in contrast to the nearly 90% actuarial survival in isolated orthotopic cardiac transplantation. However, when survival estimates were prepared for each type of mechanical support employed in conjunction with transplantation, the 1- and 2-year estimates for univentricular support were equivalent to isolated orthotopic cardiac transplantation.

The complications precluding transplantation after establishing circulatory support were numerous. Most of the patients suffered more than one complication. Stepwise logistic regression analysis indicated, in decreasing order of importance, that bleeding, neurological events, and biventricular and renal failure had significant negative effects on future transplantation. Not surprisingly, bleeding, renal failure, persistent respiratory failure, infection, and rejection negatively affected hospital discharge. Multivariate analysis indicated that bleeding, infection, and renal failure were the most important predictors of hospital death after staged transplantation. The causes of death after 30 days paralleled those of the isolated cardiac transplantation population.

COMMENT

The collective experience with VAD and TAH undeniably underscores the efficacy of mechanical circulatory support as a bridge to cardiac transplantation. Some data are beginning to suggest

improved outcomes after transplantation in supported patients. Recent results from Penn State, for example, included a 30-day mortality after transplantation of 0% in mechanically bridged patients, compared to a 14% mortality in non-bridged Status I recipients[21]. Such results are all the more encouraging when one considers that these bridged patients would most likely have died before allografting had mechanical circulatory support not been available.

While the benefit to an individual patient may be substantial, the derived benefits in broader terms are less obvious. Heart transplantation remains the final objective of VAD and TAH use today. Bridging, though, does nothing to fix the biggest problem with heart transplantation – inadequate organ supply. Indeed, bridging devices can only worsen the disparity between available organs and potential recipients. If bridging continues to generate better post-transplantation outcomes than more traditional patient management approaches, then the use of the devices may be justifiable from a public-health standpoint. One must acknowledge that these devices involve considerable expense. Analyses have shown that overall pretransplantation costs incurred by mechanically bridged patients are significantly greater than those incurred by non-bridged patients[50,51]. Certainly this relationship will reverse itself as the need for intensive, in-hospital monitoring of patients with bridging devices gives way to relatively inexpensive, out-of-hospital management. There is also some evidence that a physically active, bridged patient may recover more rapidly from the transplantation procedure than will a bedridden, physiologically 'sicker' candidate supported by inotropic agents[50]. In this manner, bridging could help to decrease the cost of treating end-stage cardiomyopathy.

Perhaps the greatest benefits from bridging to transplantation will be realized in the eventual application of permanent circulatory support systems (Chapter 78). Permanently implanted VAD and TAH are under active development, with large-animal survivals of greater than 1 year having been attained for both types of devices. The successful rehabilitation of patients supported with temporary, bridging devices has validated the experimental results obtained with permanent systems, and information gleaned from the bridging experience will make more chronic devices a clinical reality in the next 5–10 years. At that time patients with relative or absolute contraindications to transplantation will be given the opportunity to resume productive lives through cost-effective, unobtrusive, mechanical support, while scarce donor hearts can be allocated to a smaller set of younger patients who stand to benefit more from heart transplantation.

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22
Anesthetic Management, Including Cardiopulmonary Bypass

J.V. BOOTH, D.R. WHEELDON AND S. GHOSH

INTRODUCTION

Since the first successful transplantation of the human heart over 25 years ago many groups of anesthetists have described the anesthetic techniques they use for managing their patients during the recipient operation[1-11]. There has been an enormous variety of different anesthetic agents employed in transplantation, and it appears that each unit has based its regimen on experiences in patients with decompensated cardiac failure. As experience is gained in transplantation, previous boundaries are stretched, with older patients being accepted for transplantation and an increasing number of patients with relatively higher transpulmonary pressure gradients being operated on. The choice of individual anesthetic agents is in itself of relatively little consequence provided that the overall technique employed does not produce marked cardiovascular effects.

PRE-ANESTHETIC MANAGEMENT

In our practice it is rare for the recipient to be in our hospital when the donor call is received. The recipient is contacted either in his/her local hospital or at home, and then transferred to our center and prepared for operation. Most patients will therefore not have had any oral intake for 5–6 hours before induction of anesthesia. On admission a period of intense activity follows. The patient is bathed and shaved, bacteriological swabs are obtained, and a final surgical and anesthetic assessment undertaken. The circumstances surrounding heart transplantation in respect of distant organ procurement and the coordination of organ donor to recipient matching usually allows limited time for preoperative assessment and preparation of the recipient. The anesthetist can assume that the patient will have evidence of severe cardiac and respiratory dysfunction as well as secondary renal or hepatic impairment. The preoperative visit is not designed to alter therapies, which should already be optimal, but rather to assess the extent and clinical implications of the patient's organ dysfunction. The history is usually well described in the medical case notes, so patient evaluation can be limited to current cardiovascular and respiratory function and exercise limitation. A brief relevant history is taken, concentrating on drug history, drug sensitivities, previous anesthetics, the anatomy of the upper airway, and recent oral intake.

Our practice is to avoid prescribing any sedative agent in the preoperative period. Patients with low cardiac output can be very sensitive to central nervous system depressants, with resulting hypopnea or apnea. Sensitivity may be due to impaired redistribution of the drug from central to peripheral compartments because of low tissue perfusion consequent to a low cardiac output state. Alternatively, the increased depressant effects of these drugs may be due to decreased protein binding, particularly in chronic debilitating diseases. Low tissue binding allows higher concentrations of free drug at the receptor sites.

MONITORING

As for all major cardiothoracic procedures, monitoring of the patient comprises routinely: (a) continuous multi-lead electrocardiography, (b) pulse oximetry, (c) capnography, (d) inspired oxygen concentration, (e) central venous pressure, (f) systemic arterial pressure at the radial artery, (g) hourly urine volumes, and (h) core temperature. A careful aseptic technique should be employed when inserting vascular cannulae. This is especially important in these patients, whose risk of infection is heightened considerably by perioperative immunosuppresive therapy.

We routinely insert a 7.0F gauge triple-lumen central venous pressure catheter. Although we do not routinely insert a pulmonary artery flotation catheter (PAFC), we often insert the 8.5F gauge sheath through which the PAFC can be advanced if required following cardiopulmonary bypass. PAFC may increase the risk of infection in these patients[7], although some authors have questioned this[12]. A survey published in 1986 reported that 32% of hospitals in North America used PAFC routinely during heart transplantation prior to cardiopulmonary bypass, and that 44% used them after bypass[13].

Some centers now use intraoperative transesophageal echocardiography (TEE) to assess cardiac function, particularly right ventricular function[14], although the role of TEE in heart trans-

plantation has yet to be evaluated. Others have advocated the use of right ventricular ejection fraction catheters that allow assessment of beat-to-beat changes in right ventricular ejection[15].

ANESTHETIC MANAGEMENT

The risks of absolute or relative overdose of anesthetic agents, leading to further myocardial depression, during a rapid sequence induction must be balanced against the risks of pulmonary aspiration in patients with a full stomach. At Papworth Hospital we find that this is rarely a problem. Good planning almost always allows the potential recipient to have fasted before induction of anesthesia. A true rapid sequence induction is relatively contraindicated, as this may exacerbate the underlying high pulmonary vascular resistance, further depressing cardiac output and causing decompensation. If the risk of aspiration is considered high, a modified rapid sequence induction may be used that aims for cardiovascular stability. As consciousness is lost, cricoid pressure is applied, and the lungs ventilated cautiously during the transition from spontaneous to mechanical ventilation. The judicious use of H2 blockers preoperatively may also further decrease the risks of pneumonitis due to aspiration. Before cardiopulmonary bypass, cardiac function can be augmented by pharmacologic means to optimize perfusion of other organs without concern for detrimental effects on the heart in the early postoperative period.

Induction of anesthesia should be gradual and controlled to avoid any precipitous increase in the pulmonary vascular resistance or marked decrease in systemic arterial pressure. We use midazolam 0.1 mg kg^{-1}, or diazepam 0.15 mg kg^{-1}, followed by fentanyl at a total dose of 10–15 mg kg^{-1} given in increments slowly, and titrated against hemodynamic response. Muscle relaxation is achieved with pancuronium 0.15 mg kg^{-1} to 0.2 mg kg^{-1}. The endotracheal tube should have a high-volume, low-pressure cuff. The lungs are ventilated with oxygen, air and isoflurane 0.5–1% to maintain anesthesia. Alternatively, an infusion of propofol at 3 mg kg^{-1} h^{-1} is satisfactory.

Almost all the transplant recipients have been optimized on maximal medical therapy, involving potent diuretic agents, resulting in a contracted intravascular volume[16]. Hypotension is likely, with a loss of sympathetic tone and a decrease in catecholamine concentrations. This responds well to vasoconstrictors titrated carefully i.v. We use diluted phenylephrine or metaraminol with 0.1–0.25 mg increments.

Immunosuppression (with azathioprine and steroids) and antibiotics are commenced on induction. If RATG is to be given, then this is started as an infusion at the onset of cardiopulmonary bypass to control the profound fall in systemic pressure that may accompany its administration.

CARDIOPULMONARY BYPASS

We delay cardiopulmonary bypass until the donor organs are certain to arrive in the operating room, except when severe cardiovascular instability requires cardiopulmonary support. Heparin 300 units kg^{-1} i.v. is given for anticoagulation. The dose may have to be adjusted if the patient is on heparin or warfarin preoperatively. We aim to maintain an activated clotting time >400 seconds.

The aorta is cannulated higher than for routine cardiac surgery, to facilitate access to the pericardial sac. Bicaval cannulation is performed (cannulation of the inferior vena cava and superior vena cava separately), and the cavae snagged around the cannulae to prevent venous blood spillage into the atrium.

If the patient has had previous cardiac surgery the femoral artery and femoral vein may be cannulated for cardiopulmonary bypass before sternotomy. This allows rapid intravascular fluid resuscitation and the option of partial bypass in the event of massive bleeding following sternotomy. In all cases the groins should be prepared in case femoral cannulation is required. The routine at Papworth is to use a membrane oxygenator and to provide non-pulsatile perfusion with moderate hypothermia (30°C). The machine is primed with crystalloid solution to which 6000 IU of heparin are added. Pump flow is 2.41 l min^{-1} m^{-2} at normothermia, reducing to 2.0 l min^{-1} m^{-2} at 30°C.

Facilities are always available for hemofiltration and/or hemodialysis should either prove necessary[17]. Patients in congestive cardiac failure with enlarged hearts may present a large volume load for the cardiotomy reservoir at the onset of cardiopulmonary bypass.

Pressure on bypass is controlled to give a perfusion pressure between 40 and 60 mmHg. At the initiation of bypass, various factors combine to produce a fall in peripheral resistance. This is normally self-limiting, but can be progressive, particularly if the patient was receiving regular angiotensin-converting-enzyme inhibitor drugs. Vasoconstrictors such as metaraminol or phenylephrine are administered when the pressure is below 40 mmHg, and phentolamine is used as a vasodilator to control hypertension during cardiopulmonary bypass.

Rewarming is initiated during the aortic or pulmonary artery anastomosis, the aim being to achieve a blood temperature of 37°C by the time of release of the aortic clamp. Potassium is added, if required, to keep the serum potassium level above 4.5 mmol l^{-1}, as low potassium levels have been associated with an increased incidence of arrhythmias.

Discontinuation of cardiopulmonary bypass follows the same principles as during elective cardiac surgery. The lungs are ventilated with as low an inspired oxygen concentration as possible, commensurate with adequate tissue oxygenation. The heart is slowly filled to optimum pressures to avoid overdistending the ventricles while bypass flow is reduced. The transition from cardiopulmonary bypass is usually uneventful. Direct observation of the heart is often the best guide to filling of the right atrium and ventricles. This is particularly relevant, because right ventricular systolic and diastolic dysfunction is common in the early post-transplant period[18,19]. Such dysfunction is often associated with a raised pulmonary vascular resistance. Isoprenaline (isoproterenol) 0.005–0.01 μg kg^{-1} min^{-1} by infusion i.v. is used to decrease pulmonary vascular resistance whilst augmenting inotropic and chronotropic activity. Persistent right heart failure may require infusion of prostacyclin 0.1 mg kg^{-1} min^{-1} i.v.[20], intra-aortic balloon pump correction of septal wall dislocation[21,22], or inhaled nitric oxide[22,23].

POSTCARDIOPULMONARY BYPASS PERIOD

Once separated from cardiopulmonary bypass and hemodynamically stable, protamine is given to antagonize the anti-

coagulation induced by heparin. At this stage the ventricles are poorly compliant. The transplanted heart is also denervated, rendering it volume dependent and unable to respond to decreased preload by an increase in heart rate. Protamine should be administered cautiously to avoid hypotension from systemic arterial vasodilatation, raised pulmonary vascular resistance, and its negative inotropic effects[24].

The recipient may present with abnormal coagulation indices preoperatively due to hepatic congestion secondary to right ventricular failure, or anticoagulant or aspirin therapy. After surgery, if the activated clotting time returns to the recipient's baseline value, or within 10% of it, it is likely that heparin neutralization is complete. Results of a platelet count may be misleading, because although absolute numbers of platelets may be within the normal range, those platelets may be dysfunctional. Platelet dysfunction post-bypass is caused by factors that decrease the concentration of the GPIb and GPIIb receptors on the surface of the platelets. These receptors are essential for platelet-to-platelet interaction and aggregation[25]. Continued bleeding after surgery may require platelets, fresh-frozen plasma, or desmopressin (DDAVP) to be administered. Transfusion of stored blood is required when the hemoglobin concentration falls below 85 g l^{-1}.

Although we do not routinely use aprotinin for all heart recipients, it is useful when excessive bleeding is anticipated, such as in those who have had previous cardiac surgery or those who have been chronically anticoagulated. In these cases we use a loading dose of 2 million units of aprotinin given as a slow bolus injection after a test dose of 200 000 units given before cardiopulmonary bypass. A further 2 million units is added to the bypass prime, and following bypass an infusion of 500 000 units per hour is continued for 1 hour postoperatively.

Although most intraoperative anesthetic problems in the heart transplant recipient occur in the prebypass period, the incidence of ventricular arrhythmias has been reported as high as 79% after bypass[26]. A proportion of these (18%) may persist for more than 24 hours[27]. If the venous preload is adequate, there should be no decrease in systemic arterial pressure when the chest is closed.

We deliver analgesia to patients using an infusion of morphine 1–5 mg h^{-1}, initiated in the operating room just prior to transfer of the patient to the intensive-care unit. In our institution the majority of heart recipients are extubated within 6 hours postoperatively.

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23
Current Techniques of Myocardial Protection for Cardiac Transplantation

S. AZIZ AND A.L. PANOS

INTRODUCTION

Improvements in immunosuppression and pre-, peri-, and post-operative management have resulted in increased survival being obtained after heart transplantation (HTx)[1] (Chapters 43 and 44). Currently used techniques of myocardial protection for preservation of the donor heart allow only limited time for transportation between donor and recipient hospitals. Mild to moderate cardiac allograft dysfunction is not uncommon after HTx. Ischemic times greater than 5 hours are associated with increased need for inotropic support, and increased morbidity and mortality[2] (Chapters 43 and 44). Early failure of the donor heart (primary graft dysfunction) is still responsible for up to 25% of the deaths following HTx and can result from poor donor selection or inadequate organ preservation, or both[3]. The ideal method of preservation is one that will give the longest preservation time while maintaining excellent graft function. Improved methods of organ preservation should therefore demonstrate improved graft function despite a prolongation in procurement time.

Improvements in cardiac allograft preservation could: (a) increase the current 4-hour limit of ischemia; (b) decrease the incidence of primary graft dysfunction; (c) allow time to determine viability of the allograft (Although currently there is no specific test able to predict viability of the graft, future developments using various indirect methods, such as magnetic resonance spectroscopy, may be able to evaluate myocardial metabolism *in vivo*.); (d) enhance donor organ metabolic status at the end of the period of transport and prior to implantation; (e) increase the pool of available donors (by allowing the utilization of less-than-ideal organs); and (f) allow for possible future immunologic manipulation of the donor (e.g. by monoclonal antibodies, gene therapy).

In this chapter we will review the current theories and methods of myocardial protection used during HTx, and touch upon some promising new avenues of research for future development. A further review of experimental work in cardiac storage appears in Chapter 74.

HISTORY OF MYOCARDIAL PRESERVATION

Much has been learned from the principles of myocardial preservation developed for general cardiac surgery, and these have been applied to transplantation (and vice-versa). The efficacy of hypothermia as a means of decreasing myocardial injury during cardiac surgery was first demonstrated in the 1950s by Bigelow, Lewis and Taufic, Swan and others[4-10]. Topical hypothermia was also used as a means of protecting the donor heart during HTx[11,12]. Shumway and Lower were able to demonstrate that topical hypothermia alone was able to provide adequate protection during experimental orthotopic HTx[11]. The Stanford group also demonstrated that excellent results could be obtained using topical cold for routine cardiac surgery[13]. Clinically, the first human-to-human HTx was performed by Barnard in 1967. At that time, because the concept of brain death had not been introduced, the donor heart was not removed until there was electrical and mechanical silence, at which time the donor was placed on cardiopulmonary bypass and cooled prior to explantation of the heart[14].

In the initial period of HTx, in an effort to keep ischemic times to a minimum, only local donors were used, with the procurement being performed in the same hospital in an adjacent operating room. Following extensive experimental work that demonstrated the safety of longer cold ischemic times, workers at the Medical College of Virginia and Stanford University demonstrated the clinical safety of long-distance procurement[15,16].

With the reintroduction of cardioplegia for myocardial protection in cardiac surgery in the 1970s[17-19], this was also used for donor procurement. Following extensive experimental work that demonstrated the safety of longer cold ischemic times, the practice was extended to the clinical arena. In 1978 Thomas *et al.* reported the first successful long-distance procurement of a preserved heart[15]. Watson *et al.* likewise demonstrated the safety of long-distance procurement using hypothermic cardioplegia and storage in cold saline for transport[16]. They reported no differences between locally procured hearts (mean ischemic time 52 ± 12 min) and long-distance-procured hearts (mean ischemic time 154 ± 30 min) with regard to: (a) the

need for postoperative inotropic support, (b) 90-day mortality, or (c) rejection frequency. In another study Billingham *et al.* found that, although there were no functional differences between local and long-distance-procured donor hearts, there were ultrastructural differences between the two groups[20].

In comparison to advances in developing solutions for clinical preservation of other solid organs, progress in the development of solutions for myocardial preservation for HTx has been slow. Experimentally, several investigators have demonstrated the safety of preserving hearts for <24 h. However, clinically, most centers restrict their preservation times to <5 h because of an increase in morbidity and mortality that is associated with more prolonged ischemic times[2]. The reason for this disparity between experimental and clinical findings remains the topic of much discussion and debate.

THEORETICAL CONSIDERATIONS

During periods of myocardial ischemia the myocardium is damaged in a time-dependent fashion because of the imbalance between metabolic demand and substrate supply. This results in depletion of high-energy phosphates, accumulation of waste products, intracellular acidosis, membrane injury, and contractile dysfunction. If the ischemic time is prolonged, these abnormalities will not be corrected even after perfusion is re-established. As Stinson has aptly stated: 'the term "myocardial protection" is really a euphemism for minimizing myocardial injury during cardiac operations that involve an interruption of coronary flow and arrest of ventricular contractions'[21] (see Table 1).

The principles of myocardial protection have been reviewed extensively by others[22–26], but we will recapitulate some of the important highlights. The approach to myocardial preservation can best be summarized by the concept of the 'supply/demand' ratio – i.e. the ability to maintain a balance between the supply and demand requirements of the heart. In HTx, however, once the heart is explanted (and in the absence of an *ex vivo* support system) the donor heart is deprived of any source of supply of energy. The attempt to optimize the supply/demand ratio could involve: (a) supplying nutrients to the heart, or (b) decreasing the metabolic demand.

Enhancing supply: machine preservation

The concept of using *ex-vivo* perfusion to protect organs for transplantation found most applicability in renal transplantation. Although a number of papers have reported the use of *ex-vivo* perfusion for experimental HTx, few centers have ventured into this arena clinically on a consistent basis[27,28]. The obvious theoretical advantages of a continuous perfusion system include supplying substrates and removing metabolic wastes continuously. Robicsek *et al.*[29] and others[30] developed *ex-vivo* autoperfusion circuits for prolonged storage periods. The equipment needed, however, was relatively cumbersome, and this approach was not routinely adopted. Flush perfusion techniques, on the other hand, are simple, cheap and convenient, and therefore have become widely used clinically.

Decreasing demand

Efforts have concentrated upon protecting the heart by decreasing its energy requirements until perfusion is re-established. Historically, hypothermia has been the main method of decreasing myocardial metabolic demand during periods of ischemia, and all clinical methods of cardiac preservation for HTx today use hypothermia. From the moment the heart is deprived of its blood supply its metabolic supply is removed, products of metabolism accumulate, and tissue damage occurs (which is initially reversible but eventually becomes irreversible). By slowing down the rate of metabolic activity, hypothermia reduces the rate of organ deterioration that accompanies ischemia. Although hypothermia slows down the rate of degradation of the tissue, the optimal temperature is not known. Experimental work by Karck *et al.*[31] suggested that hypothermia at 4°C (as compared to 15°C)

Table 1 The stages at which injury to the donor heart may be minimized

1. Prior to explant (following brain death):
   correct effects of endocrine failure (T3, cortisol, insulin)
   optimize donor hemodynamics (volume, electrolytes, hematocrit, temperature)
   decrease inotropic support to a minimum
   administer antioxidants to the donor
2. Donor procurement:
   gentle handling
   prevent cardiac distension
   ensure prompt and efficient delivery of cardioplegia
3. Storage for transport:
   choice of cardioplegic/storage solution
4. Implantation:
   continue myocardial protection (frequent doses of cardioplegia)
   prevent cardiac distension
5. Reperfusion
   'hot shot' prior to re-establishment of antegrade flow
   prevent cardiac distension
   use of monoclonal antibodies against adhesion molecules
   use of leukocyte depletion filters
6. Early postoperative period
   avoid excessive and inappropriate use of inotropes
   avoid hypoxia

prolonged myocardial protection with respect to: (a) ATP preservation, (b) prevention of the fall in intracellular pH, and (c) enhancement of postischemic hemodynamic recovery.

A number of experiments have suggested that surface cooling alone can lower myocardial temperature. However, the cooling may not be rapid or uniform, and surface cooling alone is an inefficient method for reducing the core temperature of the heart. Others have emphasized that cooling should be accomplished as quickly as possible, but too rapid cooling can result in tissue damage.

Although hypothermia retards the development of ischemic injury it has many other effects, many of which can be damaging. The unwanted side-effects include: (a) decreasing enzyme function[32], (b) changing membrane stability[33], (c) calcium sequestration[34], (d) interfering with glucose utilization[35] and ATP generation and utilization[36], as well as (e) changes in pH[37] and osmotic homeostasis, leading to cell swelling[38].

Cardioplegia

Cardioplegic solutions were developed to produce rapid and even cooling of the heart and to enable rapid cessation of cardiac contractility (to rapidly reduce utilization of large amounts of myocardial energy reserves). The administration of cardioplegic solution is akin to the use of flush solutions used in other solid-organ preservation techniques which enable rapid, even cooling, and eliminate blood from the vascular compartment. However, in recent years the concept of using blood cardioplegia has become widespread, and has resulted in improved myocardial preservation. The importance of the chemical composition of the cardioplegic solution is well recognized. Increasingly complex preservation solutions have been developed, with special additives being used to decrease the injury resulting from the numerous insults that occur during procurement, preservation and reperfusion.

Although advances in myocardial protection for HTx have been closely linked to developments in myocardial protection for routine cardiac surgery, there are features that are unique to protection of the cardiac allograft. Once explanted from the donor, during the period of transport there is no source of collateral blood supply and thus a lack of rewarming. This has also limited the applicability of the newer concepts of myocardial protection, such as the administration of continuous retrograde cardioplegia, from being applied during donor heart transportation.

Ischemia and reperfusion injury

HTx by its nature requires a period of obligatory ischemia followed by reperfusion. The previous discussion dealt with methods to decrease this ischemic insult. The important role that free radicals, calcium overloading, neutrophils, adhesion molecules, and platelets play in mediating injury, particularly during reperfusion, has been described. With current technology we are still unable to avoid ischemia/reperfusion injury completely, but with increasing understanding of the mechanisms involved, in there exists the potential to develop further therapeutic interventions.

CURRENT CLINICAL TECHNIQUES OF MYOCARDIAL PROTECTION FOR CARDIAC TRANSPLANTATION

Most centers now use cold flush solutions to stop and cool the donor heart at the time of procurement. The ideal cardioplegic solution should: (a) decrease energy demand, (b) prevent accumulation of toxic metabolites during the period of storage, (c) maintain normal ionic composition and pH, (d) prevent cell swelling and ultrastructural damage to the cell membrane. In addition, it should (e) prevent the development of excessive vascular resistance during storage and upon establishment of reperfusion, (f) minimize the extent of reperfusion injury, and (g) prevent long-term damage to myocyte, interstitium and vasculature.

Many different cardioplegic solutions are used in HTx programs, but a number of surveys attest to the widespread use of cardioplegic solutions of extracellular-type composition (Chapter 74). Because of initial experimental evidence that the University of Wisconsin (UW) solution (an intracellular-type solution) was effective in providing myocardial protection for long periods of time, a number of centers have resorted to use of this solution, with good results. UW solution is said to be effective because it contains a number of cell impermeant agents, such as lactobionic acid, raffinose, and hydroxyethyl starch, that prevent cell swelling during ischemic storage. It also contains glutathione and adenosine, compounds that stimulate recovery of normal metabolism on establishment of reperfusion by: (a) augmenting the antioxidant potential of the organ and (b) stimulating the generation of high-energy phosphates.

Others have reported that improved results are obtained if 2,3-butanedione monoxime (a reversal inhibitor of cardiac contracture) and calcium are added to standard UW solution[39]. Wicomb *et al.* have suggested that a fresh supply of glutathione be added to UW solution prior to its use[40]. During cold storage of human cardiac allografts in UW solution, the expression of major histocompatibility complex antigens and vascular adhesion molecules on endothelial cells and myocardial cells remains unchanged[41]. The possibility that the high potassium content of UW solution could result in endothelial injury has been reported[42–44], and a higher incidence of graft coronary artery disease in patients whose hearts were preserved with UW solution (in comparison to the Stanford cardioplegic solution) has been reported[45]. However, this finding has not been confirmed by other centers that also routinely use UW solution.

OTHER FACTORS AFFECTING GRAFT VIABILITY

It is now apparent that 'protection' of the donor heart begins before its removal from the donor. A number of reports describe the beneficial effects of manipulation of the hormonal environment of the donor. In addition, attention must be focused upon correcting electrolyte, hemodynamic, and temperature parameters prior to and during the procurement process.

The ability to safely wean the donor heart from cardiopulmonary bypass is often the best indicator of the efficiency of the myocardial protection used. However, it must not be forgotten that factors other than preservation can affect the initial function of the cardiac allograft. These may include: (a) the presence of underlying donor cardiac disease (e.g. coronary artery disease or myopathy); (b) injury sustained at the time of trauma (that

resulted in brain death); (c) the deleterious effects associated with brain death; (d) inadequate donor management (e.g. unsuitable volume replacement, inefficient use of inotropic agents, inadequate prevention of hypoxic insults); (e) poor technique of donor procurement; (f) inadequate experience of the anesthetic and surgical teams; (g) suboptimal management of cardiopulmonary bypass in the recipient; (h) suboptimal steps to minimize reperfusion injury; and (i) immune-mediated injury.

The sum total of preservation injury, the aforementioned non-preservation-related injuries and the additional effects of poor donor selection may become manifest in the early postoperative period as difficulty in weaning from cardiopulmonary bypass with the need for high-dose inotropic support, intra-aortic balloon pump counterpulsation or a mechanical assist device.

Effects of brain death

This topic is discussed in Chapter 4, but in view of its relevance to myocardial preservation and its importance with regard to immediate post-transplant cardiac function, brief mention will also be made of it here. It has long been established that cardiac death eventually follows brain death. The state of 'brain death' is an interesting and physiologically unusual state[46–49]. Major changes in cardiac metabolism, histology, and function occur in brain-dead patients, and these changes may be the cause of unexplained cardiac allograft dysfunction after HTx.

Histology

The histologic changes described in brain-dead patients include hemorrhage and myocardial necrosis. Subendocardial hemorrhage is found at autopsy in patients who die of head trauma[50]. Morphological changes were also seen in the hearts of mice that had undergone the intracranial injection of a small amount of blood[51]. Novitzky *et al.* found pathologic changes affecting the myocardium, coronary artery smooth muscle cells, and conduction tissue that appeared to be mediated by the autonomic nervous system in a baboon model of brain death[52,53].

Endocrine

The cessation of brainstem function affects the anterior pituitary gland, leading to decreased ADH production, causing diabetes insipidus in 50–70% of organ donors[54]. Novitzky *et al.* reported that changes in plasma levels of triiodothyronine, cortisol, and insulin occur with brain death[53,55]. Taniguchi *et al.* noted the average time between the diagnosis of brain death and cardiac arrest was 4.3 days[56], but in patients who received hormone supplementation this interval was prolonged to 11.5 days.

Metabolism

Hearts in brain-dead subjects have been found to be energy-depleted, but could be resuscitated with substrate enhancement[57]. There is also an important shift towards anaerobic metabolism resulting in ATP consumption[48].

Function

In an experimental study using rat hearts, Galinanes and Hearse showed that 60 min after brain death a number of indicators of cardiac contractile function decreased by approximately 50%[49,58]. However, once excised and perfused *ex vivo*, the hearts recovered function identically to hearts from animals that had not been subjected to brain death. When hearts from brain-dead animals were excised, stored (6 h at 4°C), and reperfused *ex vivo* with blood, they also recovered a functional capability identical to that of control hearts. These studies indicated that hemodynamic instability in brain-dead individuals may not necessarily be an irreversible phenomenon, and that such hearts could be considered for transplantation. Clinically, a number of echocardiographic reports suggest that septal motion abnormalities may be peculiar to the brain-death state as they improve upon implantation of the heart into the recipient[59].

Cytokines

There is also evidence from clinical studies that donors have an altered state of the cytokine system, with elevated plasma concentrations of interleukin-6, 8 and soluble p55 TNF type 1, and also of C reactive protein[60]. What effect such elevations in cytokine levels have on graft function is not clear. The ability to block the effects of these cytokines would seem worthy of study.

Treatment

The brain-dead state has important metabolic, structural, and functional effects on the donor heart. We may therefore be able to improve the donor heart's metabolic state, possibly by administering hormone therapy before excision[53].

FUTURE AREAS FOR RESEARCH

Future areas for research can be considered to encompass all phases of donor selection and management, and donor heart preparation, excision, transportation, implantation, and reperfusion.

Donor selection

The development of a viability assay which could predict the ability of the heart to assume full function upon reperfusion remains an important goal. Although currently there are no such tests readily available, there is encouraging research using biochemical markers, most notably troponin T[61]. Perhaps other non-invasive predictive tests will become available using non-biochemical techniques, such as NMR spectroscopy or imaging[31,62–65].

Donor preparation

It may be possible to prevent or reverse the myocardial changes that occur with brain death. This might be pursued by the use of better 'cocktails' to correct the hormonal and metabolic state of the donor heart. Blockers of inflammation or free radical

scavengers may prove useful in preventing injuries mediated by the inflammatory system and oxygen free radicals[66–73].

Preservation solutions

Better preservation solutions may be designed to prevent free radical injury, buffer pH changes, provide metabolic substrates, or contain additives that improve preservation.

Transportation

This entire area is fertile for research. Perfusion systems and solutions may be improved to provide 'nutrition' and maybe even 'resuscitation' during transportation. However, newer techniques of *ex-vivo* perfusion, based on microperfusion, hold great promise[74]. Wicomb *et al.* have shown experimentally that hearts can be satisfactorily preserved for <24 h using microperfusion[75].

Organ freezing

The cessation of *all* metabolism in a stored organ could be achieved by freezing. Organ banks could be developed where organs could be frozen for days, weeks, or months until the need arises to use them. Advancements in the field of cryobiology, however, have to date been limited by our inability to mitigate the deleterious effects on the cells of freezing and thawing.

Implantation

Changes in the implantation procedure would center around reduction of the reperfusion injury and, ideally, 'resuscitation' of the transplanted heart. There is evidence that neutrophils play a role in reperfusion injury, and that the depletion of leukocytes by the use of filters may be useful in limiting this injury[76–79]. Alternatively, interfering with neutrophil function by blocking the adhesion molecules that participate in neutrophil activation has proved beneficial in an experimental model[76].

The use of a 'hot shot' of blood cardioplegia, either with or without substrate enhancement, has been advocated by some surgeons. This can be achieved by inserting a cannula into the coronary sinus and starting retrograde perfusion of the heart during the implantation procedure using warm blood cardioplegia[80–83]. Furthermore, such solutions might be better tailored to 'resuscitate' the heart while it is being implanted.

Extending the donor pool

With an increase in experience the initial strict criteria established for harvesting of donor hearts have been liberalized. 'Marginal' donor hearts can now often be used safely. The question has been raised (especially in countries where brain death is not accepted as a definition of death) as to whether hearts that have already arrested can be successfully transplanted. If so, what is the maximum safe period of arrest?

About 20 years ago, experiments under certain conditions demonstrated that canine hearts arrested after exsanguination could be resuscitated if the warm ischemic period did not extend >60 min[84]. If the cause of cardiac arrest was anoxia, resuscitation was less likely to be successful but could still be obtained if the ischemic period was <30 min. More recently, Gundry *et al.* have shown the feasibility of transplanting lamb hearts harvested 30 min after death from exsanguination[85]. Shirakura *et al.* have reported good function of donor hearts harvested from non-heart-beating donors that had been pretreated and cooled by cardiopulmonary bypass prior to heart procurement[86,87].

COMMENT

The ideal preservation method is one that will provide the longest preservation time while maintaining excellent graft function. Improved methods of organ preservation should demonstrate improved graft function despite a prolongation in ischemic time. Myocardial protection of the donor heart plays an important role in determining morbidity and mortality after HTx. Our understanding of the factors that are involved in minimizing cardiac injury during the periods of procurement, storage for transport, implantation, and reperfusion has increased, but remains incomplete. Indeed, progress in heart preservation has been slow when compared with other organs. Unlike other organs the heart must provide *immediate* support upon separation of the patient from cardiopulmonary bypass. Currently, all methods of cardiac preservation continue to use hypothermia to decrease metabolic activity. Better preservation would allow hearts to be procured from further afield. It must not be forgotten, however, that many factors other than preservation can affect the immediate function of the cardiac allograft.

In the future, emphasis should be placed on enhancing our understanding of hypothermia-mediated cell injury and reperfusion injury, and on the development of techniques to decrease both of these insults.

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24
Surgical Technique of Orthotopic Heart Transplantation. I: Standard Approach

D.K.C. COOPER

INTRODUCTION

There are two very different basic operations for performing heart transplantation – *orthotopic*, in which the recipient heart is excised and replaced in the correct anatomical position by the donor heart, and *heterotopic* (the so-called 'piggy-back' heart transplant), in which the donor heart is placed in the right chest alongside the recipient organ, and anastomosed in such a way as to allow blood to pass through either or both hearts. Both procedures, however, have various modifications.

In recent years, orthotopic heart transplantation has been modified to include excision of the right atrium with bicaval anastomosis (rather than anastomosis of the two right atria) and reduction in the remnant of the recipient left atrium – the so-called bicaval or 'total' technique. These 'modifications' (which in the experimental laboratory actually predate[1] the standard technique[2,3], which aimed to simplify the operation), are described in Chapter 25. Heterotopic heart transplantation can be performed to provide support for both recipient ventricles or for only the left ventricle. These techniques are outlined in Chapter 37.

In this chapter the *standard technique* of orthotopic heart transplantation that has been used successfully in clinical heart transplantation for almost 30 years will be illustrated.

With any heart transplant, ideally the recipient operation should not be begun until the donor has been carefully assessed by the transplant surgeon, and found to be suitable for transplantation. Whenever there is doubt, e.g. when there is the possibility of cardiac injury from chest trauma, the recipient operation should certainly be delayed until the donor chest has been opened and the heart inspected.

The basic technique of orthotopic heart transplantation was developed in the research laboratory in the late 1950s and early 1960s[1] (Chapter 18). It was the work of Lower and Shumway in 1960[2] which established the operation as a successful procedure in the experimental animal. The operation was first attempted clinically by Hardy and his colleagues in 1964[3] and by Barnard in 1967[4]. In 1968, Barnard[5] contributed a small but significant modification to the operative technique whereby the incision in the right atrium of the donor heart was extended from the opening of the inferior vena cava (IVC) into the base of the right atrial appendage, and not into the superior vena cava (SVC), thus avoiding the region of the sinoatrial node. The operation has remained essentially unchanged since then, and is the operation of choice in the majority of centers performing heart transplantation today.

DONOR HEART EXCISION

Heart excision is almost always part of multiorgan retrieval (and will be described as such below). The needs of the surgeons retrieving other organs must be considered at all times. Acute or unexpected failure of cardiac action may result in loss of all donated organs. Good communication between the various surgical teams is essential if retrieval of the various organs is to be satisfactorily coordinated.

With the subject supine, a median sternotomy is performed and the pericardium opened longitudinally. The heart is inspected in at-risk donors for external signs of injury caused by trauma or external cardiac massage. The coronary arteries should be palpated to exclude obvious coronary disease. The contractions of the ventricle should be observed closely and, if weak or irregular, consideration should be given to the cause and to means of improving ventricular performance.

The ascending aorta is dissected from the pulmonary artery to allow subsequent application of a cross-clamp. The SVC is mobilized up to the azygos vein to allow SVC ligation cephalad to the sinus node. Two heavy ties are placed around the SVC, but not ligated at this stage. The IVC is mobilized. (Preparations by the liver surgeons usually take longer than preparation of the heart, and are completed at this stage.) The donor is fully heparinized (25 000–30 000 units intravenously). A cannula for infusion of cold cardioplegic agent is inserted into the ascending aorta, and is maintained in place by a purse-string suture and 'snugger' or 'snare' so that it does not have to be held by one of the surgical team, thus releasing all available hands for more important activities. If the liver is to be procured, and if IVC blood is not to be drained retrogradely, the right pleural cavity is opened from sternum to IVC to allow drainage of inferior vena caval blood when the thoracic IVC is subsequently divided.

When all the other surgical teams are fully prepared, excision of the heart can proceed. The SVC is doubly ligated (or suture ligated or stapled) and divided between the ligatures. (Any indwelling central venous pressure cannula must be withdrawn high into the SVC by the 'anesthesiologist' before this vessel is ligated and divided.) The IVC is divided at the diaphragm, completing inflow occlusion, and decompressing the right side of the heart. Inferior vena caval blood from the liver (together with the liver perfusion solution) drains largely into the right pleural cavity, but it is essential to have good suction available to ensure that neither the liver perfusion solution nor warm blood contaminate the heart or interfere with the surgical field in which the cardiac surgeon is working. It is convenient to introduce a suction catheter into the IVC to avoid blood obscuring the operative field in the pericardial cavity. (If the liver is not being procured, the IVC can be clamped at the diaphragm before it is divided.) One or more pulmonary veins are incised or divided to decompress the left side of the heart. (If the lung is being procured, the left atrial appendage is opened to allow this decompression.) Adequate decompression of the heart is essential before the aorta is cross-clamped. (If the aorta is cross-clamped first, the heart may become distended and considerable injury may occur to the myocardium.) If an arterial line is in place, the pulse wave should be lost, indicating that the left ventricle has emptied. The ascending aorta is then cross-clamped at the level of the brachiocephalic (innominate) artery, and cardioplegic solution (approximately 10 ml/kg at 4°C, or 1 liter) infused into the root of the aorta. At least 1–2 liters of cold saline (at 4°C) are poured over the heart to cool it rapidly. (Perfusion of the lungs, liver and kidneys is also initiated.)

The pressure in the aorta during cardioplegia infusion should not exceed approximately 100 mmHg. This can be achieved by placing the bag containing the cardioplegic agent in a pressure bag, pressurized to 300 mmHg; experience has shown that this results in an aortic pressure in the desired range. Frequent palpation of the ascending aorta by the surgeon allows approximate estimation of the aortic pressure, and the infusion pressure should be adjusted if the pressure in the aorta becomes too high or too low. During infusion, the heart should be gently massaged at intervals to ensure adequate decompression is maintained. Cardioplegic infusion is usually complete within 3–5 minutes (although infusion of the lung preservation solution into the pulmonary artery may take longer).

During the ischemic period in which the heart is transferred to the recipient there will be no cardioplegic washout from collateral blood flow (as occurs to a cardioplegically arrested heart during open-heart surgery). It is theoretically, therefore, only necessary to give sufficient cardioplegic agent to bring about arrest of the heart. The cardioplegic infusion, however, also contributes towards rapid cooling of the myocardium, and it is therefore our policy to infuse approximately 10 ml/kg (500–1000 ml) even if the anticipated ischemic period is expected to be short.

Once the cardioplegic (and pulmoplegic) agent has been administered, the topical cold saline is sucked out of the pericardial cavity, and section of the four pulmonary veins is completed. (If one or both lungs are being retrieved then the lines of incision into the donor heart should be around the left or right pulmonary vein orifices, allowing a cuff of left atrium to be retained with the pulmonary veins (Chapter 48). Division of the aorta as high as possible, immediately proximal to the cross-clamp, and of the pulmonary artery at its bifurcation (or at the origins of its main right and left branches) completes division of the major vessels.

The apex of the heart is then lifted anteriorly, and the mediastinal tissue posterior to the atria and major vessels is divided, allowing the heart to be removed from the pericardial cavity. The approximate time taken from ligation of the SVC to completion of excision of the heart is usually 5–10 minutes.

PREPARATION OF DONOR HEART

The heart is placed in a bowl of cold (4°C) saline while it is prepared for insertion into the recipient.

The tissue between the orifices of the four pulmonary veins on the posterior aspect of the left atrium is excised, leaving one large opening (Figure 1). The edges of the left atrium usually require trimming at the time of insertion into the recipient. It is wise to inspect all four cardiac valves to exclude the presence of traumatic injury or unexpected conditions such as bacterial endocarditis[6]. (The aorta and pulmonary artery should not be shortened at this stage, as it is preferable to wait to trim them to the ideal lengths when these vessels are to be anastomosed in the recipient.) The right atrial cavity is opened, beginning posterolaterally at the IVC orifice and continuing the incision into the base of the right atrial appendage, thus avoiding the areas of the coronary sinus and the sinoatrial node[5] (Figure 2). The atrial septum is inspected for the presence of a patent foramen ovale (PFO) or atrial septal defect. Unless large, it is not necessary to close a PFO.

The heart is stored in normal saline in ice for the period of transportation. It is usual to place the heart in a bag containing 1 liter of normal saline, to place this in turn in another bag containing a further liter of normal saline, and this into a

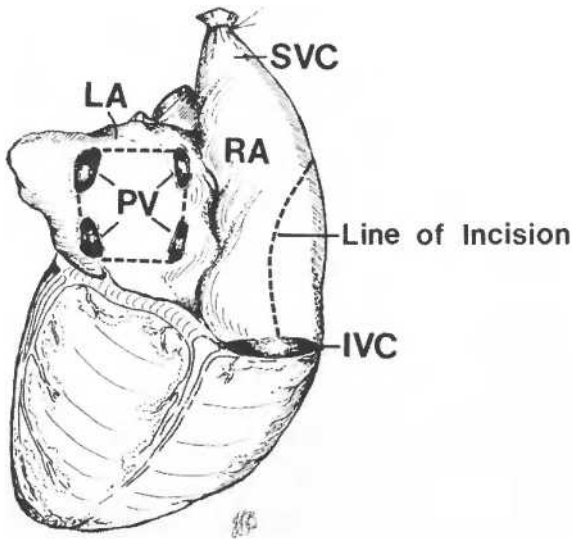

Figure 1 Excised donor heart (posterior view), showing lines of incision. (Abbreviations used in figures in this chapter are: LA = left atrium; RA = right atrium; SVC = superior vena cava; IVC = inferior vena cava; PV = pulmonary vein; RV = right ventricle; PA = pulmonary artery; AO = aorta; LV = left ventricle)

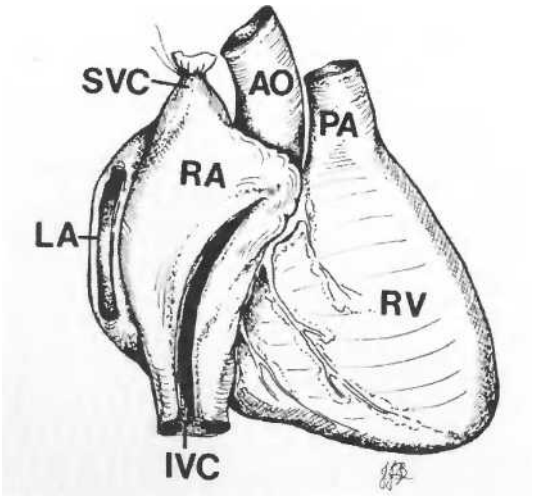

Figure 2 Donor heart (right anterolateral view) prepared for implantation

third bag containing one liter of normal saline. When these bags are placed in ice, the heart is protected from freeze injury during transportation.

The heart can then be transferred to the surgical team preparing the recipient.

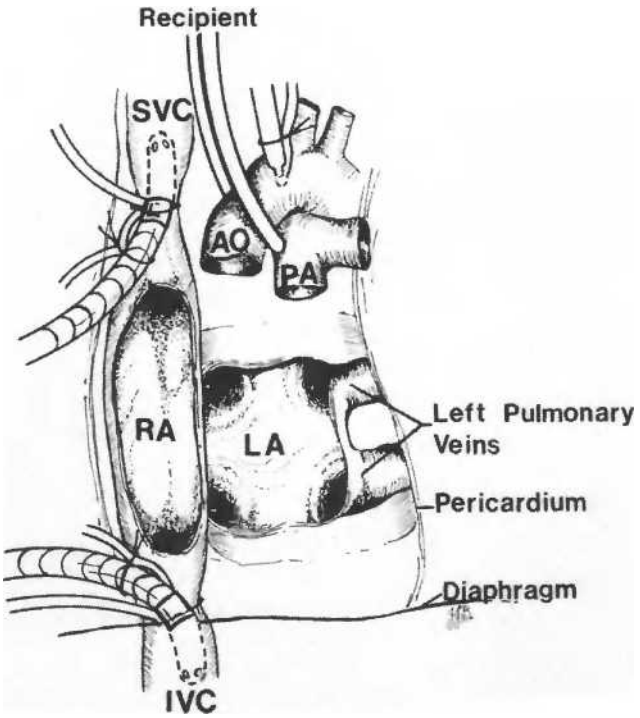

Figure 3 View of recipient pericardial cavity after excision of the recipient heart

THE RECIPIENT OPERATION

When no previous cardiac surgery has been undertaken, preparation of the groin is unnecessary. With the patient lying supine, a median sternotomy is performed, the pericardium opened longitudinally, and its edges retracted. If the patient has undergone previous cardiac surgery there are likely to be adhesions between the heart and pericardium or posterior surface of the sternum. Dissection of a poorly functioning heart under such circumstances may require considerable handling, and acute cardiac failure may intervene, placing the life of the patient at risk. It is therefore wise to prepare the groin in case access to the femoral vessels is required for urgent initiation of cardiopulmonary bypass. In hemodynamically unstable patients it is preferable to actually prepare the femoral vessels for urgent cannulation if necessary. If the femoral artery and vein have been prepared, heparin can be given and the vessels cannulated rapidly, allowing pump-oxygenator support to be begun. The dissection of the heart can then be completed without undue urgency. When this has been achieved it is usual to cannulate the SVC and IVC (in addition to the femoral vein).

Initiation of cardiopulmonary bypass

After heparinization, cardiopulmonary bypass (CPB) is initiated via cannulae inserted into the ascending aorta at or near to the level of the brachiocephalic artery, and into the SVC and IVC via the lateral wall of the right atrium (Figure 3). Snares (snuggers) or clamps are placed around the SVC and IVC to bring about total CPB. Body cooling to at least 28°C, and possibly lower (26°C), helps prevent early rewarming of the donor heart as it lies in the pericardial cavity during insertion. The aorta is then cross-clamped immediately proximal to the aortic cannula.

Excision of recipient heart

The heart is excised by dividing the right and left atrial walls (close to the atrioventricular groove) and atrial septum, leaving a cuff of atrial wall to allow easy suture of the donor heart. Both atrial appendages should be excised to prevent thrombus formation occurring in these cavities after transplantation. The aorta and main pulmonary artery are divided as close to their respective valves as possible (Figure 3). Subsequently these vessels may be trimmed before being anastomosed to their counterparts of the donor heart.

In essence, therefore, only the ventricles have been excised, with a short cuff of the two atria.

The chronological order of division of these structures is unimportant, but a simple sequence is: (a) free wall of right atrium; (b) free wall of left atrium, excluding the superior wall posterior to the origins of the aorta and pulmonary artery; (c) pulmonary artery, (d) aorta, (e) (after retracting the proximal aorta and pulmonary artery anteriorly) the remaining superior wall of the left atrium, and (f) the atrial septum.

Insertion of donor heart – order of anastomoses

The order of anastomoses of the various chambers and vessels followed by most groups for many years was: (a) left atrium (LA), (b) right atrium (RA), (c) pulmonary artery (PA), (d) aorta (AO). However, this delayed reperfusion of the donor heart until

the operation was completed by the performance of the aortic anastomosis. In recent years the desire to reperfuse at an earlier stage, and thus reduce the ischemic time, has led to variations of this sequence. The aortic anastomosis can, therefore, be carried out immediately after completion of the left atrial anastomosis. Air needles can be inserted in the apex of the left ventricle and aorta, the aortic cross-clamp can be removed, and the myocardium can be perfused while the pulmonary artery and right atrial suture line are performed.

Anastomosis of the pulmonary artery can be slightly difficult under such circumstances because: (a) access to the pulmonary arteries is partially obscured by the aorta, and (b) blood return from the coronary sinus to the donor pulmonary artery (via the right ventricle) may obscure the operative field. To avoid these additional problems the pulmonary artery anastomosis (which takes only a few minutes) can be performed before the aortic, leaving only the right atrial anastomosis to be carried out while reperfusion is continuing. Coronary sinus return may obscure the right atrial anastomosis in the region of the IVC, but this can be avoided by adequate suction.

The latter sequences (LA, AO, PA, and RA, or LA, PA, AO, and RA) have been the ones of choice (depending on the individual anatomy and need to reduce the ischemic time) at our center for the past several years. However, in this chapter the sequence used in the original technique (LA, RA, PA, AO) will be illustrated.

Anastomosis of left atria

The donor heart is placed (or held by an assistant) over the left side of the divided sternum, parallel to the remnants of the excised recipient heart. The donor heart is rotated 90–180° to the left so that its posterior surface faces anteromedially (towards the surgeon if he or she is standing to the right side of the table); the free walls of both recipient and donor left atria will then lie adjacent to each other (Figure 4). Using a double-ended 4/0 polypropylene suture, the left atrial walls are anastomosed by a continuous suture, beginning at the base of the donor left atrial appendage and at a point close to the caudal end of the recipient left superior pulmonary vein (Figure 4). The suture can be tied immediately after drawing the heart into the pericardial cavity or, alternatively (if it is chosen not to ligate the suture), at a convenient stage the donor heart is drawn down into the pericardium and the suture tightened. The suture is continued around the superior and inferior borders of the left atrium on to the atrial septum, and tied in the middle of the septum (Figure 5).

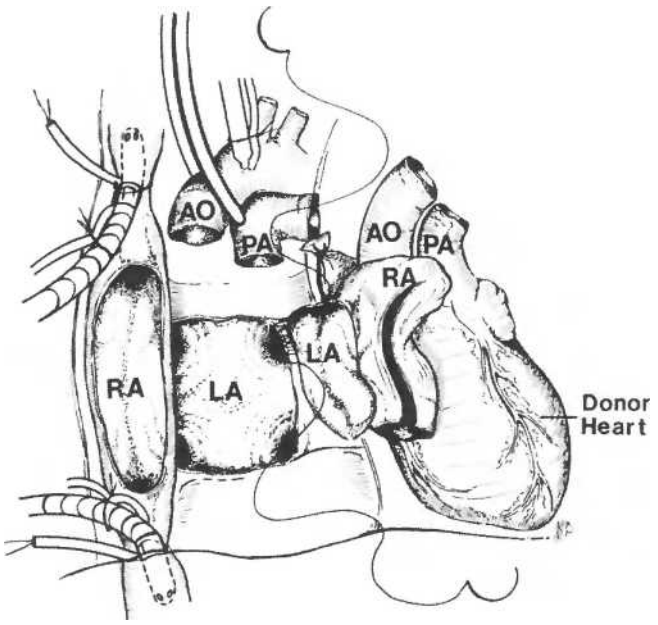

Figure 4 Donor and recipient hearts, showing the beginning of the anastomosis between the two left atria

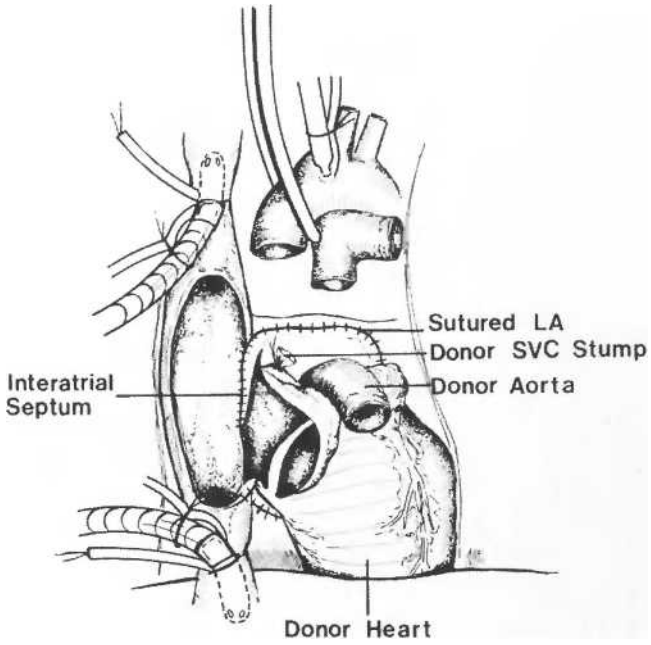

Figure 5 Completed left atrial free wall suture line; the anastomosis between the two septa is being performed

It is essential to maintain myocardial temperature as low as possible, preferably below 15°C, throughout the ischemic period, though tissue damage from freezing must be avoided. Between the performance of each suture line, therefore, the pericardium should be temporarily irrigated with cold saline to maintain a low myocardial temperature, or, ideally, a system of continuous myocardial cooling should be utilized. (Signs of ventricular myocardial activity rarely occur, but if such activity is seen or detected on the electrocardiogram, then further cardioplegic agent should be administered.)

At this stage, as discussed above, if it is decided to minimize the donor heart ischemic time, the surgeon can immediately progress to the aortic anastomosis (Figure 6).

Anastomosis of right atria

The two right atria are anastomosed using a double-ended suture of 4/0 polypropylene (or of 5/0 polypropylene if the right atrial walls are particularly thin). The suture is initially placed at the mid-point of the donor septum and at a convenient point in the

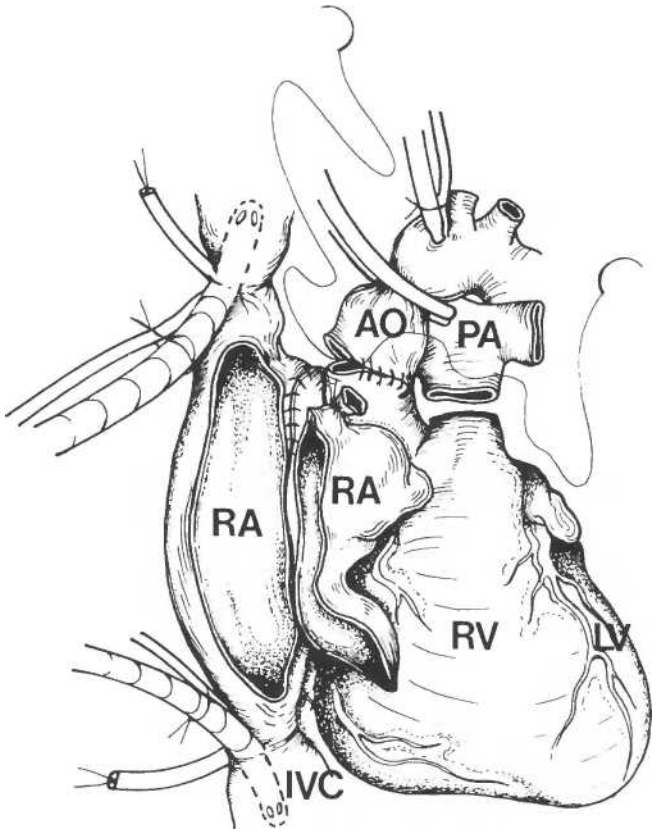

Figure 6 Alternative approach that can be utilized to minimize the donor heart ischemic time. The left atrial anastomosis has been completed. The aortae are being anastomosed, after which the aortic cross-clamp can be removed and the myocardium reperfused. The pulmonary artery and right atrial anastomoses can be performed while reperfusion is continuing

posterior lip of the incision in the recipient right atrium (usually slightly caudal to the mid-point). The anastomosis is continued first inferiorly, as this is the more difficult area in view of the small cuff of recipient right atrium which remains in the region of the IVC. Subsequently, the superior anastomosis is completed, and the two ends of the suture (inferior and superior) tied at the mid-point of the right atrial free wall (Figure 7). The atrial septum has therefore been sutured twice, once on the left atrial side and once on the right.

Anastomosis of pulmonary arteries

The two pulmonary arteries are then trimmed to their required lengths and anastomosed using a continuous suture of 4/0 polypropylene (Figure 8). It is important to trim the pulmonary arteries to the correct length. If they are left too long, kinking of the pulmonary artery can occur after anastomosis, and can be partially obstructive to blood flow.

Anastomosis of aortae

The aortae are similarly trimmed and anastomosed by continuous suture using 4/0 polypropylene (Figure 8).

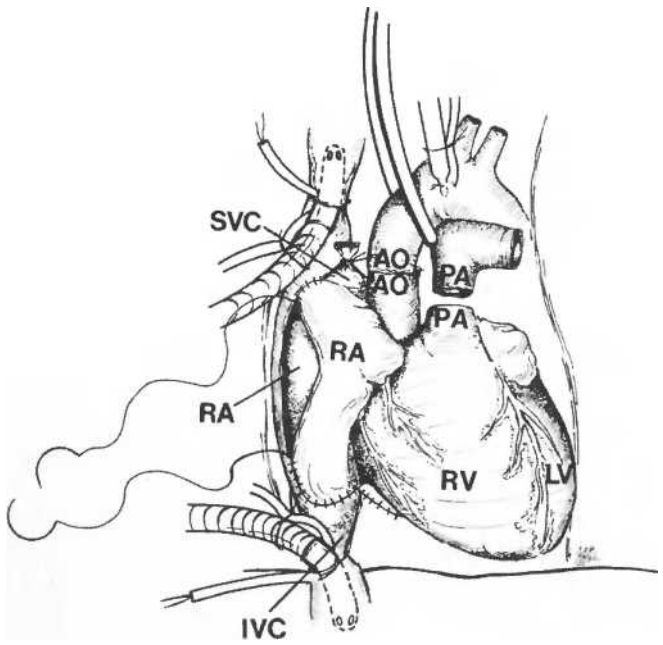

Figure 7 The septal anastomosis has been completed; the free walls of the two right atria are being anastomosed

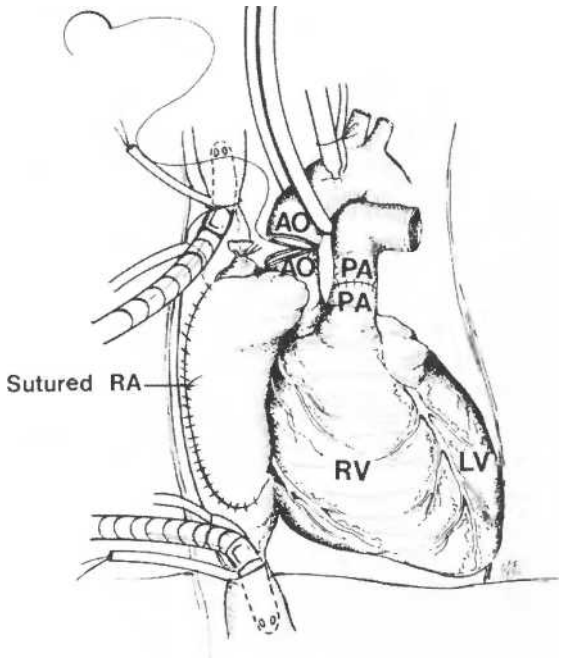

Figure 8 The right atrial and pulmonary artery suture lines have been completed; beginning of aortic anastomosis

Air needles are then placed in the apex of the left ventricle, anterior wall of the right ventricle, ascending aorta and main pulmonary artery. (Additionally, the left atrial appendage can be incised.) The SVC and IVC snares or clamps are released and the heart is gently massaged to expel air from the ventricles and major vessels. The lungs should be gently ventilated to increase the venous return to the left side of the heart, thus expressing

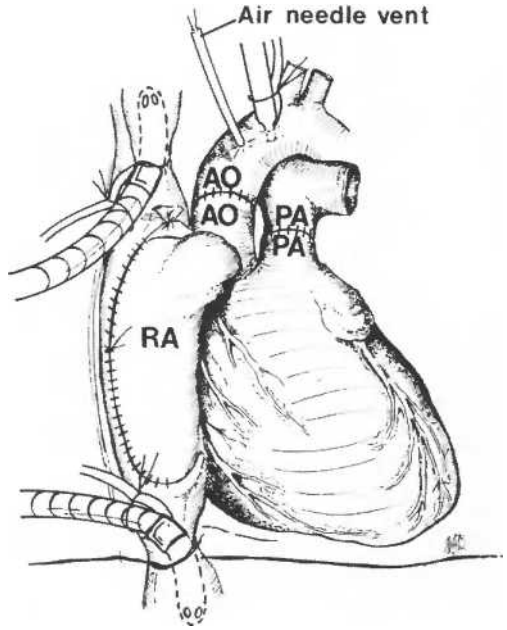

Figure 9 The aortic anastomosis has been completed and the aortic cross-clamp removed. An aortic air needle vent has been inserted. Similar needle vents should be placed in both ventricles and main pulmonary artery

further air, though care must be taken to ensure that the heart is not over-distended. The aortic cross-clamp is then released, allowing the coronary arteries of the donor heart to be perfused once again with oxygenated blood (Figure 9). Further efforts are made to expel air from both ventricles and major arteries.

Total body rewarming can now begin. Ventricular pacing wires are routinely applied in case temporary heart block or bradycardia ensues; in such cases the ventricles are paced to stimulate ejection of blood and ventricular decompression. A period of asystole is, in fact, not uncommon, particularly if the ischemic time has been prolonged. Temporary pacing may therefore be required. In many cases, however, vigorous ventricular fibrillation or spontaneous coordinated myocardial contractions occur. If ventricular fibrillation is present, electrical defibrillation will be necessary. Further vigorous efforts to expel air from the cavities of the heart should then be repeated. Until coordinated contractions are sufficient to eject blood and thus decompress the ventricles, the heart should be manually decompressed gently at intervals if distension occurs.

In our experience it is advisable to begin an inotropic infusion before discontinuation of cardiopulmonary bypass (CPB). Our own choice is isoproterenol, as this is the most efficient at increasing cardiac rate. A rate of 110 beats/minute is probably the most efficient in the early postoperative period as the donor heart that has been ischemically stored is more dependent on rate than stroke volume for its cardiac output. The infusion rate can be modified as necessary when cardiac function is assessed post-CPB. Many of these patients, particularly those who have been in hemodynamic distress pretransplantation, remain significantly vasodilated in the early post-transplant period, leading to difficulty in obtaining an acceptable systemic blood pressure. A low-dose phenylephrine (neosynephrine) infusion can be beneficial at such a time, and if doses of less than approximately 1.5–2.0 $\mu g\ kg^{-1}\ min^{-1}$ are infused, it has not been found to be detrimental to renal blood flow. By providing a little vasomotor tone, such doses of phenylephrine may significantly increase blood pressure.

It has been our policy to provide at least 30–60 minutes of pump-oxygenator support after release of the aortic clamp, to allow full recovery of the donor heart from its ischemic episode, before challenging it with responsibility for support of the circulation. During this period a careful check for bleeding is made on all suture lines, and further sutures inserted if necessary.

Discontinuation of pump-oxygenator support

When myocardial function is clearly satisfactory, and adequate rewarming has taken place, the ventricular and pulmonary artery air needles are removed, and their insertion sites oversewn with 4/0 or 5/0 polypropylene. The SVC cannula is withdrawn into the right atrium, and the IVC cannula removed. CPB is discontinued, and, after a few minutes observation, the aortic air vent is removed and its site oversewn. If cardiac performance is satisfactory, then the aortic and SVC cannulae are removed and protamine sulphate administered (Figure 10).

Two drains are inserted, one into the pericardial cavity posterior to the heart and the other into the anterior mediastinum; the pericardium may be left entirely open, though it has been our policy to close it partially, particularly over the aorta, as this provides a barrier to sternal infection tracking posteriorly to the region of the aortic suture line. Complete closure, even when the pericardial cavity is very large, should probably be avoided as a safeguard to help ensure that early or late tamponade does not occur. The sternum is reunited with at least six stainless-steel wire or other strong sutures, and the tissues anterior to the sternum are repaired.

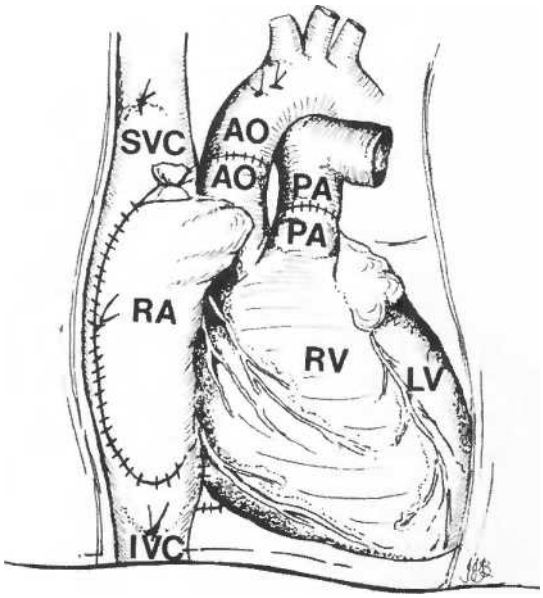

Figure 10 Completed operation. Arterial and venous cannulae have been removed

COMMENT

The two potential major complications of this operation are bleeding, in view of the extensive areas of anastomosis, and systemic air emboli. Great care must be taken to dispel all air from the left atrial and ventricular cavities before the donor heart resumes coordinated contractions after reperfusion.

Every effort must be made to maintain donor myocardial temperature as low as possible (yet avoid freezing injury) during transfer of the heart from donor to recipient, and this should be continued until blood reperfusion is commenced. The limitations of simple hypothermia and other storage methods in maintaining myocardial viability are discussed elsewhere (Chapters 23 and 74).

Unfortunately, inadequate donor heart function, or even nonfunction, is still reported, and may be a consequence of the effects of the agonal period or brain death on the myocardium, or result from inadequate preservation during transportation. If neither inotropic or intra-aortic balloon support retrieve the situation, then the only options open are to continue pump-oxygenator support or provide some other form of mechanical assistance until a second donor heart becomes available. Although rarely necessary, on occasion we have found the intravenous administration of triiodothyronine (T_3) to the recipient to be beneficial when all other measures had failed[6]. This form of therapy, the background to which is discussed in Chapter 4, remains controversial however.

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25
Surgical Technique of Orthotopic Heart Transplantation. 2: Bicaval 'Total' Approach

G. DREYFUS

INTRODUCTION

Since 1960, orthotopic heart transplantation (OHT) has been performed according to the technique of Lower and Shumway[1] (Chapter 24). Barnard introduced a small but useful modification whereby the sinoatrial node could be more readily avoided by opening the right atrium from the inferior vena cava to the right atrial appendage (Chapter 24). In 1990, Reitz[2] introduced the domino procedure by using the native heart of a patient undergoing heart–lung transplantation. This was the first approach to bicaval anastomosis with end-to-end anastomoses of both superior (SVC) and inferior (IVC) venae cavae. In 1989 Yacoub and Banner[3] had reported modifications to the standard technique by removing both native atria. Finally, in 1991 the 'total' orthotopic heart transplant technique was first reported[4].

There are therefore two different methods of performing OHT: (a) the standard technique, which can be considered as a biventricular transplant (Chapter 24) and (b) the alternative technique of total OHT, which is an anatomical transplant of both atria and ventricles. In this chapter the major differences in the technique of total OHT (when compared with the standard procedure) will be outlined. (For other details, the reader is referred to Chapter 24)

DONOR ASPECTS

Donor heart excision

Harvesting of the donor heart is very slightly modified from the standard technique, as both venae cavae should be maintained as long as possible. Both SVC and IVC should be completely transected, the SVC intrapericardially just below the azygos vein and the IVC proximal to the diaphragm. Just before clamping the aorta, one pulmonary vein should be incised to decompress the left side of the heart. The aorta and pulmonary artery should then be transected, leaving the donor heart attached only by the pulmonary veins. The heart is then elevated, allowing direct vision of the remaining pulmonary veins, which are transected intrapericardially at their entry into the left atrium (Figure 1).

Multiple organ procurement

Multiple organ procurement involving procurement of the lungs or liver may impair the integrity of the left atrium or IVC, respectively. If the liver is being excised, the donor IVC is often divided near or at the junction of the IVC with the right atrium. The donor free margin of the IVC (for subsequent suture to the recipient) may therefore consist of right atrial wall. The length of donor IVC available for anastomosis is never a problem as this anastomosis (in the recipient) can be performed between the right atrium (RA) of the donor and the remnant of the recipient IVC/RA (being more of an atrio-atrial anastomosis than a cavo-caval anastomosis). Therefore, if the liver team requires a long IVC, this does not preclude total OHT.

Problems can arise, however, with retrieval of a single lung or both lungs from the same donor. Depending on the lung transplant surgeon's skills and habits, the amount of left atrial cuff removed with the lung(s) will vary. This is obviously of the utmost importance to the possibility of performing total OHT, because sufficient posterior left atrial wall must remain with the heart to allow the total technique, even if the origins of the pulmonary veins have been excised with the lungs. However, at our own center, this technique aspects has never made the total OHT technique impossible to perform.

Preparation of the donor heart

In contrast to preparation of the donor heart for the standard procedure, both the SVC and the IVC are left open, with neither being ligated or oversewn. When the heart is harvested in the absence of lung retrieval, the left atrium clearly shows the origins of the four pulmonary veins (Figure 1). The bridging tissue between the left superior and inferior pulmonary veins is resected (as it is on the right), thus creating single left and single right pulmonary vein orifices (Figure 2). These orifices should be at least as wide as the mitral annulus in order to avoid restriction after anastomosis. The aorta is dissected from the pulmonary artery. Our own preference is to transect the pulmonary artery

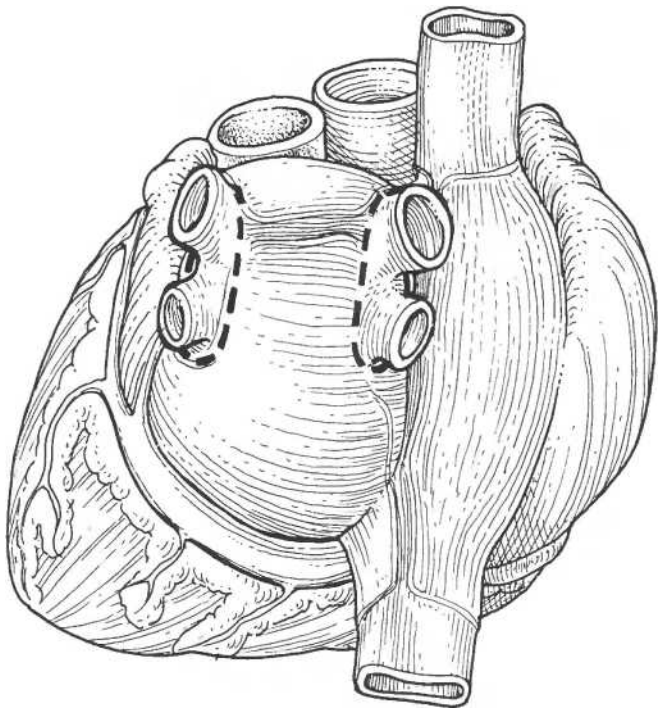

Figure 1 The excised donor heart. Note the integrity of all four pulmonary veins and the lengths of both superior and inferior venae cavae. The dotted lines demarcate the tissues to be excised to create two pulmonary venous orifices

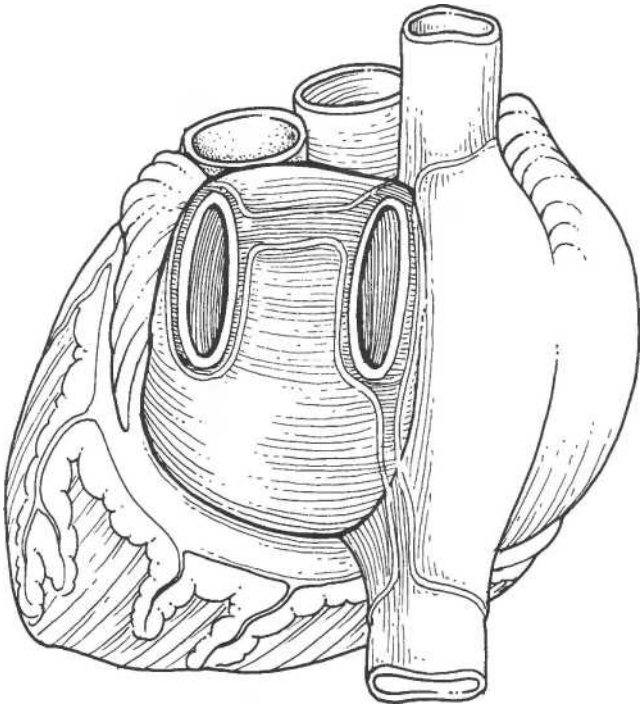

Figure 2 Preparation of the donor left atrium. The tissue bridge between the superior and inferior pulmonary veins is resected on each side, creating large right and left pulmonary orifices.

approximately 1 cm distal to the pulmonary valve (in order to avoid kinking after anastomosis to the recipient pulmonary artery).

THE RECIPIENT OPERATION

Cardiopulmonary bypass is initiated in a standard manner except that the SVC should be cannulated about 2 cm above its entry into the right atrium. The IVC should be cannulated via the lateral wall of the right atrium as close as possible to the diaphragm. After institution of cardiopulmonary bypass, both venae cavae are snared (snugged).

Excision of recipient heart

Excision is carried out as a two-step procedure. Firstly, the right atrium is opened as posterior as possible, close to both cannulae. The atrial septum is incised, which allows the left ventricle to

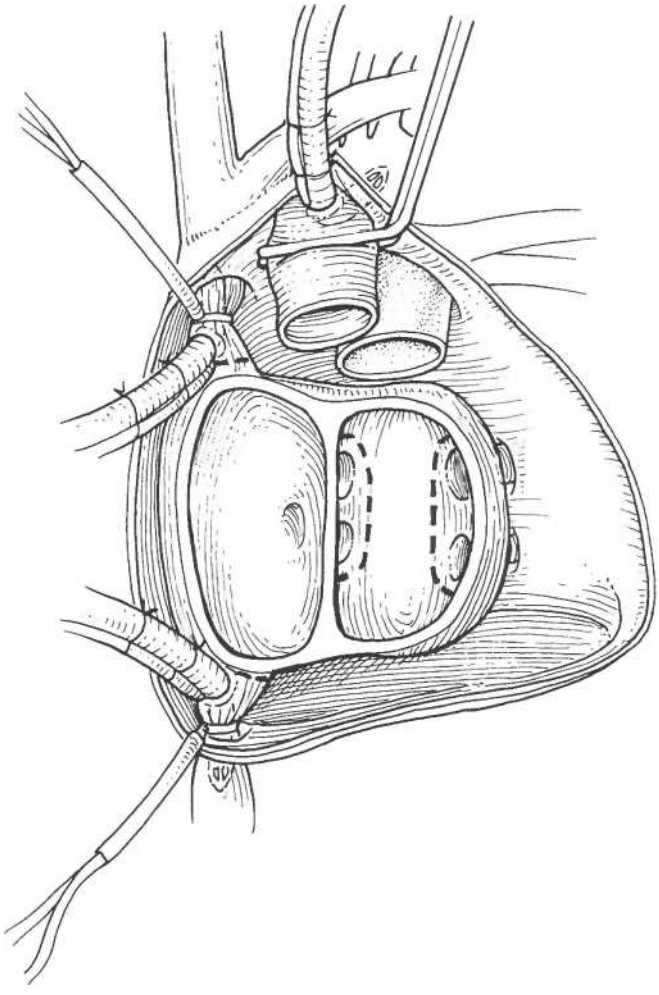

Figure 3 Excision of recipient heart. As a first step the heart is removed as for a standard orthotopic heart transplantation. This consists of excising both ventricles and all four valves. The dotted lines demarcate the left atrial venous cuffs that will remain *in situ*

empty via the mitral valve and left atrium. The aorta and pulmonary artery are transected. Finally, the left atrium is trimmed posterior to the left atrial appendage (Figure 3). At this point the recipient explantation follows that of the standard OHT technique.

Secondly, the posterior walls of both atria are excised. On the right this is performed by transecting both the SVC and IVC at their junctions with the right atrium. The right atrium should not be divided too close to the IVC cannula, as this may make the subsequent anastomosis with the donor IVC difficult. Therefore, the IVC cuff is, in fact, a right atrial cuff preserving at least 6–10 mm of atrial tissue beyond the IVC cannula. The right atrium remains attached only by the residual atrial septum. The posterior wall of the left atrium is freed from its pericardial attachment. This allows direct vision of the origins of the right and left pulmonary veins. The posterior aspect of the left atrium is trimmed, leaving a cuff on each side which includes the origins of the superior and inferior pulmonary veins. On the right side, the redundant left atrial wall is removed together with the remains of the atrial septum (Figure 4).

If the native left atrium is particularly large (as in dilated cardiomyopathy), or when there has been harvesting of a lung or both lungs from the same donor, care must be taken to leave large cuffs around the pulmonary veins.

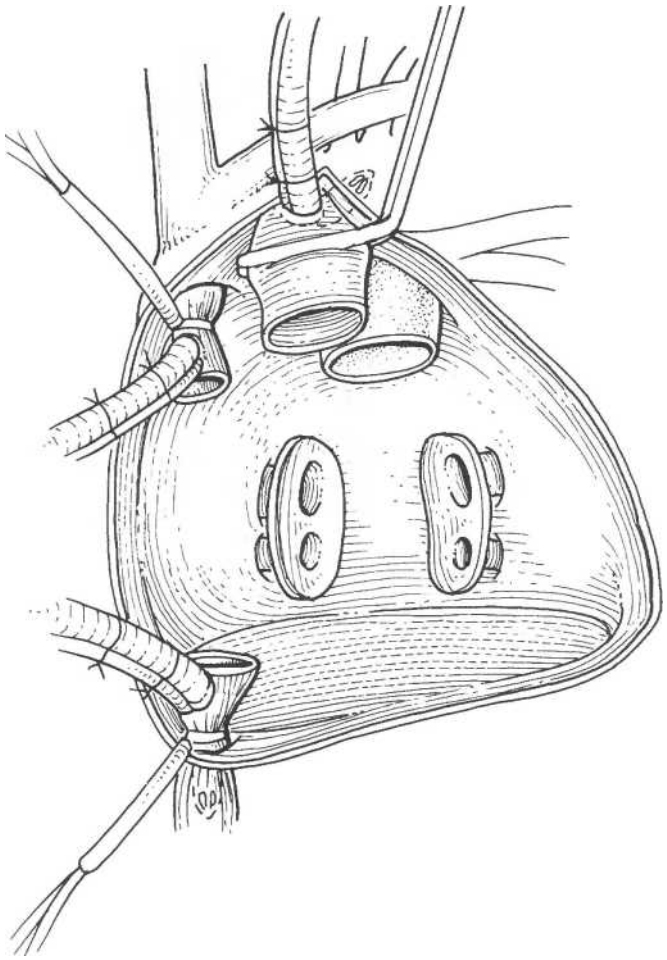

Figure 4 Excision of recipient heart. As a second step the remnants of both right and left atria are excised, leaving only two pulmonary venous cuffs and two caval cuffs

Anastomosis of left atria

As it is the most posterior of the suture lines that need to be performed, the first anastomosis is that between the left pulmonary venous orifice of the donor left atrium and the cuff around the recipient left pulmonary veins. To perform this, the donor heart is placed in the anatomic position. Two stay sutures are inserted to identify the cranial and caudal aspects of the rim of the donor left pulmonary venous orifice and of the corresponding recipient left atrial cuff. With the donor heart placed on its right side, and with its apex directed towards the right side of the pericardium (i.e. towards the surgeon, who is standing on the right side of the patient), the posterior wall suture line is performed in an end-to-end fashion (Figure 5). A running 5/0 polypropylene suture is begun inferiorly. Once the posterior wall is completed, the anterior wall suture line is carried out in the same manner.

The donor heart is then rotated to the left. Anastomosis of the recipient right pulmonary venous cuff with the rim of the donor right pulmonary venous orifice is performed in the same way as on the left (Figure 6).

These two anastomoses have to be performed with great care, as access to this region will be poor once the operation has been

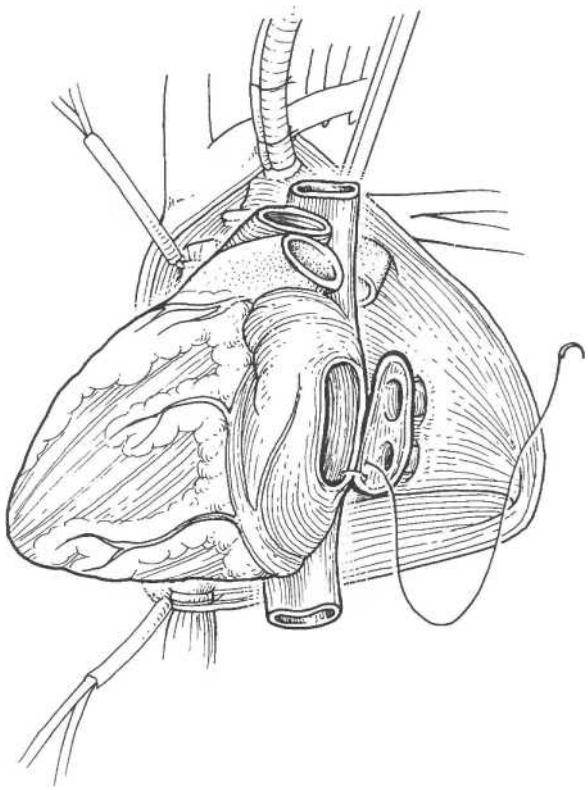

Figure 5 Anastomosis of left atria. The donor heart is rotated onto its right side with its apex directed to the right. The suture line begins inferiorly on the posterior aspect of the recipient left pulmonary venous cuff and rim of the donor left pulmonary venous orifice.

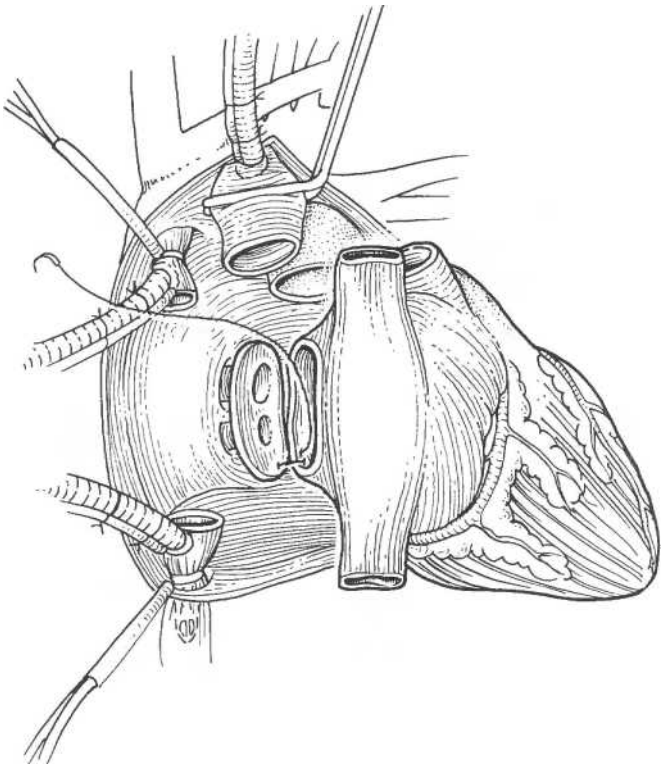

Figure 6 Anastomosis of left atria. The donor heart is rotated to the left. Anastomosis of the left pulmonary venous orifices has been completed. The recipient right pulmonary venous cuff is being anastomosed to the rim of the donor right pulmonary venous orifice.

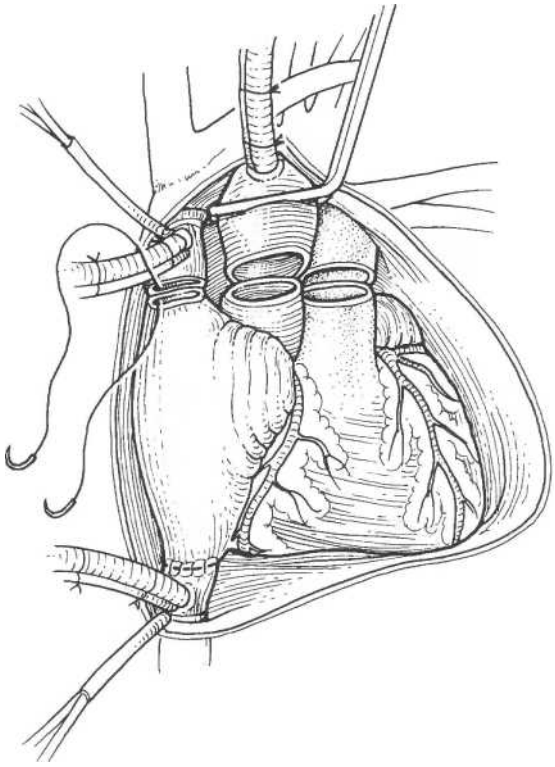

Figure 7 Anastomosis of venae cavae. The anastomoses of the venae cavae are performed in an end-to-end fashion. The IVC anastomosis is completed, and the SVC anastomosis is beginning

completed. However, if an extra suture requires placement, it has always been possible to gain access to the left atrial anastomosis either by retracting the ascending aorta forward or to one side, or by elevating the left ventricle.

Anastomosis of venae cavae

Both the SVC and the IVC are anastomosed in an end-to-end fashion (Figure 7). The IVC anastomosis is more of an atrial anastomosis than a caval anastomosis, as cuffs of right atrial tissue have been left both in the recipient and with the donor heart. This anastomosis is performed using a running 4/0 polypropylene suture. The recipient contribution to the SVC anastomosis is the recipient SVC, but the donor contribution can be either SVC or SVC-entry site into the right atrium (depending on the extent of dilatation of the native right heart and the size of the donor heart). (The presence of atrial cuffs in the recipient and on the donor heart has been recommended when there is considerable size mismatch between donor and recipient right atria[5].) The SVC anastomosis is performed with a running 5/0 polypropylene suture, and either can be performed immediately after the IVC anastomosis or, alternatively, in order to decrease ischemic time, can be delayed until the aortic anastomosis has been performed and the aortic cross-clamp has been removed (see Chapter 24). If the SVC anastomosis is to be delayed while the aortae are anastomosed, a clamp is placed on the donor SVC to prevent blood obscuring the field.

Anastomosis of pulmonary arteries

Both donor and recipient pulmonary arteries are trimmed to adequate length. Our personal preference is that the *donor* pulmonary artery should be cut short (just 1 cm above the pulmonary valve) in order to avoid any kinking of the pulmonary artery after anastomosis. (Alternatively, the *recipient* pulmonary artery can be cut short, although this renders access to the anastomotic site slightly more difficult.) We perform this anastomosis using a continuous 5/0 polypropylene suture (Figure 8).

Anastomosis of aortae

The aortae are trimmed to the required length and anastomosed using a running suture of 4/0 polypropylene (Figure 8). (This anastomosis can be performed before the pulmonary artery anastomosis if it is desired to reduce graft ischemic time.)

De-airing of the heart

As all cavities have been opened, de-airing of the transplanted heart remains crucial. Air needles should be placed after the SVC and IVC snares are released and ventilation is reinstituted. Our own routine is to place an air needle: (a) into the roof of the left atrium, (b) at the apex of the left ventricle, and (c) into the ascending aorta. A pad is placed posterior to the left ventricle, elevating the apex upwards to allow air to escape. Only then is the

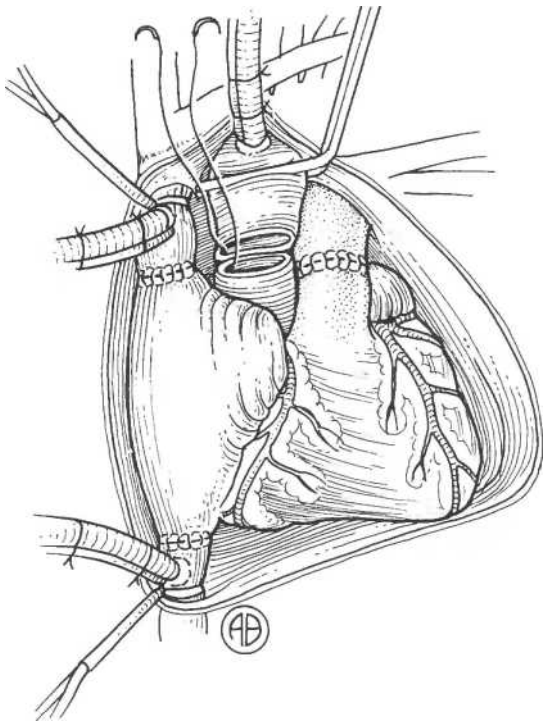

Figure 8 The pulmonary arteries have been anastomosed. The aortic anastomosis is beginning.

aortic cross-clamp released to allow reperfusion of the heart. When the heart either defibrillates spontaneously or is electrically defibrillated, both aortic and left ventricular needles are placed on suction for a few minutes to expel all air from the left side of the heart and to avoid distension.

Routinely, we initiate an isoproterenol infusion (at a dose of 0.05 $\mu g\ kg^{-1}\ min^{-1}$) at 10 min before unclamping the aorta. Pacemaker wires are routinely applied (even when the heart resumes sinus rhythm) to both ventricles and both atria, to allow sequential atrioventricular pacing if required (to prevent loss of atrial contraction if atrioventricular block occurs).

Discontinuation of pump-oxygenator support

The protocol for this part of the operation varies considerably between centers. As an example our own preference will be outlined. Both left and right ventricular function are monitored routinely by transesophageal echocardiography (TEE). After discontinuation of cardiopulmonary bypass we aim for a systolic blood pressure >100 mmHg, a heart rate <120/min, and a nondistended right ventricle (as judged by TEE). If hemodynamic status is compromised, pump-oxygenator support is resumed while pharmacologic adjustments are made (by increasing isoproterenol and adding epinephrine if necessary). When the right ventricle is distended, with or without pulmonary hypertension, we routinely use inhaled nitric oxide to assist in weaning from cardiopulmonary bypass.

Completion of operation

All suture lines are checked for bleeding, and extra sutures placed if required. (Access to the pulmonary venous anastomoses is poor.) The air needles are removed and the sites oversewn, if necessary. A metallic clip is placed at the margin of the apex of the right ventricle (just to the right of the left descending coronary artery) to assist in the performance of endomyocardial biopsy post-transplantation. The aortic and venous cannulae are removed. Protamine sulfate is administered. The pericardial cavity is drained using apical and basal drains. The sternum is closed.

COMMENT

The total technique can be considered an anatomical heart replacement. If care is taken when performing the SVC anastomosis to avoid a purse-string effect, the incidence of SVC stenosis is negligible. At our own center this operation has now been performed for many years with no incidence of SVC stenosis. However, the total technique is only an alternative to the standard technique, which has shown its efficacy for 30 years. Furthermore, the total approach is technically more demanding than the standard approach and, in general, takes rather longer to perform[6]. Others have modified the total approach, utilizing, for example, a single cuff of recipient left atrium incorporating all four pulmonary veins[7,8].

The bicaval anastomosis allows maintenance of the integrity of atrial conducting pathways, improving the likelihood of obtaining sinus rhythm, which is important for good early hemodynamics. A significant decrease in arrhythmias with improved atrial function has been shown after the bicaval technique[5,9]. The incidence of tricuspid regurgitation, and even of mitral regurgitation, is also reported to be lower than with the standard approach[6,10–12]. Early or late hemodynamic superiority, however, cannot yet be demonstrated conclusively, as many different factors (e.g. ischemic time, pulmonary vascular resistance, body surface area mismatch, etc.) play a role. Both exercise duration and exercise capacity, however, have been demonstrated to be improved in one study[10], and a higher cardiac index documented in the early postoperative period in another[5].

The introduction of TEE as a routine form of monitoring is fairly recent. It has been utilized by Angermann *et al.*[13] to demonstrate imperfections of the standard OHT technique. The following observations were made: (a) the atrial suture lines protrude into the respective atria, increasing the risk of thrombus formation (which proved more frequent than anticipated); (b) the presence of large recipient atrial remnants results in an abnormally large atrial volume (with an anteroposterior diameter up to twice the normal size); (c) the synchronous contractions between the recipient and donor atria lead to (i) pseudoaneurysmal behavior of the recipient atria and (ii) asynchronous opening and closing of both mitral and tricuspid valves.

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Immediate Postoperative Care and Potential Complications

D.K.C. COOPER AND N.M. LIDSKY

IMMEDIATE POSTOPERATIVE CARE

The immediate postoperative care of a patient who has undergone heart transplantation, whether it be orthotopic or heterotopic, is similar to that of any patient who has undergone open-heart surgery. Precautions need to be taken, however, to minimize the risk of infection, and in general in the first few postoperative days there is greater concern over potential pump dysfunction and chronotropic inadequacy when compared with other cardiac surgical procedures. Maintenance immunosuppressive therapy is begun immediately before the operation and is continued afterwards.

Patient monitoring

The patient will return from the operating room intubated and ventilated with a volume-cycled ventilator capable of providing intermittent mandatory ventilation and positive end-expiratory pressure. *In-situ* arterial and central venous (CVP) cannulae allow monitoring of pressures, and other intravenous lines allow replacement of blood and fluid, as well as administration of vasoactive drugs if necessary. A urinary catheter and electrocardiogram (ECG) electrodes are also in position. A central temperature probe (blood, bladder or rectal) may be *in situ*. Drainage catheters will drain both the pericardial cavity and the anterior mediastinum. If either or both pleural cavities have been opened, it is preferable to drain these cavities independently.

A Swan–Ganz catheter to monitor cardiac output and pulmonary capillary 'wedge' pressure (or an oximetric catheter to give additional continuous information on the systemic venous oxygen – SVO_2) is an advantage, but not essential. Following orthotopic transplantation, if donor heart function is clearly satisfactory, we do not feel that measurements of left heart pressures or cardiac output are necessary. Such observations may be essential, however, if donor heart function is poor, or there is significant pulmonary vascular disease. The complex anatomy of heterotopic heart transplants renders measurement of cardiac output difficult, but information gained on pulmonary artery and 'wedge' pressures may prove valuable.

The nursing staff will monitor (either continuously or at frequent intervals) all vital signs, which will include arterial and central venous pressures, heart rate(s), peripheral pulses, temperature, respiratory rate and ventilator parameters. They will also keep careful records of blood loss from the drains, urinary output, and blood, plasma, and fluid input. Measurement of arterial blood gases, serum electrolyte levels (particularly potassium) and blood glucose will be carried out at intervals as in any patient who has undergone open-heart surgery, and appropriate steps taken to maintain these parameters within the normal ranges.

Most centers involved in heart transplantation consider it an advantage to extubate the patient as soon as possible, to minimize the risk of pulmonary infection and barotrauma. It is argued that the presence of an indwelling endotracheal tube, which prevents the normal cough reflex and mucociliary tracheal clearance, and provides a conduit for entry of microorganisms is, together with the intermittent introduction of tracheal suction catheters, a major factor that predisposes to infection. Extubation should be carried out as soon as the patient is awake enough to cooperate with the nursing and physiotherapy personnel, and can cough when requested, as long as his or her hemodynamic state is satisfactory. This typically occurs within 24 hours of admission to the intensive-care unit, and sometimes almost immediately. The parameters and guidelines for weaning from the ventilator and extubation are the same as for any patient who has undergone open-heart surgery. These criteria are standard, and include: (a) acceptable gas exchange on an F_iO_2 <50% with PEEP <5 cmH_2O; (b) normal pH and PCO_2 during a trial of spontaneous respiration (i.e. CPAP with or without low levels of pressure support); and (c) no signs of excessive work of breathing, such as tachypnea, tachycardia, or use of accessory muscles. Early postoperative pulmonary infections may occur in patients in whom, for one reason or another, there has been a delay in extubation until 48 hours have elapsed, but fortunately remain relatively uncommon. There is, however, a higher risk of pulmonary infection should the patient require ventilation beyond this time.

Chest drains are removed when there is no risk of further bleeding and, in heterotopic heart transplant patients, in the absence of a right pneumothorax or significant pleural effusion.

Removal can generally be carried out within 48 hours, but we have experienced no complications from leaving the drains in longer, if indicated.

Initially, chest radiographs are taken daily until the chest drains have been removed and the patient is being mobilized, and then at less frequent intervals (unless otherwise indicated) to augment the clinical examination of the chest. The radiographs are taken primarily to confirm bilateral lung re-expansion and absence of pleural effusion, particularly after heterotopic transplantation, and also to exclude the presence of areas of consolidation that might suggest infection. The proper position of central venous or Swan–Ganz catheters needs radiographic confirmation.

Precautions to prevent infection

Meticulous attention to sterility is required by the nursing staff for all procedures affecting the patient. All personnel attending the patient should wash their hands on entering the room. At most centers, however, it is no longer felt necessary for personnel to wear face masks when attending the patient. Staff with obvious infection should not be involved in the care of heavily immunosuppressed patients, and nursing staff should ideally not be caring for two patients if one of them has a significant infection, for fear of cross-infecting the other patient.

It is used to be our policy to send several specimens each day for laboratory study to monitor possible early infection. Tracheal aspirate or sputum, blood, urine and swabs from the throat and from around the various drains and cannulae were sent daily for the first few days for culture to exclude significant bacterial growth. Urine and throat swabs (or gargle culture media) were sent for virological studies. Blood cytomegalovirus titers were estimated weekly. This intensive monitoring for infection was not found to be cost-effective, and has been discontinued. Specimens are sent now only when clinically indicated.

The patient should preferably be nursed in a clean-air environment. The number of hospital personnel entering the room should be kept to a minimum, and at this stage only close relatives should be permitted to visit the patient. We no longer believe that it is essential for personnel to change into operating-room garments or cover their outer clothing with a clean surgical gown, but frequent handwashing is essential. If blood is required for transfusion, it should ideally be CMV-negative, leukocyte-poor, and irradiated to reduce the risk of transfer of CMV.

All arterial and intravenous cannulae are removed as soon as possible, to reduce the risk of introducing infection into the blood either through or alongside the cannulae. A CVP line can be kept *in situ* for several weeks, if require, in patients who require such a line for any purpose, such as the daily intravenous administration of antithymocyte globulin, but meticulous care of the skin entry site must be maintained. The surrounding skin should be cleaned with iodine or other effective antiseptic, and a new sterile dressing applied daily or on alternate days. The literature concerning routine (e.g. weekly) elective prophylactic replacement of central venous catheters is inconclusive as to whether it has a beneficial influence on the rate of infection. We are, however, aggressive in monitoring for any clinical features of infection associated with the presence of a venous cannula, whether these are local (at the site of infection) or systemic (e.g. when there are positive blood cultures or fever of uncertain origin). If infection is suspected, a new cannula should be inserted in an alternative site. The tips of all removed cannulae should be sent for microbiological culture.

The sternal wound dressing should be changed daily and the wound inspected for signs of infection.

Respiratory therapy and physical rehabilitation

To ensure early re-expansion of the lungs, and to keep the airways clear of secretions, respiratory therapy is commenced within 6 hours of operation (as long as the patient is hemodynamically stable), and continued every 4–6 hours for the first 1–2 days, and then as often as necessary until the patient is fully mobilized, at which time it may be reduced or discontinued altogether. In the event of hemodynamic instability, initiation of respiratory therapy should be delayed or at least modified. In the intubated patient, respiratory therapy takes the form of vibration of the thoracic cage, postural drainage, and hyperinflation of the lungs by manual Ambu bagging to expand the lungs and loosen secretions, followed by tracheobronchial suction. In addition, it is advantageous to utilize 3–5 cmH_2O PEEP to prevent lung derecruitment, as this amount of PEEP represents the equivalent of 'physiologic PEEP' in the non-intubated state. A mucolytic agent may be injected into the airways of patients with viscid secretions, as well as bronchodilators when indicated.

After extubation, the nursing staff should encourage incentive spirometry and assisted coughing every hour when the patient is awake. If the effort on incentive spirometry is poor or marginal (generally <75–1000 ml per effort in an average-sized individual), IPPB therapy can be prescribed until respiratory muscle strength improves or other contributory factors, such as chest wall pain, are adequately addressed. In some extubated patients, use of CPAP or BiPAP, followed by assisted expectoration, may be indicated to prevent atelectasis and to keep the airways free from excessive secretions.

Within 24 hours of operation, if the patient is hemodynamically stable, passive muscular exercises are introduced, particularly to the legs to prevent deep-vein thrombosis and to strengthen the musculature. Active exercises, such as straight-leg raising, are introduced as soon as the patient can cooperate. As soon as possible, usually within 48 hours, the patient should be assisted to sit and stand out of bed at intervals of a few hours, and encouraged to become fully mobile over the next few days.

These patients have frequently been relatively inactive, or even bedridden, for some weeks or months before transplantation due to their underlying myocardial failure. Their muscles may have become weak and atrophied. Furthermore, the corticosteroids given to prevent rejection, particularly if given in large doses, contribute in most patients to a further wasting of the musculature that is so extreme in some to warrant the description of 'steroid myopathy'. A similar form of myopathy is believed to occur in hypomagnesemic patients receiving cyclosporin. Frequent and regular attention is therefore required from the cardiac rehabilitation staff if this muscle wasting is to be kept to a minimum and eventually reversed.

Each patient is prescribed a program of exercises which he or she is expected to perform frequently (e.g. every 2 hours) throughout the waking hours. This exercise program especially concentrates on strengthening the legs. Walking is probably the best exercise at this stage, but is frequently restricted by the pres-

ence of monitoring leads and intravenous infusions. Static dynamic bicycle riding is a good alternative, and is generally begun under the therapist's supervision within the first 3 or 4 days, as long as the patient's general condition does not contraindicate this more vigorous form of exercise. Initially, the bicycle workload is set low, and the period of time the patient spends on it restricted to 2 minutes, but both factors are steadily increased as the patient's recovery continues. Bicycle riding is ideally carried out four times each day, and the period of exercise increased up to 15 minutes per session.

During a moderate acute rejection episode, when the myocardium is edematous and undergoing cellular infiltration and myofiber injury, we believe it is only sensible to restrict the exercise of the patient in an effort to minimize permanent myocardial damage and potentially dangerous dysrhythmias. Vigorous exercise, such as bicycle riding, should be omitted, though gentle exercise and walking may continue. When rejection is severe, particularly if it is causing hemodynamic instability, then bedrest is mandatory.

Fluid balance and dietary guidance

Once the initial post-cardiopulmonary bypass diuresis is completed, it is easy for the patient to become overloaded with fluid, leading to increased right and left heart pressures, which can lead to graft dysfunction, and peripheral and pulmonary edema. This can result from the relatively large number of intravenous infusions and drugs that are required, and can be exacerbated by the patient's usual thirst (and desire to quench it) that occurs after extubation. The use of loop diuretics may be necessary to normalize volume status; intermittent intravenous boluses of furosemide, bumetanide, and/or ethacrynic acid usually suffice. Hemodynamic status should be monitored carefully during such therapy.

A balance has to be sought between ensuring that the patient does not become overloaded (which might lead to right heart dysfunction) and yet does not become hypovolemic (which might exacerbate the renal dysfunction that is commonly seen in patients who have been in cardiac failure pretransplant and who are receiving cyclosporin).

In the early postoperative period the diet should be fairly liberal, although salt intake should be restricted. Once the patient is eating satisfactorily, attention should be paid to ensuring that he or she receives a low-fat, low-cholesterol diet (Chapter 16).

Prevention of psychological isolation and boredom

It is rare today for a patient to require isolation in an intensive-care unit for more than a few days, but should the patient need physical isolation for longer, it is important to prevent boredom by providing such facilities as television and/or video, which the patient may make use of if he/she feels inclined. The occupational therapist may play an important role at this stage. The medical and nursing teams must watch for symptoms or signs of depression, as this can occur in this patient population and may occasionally be severe enough to require specific psychotherapy and/or pharmacologic intervention.

DRUG THERAPY OTHER THAN IMMUNOSUPRESSION

Infection prophylaxis

Prolonged prophylactic antibiotic therapy is to be avoided in the immunosuppressed patient, as there is a greater risk of this leading to the growth of resistant bacteria or fungi. Patients should receive a suitable antibiotic (e.g. a cephalosporin) at the time of induction of anesthesia, with further doses after operation to cover the first 24 hours. Further antibiotics should be given only when signs of clinical infection are present, and when the causative organism has been identified, if this proves possible.

Nystatin mouthwash ('swish and swallow') is given after meals and at night to prevent oral or esophageal candida infection. Trimethoprim sulfamethoxazole (Bactrim) is begun within a few days as prophylaxis against *Pneumocystis carinii* infection. Bactrim prophylaxis has virtually eradicated this life-threatening infection.

Cytomegalovirus infection remains the most frequent post-transplant infection, though it usually does not develop during the first post-transplant month. Prophylaxis is generally considered to be beneficial, either by preventing the infection or by delaying its time of onset and its severity. The ideal prophylactic regimen has not yet been determined, but intravenous ganciclovir, oral acyclovir, and intravenous immunoglobulins are all advocated by different groups. Many various combinations of these options have been adopted, and there is no general agreement on the optimum regimen. The CMV status of donor and recipient may be important in the timing and specific nature of the anti-CMV prophylactic regimen chosen (Chapters 32 and 57).

Our own policy has been to initiate oral acyclovir therapy (800 mg t.i.d. or q.i.d., depending on the size of the patient) before the patient leaves hospital, and to continue this for 3 months. This is combined with intravenous administration of commercially available immunoglobulin (500 mg/kg) or CMV hyperimmune globulin (150 mg/kg) on two occasions on post-transplant days 7 and 35. In patients deemed to be at high risk, for example those who have received excessive immunosuppressive therapy for acute rejection episodes, we prefer a 2–3-week course of intravenous ganciclovir as prophylaxis, which can be continued orally (or switched to oral acyclovir) for 3 months. The doses of both acyclovir and ganciclovir need to be adjusted in patients with impairment of renal function. This important topic is discussed more fully in Chapters 11, 32 and 57.

Vasoactive drug therapy

If inotropic support is indicated, isoproterenol hydrochloride has the advantage of increasing donor heart rate, which is considered desirable as the transplanted heart depends significantly on an increase in heart rate to increase cardiac output in the early postoperative period. When cardiac function is poor, additional inotropic agents (e.g. dopamine or dobutamine) may be required or exchanged for isoproterenol. Epinephrine may be life-saving in occasional cases. Phenylephrine hydrochloride by continuous intravenous infusion in low doses ($<2.0\ \mu g\ kg^{-1}\ min^{-1}$ per hour) is frequently beneficial in increasing peripheral vasomotor tone sufficiently to maintain an adequate blood pressure, without being detrimental to the blood flow to such essential organs as the kidney. Higher infusion rates, however, should be avoided.

With the exception of isoproterenol therapy being administered for its chronotropic effect only, if multiple agents are required for more than brief periods, we advocate the insertion of a Swan–Ganz catheter for meticulous hemodynamic monitoring, with the aim of using the least toxic regimen that will normalize the circulatory status. The technology is now available to continuously monitor cardiac output, cardiac index, stroke volume, right ventricular ejection fraction, systemic and pulmonary vascular resistances, and systemic venous oxygen (SVO_2). The principal parameters indicating potential inadequacy of vital organ perfusion are cardiac output, arterial blood pressure, and SVO_2, and these are supplemented by physical and laboratory measures, such as urine output, mental status, capillary refill and serum lactate level.

The stimulatory effect of calcium on the myocardium should not be ignored, and the intravenous administration of 500–1000 mg of calcium chloride or gluconate not only increases myocardial contractility (and blood pressure) but may also improve conduction, particularly in cases of heart block or bradycardia, as well as possibly enhance peripheral vasomotor tone in cases of systemic hypotension.

Intravenous vasodilator therapy with, for example, sodium nitroprusside or nitroglycerine, may be indicated in the early postoperative period to reduce afterload, as in any patient who has undergone open-heart surgery. Other agents that can be administered intermittently in less urgent situations include intravenous hydralazine and nicardipine.

Cyclosporine therapy may result in hypertension, though it is relatively unusual for this to be a problem within the initial few postoperative days. Nifedipine (10 mg given sublingually) is usually effective in normalizing the blood pressure rapidly, and frequently in maintaining it at a desirable level for several hours, if hypertension becomes dangerously high. If the patient is absorbing drugs through the gastrointestinal tract, nifedipine (beginning at 10 mg every 8 hours) or other suitable antihypertensive therapy is generally effective in controlling hypertension continuously and, in our experience, is often associated with an increase in diuresis, presumably through a vasodilatory effect on the renal arteries.

The role of prostaglandins (eicosanoids) in the treatment of pathophysiologic states of the cardiovascular system is being increasingly investigated. Their metabolism, physiology, and pharmacology in relation to the cardiovascular system have been reviewed[1]. If severe right heart failure is present after orthotopic transplantation, and if this failure is secondary to a high PVR, prostaglandin E1 (PGE1) has been shown to have a beneficial effect, often dramatic, when given through a central venous catheter[2,3] (Chapter 20). PGE1 can be administered as an infusion of 500 μg in 100 ml of normal saline at a rate sufficient to bring about a reduction in systemic pressure, which is usually within the range of 0.1–0.4 mg/kg per minute. The patient should also be receiving 100% inspired oxygen. Pulmonary hypertension is relieved in large part by the vasodilating properties of the drug. Theoretically, 95% of injected prostaglandins is extracted by the lungs in a single pass through the pulmonary circulation[4], so that systemic hypotension requiring high-dose vasopressors can usually be avoided. PGE1 inhibits the release of thromboxane, a potent vasoconstrictor and potentiator of platelet aggregation[5], and has several other effects which make it of value in this situation[6]. If PGE1 is to be used, the insertion of a Swan–Ganz catheter is essential. (Prostacyclin (PGI_2) may prove to be more valuable for right ventricular failure, but in the USA is still at this time undergoing FDA investigation.)

It has long been known that plasma free triiodothyronine (T_3) is reduced in patients on cardiopulmonary bypass[7–9]. Laboratory and clinical research increasingly indicates an inotropic effect of T_3 following a period of myocardial ischemia on cardiopulmonary bypass[9,10]. Although this form of therapy remains highly controversial, it should be considered after heart transplantation if all other therapy (short of the placement of a mechanical assist device) appears to be failing. An initial dose of 0.1–0.2 μg/kg is usually sufficient, with further doses of 0.1 μg/kg at 4- or 8-hourly intervals, depending on hemodynamic response, for 24 hours.

If vasoactive drug therapy fails to support either right or left heart circulations, then some form of mechanical assistance will be required. This may take the form of an intra-aortic balloon pump in patients with predominant left ventricular failure, or a mechanical assist device for either left or right ventricular failure. Our general philosophy is to institute such mechanical therapies early, in order to prevent a deteriorating cascade of further ventricular overload and enlargement which leads to worsening pump function. If a period of such support is unsuccessful in allowing donor heart recovery, then retransplantation may be the only remaining alternative.

Pain relief and sedation

Large doses of morphine and other central nervous system or respiratory depressant drugs should be avoided. Fortunately, median sternotomy is not associated with a great deal of pain, and small doses of morphine (1–2 mg i.v.) or similar analgesic (e.g. buprenophine 0.1–0.2 mg i.v.) are usually all that is required to keep the patient comfortable. Such doses rarely cause significant respiratory or hemodynamic depression. Fentanyl, given continuously as an intravenous infusion or as intermittent boluses, has gained increasing popularity. Rarely, a morphine sulfate patient-controlled analgesic pump may be required. Non-steroidal anti-inflammatory agents should generally be avoided because of the increased risk of gastric irritation and bleeding.

After extubation, adequate pain relief or, preferably, pain avoidance is essential if the patient is to be encouraged to cough adequately to prevent secretion accumulation in his/her airways. It is unusual, however, for a patient to require more than the above doses of analgesia and, in many, mild oral analgesics may suffice.

Anxiety may be a problem in some patients, particularly while still intubated. Small doses of diazepam (1–2 mg) or similar relaxant, given intravenously, should be administered when necessary. Midazolam and lorazepam are good alternatives.

Dysrhythmias

Complete atrioventricular (AV) dissociation (third-degree heart block) is not uncommon in the immediate post-transplant period. Data suggest that approximately one patient in 10 will demonstrate a prolonged period of heart block. Pacing wires should therefore be inserted into the right ventricular myocardium in

every patient at the time of surgery, and should be tested in the operating room. In those with AV dissociation, pacing should be continued until the heart recovers. As heart block can clearly be life-threatening in such patients, two myocardial pacing wires should be inserted and one non-active wire should be inserted in the skin. In the event that one myocardial wire becomes ineffective, for whatever reason, the second wire may prevent a catastrophe. It is essential to test the pacemaker's efficiency in the operating room and to ensure that the wires and box are well protected when the patient is moved from the operating room to the recovery room, or from the recovery room to the intensive-care unit.

The continuous infusion of intravenous isoproterenol may diminish the period of time that the patient is pacemaker dependent. Once a spontaneous rapid rate has been achieved, then the pacemaker should be set on demand on a low rate (e.g. 60–80/min) in case heart block recurs.

There have been reports that the oral administration of slow-release theophylline may stimulate a change from block to sinus rhythm in some patients. The recommended oral dose is 150 mg every 12 h, and this dose should be titrated to keep the heart rate above 90 beats/min for at least 24 h. At that time the dose can be weaned slowly, and discontinued if the heart rate stays above 80–90 beats/min. A usual dose of 150–300 mg every 12 h is required. Rarely, dosing every 8 h is required. Theophylline levels should be measured throughout this therapy, and should be maintained between 10 and 15 μg/ml.

This therapy should be used with some caution, however, as potential complications of theophylline include seizures, interaction with H2 blockers, and tachycardia. These complications can generally be avoided if the blood levels are measured at fairly frequent intervals and maintained within the desired levels. However, even at these levels some patients complain of a feeling of nervousness and anxiety, and occasionally the drug cannot be tolerated for these reasons.

In our experience theophylline has occasionally appeared to stimulate an increase in heart rate, but not invariably so. If complete AV dissociation continues for more than 3–4 days despite isoproterenol and/or theophylline therapy, or a marked bradycardia (heart rate <50/min) persists when isoproterenol is discontinued, despite theophylline therapy, it has usually been our policy to proceed to insertion of a permanent pacemaker. This is done partly to ensure the safety of the patient, as temporary pacemaker wires always place the patient at some risk through detachment. Furthermore, if the patient's discharge home is delayed purely because of continuing bradycardia or pacemaker-dependence, then we have found it more cost-effective to insert a pacemaker at an early stage, allowing the patient to be discharged home 24 h later.

In the majority of these patients the heart rate subsequently increases, and they no longer become pacemaker dependent. However, the incidence of a return of heart block in the future or temporary bradydysrhythmias is uncertain, and we believe it places the patient at less risk if a permanent pacemaker has been inserted in such cases.

Serious rhythm disturbances are relatively common in donor hearts that have suffered damage from prolonged ischemia during implantation. Following heterotopic heart transplantation, dysrhythmias of the recipient heart may occur, though these can often be ignored, unless they lead to hemodynamic embarrassment of the donor heart, which is rare.

When prescribing therapy, it should be remembered that the response of the denervated donor heart to therapeutic agents that have an effect in correcting dysrhythmias may differ from that of an innervated heart (Chapter 27). Calcium antagonists, such as verapamil hydrochloride, and beta-blocking agents have a greater effect on the denervated heart than on the innervated heart, whereas the donor heart is less sensitive to some other drugs – for example, digoxin.

Fluid retention

Diuretic therapy is almost always necessary during the first few postoperative days, particularly in patients who were in severe cardiac failure (and fluid overloaded) before operation. The fluid-retention effect of corticosteroids is a factor in many patients. A temporary loss of right and/or left ventricular compliance immediately after transplantation also leads to raised jugular and/or pulmonary venous pressures, which in turn may result in peripheral and pulmonary edema.

There is usually a good initial diuresis as the patient excretes the 5% dextrose cardiopulmonary bypass machine prime given at the time of surgery. This frequently continues for several hours. If the immediate post-cardiopulmonary bypass diuresis is poor, consideration should be given to its cause. If the patient is hypotensive or hypovolemic, then these factors should be corrected by increased inotropic therapy and/or phenylephrine infusion and/or fluid or blood infusion. However, a diuretic such as furosemide or bumetanide is frequently necessary. A relatively large dose may be required in patients who have been taking large and frequent doses pretransplant. Mannitol (50 g i.v. given rapidly as a bolus) is frequently helpful in resistant cases, although care has to be taken in patients who are already hypervolemic, as pulmonary edema can be induced. Ethacrynic acid is also frequently successful in patients in whom furosemide fails. Intravenous diuretic therapy is clearly preferable in the early post-transplant period when gastrointestinal absorption is poor, and when a rapid response is required. Occasionally, we utilize a continuous intravenous infusion of furosemide, as the resulting diuresis is reliable, titratable, and more dose-effective. This is desirable in patients with marginal hemodynamics. Oral diuretic therapy can be initiated later if necessary. Some centers advocate 'renal dose' dopamine infusion at 0.5–2.0 μg/kg per minute.

Cyclosporin (CsA), particularly if given intravenously, may occasionally be associated with an acute oliguria, which may respond to diuretic therapy, though in addition, reduction in the CsA dosage (or even omission of this drug) may be required for a period of time. It may be difficult to distinguish between oliguria from CsA toxicity and acute tubular necrosis from other causes. Measurements of CsA levels are clearly helpful in differentiating these conditions. Additionally, CsA is sometimes associated with a relative mineralocorticoid deficiency, which can lead to hyponatremia and hyperkalemia. These abnormalities are not usually severe, and generally require no specific therapy; however, when necessary, they can be treated with drugs such as fludrocortisone acetate.

Prevention of peptic ulceration

Patients undergoing major surgical procedures and receiving corticosteroids are clearly at risk of developing peptic ulceration. An H2 antagonist or antacid is begun immediately after operation to help prevent gastric erosion or stress ulcer, and should be continued at least during the first 3 months after transplantation, by which time steroid dosage should have been substantially reduced. More powerful agents, such as omeprazole, may be indicated in patients deemed to be at high risk (e.g. judging by previous history), or in those who develop any features of peptic ulceration (e.g. falling hemoglobin associated with stool positive for blood). Therapy should be continued in those considered at high risk until steroids have been discontinued or at least reduced to a low maintenance level (e.g. <0.1 mg/kg per day). (Occasionally, endoscopy is required to locate the source of upper gastrointestinal tract bleeding.)

Anticoagulation

Full anticoagulation is unnecessary in patients with orthotopic heart transplants unless there are other specific indications. Some groups prescribe an anti-platelet agent for the first 6 weeks, and others continue such agents indefinitely in the hope of delaying or preventing the development of graft vasculopathy (chronic rejection) (Chapter 35). Aspirin therapy should ideally be avoided in the early post-transplant period, for fear of inducing peptic ulceration and bleeding complications.

In over 200 consecutive heart transplants at our center we have documented one small cerebral embolus (presumably from thrombus developing on the left atrial suture line) which fortunately resolved rapidly and resulted in no permanent neurological deficit. This patient was subsequently anticoagulated for 6 months. The need to anticoagulate all patients with heterotopic heart transplants is discussed in Chapter 37.

Prevention of graft vasculopathy (chronic rejection)

It is generally considered that a diet low in lipids may reduce the rate of progression of chronic rejection (accelerated graft arteriosclerosis), particularly in patients with hyperlipidemia (Chapter 35). This diet should be initiated as soon as practicable in the postoperative period (Chapter 16).

Hypercholesterolemia

In patients with underlying ischemic heart disease who are known to be hypercholesterolemic, therapy with a cholesterol-reducing agent may reduce the rate of progress of atheroma in peripheral vessels. It is rarely indicated to begin this therapy in the early postoperative period, and generally it would seem wise not to add non-essential drugs until the patient is successfully through the critical initial month or so after transplantation.

Some of the newer agents, such as lovastatin, however, should be used with caution, in view of the risk of rhabdomyolysis which has been reported when they have been used in association with CsA[11,12]. Dosages of lovastatin of up to 40 mg/day, however, seem well tolerated, and rarely cause problems when given to patients also receiving CSA.

Whether such cholesterol-lowering therapy slows the development of graft arteriosclerosis remains in doubt, and is discussed further in Chapter 35.

Antituberculous therapy

Long-term antituberculous therapy is essential in patients who have contracted this disease in the past. In patients with features on chest radiographs of previous tuberculosis infection it has become our policy to administer isoniazid for at least the first 6 months as prophylaxis against recurrence. As with other drugs that are not critical during the first few days, it is probably wise to withhold this drug until the patient's hemodynamic, renal and hepatic status is good.

Mineral replacement therapy

Many patients who undergo heart transplantation have osteoporosis induced by inactivity and diuretic therapy. Further bone loss may occur from corticosteroid therapy. An attempt should be made to prevent or reduce such loss by dietary supplements of calcium, possibly given in association with vitamin D supplementation.

CsA neurotoxicity is associated with a low serum magnesium[13,14]. Hypomagnesemia is common in heart transplant patients receiving CsA, and it is probably beneficial to administer dietary supplements of magnesium to all patients at least during the first several weeks or months.

MAINTENANCE IMMUNOSUPPRESSIVE DRUG THERAPY

Immunosuppressive drug therapy should be begun immediately pretransplant, and continued in the post-transplant period according to the protocol of the individual center. This topic is discussed in Chapter 8.

EARLY POSTOPERATIVE COMPLICATIONS

Any of the complications of open-heart surgery can, of course, occur following orthotopic or heterotopic heart transplantation. Technical complications are today fortunately rare.

Hemorrhage

Hemorrhage is a potential early complication that may occur following the operation of either orthotopic or heterotopic transplantation. In both procedures there are long suture lines involving both low pressure venous systems and high pressure arterial systems. With care, however, postoperative bleeding should not be a major problem, though it is more likely to occur in patients who have undergone previous cardiac surgical procedures, and in those with coagulopathies, possibly associated with impairment

of hepatic function from long-standing right heart failure or from pretransplant anticoagulant therapy.

Technical complications

In the preparation of the donor heart for both orthotopic and heterotopic transplantation, care must be taken to avoid the region of the sinoatrial node. In heterotopic heart transplantation the recipient sinoatrial node must also be preserved.

Inadequate surgical technique may lead to narrowing at anastomotic suture lines, the pulmonary artery anastomosis possibly being the most vulnerable.

Wound infection

Wound infection is fortunately relatively rare after major cardiac surgery, including transplantation, but can, of course, be potentially disastrous in the immunosuppressed patient. Its treatment in the transplant patient follows the guidelines for any patient who has undergone open-heart surgery.

Systemic and pulmonary emboli

Patients with poorly functioning orthotopic heart transplants (e.g. from acute or chronic rejection) are at risk of developing ventricular thrombi, which may be embolized into the pulmonary or systemic circulations. Pulmonary emboli have also been reported arising from a residual recipient right atrial appendage following orthotopic transplantation[15]; this appendage should be excised at operation.

A fatal paradoxical cerebral embolus (via an unnoticed patent foramen ovale in the donor atrial septum) resulted from a deep-vein thrombosis in one of the patients in the Cape Town experience; closure of a large patent foramen ovale in either donor or recipient atrial septum should therefore be carried out at operation.

Complications of immunosuppressive therapy

Both early and late complications of immunosuppressive therapy are discussed in Chapter 8. Complications such as acute tubular necrosis and bleeding peptic ulceration, that may occur after any major surgical procedure, may be even more problematic in patients receiving drugs such as CsA and corticosteroids.

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Physiology and Pharmacology of the Transplanted Heart

J.B. YOUNG

INTRODUCTION

Heart failure is an extraordinarily complicated milieu. However, at the center of the difficulty is substantive impairment of cardiac pump function. Though the heart failure syndrome is characterized by profound perturbation of hormonal, humoral, neurologic, and cytokine systems, the root difficulty is impaired cardiac filling, decreased forward blood flow with peripheral organ hypoperfusion, and structural remodeling (hypertrophy and chamber dilatation)[1-3]. Many diseases cause myocardial injury that can impair normal cardiac contraction and relaxation. Therapies today have focused largely on ameliorating background difficulties prompting cardiac injury, and a great number of treatments used today attempt to interdict effects on perturbed humoral systems[4,5]. However, it has become clear that attenuation of peripheral physiologic abnormalities engendered by advanced heart failure cannot, alone, dramatically alter prognosis[6]. Replacement of the malfunctioning pump is necessary to significantly change long-term outlook.

Indeed, few things are more dramatic in medicine than observing the functional improvement that occurs when patients with advanced heart failure or cardiogenic shock undergo successful heart transplantation (HTx) with reversal of the heart failure syndrome[7]. Time has proven that HTx affords substantial physiologic rehabilitation in patients with severe heart failure. Functional limitations directly related to hemodynamic abnormalities improve dramatically and often resolve completely. It is clear that, in the strictly selected patient, HTx is vastly superior to any other therapy available to improve functional capacity and prolong life. Still, it is important to understand that cardiac allografts do not function entirely normally, and exercise tolerance in transplant recipients is less than one might anticipate[8]. Furthermore, because the transplanted heart represents a denervated organ system, response to many pharmacologic agents is not comparable to that noted in patients or individuals at large. It is important to understand the implications of altered cardiac physiology after HTx, particularly its relation to exercise physiology and pharmacotherapeutic intervention.

PHYSIOLOGY OF THE CARDIAC ALLOGRAFT

Factors affecting cardiac allograft function

Table 1 summarizes many factors which lead to alteration of cardiac allograft function. They include characteristic hemodynamic perturbations observed in HTx recipients, the important issue of afferent and efferent allograft denervation which persists after HTx, myocardial injury and maladaptation that can occur at the time of organ harvest, subsequent rejection injury, and preexisting donor cardiac pathology. It is fair to say that all cardiac allografts are abnormal to some extent and, with time, deterioration occurs because of rejection, drug toxicity, the often-present hypertension, and allograft vasculopathy (chronic rejection). The slope of functional deterioration is determined by static effects such as denervation and donor/recipient body size mismatch at implant, as well as dynamic factors such as intensity, severity, and chronicity of rejection and hypertension.

With respect to hemodynamic factors, one must remember that size of the donor, in relationship to size of the recipient, is extraordinarily important. Obviously, a heart geared to supplying output to an individual with a body surface area of 1.5 m^2 would behave differently when the demands of an individual of 2.5 m^2 present. Both afferent and efferent cardiac receptors are eliminated when the native heart is extirpated. Interdiction of normal neurohumoral control affects the manner in which cardiac output is regulated. Myocardial injury due to inflammatory assault is a constant issue in HTx recipients. This, combined with baseline injury occurring during donor organ preservation and recovery, complicated by acute and chronic ischemia (which seems ever present in cardiac allografts because of allograft vasculopathy), coupled with ventricular hypertrophy (developing because of hypertension), can cause significant organ dysfunction.

Insight gained from historic transplant paradigms

Initial models of HTx gave great insight into the function of these allografts. Indeed, Mann *et al.*[9] prophesied in 1933 that circulatory physiology after HTx would be 'excellent' if the 'bio-

Table 1 Factors affecting function of the transplanted heart

Hemodynamic issues
Donor/recipient atrial asynchrony
Early postoperative restrictive physiology
Late occult restrictive physiology

Allograft denervation
Afferent denervation
- Altered reflex control of peripheral vasocontraction/vasodilatation
- Altered Na^+/H_2O regulation via central-nervous-system-dependent vasopressin, renin, angiotensin, aldosterone secretion
- Absence of anginal syndrome during ischemia

Efferent denervation
- Absent vagal nerve control
- Rapid heart rate at rest
- Attenuated heart rate response to exercise

Hypersensitivity to circulating catecholamines
Exaggerated response to acetylcholine

Altered hormonal milieu
ANP secretion enhanced
Elevated exercise circulating catecholamines
Increased circulating paracrine peptides (endothelin)

Myocardial injury/maladaptation
Organ preservation and recovery injury
Intraoperative complications
Rejection
Ventricular hypertrophy
Hypertension (increased ventricular wall stress)
Allograft arteriopathy (ischemia)

Donor-related issues
Effect of brain death
Donor/recipient size mismatch
Age-related diastolic dysfunction
Pre-existing arteriosclerosis
Pre-existing hypertrophy
Pre-existing myopathy
Pre-existing structural heart disease

Modified from ref. 8.

logic factors' that invariably caused organ demise during experiments could be identified and controlled. Function of the transplanted heart was known at that time to be impaired virtually immediately postimplantation. It is likely that cell and humoral rejection caused rapid organ demise with systolic and diastolic dysfunction. Autotransplantation models, however, to effect complete cardiac excision with subsequent reimplantation, demonstrated the impact of cardioplegia, ischemia, denervation, and technical difficulties on replacement of the heart[10–13]. Indeed, these experiments were often performed to study the effects of innervation and denervation on the heart.

Observations after autotransplantation included the fact that right and left heart filling pressures were elevated (even in the absence of other known difficulties), gradually returning to more normal levels with time[14,15]. Interestingly, exercise tolerance in canine paradigms of autotransplantation approached that of control animals during follow-up[12,13]. Autotransplantation models also demonstrated that total blood volumes increased substantively compared with values obtained in control dogs[16,17]. A blunted diuretic and natriuretic response to volume expansion was also shown in these denervated canine auto-heart-transplant preparations[16]. It became apparent quite early that interruption of afferent neurocardiac innervation mediated, at least in part, circulatory system volume homeostasis. By interrupting the afferent neurofibers to the heart, a decrease in the opposition of sympathetic renal stimulation became apparent. This engendered a new volume steady state that subsequently translated into fluid retention with altered cardiac loading conditions[15–17]. We will subsequently detail these issues in human HTx, but it is apparent that the global circulatory milieu of cardiac allograft recipients is one of volume expansion. Very important was the observation that denervation of the heart caused myocardial catecholamine levels to diminish quickly[18,19] (as early as 1 week after autotransplantation), and this likely leads to loss of feedback inhibition of circulatory catecholamine increase. It is still important to remember that often transplanted dogs were able to exercise substantially, and for long periods, despite their cardiac physiology derangements.

Hemodynamics in human heart transplant recipients

Despite the fact that cardiac allografts are usually functioning more normally than the organs they replaced, hemodynamic derangement, sometimes extremely subtle, is generally apparent. Early reports of HTx in humans gave some insight into this, but these observations were confounded substantially by rejection. Initial studies[20] suggested that cardiac output was generally depressed in the early postoperative days, and that high central venous pressure was essential to maintain adequate flow. Atrial dynamics were quite abnormal[21–24], seemingly because of the mid-atrial anastomoses between donor and recipient hearts. This approach to allograft implantation allows varying portions of donor and recipient atria to be present, and the native atria do not contract synchronously with the allograft atria. Native sinus node electrical activity is not transmitted across the atrial suture line. Consequently, the normal atrial contribution to net stroke volume is generally less in the HTx patient than in normal subjects. Indeed, normally one would expect 15–20% of net stroke volume to be contributed by atrial systole. This does not appear to be the case in HTx recipients.

Recent reports suggest that improved left atrial transport and function can be achieved following orthotopic HTx by utilizing a bicaval and pulmonary venous anastomosis technique, the so-called, 'total' orthotopic HTx (Chapter 25). Freimark *et al.* recently reported[25] that HTx with this approach avoids the large atrial anastomoses of the standard biatrial implantation method. This group, utilizing Doppler echocardiographic techniques, suggested that greater ejection force and more normal atrial volume transmission could be achieved with this surgical approach.

Indeed, left atrial dimensions in the bicaval model are much smaller than in the biatrial anastomosis group, and the atrial ejection force is higher.

Early reports summarizing hemodynamics after HTx[26,27] generally noted that intracardiac pressures were normal at rest, but ventricular end-diastolic pressure increased rather dramatically during exercise. In fact, an evolutionary pattern of change in hemodynamics is characteristic of patients followed long term after HTx while receiving cyclosporine-based immunosuppressive protocols[28–30]. Again, complicating factors such as rejection and systemic hypertension have been emphasized in these reports. What has emerged is the fact that restrictive hemodynamic patterns are apparent early after HTx, but generally resolve within a short period of time (days or weeks)[30,31]. Despite the fact that the overt myocardial restrictive patterns disappear quickly, a subclinical and latent or 'occult' restrictive hemodynamic state persists. To observe this pattern, acute volume challenge is sometimes required[30]. A subtle abnormality is present in virtually every HTx recipient. The significance of this is not clear, but certainly it impacts on cardiac filling and likely contributes to biophysical remodeling. Persistently impaired ventricular filling, that is significant and overt, late after HTx can be seen in 10–15% of patients, and has been related to substantive allograft rejection[32,33].

It is important to remember that donor–recipient size mismatch may account for some of the restrictive hemodynamic patterns. Generally, donors are at least 70–80% of recipient weight, but variability can be greater. This would imply that most donor hearts are less than the desirable size for recipients. Hosenpud *et al.*[34] reported a significant negative correlation between donor–recipient weight ratio and resting heart rate, right atrial filling pressure, and pulmonary capillary wedge pressure at the 90-day observation point post-HTx. Individuals receiving a heart from a donor weighing substantially less had higher resting heart rates and ventricular filling pressures than those of other patients.

Many echocardiographic studies have been used to gain insight into anatomic and functional constraints of the cardiac allograft[32,35,36]. In studies performed when rejection (by endomyocardial biopsy) is not apparent, ejection fraction usually remains within normal limits over long-term (2–4-year) follow-up, but a substantially increased cardiac volume and end-systolic wall stress are apparent even in the absence of increased myocardial mass[31]. St Gore *et al.*[37] utilized Doppler echocardiographic techniques early after transplantation to assess left ventricular diastolic function serially and noted findings (isovolumic relaxation time, pressure half-time, and time-to-peak ventricular filling) that were suggestive of restrictive myocardial physiology. This compares nicely with right heart hemodynamic observations previously noted. Echocardiographic studies also demonstrated that these restrictive hemodynamic patterns normalize over the first postoperative month[30,31,35], and that the findings do not correlate well with preoperative pulmonary pressure or vascular resistance. Additionally, the restrictive myocardial changes were not influenced by cardiopulmonary bypass time, total ischemic time, or donor age.

In the absence of acute rejection, normal values for left ventricular ejection fraction during long-term follow-up are also, seemingly, normal. An elegant fluoroscopic analysis of radiopaque myocardial markers surgically implanted at the time of HTx[38,39] demonstrated that shortening fractions were within normal baseline ranges (obtained from non-transplant controls) and that an appropriate increase in cardiac output occurred during low-intensity exercise, resulting from augmentation of end-diastolic volume, with a subsequent increased left ventricular stroke volume. Utilizing resting radionuclide blood pool and angiography, Verani *et al.*[40] demonstrated that systolic ventricular performance of the transplanted heart, with respect to both right and left ventricular ejection fraction, was comparable to that of controls. Resting peak diastolic filling rates and time-to-peak diastolic filling rate were also normal. However, with exercise, significant increases occurred in left and right ventricular ejection fraction and peak diastolic filling rate, but the increases were significantly lower than in normal subjects. Again, these data correlate nicely with invasive hemodynamic and echocardiographic observations regarding cardiac function that indicate that cardiac allografts have mildly impaired ventricular function. Generally, stressing maneuvers are required to unmask these findings.

Though the effects of denervation on the cardiac allograft will be discussed separately, one consistent characteristic is that, without tonic vagal input, resting pulse rates are generally higher than noted in normal subjects[41]. Furthermore, rate acceleration with exercise is slower than in normal controls with lower peak heart rates achieved at similar degrees of exercise[35]. The higher resting pulse noted in cardiac allograft recipients does not respond to physiologic stimuli such as carotid sinus massage, Valsalva maneuver, or innervation-dependent pharmacologic therapy such as atropine infusion.

The exercise response of the cardiac allograft

Patients with cardiac allografts have diminished maximal exercise tolerance when compared with that of normal subjects. This is likely one effect of a subnormal ejection fraction and cardiac output during increased workload, as well as of an exaggerated increase in intracardiac volume[40,42–44]. The findings are generally subtle, and the longer the time period between HTx and observation, the less significant the hemodynamic perturbation may be, particularly if patients train aerobically. Elevated intracardiac filling pressure during exercise, in the setting of normal or reduced left ventricular volume, suggests that the cardiac allograft performs on a ventricular pressure–volume curve that is steeper and shifted to the left. This particular hemodynamic observation suggests that allograft ventricles are less compliant. Furthermore, allograft cardiac performance seems uniquely dependent on loading conditions, which in turn are clearly related to postural volume changes[42–44]. Rudas *et al.*[43,45] evaluated upright exercise hemodynamics 3 and 12 months after HTx, noting attenuated heart rate responsiveness, reflected by a slower heart rate acceleration, but rapid deceleration post-exercise after the first year. Compared to baseline studies, however, initial heart rate increase at 1 year seemed faster. Pulmonary capillary wedge pressure was lower at 1 year versus 3 months, both at rest and at peak exercise, with right atrial pressure also significantly lower at 1 year during exercise. This observation should be placed into the perspective of reports of exercise-induced hypoxemia in HTx recipients, because not all of the exercise and resting hemodynamic impairment noted in cardiac allografts is due entirely to changes within the heart.

Braith *et al.*[46] studied whether HTx was associated with an adverse effect on pulmonary diffusion capacity, and investigated the potentially deleterious effects of impaired pulmonary diffusion on arterial blood gas dynamics during exercise. The possibility exists that abnormal pulmonary diffusion occurred because of cyclosporine therapy, or because of pre-existing conditions known to affect diffusion adversely (such as congestive heart failure, primary lung disease, or pulmonary infection in an immunocompromised host). Patients underwent pulmonary function testing with bicycle ergometric exercise 3 months before and 18 months after HTx. Significant improvement in forced vital capacity, forced expiratory volume in 1 second, and diffusion capacity occurred; however, post-HTx vital capacity, forced expiratory volume, and diffusion were lower when compared with matched control subjects. Changes in arterial blood gases were similar among groups at 40% of peak power output, but at 70% of peak power output, arterial blood gases and blood pH were significantly lower in allograft patients having low diffusion capacities than in patients with normal diffusion and in control subjects. Cardiac index did not differ between the transplant patients with normal or low diffusion at rest or during exercise. Post-transplantation mean arterial pressure was related to exercise-induced hypoxia. It appears that abnormal pulmonary diffusion observed in patients before HTx can persist after the operation, with or without restrictive or obstructive ventilatory defects. When diffusion at rest is 70% of predicted, HTx recipients frequently experience exercise-induced hypoxemia. Obviously, this may have an effect on overall exercise capacity. Whether this is due to new infection, primary pulmonary disease, or a residual effect of heart failure is not clear[47–49].

Relating exercise performance post-HTx to other determinants of mobility, such as peripheral muscle function and performance, is also important. Patients with advanced heart failure often have skeletal muscle atrophy with metabolic abnormalities of muscle function[50–52]. Wilson *et al.*[52] recently studied this issue. Because the exertional dyspnea and fatigue associated with heart failure is frequently attributed to circulatory dysfunction, heart failure patients underwent hemodynamic monitoring during a maximal treadmill exercise test. The level of exercise intolerance perceived by patients with heart failure had little or no relation to objective measures of circulatory, ventilatory, or metabolic dysfunction apparent during the aerobic stress. This being the case, simply altering blood flow and pressure to more normal levels after HTx may not entirely address patient complaints of exercise intolerance. Intrinsic muscle oxidative problems may actually be exacerbated by immunotherapy post-transplant. Exercise testing focusing on peak oxygen utilization and time taken to reach anaerobic threshold is, then, often impaired post-transplant because of residual peripheral skeletal muscle difficulties originally created by the heart failure syndrome[50,51]. With increasing exertion, anaerobic metabolism is common quite early, and several studies have demonstrated that peak exertional oxygen utilization after HTx is surprisingly low. Savin *et al.*[53] demonstrated that HTx patients had higher peak lactate levels and ventilatory equivalents, but lower peak oxygen uptake and peak work rates, than those of normal control subjects. Exercise after HTx eventually induces tachycardia and an increased contractile state, but this takes time to develop and is seemingly related to the effects of increased circulating catecholamine levels. The dynamics of this exercise response may account for the fact that the maximal stress cardiac output after HTx is generally lower than seen in normal subjects[38,40,53]. However, transplant patients are capable of participating in most desired physical activities, and in comparison to their pretransplant state are dramatically improved.

The effects of cardiac allograft denervation

Human donor heart cardiectomy with subsequent HTx creates both afferent and efferent cardiac denervation[11,12,15]. Interruption of the afferent nervous pathways alters cardiovascular homeostasis by impairing renin–angiotensin–aldosterone regulation, and impeding the normal vasoregulatory response to changing cardiac filling pressure[54]. Loss of afferent nervous signaling is also the likely reason complaints of angina pectoris are absent in HTx patients experiencing ischemia or myocardial infarction. Chest pain in the cardiac allograft recipient is most often due to other etiologies[55].

Efferent cardiac innervation mediates sympathetic and parasympathetic nervous system effects on the heart. The absence, therefore, of vagal-mediated parasympathetic influences causes resting heart rate to be higher, and eliminates the influence on the heart of vasosignaling from the central nervous system[55–57]. Loss of autonomic innervation blunts the usual rapid changes in heart rate and contractility noted normally during exercise, hypovolemia, or vasodilation[35]. Also important is the fact that administration of beta-adrenergic blocking agents may have deleterious effects on the denervated heart. Because the denervated cardiac allograft relies on enhancement of ventricular performance through stimulation of myocardial beta-adrenergic receptors by circulating catecholamines[56,58], beta-blockade may blunt exercise-induced augmentation of cardiac function. Indeed, in a study by Verani *et al.*[58], beta-adrenergic blockade by propranolol administration produced a decrease in ventricular performance at rest, characterized by lower stroke volume index, lower cardiac index, and lower ejection fraction in both HTx patients and control subjects. Though the changes were similar in both groups, ejection fraction was significantly lower in the HTx patients. As the ejection fraction decreased, end-systolic volume increased substantially. In normal subjects there was a reduction in heart rate, but there was only a minimal reduction in ejection fraction and no change in end-systolic volume. These observations emphasize that, in the denervated HTx patient, circulating hormones appear crucial to maintaining reasonable exercise performance. Use of beta-blockers should be judicious.

The response of the denervated heart to other types of stress is also important to review. A canine model of the denervated heart studied by Tsakiris *et al.*[56] demonstrated that acute hypertension was well tolerated with only a slight decrease in cardiac output and a small increase in left ventricular end-diastolic pressure. Hypotension was less well tolerated with minimal reflex increase in cardiac output because of little, and late, heart rate responsiveness. Baroreflex-induced volume regulation after HTx has been demonstrated by Mohanty *et al.*[57] to be impaired. Central volume reduction (created by placing the lower body of the patient in a negative-pressure chamber) produced minimal reduction in forearm blood flow and only a slight increase in forearm vascular resistance (because HTx permits portions of

the native atria, with their accompanying sympathetic and parasympathetic innervation, to remain intact). This observation suggests that nerves arising in the ventricle rather than in the atrium or pulmonary vasculature constitute the afferent limb of this reflex. Additionally, it has been suggested that the inability to reduce blood flow (by vasoconstriction) to non-working muscles plays a role in limiting the maximal exercise capacity of HTx recipients[35]. Scherrer *et al.*[59] suggested that cyclosporine-induced hypertension (noted so frequently in HTx recipients) is associated with increased peripheral sympathetic nerve discharge, and that this effect may be exaggerated in HTx patients because of cardiac denervation.

There is evidence that reinnervation occurs in some patients after HTx. It has been demonstrated by immunohistochemical staining that human cardiac allografts contain viable intrinsic nerve fibers, though they remain extrinsically denervated[60]. Although ischemia-induced chest pain in the form of classic angina pectoris is said not to occur in HTx recipients, reports of this occurring in relation to ischemia are available[55]. This suggests that at least some HTx recipients undergo partial afferent reinnervation. Stark *et al.*[55] demonstrated a tyramine-induced cardiac epinephrine response suggesting reinnervation in two patients with angina pectoris and allograft arteriopathy. Wilson *et al.*[61], in a study of norepinephrine release in response to tyramine and sustained hand grip, concluded that it was likely that sympathetic reinnervation commonly occurred late after HTx, but that the pattern of reinnervation was widely variable.

More recently, Burke *et al.*[62] evaluated the functional effects of neuron reinnervation late (> 1 year) after HTx. Left ventricular and coronary hemodynamics in HTx and control groups were studied after stimulating sympathetic neurons with an injection of tyramine into the left coronary artery. Reinnervation was defined as the ability to measure cardiac norepinephrine release after the tyramine injection. Left ventricular pressure was also measured before and after tyramine infusion, as was coronary blood flow velocity, and coronary artery cross-sectional area (calculated by quantitative coronary angiography). In patients studied early after HTx, as well as those studied late, denervation was defined as no change in left ventricular function in response to tyramine. In HTx patients with reinnervation, left ventricular dP/dT rose significantly, but less than in control subjects. This serves to confirm the fact that cardiac allografts function abnormally, but subtly so, when compared to normal hearts. In both early- and late-denervated patients there was no change in coronary blood flow velocity in response to tyramine. These investigators concluded that, by stimulation of reinnervating sympathetic neurons with tyramine, a significant but subnormal increase in dP/dT and a transient decrease in coronary blood flow occurred, suggesting that reinnervating sympathetic neurons can produce physiologically meaningful changes in ventricular function and coronary artery tone. How frequent and what the physiologic significance is of reinnervation remains unclear.

Endocrine issues and the heart transplant

Atrial natriuretic peptide (ANP) is synthesized, stored, and released from myocytes of mammalian hearts, and has peripheral effects. The heart is thus an endocrine gland. Several observations in cardiac allograft recipients have given insight into the role cardiac innervation plays in the endocrine function of the heart. One hypothesis is that cardiac autonomic nerves trigger the release of ANP. Pepke-Zaba *et al.*[63] measured plasma ANP concentrations in the right atrium and main pulmonary artery, together with determination of pulmonary hemodynamics, in HTx recipients undergoing graded submaximal bicycle exercise testing during right heart catheterization. Pulmonary artery blood samples and hemodynamic measurements were obtained at rest, during peak exercise, and after recovery. Exercise significantly increased ANP levels in both the right atrium and the main pulmonary artery. It would appear that HTx recipients still retain the ability to increase ANP release in response to exercise, and that the mechanisms underlying ANP release are not entirely dependent upon the integrity of cardiac innervation. Unfortunately, these investigators did not determine whether partial reinnervation of allografts had occurred.

More recently, Bussieres-Chafe *et al.*[64] attempted to relate the influence of body posture and central hemodynamics on plasma levels of ANP. Central hemodynamics, mixed expiratory gas and ventilatory measurements, and venous blood for ANP determination, were obtained at rest and during graded cycle exercise. They also noted a change in exercise ANP from rest to exercise, and this change correlated with increases in pulmonary capillary wedge pressure, systolic pulmonary artery pressure, and right atrial pressure. There was no correlation between changes in ANP and peak oxygen consumption, heart rate, or mean arterial blood pressure. They concluded that in HTx recipients exercise is a stimulus for ANP secretion, and that augmentation in plasma ANP levels during exercise is modulated by changes in central hemodynamics. Again, the degree of reinnervation of these hearts was not determined.

Along these lines, Ationu *et al.*[65] studied expression of brain natriuretic peptide post-HTx. Observations in this group were determined over a broader time period (1–74 weeks post-HTx), and it was noted that a significant positive association between ventricular natriuretic peptide and time after transplant was apparent, suggesting that either a functional adaptation of the transplanted heart was occurring, or effects of reinnervation were present. The fact that, in HTx patients, plasma ANP levels may be elevated higher than expected by atrial stretch alone is a fascinating observation needing further clarification. The issue of immunologically mediated ANP secretion has not been resolved.

Other aspects of paracrine system perturbation are apparent in HTx patients. Indeed, cyclosporine-treated individuals exhibit a high incidence of systemic hypertension, and endothelin, a potent vasoconstrictor peptide of endothelial origin, may be important. Haas *et al.*[66] evaluated whether immunoreactive endothelin-1 detected in circulating plasma of HTx recipients was elevated, and if levels correlated with hemodynamic characteristics, cyclosporine concentration, or renal function. Indeed, plasma endothelin-1 was increased in the HTx population and this increase persisted during short-term follow-up. Endothelin-1 did not correlate with hemodynamic variables, serum creatinine, or cyclosporine levels. It seems, then, that endothelin-1 is increased after HTx for some reason, but there is no association with hemodynamics, renal function, or cyclosporine levels. This observation remains of

some concern because of the rather substantive hemodynamic effect of this paracrine peptide.

Electrocardiographic and electrophysiologic changes

Serial electrocardiographic (ECG) changes have been noted in the HTx recipient and, indeed, the earliest method of monitoring for HTx rejection utilized quantification of ECG voltage[67,68]. It is now generally accepted that this finding is not sensitive or specific enough to adequately diagnose allograft rejection. However, early observations noted substantive decrement in QRS voltage when rejection occurred. This undoubtedly reflected the attenuated electromechanical coupling that occurred when rejection was significant. Cyclosporine is said to alter this process.

Plainer ECG abnormalities are frequently noted post-HTx. Leonelli *et al.*[69] indicated that almost three-quarters of first postoperative ECG evidenced changes that would not be expected in normal patients, with a predominance of right bundle branch block being noted. Patient age, donor age, ischemic time, and prior drug therapy in recipients did not significantly differentiate between HTx recipients who had normal (as opposed to abnormal) early postoperative ECG. There are, seemingly, evolutionary changes taking place during the initial post-HTx hospitalization. Patients having progressive deterioration of the conduction system (manifest by widening QRS complexes) or worsening preexisting conduction defects have a higher early mortality. These changes therefore become an important prognosticator.

Approximately 20% of HTx patients during early postoperative follow-up demonstrate sinus node dysfunction with slow or no spontaneous depolarization[41,70]. Most frequently, these patients have junctional rhythms, with a lower resting heart rate (usually <70 beats/minute) than that of the majority of HTx patients[41]. Sinus node dysfunction may be caused by ischemic injury during graft harvesting, but rejection or, later, allograft vasculopathy may play a role. Furthermore, sinus node dysfunction has been described in patients who died in a sudden and unexpected fashion early and late after HTx[71]. Indeed, some patients have required permanent pacemaker implantation for persistent sinus node dysfunction[41,72]. In addition to the usual indications for permanent pacemaker implantation, some believe permanent pacing is indicated in HTx patients with unexplained recurrent syncope, or near syncope (particularly in the setting of allograft vasculopathy), as sinus node dysfunction may be intermittent and difficult to document[71].

Electrophysiologic studies performed after HTx demonstrate that atrioventricular node conduction times are similar to those of normal subjects both at rest and during pacing[73,74]. Atrial–His and His–ventricular intervals are also normal. Although the atrioventricular node alters conductivity relative to the rate of stimulation, this requires more time to occur in the transplanted heart[74]. Taken together, these observations emphasize that atrioventricular node impulse transmission control is an intrinsic function, with autonomic innervation enhancing this activity rather than being essential for underlying function.

It has been suggested that changes in atrial or ventricular pressure or chamber size modulate changes in electrophysiologic properties. Ellenbogen *et al.*[75] studied the coupling of mechanical and electrical alterations (termed mechano-electrical feedback) in the transplanted heart. This was done to avoid confounding influences of the autonomic nervous system on electrophysiologic measurements. Right atrial and ventricular pacing thresholds were measured using temporary epicardial pacing wires, with right ventricular monophasic action potential duration noted at 90% repolarization during right ventricular pacing at 600 and 400 ms. Also observed were donor heart rate, systolic, diastolic, and mean arterial and central venous pressures. Lower-body negative pressure was applied to change central volume and cardiac size. Lower-body negative pressure did not result in a significant change in any electrophysiologic variable despite significant changes in right atrial pressure. Thus, in the denervated transplanted human heart, unloading of the right heart results in no, or minimal, changes in atrial or ventricular pacing thresholds or ventricular monophasic action potential duration.

Ventricular arrhythmias in the HTx patient are not common. Arrhythmias tend to be either bradyarrhythmias or supraventricular tachycardia that is most often seen early post-transplant and usually in the setting of allograft rejection. Animal paradigms have suggested that cardiac denervation is an antiarrhythmic maneuver, particularly in terms of ischemia-related ventricular arrhythmias[76]. Indeed, there appears to be a low prevalence of ventricular tachycardia in long-term survivors of orthotopic HTx, with most agreeing that ventricular arrhythmias, when noted late, are associated more with development of allograft vasculopathy[75,77,78] and, when seen early, with acute rejection. The relationship of ischemic disease in the transplanted heart to bradyarrhythmias may actually be more important and ominous. Bradyarrhythmias should be considered when HTx patients complain of non-specific 'spells' or presyncopal episodes[71]. Importantly, it is an anecdotal impression that HTx patients do not sense their arrhythmias with the same frequency or intensity as non-transplant patients.

PHARMACOLOGY AND THE CARDIAC ALLOGRAFT

Primarily because of denervation, drugs affecting physiologic responses through autonomic nervous system stimulation are not usually useful in the transplanted heart. Atropine, for example, with its effects mediated by a parasympatholytic mechanism, does not speed the allograft ventricular rate when bradycardia is present[79]. Atropine is, mostly, a useless drug in HTx patients when bradycardia, sudden heart block or asystole develop. Direct-acting beta-adrenergic stimulating drugs such as isoproterenol or epinephrine must be used in these circumstances[79-81]. Likewise, edrophronium, a cholinesterase inhibitor, has no effect on heart rate[80]. Sympathetic agents, having direct effects on beta-adrenergic myocardial receptors, induce normal or even exaggerated effects on heart rate and cardiac contractility. Indeed, cardiac denervation may lead to an increased sensitivity of the denervated allograft to parenterally administered beta-adrenergic agents[81]. Exaggerated sensitivity to acetylcholine in denervated cardiac paradigms has also been observed[82]. Very importantly, because acetylcholine and the endogenous nucleoside adenosine have similar cardiac electrophysiologic effects, this drug must be used cautiously because of the greater changes noted in HTx recipients with denervated myocardial autonomic nodes[83]. The fact that a denervated sinus

node drives intrinsic heart rate means that care should be exercised to prevent bradyarrhythmia when the agent is used during diagnostic scintigraphic study, or to attempt cardioversion in patients having paroxysmal supraventricular arrhythmias (usually in the setting of rejection).

Because the electrophysiologic effects of digoxin are primarily on sinoatrial and atrioventricular nodes, but are mediated by sympathetic and parasympathetic nervous signaling, this agent has little electrophysiologic activity in the transplanted heart[84]. The inotropic effect of digoxin (which is not mediated by the autonomic nervous system) seems to remain intact. Utilizing digoxin to induce atrioventricular block in patients with atrial fibrillation after HTx is not usually effective. Furthermore, the effects of digoxin on baroreceptor mechanisms of blood pressure control will be minimal. Digoxin's role in patients developing systolic ventricular dysfunction after HTx (usually due to acute rejection (early) or allograft vasculopathy (late)) remains undefined, but is probably not great.

Certain antiarrhythmic agents (class IA drugs, such as quinidine and dysopyramide) have vagolytic effects that increase resting heart rate in non-transplant patients[85,86]. These changes are not observed in the denervated cardiac allograft and, instead, decreased sinus rate and increased atrioventricular conduction times often result when these drugs are administered.

Calcium channel blockers demonstrate attenuated responses with respect to electrophysiologic and electrocardiographic changes when compared to their effects in non-transplant patients. Diltiazem, a calcium channel blocker frequently used to control hypertension post-HTx (and believed by some to be effective in attenuating allograft vasculopathy), does not cause substantial decrease in heart rate in these patients[87,88]. Verapamil produces a slight increase in atrial–His interval[87]. The dihydropyrimidine calcium channel blocker, nifedipine, produces a minimal reflex increase in heart rate coincident with decrement in blood pressure, and has been shown to produce a slight decrease in the atrial–His interval[87].

HETEROTOPIC HEART TRANSPLANTATION

Heterotopic, or so-called 'piggy back', HTx has been performed much less frequently than orthotopic HTx, comprising <2% of all HTx reported to the Registry of the International Society for Heart and Lung Transplantation[89]. Nevertheless, there may be an important therapeutic place for this procedure and, particularly as donor-to-recipient size mismatch becomes problematic, it is likely to be performed more frequently[90–92]. The concept of utilizing a heterotopic HTx procedure is, in effect, using the allograft as a 'biologic' ventricular assist device[91] (Chapter 37). Generally, heterotopic HTx has been performed in the setting of very elevated pulmonary artery pressure, or when the donor size seems inadequate in relation to that of the potential recipient. Central circulatory hemodynamics are difficult to assess, since the heterotopic implant is placed in parallel to the existing circulation. Therefore, hemodynamic function of the heterotopic heart involves additional physiologic variables, such as the contribution to overall cardiac output by the native heart and the different loading conditions (such as pulmonary hypertension and ability/inability to fill the heterotopic right heart system). Because both donor and native hearts are beating in parallel but not synchronously, hemodynamic assessment of the relative contributions of the two hearts is difficult. However, heterotopic implants have been demonstrated to be capable of completely supporting a patient's circulation when the native heart becomes asystolic or develops ventricular fibrillation[91]. Additionally, with time, reduction in pulmonary hypertension may occur, and right heart hemodynamics that are similar to those following orthotopic HTx have been observed[92]. Heterotopic HTx can provide hemodynamic support sufficient to ameliorate many of the abnormalities seen in patients with advanced left ventricular dysfunction, and of considerable interest is the potential for the heterotopic HTx to reverse the biologic dysfunction of a dilated, failing native heart.

CONFOUNDING ISSUES REGARDING PHYSIOLOGY OF THE TRANSPLANTED HEART

Factors which may significantly alter function of the transplanted heart should always be considered. Possibly the most important is cell-mediated or humoral rejection, with its subsequent myocarditis. Mononuclear cellular infiltration and humoral antibody production, with concomitant complement system activation, can profoundly impair cardiac contractility, as well as affect coronary blood flow. The severity of these difficulties relates to the timing and dynamics of immunologic activity. During acute rejection, coronary vascular reserve is compromised and varying degrees of systolic and diastolic ventricular dysfunction can be observed. Profound reductions in cardiac output and ejection fraction with concomitant acute rise in ventricular filling pressure are frequently noted when rejection develops rapidly and is fulminant. Treatment of rejection often reverses these abnormalities, resulting in improved graft function[93,94]. However, permanent biventricular diastolic dysfunction may be the result of frequent, severe episodes of histologic rejection. It has been postulated that the restrictive hemodynamic patterns noted after HTx relate to histologic signs of rejection. Other long-term changes include increased left ventricular afterload and hypertrophy due to hypertension (which in turn contributes to the remodeling process and functional alteration noted in the long term).

Unfortunately, allograft vasculopathy remains frequent in HTx patients, and likely contributes to functional graft impairment. Left ventricular systolic dysfunction can occur in the setting of graft ischemic disease, and coronary angiography defines a high-risk subgroup for subsequent events such as acute myocardial infarction and heart failure[95]. Additionally, sudden cardiac death syndrome (frequently from bradyarrhythmic death) may be related to allograft vasculopathy.

Pre-existing disease in donor hearts may also account for dysfunction after transplantation. Older donor hearts, for example, may be at risk for having occult, but significant, coronary artery disease. Hypertension in the donor may have led to increased ventricular mass and diastolic dysfunction. It has recently been suggested that extending donor age to at least 50 years seems reasonably safe, but caution needs to be exercised when older donor hearts requiring significant inotropic support are used, particularly if the anticipated ischemic time is long[96]. This may put the patient at increased risk of receiving a poorly functioning allograft, with the attendant higher morbidity and mortality.

Confounding issues, such as pre-existing disease in the donor heart and rejection, may be important factors in failure of the allograft to perform adequately. Function of the heart is critically dependent upon eliminating or controlling these factors. Whenever one is evaluating function of a transplanted heart, the patient's status with regard to these confounding issues must be considered.

COMMENT

The transplanted heart is a denervated preparation that, in the absence of substantive rejection, significant allograft vasculopathy or hypertension, performs at rest in a similar but not identical fashion to a normal, healthy heart. Diastolic dysfunction is common early after HTx and may recur at later stages in some patients. This difficulty may be due to repeated episodes of acute rejection, hypertrophy and remodeling secondary to hypertension, or significant allograft vasculopathy. Cardiac reserve during exercise is usually adequate, but generally less than that seen in normal hearts. Augmentation of cardiac performance during stress is apparent, and is related to endogenous elevation of catecholamines and changes in diastolic loading conditions. In view of the preoperative functional limitations obvious in patients with advanced heart failure, cardiac allografts demonstrate a welcome degree of 'normal' physiologic function.

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28
Pathology of Cardiac Allograft Rejection. 1: Vascular (Microvascular)

E.H. HAMMOND

INTRODUCTXION

Pathologic descriptions of allograft rejection of all solid organs have long recognized vascular involvement in the rejection process. Renal allografts, the earliest allografts to be studied, commonly display vasculitis involving the arteries and arterioles of the kidney cortex with or without cellular infiltrates invading tubules[1-3]. This vascular inflammatory process, often termed acute vascular rejection, is frequently associated with allograft loss in spite of increased immunosuppressive therapy[2,3]. Similarly, arteritis has been identified in cardiac allografts on endomyocardial biopsy, and has been associated with poor allograft survival[4,5].

In all these studies, emphasis has been placed on involvement of the arterioles and arteries rather than on capillaries or venules. Since venules and capillaries are the sites of lymphocyte trafficking, as well as being the most important sources of tissue oxygenation, it is also important to consider the changes in these vascular structures in allograft rejection[6,7]. Studies of allograft rejection in animals have shown that the microvasculature is the earliest structure to be destroyed during rejection[8,9]. Destruction of the microvasculature of human skin allografts has also been shown to be the central event in first-set rejection; in fact, the destruction of the capillary bed is likely more damaging to the graft than piecemeal destruction of the graft parenchyma[10]. Although endothelialitis of venules is considered evidence of moderate acute rejection in the liver, and capillaritis is considered as an important component of acute allograft rejection in the lung, the importance of microvascular changes in cardiac allografts has been ignored until recently[11-16].

Microvascular rejection is also the morphologic picture of rejection seen in treated and untreated experimental cardiac xenografts. Studies in several different animal model combinations, including porcine heart to primate models, have documented that hyperacute microvascular rejection is associated with vascular accumulation of immunoglobulin and complement, and results in rapid loss of the xenograft[17-20].

In this chapter, pathologic features of rejection involving the microvasculature (capillaries and venules) are described as they have been studied and reported from the Utah Transplantation Associated Hospitals (UTAH) Cardiac Transplant program (530 patients receiving cardiac allografts between 1985 and 1995). Most of these patients were treated with immunoprophylactic protocols, including anti-lymphocyte globulin (ALG) or murine monoclonal antibody against the CD3 receptor (OKT3)[21,22].

The morphologic observations in this chapter detail the types of microvascular changes that can be encountered in endomyocardial biopsies (EMB), explanted hearts, and autopsies from such patients[14,16,23]. The histologic appearance of acute cellular rejection (Chapter 29) and the typical appearance of coronary artery vasculopathy (Chapter 33) are addressed elsewhere. A chronic generalized form of microvascular rejection leading to heart failure in transplant patients is described here, since this type of chronic vasculopathy is frequently observed in patients with microvascular rejection. This process has been designated 'global myocardial ischemic damage'[16].

In all histologic investigations it is important to remember that our observations are static, and that the rejection processes are dynamic. Thus, the real importance of types of infiltrating cells in a biopsy or the presence of immune complexes, *if not demonstrated consistently over time*, may not be relevant to the mechanism by which rejection occurs. Earlier publications have focused on the relevance of CD8- or CD4-positive lymphocytes in the rejection process involving vessels[24-26]. These observations are flawed because they try to relate human vascular changes to those of well-characterized animal models. The relevance or any finding, isolated in time, to long-term outcome of a graft must be shown with clinical correlative studies. Of course, the impact of immunosuppression, and its effectiveness in treatment of rejection, must also be taken into account. This is a particular problem in relating the experience in untreated experimental allografts to those of treated human ones[8,9,26-28].

IMMUNOCYTOCHEMICAL AND MORPHOLOGIC DETERMINANTS OF MICROVASCULAR REJECTION

Initial criteria for the diagnosis of microvascular rejection of cardiac allografts were the demonstration of immunoglobulin and

complement components co-localized to the microvasculature of frozen sections of EMB samples[13]. These features were deemed diagnostic because they correlated with subsequent hemodynamic compromise of the allograft in the absence of cellular infiltrates diagnostic of cellular rejection[13,14,23]. Frequently the biopsies also exhibited vascular and interstitial fibrin accumulation[13,16,23].

Since the original publication of the first prospective series of patients with microvascular rejection, other investigators have confirmed and extended these observations to include other immunocytochemical markers of microvascular alteration[29–34]. Faulk and Labarrere have reported elegant studies documenting the relevance of immunocytochemical studies of anticoagulant and fibrinolytic pathway markers in assessing microvascular damage. They have noted that antithrombin III (ATT), a marker of the natural anticoagulant pathway, is normally expressed by the arterioles and venules, and is lost in instances of microvascular damage[29]. Furthermore, tissue plasminogen activator (tPA), normally present in smooth muscle cells of myocardial arterioles, is similarly lost in allograft microvascular injury. Associated with this loss of tPA is the accumulation of microvascular complexes of tPA with plasminogen activator inhibitor-1 (PAI-1)[15,29,30]. Fibrin and plasmin are found in the interstitium of such biopsies. Whether these changes of anticoagulant and fibrinolytic pathway expression are results of, or precursors to, a vascular endothelial-specific immune response is unknown. However, the alteration of the expression of these molecules clearly precedes permanent microvascular injury of cardiac allografts and has been correlated with cardiac allograft loss[29,30].

Lones and his colleagues have also noted that EMB with microvascular rejection have a large number of intravascular macrophages accumulating in the microvessels, which are often confused with activated endothelial cells[31]. Immunocytochemically, using antibodies directed against macrophage antigens KP-1 and CD68, these cells can be shown to be macrophages. Antibody directed against factor-VIII-related antigen (FVIII ra) or CD34 can be used to highlight endothelial cells[16,31,35,36]. The prominence of intravascular macrophages has been documented by ultrastructural examination of EMB[32,33]. The association of prominent intravascular macrophages and dysregulation of fibrinolysis and anticoagulation is potentially important to our understanding of this process: macrophages are able to alter fibrinolytic processes directly and indirectly, at least in atherosclerotic lesions. Tipping *et al.* have reported that macrophages from these lesions are able to make PAI-1 and also stimulate production of PAI-1 by endothelial cells (but not stimulate tPA production), probably through augmented secretion of interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α)[37].

In the following classification of microvascular rejection, both the light microscopic and immunocytochemical findings will be described, since both are used to arrive at the appropriate classification. The methodology used for immunocytochemistry by immunofluorescence is the standard methodology used for investigation of renal biopsies and includes: (1) direct immunofluorescent examination of frozen unfixed endomyocardial biopsy sections with IgG, IgM, C3, C1q and fibrin, and (2) indirect determination of the presence of HLA-DR (MHC class II), ATT, and tPA[30,38].

It is not recommended that the sections be stained with antibody directed against the MHC class I backbone determinates since they are so pervasively present[39]. We do not recommend that EMB be routinely evaluated for immunophenotyping of lymphocytes, since studies of this type have not yielded clinically useful information[24–26]. Immunocytochemical demonstration of macrophages using antibody directed against KP-1 or CD68 may be a useful adjunct to show the intravascular localization. Antibody directed against FVIIIra or CD34 may also be used to highlight the endothelial cell changes in these biopsies. Since these later antibodies are used to extend histologic observations, they are best performed on unstained sections of paraffin blocks of the biopsies by immunoperoxidase techniques[16,35,36].

Other immunocytochemical studies may prove useful in the future. It has been suggested that the evaluation of biopsies for macrophage activation (CD14) and markers of lymphocyte activation, IL-2R (CD25) may yield important information relevant to prediction of rejection[40,41]. Others have evaluated vascular adhesion marker expression in cardiac allografts. A recent longitudinal study of 20 allograft recipients suggested that increased expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on the microvasculature is found temporally related to cellular rejection[42].

VCAM expression was restricted to venules surrounded by infiltrating CD3-positive lymphocytes, which may provide a necessary co-factor for its expression. The temporal relationship of ICAM-1 to rejection events is consistent with the proposed role of this adhesion molecule as a mediator of high-affinity binding and transmigration events, as well as its co-stimulator function in antigen-dependent activation of T lymphocytes[43]. Since ICAM can also be induced in ischemic injury, its presence may be somewhat non-specific. *In vitro* studies have shown that anti-HLA antibodies can also stimulate ICAM expression by cultured endothelial cells[44]. By contrast, endothelial leukocyte adhesion molecule-1 (ELAM-1 or E-selectin) preceded cellular rejection and was only expressed transiently. Its expression was highly correlated with subsequent rejection. In animal models of allograft rejection, ICAM-1 and VCAM-1 expression has been demonstrated during rejection using PER to detect mRNA; antibodies directed against VCAM-1 were efficacious in abrogating the rejection[45–48]. This finding is confirmatory of the relationship of lymphocyte adhesion and infiltration, but is not diagnostically useful.

If up-regulation of these adhesion molecules can be consistently demonstrated before infiltration of lymphocytes, or can be correlated with need for treatment, they may become important monitoring criteria. Similarly, studies in human and animal allografts have attempted to characterize cytokine expression using PER to detect mRNA. Even in isografts, mRNA of IL-1, IL-6, TNF, LT, and TGFb can be found. By contrast, mRNA of IL-2, IL-4, and interferon-gamma (IFN-γ) were found only in allografts. Use of this information in monitoring human allografts for rejection must await further study[49,50].

The archiving of frozen tissue on all EMB will be crucial to these approaches, since all of these markers can be found only on frozen sections using immunofluorescence, immunoperoxidase, or PER techniques.

These studies highlight the crucial importance of the endothelial cell in all rejection processes. Expression of adhesion molecules, cytokines, ATT, and PAI-1 is limited to microvascular

endothelium. Studies have shown that lymphocytes adhere only to these structures within the myocardium[43]. In addition, recent experimental evidence has shown that endothelial cells, stimulated by IFN-γ possess the necessary signaling capacity to cause T lymphocyte proliferation, without the addition of macrophages[44].

CLASSIFICATION OF MICROVASCULAR REJECTION

Microvascular rejection can be evaluated descriptively or semiquantitatively by immunocytochemistry. Categories are described below on the basis of histologic and immunocytochemical findings. Such features have been seen in human allografts as well as treated animal xenografts in which acute vascular rejection has been prolonged to several days or weeks[17–20].

The value of a classification of microvascular rejection is unknown. It is offered here to provide a framework for evaluation of biopsies. Criteria are summarized in Table 1. Specifically, there may not be any significance to the designation of *mild* versus *moderate* microvascular rejection. Morphologically they appear as a spectrum of rejection of increasing severity: they are distinct from *severe* microvascular rejection which is identical morphologically to severe cellular rejection (International Society for Heart and Lung Transplantation (ISHLT), grade 4)[51,52]. The designation of *equivocal* is offered to explain changes that may be found which are not diagnostic but may cause confusion.

No evidence of microvascular rejection

In this category are included all EMB which show no light or immunofluorescent evidence of vascular rejection. By light microscopy such biopsies show no endothelial cell swelling, endothelial cell necrosis, thrombosis or inflammatory infiltrates in the walls of microvessels. Interstitial edema and hemorrhage are not present (Figure 1).

By immunofluorescence, negative biopsies show no significant MHC class II expression by endothelial cells. Furthermore, no vascular accumulation of immunoglobulin or complement components is detected. Leakage by fibrin and albumin, which would indicate increased vascular permeability and endothelial cell activation and/or injury, is not seen. Examination of the biopsy for evidence of anticoagulant pathway expression shows strong uniform ATT expression of arterioles and venules without expression by capillaries (Figure 2). This is the normal pattern of expression in the heart[29]. Tissue plasminogen activator (tPA) is expressed exclusively by smooth muscle cells of the arterioles within the heart, also the normal pattern of expression[30]. The sites of these substances in allografts without rejection have been confirmed by double-labeling experiments[29,30].

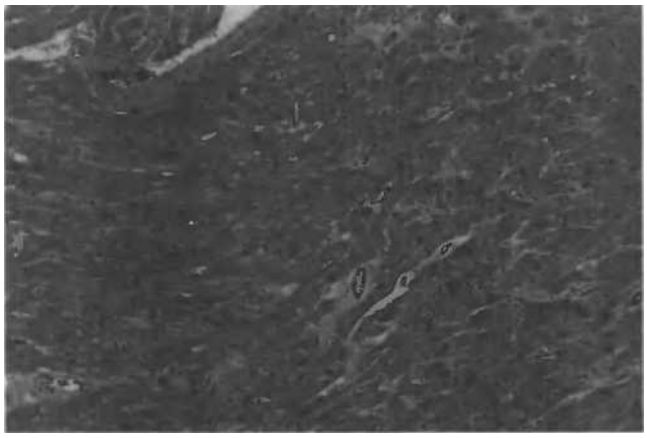

Figure 1 Photomicrograph showing the histologic features of an allograft biopsy without rejection or microvascular alteration. The capillary and arteriolar endothelial cells are flat. No interstitial edema or hemorrhage is present (magnification × 75). Used with permission from ref. 16

Equivocal evidence of microvascular rejection

By light microscopy, equivocal biopsies show histologic endothelial cell activation or damage with/without associated edema or hemorrhage. No inflammation or thrombosis is demonstrated in the walls of any capillaries, venules, or arterioles within the sample. Histologically, such biopsies are ISHLT grade 0. Since these features are very subjective, microvascular rejection can only be suspected on the basis of these findings (Figure 3). Similar equivocal findings can be seen in patients with systemic viral illnesses, especially those caused by cytomegalovirus[16,38].

Table 1 Vascular rejection criteria using immune complexes, fibrin, ATT and tPA

ISHLT Grade	*Vascular grade*	*Light microscopy*	*ATT/tPA*	*Fibrin*	*Ig/C*
0	Negative	Negative	Normal	0	0
0	Equivocal	Endothelial activation, edema, hemorrhage	Normal or equivocal	0	Ig or C
0	Mild	Endothelial activation, edema, hemorrhage, or focal vasculitis	Normal or absent	0 – 1+	Ig and C in BV 1+
0	Moderate	Endothelial activation	Absent	1 – 3+, also interstitial	Ig and C in vessels 2+
4	Severe	Diffuse aggressive polymorphous ± edema ± hemorrhage ± vasculitis	Absent	2 – 3+, vessels, interstitial with necrosis	Ig and C in vessels and interstitial

ATT = antithrombin III; tPA = tissue plasminogen activator (see text for explanation); Ig = immunoglobulin; C = complement

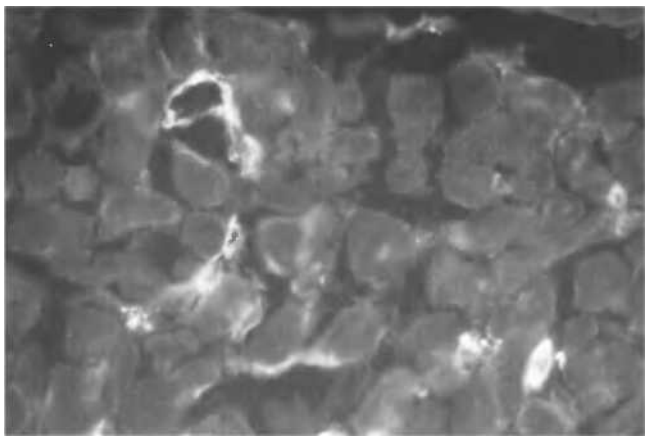

Figure 2 Photomicrograph of the usual appearance of antithrombin III staining of the vasculature of a normal endomyocardial biopsy. Strong staining by arterioles and venules is seen (× 187)

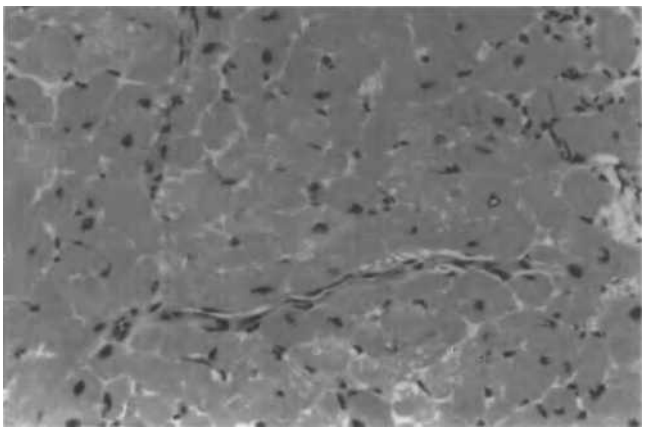

Figure 3 Equivocal histologic evidence of microvascular rejection or damage. Biopsy shows endothelial activation characterized by swelling of the capillary endothelium. No cellular rejection is seen (H&E × 187). Used with permission from ref. 16

Another equivocal histologic feature is the presence of focal myocyte necrosis or healing necrosis with granulation tissue. Focal ischemic injury is commonplace in the first weeks post-transplant in some transplant programs, and cannot be used to diagnose microvascular rejection. By contrast, the finding of myocyte necrosis, of either coagulation or contraction band type in the interval of months to years post-transplant, is a feature strongly suggestive of either allograft vasculopathy or global ischemic damage which is a consequence of long-standing microvascular rejection[14,16].

By immunofluorescence, such equivocal biopsies may show microvascular accumulation of immunoglobulin or complement components, but not both (Figure 4). Furthermore, vascular leakage may be demonstrated by seeing extravasated albumin in the absence of vascular immune complexes[38]. These features indicate that diffuse vascular permeability is present. Thus, these features are quite non-specific and cannot be used to specifically diagnose vascular rejection or vascular damage. MHC class II

Figure 4 In this immunofluorescent photograph, equivocal staining of vessels is seen with fluoresceinated antibody directed against IgG. Compare with the staining present in mild or moderate vascular rejection, seen in Figure 10 (× 178). Used with permission from ref. 16

antigen expression may be up-regulated on the microvasculature. Expression of ATT is of equivocal significance if loss of ATT is from venules but not arterioles. Tissue plasminogen activator may show focal loss from arteriolar smooth muscle[29,30].

These changes are ubiquitous in the first weeks post-transplant in patients undergoing induction immunosuppression with monoclonal anti-CD3 (OKT3) which has been shown to produce transient lymphocytic activation and release of cytokines, such as tumor necrosis factor (TNF) and interleukin-1[53,54]. Since these factors lead to vascular permeability and endothelial activation, it is not surprising that patients show these features during OKT3 therapy[54–58]. The clinical syndrome associated with OKT3 can be abrogated by treatment of patients with antibody directed against TNF[46,47]. This provides evidence of the importance of this cytokine in mediating these effects.

Mild microvascular rejection

The presence of leukocytoclastic vasculitis, in the absence of cellular rejection, qualifies the EMB for a diagnosis of mild vascular rejection. This vasculitis involves venules and capillaries, but spares arterioles. The vessels may show prominent accumulation of nuclear dust, and the invading inflammatory cells are lymphocytes and macrophages and rarely neutrophils (Figure 5). Alternatively, the biopsy may be deceptively innocuous, showing only interstitial edema and/or hemorrhage with endothelial cell activation (which may be partially activated macrophages) and no inflammation (Figure 6). This is the type of biopsy in which immunofluorescence is particularly useful, since one can be misled by the light microscopic appearance, which is indistinguishable from the appearance of equivocal biopsies.

By immunofluorescence the EMB will show co-localization of immunoglobulin and complement components in capillaries and venules, with possible intravascular localization of small amounts of fibrin (Figure 7). Rarely, vasculitis can be caused by cellular immune mechanisms and, in such cases, immune complexes in vessel walls are not demonstrated. We have seen this pattern of

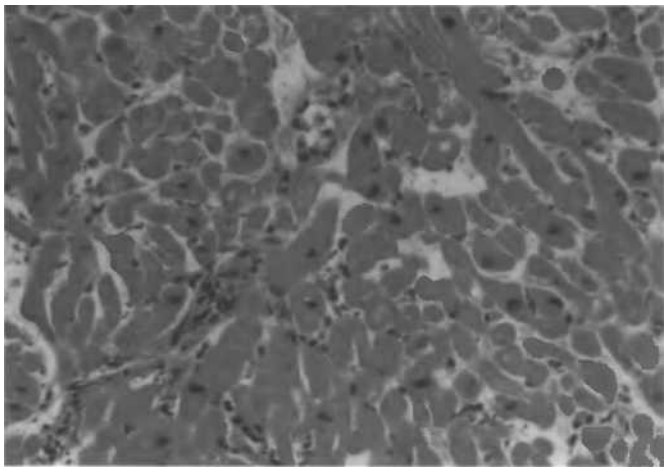

Figure 5 In addition to endothelial activation, capillaries in this biopsy show vasculitis. No cellular rejection is seen. Interstitial edema is obvious (H&E × 175)

findings in only three of 75 patients with vascular rejection, but it has been more frequent in the experience of others, especially in the absence of immunoprophylactic therapy[15,59,60].

Biopsies with mild vascular rejection consistently demonstrate alterations of the natural anticoagulant and fibrinolytic pathways. The microvasculature exhibits partial loss of ATT (on venules) and tPA (in smooth muscle cells of arterioles) or complete loss of ATT on all arterioles and venules and loss of smooth muscle tPA[15,29,30]. Up-regulation of MHC class II is also uniformly seen[16,61].

Moderate microvascular rejection

In this category, vasculitis may be extensive and arteriolitis may be found (Figures 8 and 9). Alternatively, patients with moderate microvascular rejection may show no vasculitis and may only show severe interstitial edema with a blue fibrillar appearance of

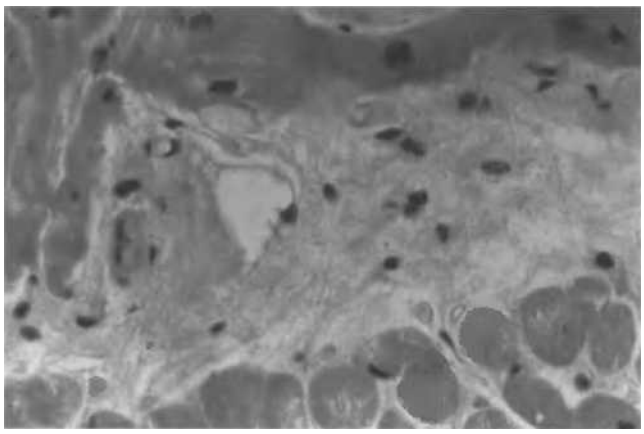

Figure 6 In this photomicrograph, at high magnification, an edematous area of interstitium is visualized. The fibrillar character of this edematous change is evident. Patient had positive immunofluorescence for immunoglobulin and complement (H&E × 30). Used with permission from ref. 16

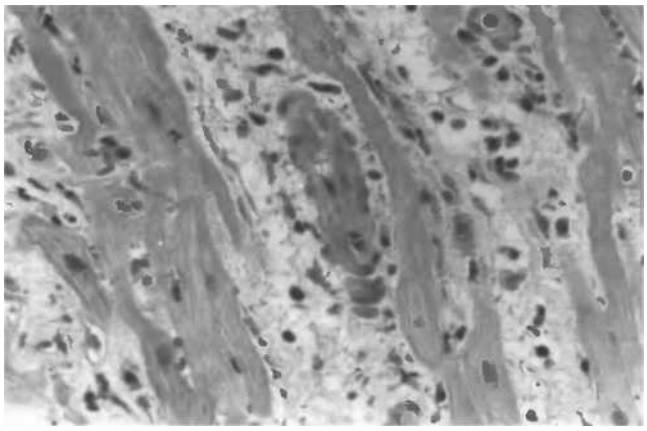

Figure 8 Biopsy with moderate vascular rejection. Capillaries, venules and arterioles, such as this one, had vasculitis. Interstitial edema and hemorrhage are seen. Particulate debris surrounding the arteriole is apparent. No cellular rejection is present (H&E × 300). Used with permission from ref. 16

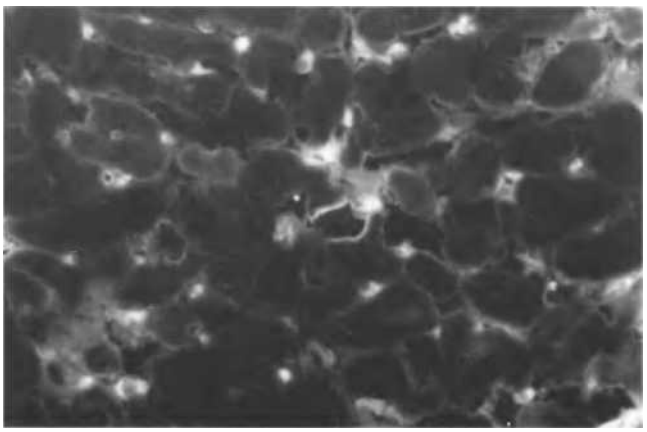

Figure 7 Frozen myocardium from patient with mild vascular rejection. Tissue was reacted with fluoresceinated antihuman IgG. There is a punctate pattern of staining, indicating vascular localization in round vessel profiles. Complement was present in an identical distribution. Compare with Figures 4 and 10 (× 178). Used with permission from ref. 16

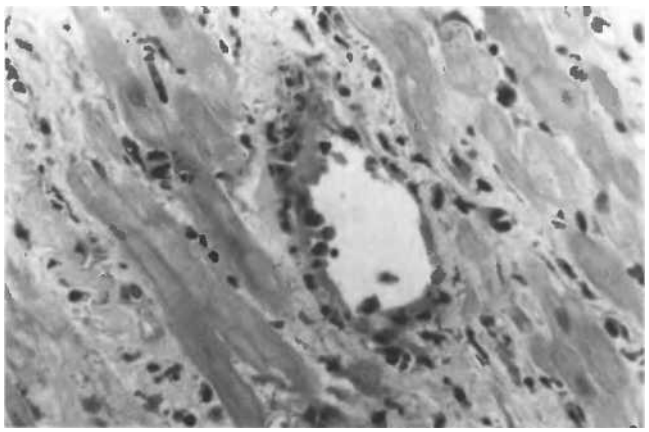

Figure 9 Moderate vascular rejection. This venule shows obvious inflammation. Interstitial edema is prominent. The immunofluorescence examination showed vascular accumulation of IgG and complement in capillaries and fibrin within the interstitium (see Figure 10). Used with permission from ref. 16

the interstitium, which has been shown to be associated with fibrin accumulation (Figure 6). In such patients it is critical to review the previous biopsy, to see whether or not the process is worse or better, in order to make an adequate assessment.

By immunofluorescence, moderate microvascular rejection usually shows obvious accumulations of immunoglobulin and complement components diffusely within capillaries and venules (Figure 10). In some cases, particularly in long-standing vascular rejection, only intravascular and interstitial fibrin is detected[14,16] (Figure 11). In biopsies of patients with moderate microvascular rejection, ATT and tPA are usually completely lost, and tPA–PAI-1 complexes are often present. This pattern of expression is often associated with clinical hemodynamic compromise[15,29,30] (Figure 12). Immunofluorescence is helpful in this setting because light microscopy may merely show interstitial edema without evidence of vasculitis. Such EMB may show piecemeal myocyte necrosis or subendocardial infarction, either

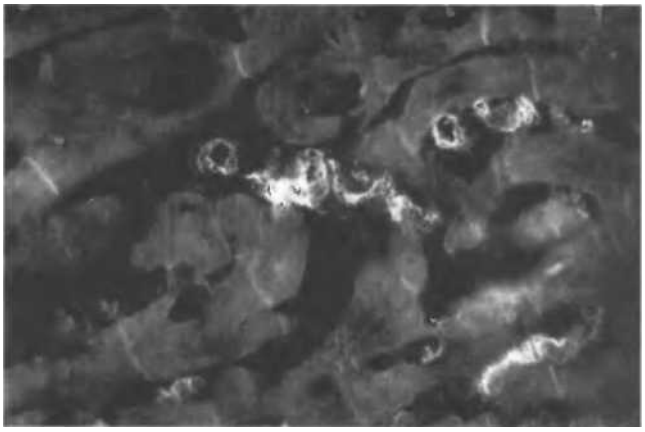

Figure 10 Immunofluorescent findings in moderate vascular rejection. Capillaries showed co-localized IgG and C3. The complement staining is shown here. Fibrin was abundant in the interstitium, and antithrombin-III was not found on arterioles or venules, consistent with moderate vascular rejection (× 178). Used with permission from ref. 16

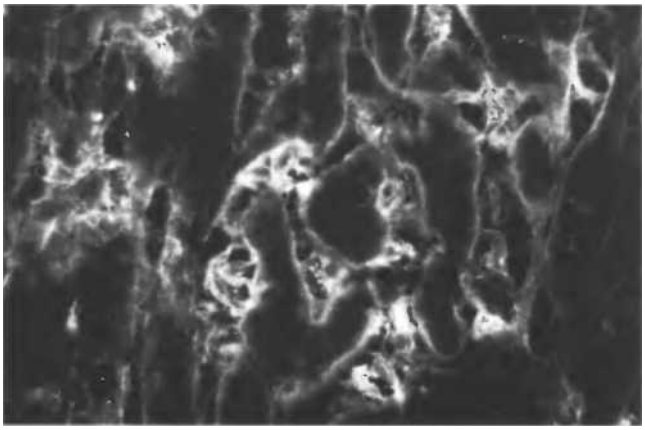

Figure 11 Fibrin localized by immunoflurescence is illustrated. Frozen myocardium is stained with fluoresceinated antibody directed against fibrin. Same biopsy as Figure 10 (× 178). Used with permission from ref. 16

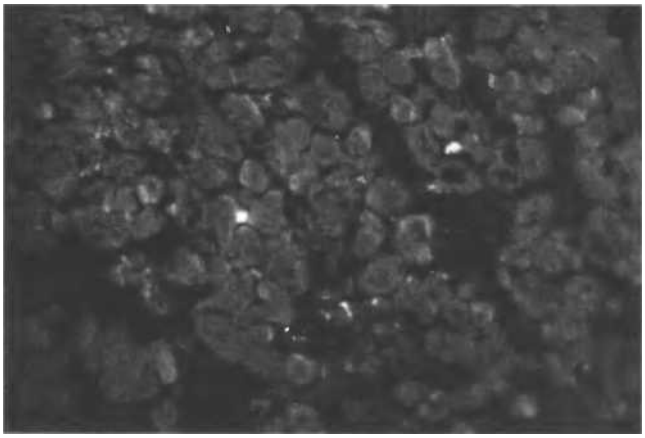

Figure 12 This photomicrograph of tissue reacted with antibody against antithrombin-III shows no staining of venules in the biopsy. Only two fluoresceinated dots are seen, which are artifactual. Compare with Figure 2, the normal distribution for ATT. Both slides were exposed for 30 seconds at the same magnification (× 178)

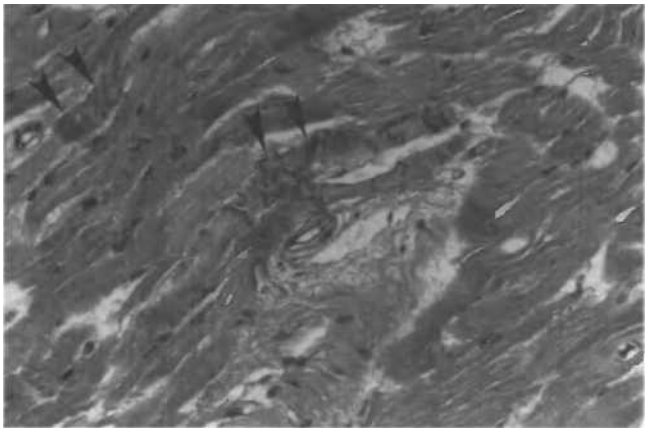

Figure 13 This biopsy from a patient with persistent vascular rejection for several weeks shows contraction band necrosis of myocytes without surrounding inflammation (arrows, H&E × 300). Used with permission from ref. 16

of which is evidence that larger arterioles, not included in the biopsy, may have vascular compromise (Figure 13). This is particularly true in the early months post-transplant, when it is very unlikely that the process could be related to epicardial coronary vasculopathy. Myocyte necrosis without inflammation, detected in the first few weeks post-transplant, may be caused by prolonged ischemic time or perisurgical hypoxia[62]. In our program such myocyte necrosis is very uncommon; other programs have reported that as many as 80% of early biopsies show this feature.

Severe microvascular rejection

Severe microvascular rejection is morphologically indistinguishable from severe cellular rejection (ISHLT grade 4). It is the end-result of any severe rejection process. The EMB shows a diffuse, mixed leukocytic infiltration including neutrophils and eosinophils (Figure 14). Myocyte necrosis and interstitial edema and hemorrhage may be prominent. Vasculitis is obvious.

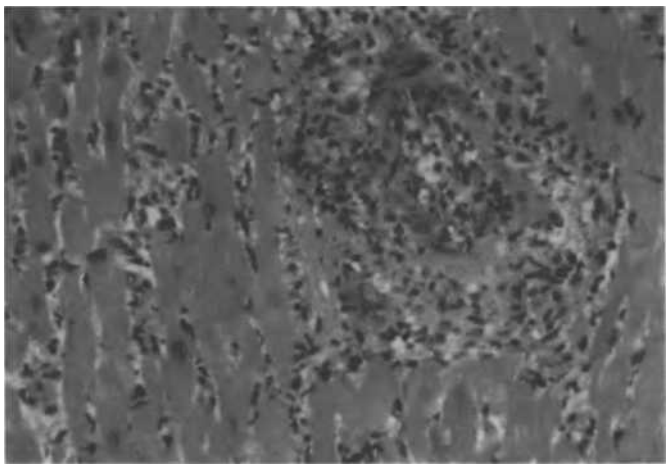

Figure 14 Histologic appearance of severe cellular and vascular rejection: arteriolitis is seen as well as other vasculitis. There is a mixed cellular infiltrate with myocyte necrosis and prominent intercellular edema. Immunofluorescence showed loss of antithrombin-III and extensive fibrin, immunoglobulin and complement in vessel walls (H&E × 175)

Immunocytochemically, biopsies with severe cellular/vascular rejection will often have vascular deposits of immunoglobulin and complement, as well as interstitial and vascular accumulation of fibrin. Complement components may also be distributed in the interstitium. Examination of biopsies for ATT and tPA will show loss of these reactants. Some tPA–PAI-1 complexes may be present[15,29,30]. The microvasculature may paradoxically show lack of MHC class II expression, resulting from the relentless vascular injury. This can be highlighted by immunoperoxidase staining of vessels with factor VIIIra[16]. In severe rejection, endothelium is ragged or frayed, or may show areas where endothelial cells are missing[14,16] (Figure 15).

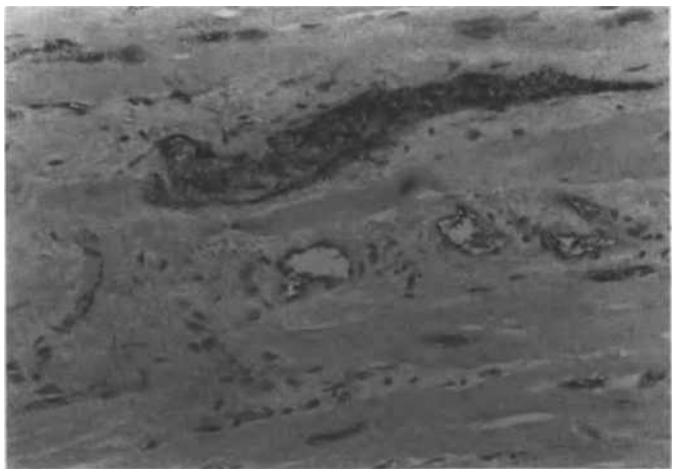

Figure 15 Vessels in this case of moderate microvascular rejection show ragged endothelial surfaces in this factor-VIIIra-stained section. The vessels from a biopsy with severe microvascular or hyperacute rejection would appear identical. (Immunoperoxidase method, counterstained with hematoxylin × 280). Used with permission from ref. 16

Severe hyperacute (microvascular) rejection

Hyperacute rejection of cardiac allografts is very rare and catastrophic. Acute cardiac dysfunction results from deposition of preformed antibody (IgG or IgM) and complement components in the microvasculature of the allograft[63]. This process has been reported to occur even in the presence of a negative lymphocytotoxic crossmatch[64]. Allograft dysfunction results from endothelial damage, vascular permeability, interstitial edema and hemorrhage, which cause myocardial ischemia. If the process persists for several hours an infiltrate of neutrophils within and around vessels can be seen.

Untreated xenotransplantation routinely leads to hyperacute rejection in discordant animal species where it has been investigated most thoroughly[17–20] (Chapter 81). The pathologic process is characterized by prominent interstitial edema followed by interstitial hemorrhage and swelling of the capillary and venular endothelium. Inflammatory infiltrates are not a feature, because of the rapid time-course of the process which leads to xenograft loss within minutes or hours. Hyperacute rejection is mediated by deposition of xenospecific antibodies in the donor heart. If the process is abrogated by depletion or inhibition of natural antibodies, or inhibition of complement activation, the histologic findings often include inflammation and venular thrombosis. Ischemic myocyte injury with myocytolysis and eventual coagulative necrosis is seen in xenografts surviving several weeks. A pattern of rejection identical to severe mixed acute cellular and vascular rejection (ISHLT grade 4) is observed in xenografts in which complement function returns (after 25 days)[17,19].

RELATIONSHIP OF MICROVASCULAR REJECTION TO ISHLT GRADING

The current ISHLT grading scheme for cardiac allograft rejection does not include provision for grading of the above-described processes[51,52]. The relationships between these microvascular grades of rejection and the ISHLT grading schema (revised in 1995) are shown in Table 2[52]. Because features of the microvasculature are ignored in this grading schema, the presence of microvascular changes causes difficulty in interpretation, and leads to interpretive disagreements, even among experienced pathologists[65]. However, since the incidence of microvascular rejection and the proper treatment for this pathologic entity are

Table 2 Comparison of UCTP and ISHLT grades

Cellular rejection: variations of ISHLT and UCTP grades
Focal mild rejection: ISHLT 1A
Mild rejection: ISHLT 1B and 2
Moderate rejection: ISHLT 3A or 3B
Severe rejection: ISHLT 4
Vascular rejection: variations of ISHLT and UCTP grades
Mild vascular rejection: ISHLT 0
Moderate vascular rejection: ISHLT 0
Severe vascular rejection: ISHLT 4
Mixed rejection: all considered as corresponding ISHLT cellular grade

UCTP = Utah Cardiac Transplant Program; ISHLT = International Society of Heart and Lung Transplantation

unknown, it is of less importance that a uniform grading schema for these changes be adopted. It was felt by the panel of pathologists, including this author, that these features should be further studied before being incorporated into a grading schema to be uniformly adopted.

Furthermore, it may not be useful at this time to adopt a classification of microvascular rejection into mild, moderate and severe subcategories, since no outcome or treatment differences between the groups have been established. Acute increases in immunosuppressive therapy are not undertaken on the basis of this information unless hemodynamic compromise is present[66]. Such hemodynamic compromise is much more common in patients with multiple episodes of microvascular rejection (vascular rejecters). These patient also have associated echocardiographic evidence of diastolic dysfunction[67].

CLASSIFICATION OF MIXED (CELLULAR AND VASCULAR) REJECTION

Mixed cellular and microvascular rejection may occur in an EMB simultaneously[13,14,16]. Independent grades of each process are assigned in our program. The cellular grading criteria are shown in Table 2, along with the corresponding ISHLT grades according to the revised ISHLT grading schema. Vascular grades are assigned according to the criteria described in Table 1. Mixed rejection, as defined in this chapter, is not recognized in the ISHLT schema; such biopsies are designated only by their cellular grade. Vasculitis is often ignored in the ISHLT schema except in severe rejection (grade 4), where it is the rule[52] (Figure 16).

DESIGNATION OF DOMINANT PATHOLOGIC REJECTION PATTERN

We have found it prognostically useful to designate patients according to their predominant form of histologic rejection[13,14,16,23]. Three separate clinical correlation evaluations of our patients have shown that these designations by predominant rejection type are prognostically important[13,14,23]. Patients with microvascular rejection have a significantly worse survival than patients with cellular or mixed rejection patterns, prospectively assigned in the first 3 months post-transplant (Figures 17 and 18)[14,23].

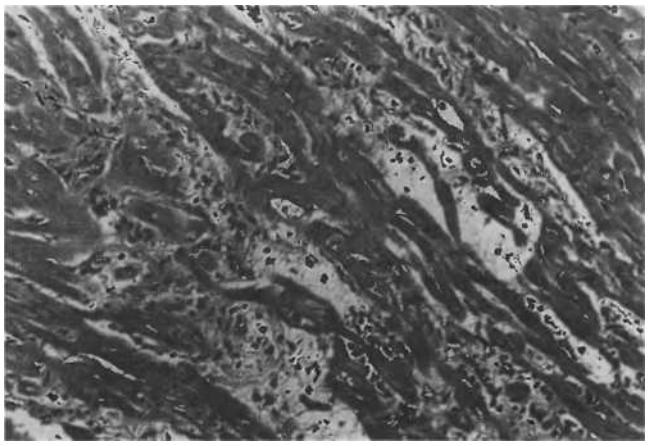

Figure 16 Histologic picture of moderate mixed rejection. Vasculitis and a space-occupying infiltrate of cells are seen, associated with myocyte damage. By ISHLT grading this biopsy would be considered 3B or 4, depending on the extent of the involvement of the biopsy with this process (H&E × 178). Used with permission from E.H. Hammond, editor. Solid Organ Transplantation Pathology vol.30 in series, Major Problems in Pathology, 1994

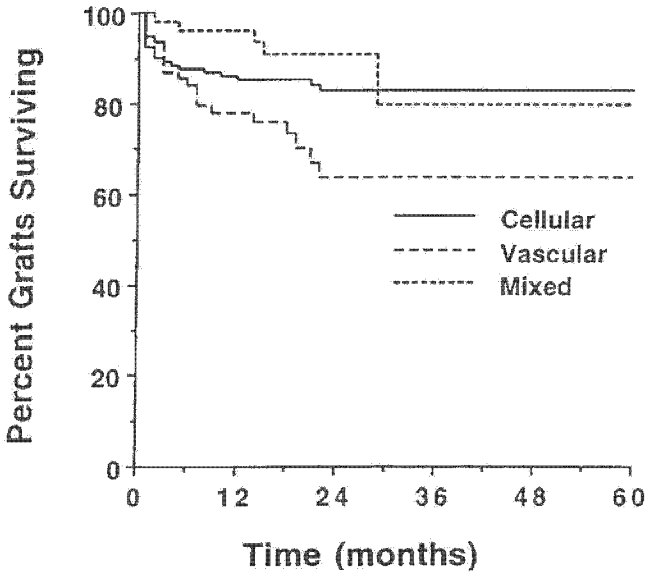

Figure 17 Kaplan–Meier graft failure curves are shown for each type of rejection group. Tarone Ware test was significant ($p = 0.027$) and the proportional hazard regression model showed the patients with vascular rejection had significantly different survival ($p = 0.012$) from patients with combined cellular and mixed rejection. Used with permission from ref. 14

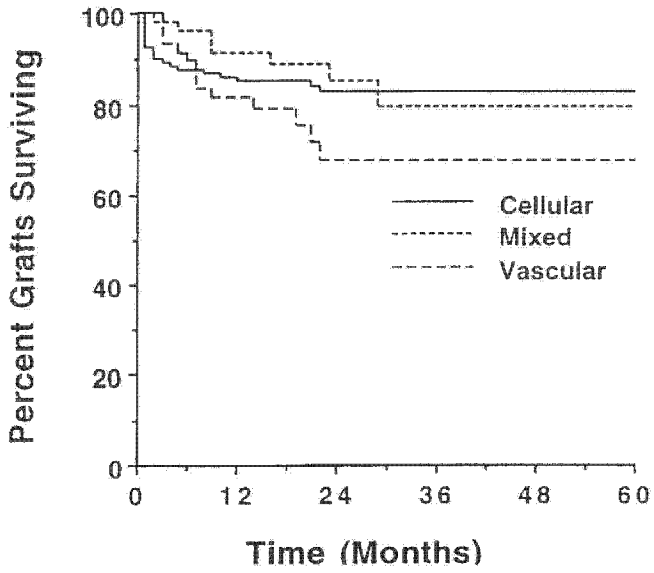

Figure 18 Kaplan–Meier graft failure curves are shown for each type of rejection group when OKT3-sensitized patients were eliminated from the analysis. Tarone Ware test for these data was not significant ($p = 0.223$). Used with permission from ref. 14

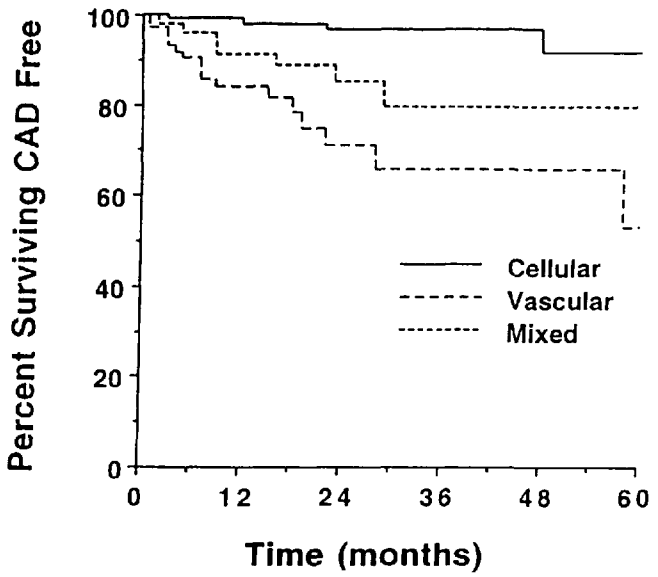

Figure 19 Kaplan–Meier coronary-free survival curves are shown for each type of rejection group. Tarone Ware test was significant among groups regardless of whether OKT3-sensitized patients were included ($p = 0.014$). The proportional hazard regression model showed that patients with the vascular pattern ($p = 0.0001$), as well as those with the mixed pattern ($p = 0.014$), were significantly different from cellular pattern patients. Used with permission from ref. 14

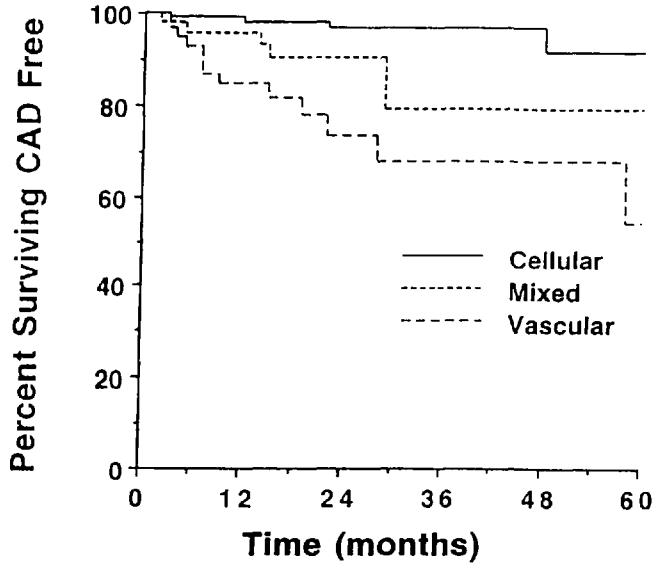

Figure 20 Kaplan – Meier coronary-free survival curves are shown for each type of rejection group. Tarone Ware test was significant among groups ($p = 0.001$). OKT3-sensitized patients were excluded from this analysis. The proportional hazard regression model showed that patients with the vascular pattern ($p = 0.0001$), as well as those with the mixed pattern ($p = 0.011$), were significantly different from cellular pattern patients. Used with permission from ref. 14.

Interestingly, although patients with mixed rejection have a survival rate similar to that in patients with cellular rejection, they have a 4-fold higher risk of developing allograft coronary artery disease; vascular rejection patients have an 8–9-fold greater risk (Figures 19 and 20). These risks are irrespective of the time post-transplantation[14]. The relationship of these rejection patterns to the occurrence of coronary artery vasculopathy is very impressive, especially when reviewed in the light of other published associations of coronary artery vasculopathy risk. Modest increases in risk of allograft coronary artery disease have been associated with: (a) degree of HLA mismatch, (b) HLA antibodies, (c) antiendothelial antibodies, or (d) panel-reactive antibodies detected in the serum of the patient post-transplant, (e) CMV infection, (f) donor–recipient mismatching for sex, (g) cellular rejection frequency, and (h) forms of hyperlipidemia (increase in total cholesterol, LDL cholesterol, or elevated plasma triglycerides). These data were recently reviewed by Marboe[68].

Faulk and Labarrere have presented compelling evidence that abnormalities in the expression of natural anticoagulant and fibrinolytic molecules are also associated with early graft loss; this loss, however is often very early, and is associated with acute myocardial infarction rather than diffuse coronary artery vasculopathy[29,30].

ALLOGRAFT CORONARY ARTERY VASCULOPATHY – RELATIONSHIP TO DIFFUSE CHRONIC VASCULOPATHY (GLOBAL MYOCARDIAL ISCHEMIA)

Elsewhere in this book are descriptions of the pathologic (Chapter 33) and clinical (Chapter 35) aspects of allograft coronary artery vasculopathy. One form of this vasculopathy will be discussed in detail here because of its prevalence in patients with multiple episodes of microvascular or mixed cellular and microvascular rejection. This form of chronic vasculopathy, studied in detail in explant and autopsy hearts from our transplant patient population, involves penetrating arteries and arterioles within the myocardium as well as the epicardial coronaries. The history and pathologic features are quite specific when present. The patient develops evidence of heart failure manifested by a decreasing left ventricular ejection fraction and the usual symptoms and signs. EMB is performed to rule out acute rejection as a cause of the patient's clinical symptoms.

On EMB a distinctive group of morphologic features is demonstrated. The biopsy shows focal areas of myocyte dropout in which myocytes are replaced by loose connective tissue and eventually collagen. Surrounding myocytes are often hypertrophied and vacuolated. Inflammatory cells such as lymphocytes and macrophages may be seen, but they are limited to the areas of myocyte dropout or scarring. Capillaries are difficult to find, and no endothelial activation is present (Figure 21)[14,16,69,70].

The patchy nature of this process, and its predilection for the subendocardial region, suggests that the myocyte loss is due to generalized microvascular damage, which includes small arteries and arterioles outside of the field of examination on endomyocardial biopsy. This observation has been documented on autopsy and explant evaluation of such hearts[14,16]. The changes are also seen in patients with allograft coronary vasculopathy. If these changes are encountered on repeated EMB in patients with slowly worsening cardiac function, they are an ominous sign. Such

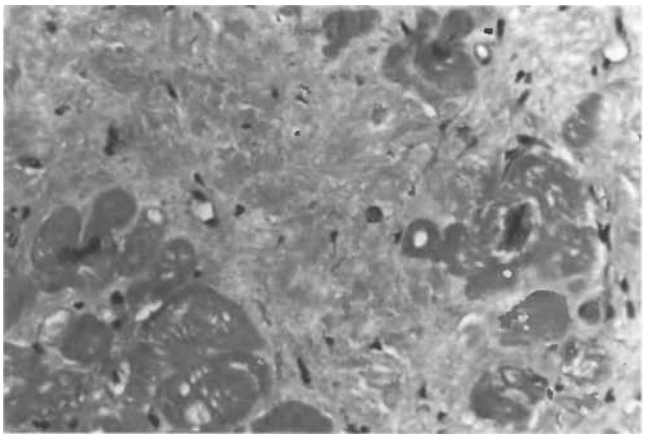

Figure 21 Histologic findings in a biopsy with changes suggestive of allograft coronary vasculopathy or global myocardial ischemia. Biopsy shows irregular areas of scarring surrounded by hypertrophied myocytes, suggesting that this area was a microinfarct which was healed. These small scarred regions were not in relationship to the endocardium as expected for biopsy site remnants. Note the prominent vacuolization of the surrounding myocytes. Patient had a history of repeated episodes of microvascular rejection over several years post-transplant (H&E × 178). Used with permission from ref. 16

patchy myocyte loss is distinctive from the loss usually associated with myocardial infarction, in which large zones of myocyte necrosis are ultimately replaced by dense scarring. When we recognize this change suggestive of diffuse myocardial ischemia at intervals after the first few months post-transplant, we note it in our reports. All patients in whom this finding was present on biopsy, and who have come to allograft replacement or autopsy, have had global myocardial ischemic damage in the heart as the predominant explanation of their cardiac failure[14,16,70]. Coronary artery involvement with vasculopathy was often also seen, but areas of complete occlusion of coronary vessels were absent (Figure 22)[14,16] (personal unpublished observations).

By immunocytochemistry, patients with global myocardial ischemia or allograft coronary vasculopathy usually show generalized increased MHC class II (HLA-DR) staining of microvasculature of EMB in the absence of other pathologic evidence of acute rejection[14,16]. The immunocytochemical findings of microvascular immunoglobulin and complement components may be decreased from previous biopsies, or may be totally absent. ATT is often absent in the microvasculature and tPA is undetectable in smooth muscle. This pattern of ATT and tPA expression was detected in seven of 11 hearts examined at autopsy in which global ischemic changes were the predominant cause of heart failure (unpublished observations). We have seen biopsies from several patients who lack microvascular damage but have coronary vasculopathy; ATT and tPA staining were not diminished. The amount of fibrin staining is often decreased over previous biopsies.

We believe that these altered morphologic expressions in the microvasculature may be the results of attempted repair of chronic damage of the microvasculature. In such patients it is only the observation of serial EMB, plus a careful examination of the light microscopic findings, which will lead to the correct pathologic diagnosis of global myocardial ischemia.

Ultrastructural observations of EMB from patients with histologic evidence of chronic damage of large and small blood vessels have shown changes analogous to those found in ischemic hearts of experimental animals; these hearts show a prominent loss of actin over myosin in myofilament bundles that are intact, giving a coarse appearance to the myofilaments (Figure 23)[16,70]. In addition, large numbers of myocyte cytoplasmic organelles are often scattered around in the interstitium, associated with a patchy and haphazard collection of collagen fibrils. The vessels usually have irregular profiles and may show irregular endothelial cell swelling[16,70].

Figure 22 This epicardial coronary artery was sectioned at the time of autopsy 4 months after transplant. The patient had multiple episodes of acute microvascular rejection which were not associated with hemodynamic compromise and were not treated prior to death. Marked narrowing of the vessel with subintimal fibrosis is seen. Numerous inflammatory cells were found in this region, consisting of macrophages and lymphocytes (H&E × 75). Used with permission from ref. 16

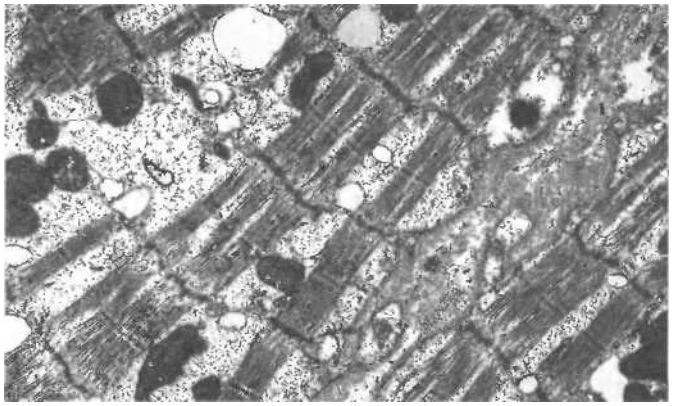

Figure 23 Ultrastructural appearance of myocytes from a biopsy showing histologic evidence of global myocardial ischemia. Myofilament structure appears coarse, due to the predominant loss of actin filaments. Original print magnification × 10,000. Used with permission from ref. 16

DRUG-INDUCED VASCULAR PROCESSES SIMULATING VASCULAR REJECTION

In patients treated with antibodies raised in various animal species, such as horse, rabbit, sheep or mice, an immune-complex-mediated response to these foreign proteins may develop[71-73]. In patients who develop these reactions the immunosuppressive function of the monoclonal antibody is abrogated by the production of antibodies against the immunosuppressive agent. Such patients may show early rejection because of lack of immunosuppression[74]. Alternatively, patients may show a serum-sickness-like process within their cardiac vessels related to the deposition of immune complexes of the monoclonal antibody and the host antibody directed against it[75]. Usually these antibodies are anti-idiotypic antibodies directed against the foreign protein.

In our institutions, where the vast majority of patients have been treated with OKT3 (a mouse monoclonal antibody), all patients are routinely evaluated by immunocytochemistry for the accumulation of mouse immunoglobulin (the antigen) in cardiac vessels[76]. Typically, the efficacy of OKT3 therapy is monitored by following total daily T lymphocyte counts or, in the case of OKT3, CD3 lymphocyte counts. This method is an insensitive way to detect early anti-idiotypic antibody accumulation. A better method is the flow cytometric assay described by Wittwer *et al.*, which detects declines in steady-state plasma levels of the OKT3 (due to complexing of antigen with anti-idiotypic antibody) 1–3 days earlier than the return of $CD3^+$ cells in the serum[77]. The value of early detection of OKT3 sensitization (anti-idiotypic antibody activity sufficient to cause plasma OKT3 levels to fall significantly) is that the patient can be immediately withdrawn from OKT3 therapy and placed on alternative immunosuppression.

We have demonstrated that maintaining the patient on OKT3 in the presence of anti-idiotypic antibodies leads to humorally mediated microvascular alterations in every case[76]. Since this is associated with severe hemodynamic consequences, it should be avoided. A subsequent study, in which patients sensitized to OKT3 had therapy promptly aborted, has led to dramatic improvement in allograft survival[78]. Eleven of 12 patients shown to be sensitized, and removed from therapy at the time of sensitization, have not lost their allografts acutely. In this later report, patients without OKT3 sensitization during induction treatment also did not develop sensitization if retreatment was necessary. This is another situation in which routine immunocytochemical monitoring is very helpful in understanding the nature of the immunologic response.

Morphologically, EMB have features of microvascular alteration identical to those found in vascular rejection patients. The only different feature is the presence of mouse, horse or rabbit immunoglobulin in a distribution identical to the human immunoglobulin and complement components which serves to distinguish it from vascular rejection. There are no direct data concerning the distribution of altered ATT and tPA expression in these patients, since most of the sensitized patients were studied prior to routine use of these reagents. However, fibrin was found in an interstitial and vascular distribution in all of the biopsies, suggesting that ATT and tPA would be expected to be missing from the microvasculature[15].

PATHOGENESIS OF MICROVASCULAR REJECTION

The changes described in this chapter suggest that endothelial cell activation, vascular permeability, and subsequent myocyte degeneration are prominent features in patients displaying light microscopic and immunocytochemical alterations associated with microvascular rejection. These morphologic features suggest that the endothelial cell plays a pivotal role in this rejection process. In-depth investigations of endothelial cell biology have shown that, rather than being non-specific targets of injury, endothelial cells are capable of many important functions which can be altered in the allograft[55-58,79-83].

Endothelial cells provide a natural anticoagulated surface through their binding of antithrombin III and thrombomodulin, although the evidence in cardiac transplants suggests that the former pathway is more important than the latter[15,29]. Endothelial cells produce diverse cytokines which can modulate the biologic behavior of cells in the myocardial tissue. These cytokines are produced in inflammation, ischemia, and many other circumstances commonly operative in transplantation, such as infection and lymphocyte activation[55]. There is good evidence that endothelial activation can be the result of immunosuppression or immunoprophylaxis utilizing monoclonal anti-T-cell antibodies. The therapy can generate T cell activation, as a result of interaction of the antibody and the CD3 or T cell receptor antigen on the lymphocyte surface[54,55,74,84]. A predominant cytokine released is tumor necrosis factor alpha; antibody directed against this cytokine is effective in abrogating the first dose response commonly seen in these patients[85,86]. Other reports suggest that such lymphocyte activation leads to increased release of IL-2 and IFN-γ, which promotes further endothelial cell activation, as has been elegantly shown by the *in vitro* studies of Pober and colleagues[55-58].

We have observed that patients with the morphologic pattern of microvascular rejection (vascular rejecters) include patients sensitized to OKT3 while undergoing immunoprophylaxis. This type of microvascular rejection is related to humoral immune responses with probable altered B-lymphocyte immune regulation or polyclonal B-lymphocytic activation[52,54]. The morphologic changes are very similar to those described in reports detailing humorally mediated vascular rejection in: (a) renal transplant recipients, (b) patients with serum sickness, and (c) experimental animals with leukocytoclastic vasculitis, (d) the Arthus phenomenon, and (e) animals rejecting xenografts in which there has been a decrease in antibody levels or complement activity[17,79-81,87-89]. The pathogenic mechanisms responsible for these vascular inflammatory processes (where an antigen–antibody complex process is definitely implicated) involve complement activation, cytokine release, and chemotaxis and activation of neutrophils and macrophages.

Recent work has shed light on the pathogenesis of this process in our allograft population. We examined the incidence of vascular rejection pattern related to the duration of OKT3 induction immunoprophylaxis (Table 3). We found a statistically significant association of the vascular rejection pattern with duration of OKT3 greater than 7 days. The percentage of patients developing microvascular rejection increases in direct proportion to the length of OKT3 therapy. Unfortunately, the number of patients treated with standard triple immunosuppression is too small to

Table 3 Relationship of duration of OKT3 induction to vascular rejection pattern

Duration of OKT3 prophylaxis[*]	*Total no. of patients*	*Vascular rejection pattern (percentage of total)*[†]	*Lost grafts*
7 days	53	6 (11)	3/6
10 days	26	8 (31)	4/8
14 days	208	56 (27)	24/56
21 days	13	6 (46)	4/6

[*] Duration of OKT3 induction determined retrospectively by chart review (Dr Hong Ma). Patients sensitized to OKT3 as determined by the flow cytometric assay were excluded from this analysis. Sensitization also documented by chart review.

[†] Vascular rejection pattern prospectively determined during first 3 months post-transplant. Patient had at least three episodes of vascular rejection without cellular rejection and prior to other rejection episodes, including cellular infiltrates. See text.

include in the analysis; the number of these patients with the vascular rejection pattern was not significantly different from the patients treated with 7 days of OKT3 immunoprophylaxis. This association, in multivariate analysis, was independent of: (a) patient sex, (b) age, (c) positive crossmatch, and (d) positive panel-reactive antibody status, which were all previous predictors of vascular rejection pattern in this patient population. Patients with OKT3 sensitization were also excluded from the analysis[90]. The association of vascular rejection pattern with durations of OKT3 immunoprophylaxis >7 days suggests that anti-idiotypic antibody production, which occurs to some degree in all patients after 7 days of induction, synergizes with the humoral rejection response so that it becomes the predominant pattern in susceptible patients.

An alternative hypothesis is that the OKT3 induction for >7 days alters cytokine production, such as TNF. It is well recognized that TNF is the cytokine responsible for the first-dose OKT3 reaction. Recent studies have shown that TNF may play a significant role in allograft rejection[46,47,49]. TNF recruits cells to the site of antigenic challenge, activates immunocompetent cells, augments the expression of MHC class I and class II antigens, and induces the production of other cytokines. Lymphocytes, macrophages, and endothelial cells are all able to produce TNF-α, and are activated by its actions. Elevation of mRNA for TNF-α is detected in rat cardiac allografts in advance of clinical rejection[46,47]. Antibody against TNF-α has also been shown to be effective, in combination with cyclosporin, in prolonging rat cardiac allograft survival. Elevation of circulating levels of TNF has been detected in cardiac and renal transplant recipients[91,92,93].

Thus, it is likely that TNF is produced concomitantly with each dose of OKT3 that is given, so that the amount of TNF produced in patients induced for 14 or 21 days is significantly greater than in patients receiving the drug for only 7 days. Furthermore, activation of macrophages and endothelial cells, which promote further TNF production, is accelerated by binding of these cells to immune complexes, like complexes of OKT3 and its anti-idiotypic antibody. Thus, circumstances are favorable for the increased production of TNF in patients receiving prolonged OKT3 induction. TNF enhances immunologic responsiveness, and would be expected to accelerate rejection responses in patients as it has been shown to do in experimental animals. If this hypothesis is correct, antibody directed against TNF-α may be an effective strategy to prevent microvascular rejection in this patient population.

The likelihood that microvascular rejection is mediated by TNF or other cytokines in addition to humoral immune mechanisms cannot be differentiated by the present or previous studies[79,83,87,88]. We have consistently seen up-regulation of HLA-DR on the large and small vessels of the allograft which, at least in experimental situations, is produced exclusively by interaction of endothelial cells with IFN-γ[79]. This finding, as well as the prominent fibrin deposition and endothelial activation, suggests that cytokine-mediated (delayed-type) hypersensitivity may be implicated in this process[10,94].

The consequence of either a humoral or cellular immune response directed against the vascular endothelium would ultimately be compromised myocardial oxygenation. Important inflammatory participants in this process include neutrophils and macrophages which can be activated by immune complexes, cytokines, complement components, endotoxin, and platelet-activating factor[77–79]. Such activation can result in the production of various leukotrienes, arachidonic acid metabolites, and a variety of cytokines which lead to vascular permeability, leukocyte adherence via various specifically induced adhesion molecules, and the activation of proteolytic enzymes such as protein kinase C. The ability of endothelial cells to express adhesion molecules (ELAM-1, ICAM-1, VCAM-1) in response to inflammatory stimuli or cytokine release, such as IL-1, can create the morphologic expression of vascular rejection, including endothelial activation and capillaritis. The consequences of this process include compromised myocardial oxygenation, diffuse myocardial damage, hemodynamic compromise, and allograft loss.

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29
Pathology of Cardiac Allograft Rejection. 2: Acute Cellular

A.G. ROSE

INTRODUCTION

Cardiac transplantation has become firmly established as a treatment for terminal cardiac failure. The early experimental work in animals[1–7] that preceded the first human-to-human cardiac transplant in 1967 is now mainly of historical interest. Thomson[8] documented the pathological findings in the donor heart of Louis Washansky, who was the recipient in this first historic operation. Shortly thereafter Lower *et al.*[9] reported their experience with human cardiac transplantation.

Despite advances in patient selection, donor heart procurement and preservation, and immunosuppressive therapy, acute rejection remains an important cause of graft loss and recipient mortality.

MACROSCOPIC APPEARANCE OF ACUTE REJECTION

Mild or moderate acute rejection usually fails to produce detectable naked-eye changes in the donor heart apart from an increased organ mass (Figure 1)[10,11]. Cyclosporine-induced systemic hypertension may also contribute to the cardiac hypertrophy. Such hearts are seldom examined pathologically unless the recipient has died of other causes.

Severe acute rejection may produce a swollen, mottled myocardium with scanty subendocardial hemorrhages. A heterotopic transplant which has undergone irreversible, severe rejection may not lead to the death of the recipient if the latter's own heart has enough residual function to support the circulation.

Figure 1 Transverse slices of recipient heart (left) and donor heart (right) 21 days after heterotopic transplantation. The severely rejected donor myocardium presents a pseudohypertrophied appearance. (Reproduced with permission from ref. 10)

Explanted heterotopic grafts usually show very severe acute rejection since immunosuppression is often reduced in the period between cessation of graft function and surgical excision of the graft. Such hearts (Figure 2) have a severely hemorrhagic, mottled appearance. Geographic zones of pale-colored, focal infarction stand out against the plum-colored, hemorrhagic, but still viable myocardium. Stasis thrombi may be present within the cardiac chambers. Orthotopic transplants that have failed primarily due to acute rejection usually show less obvious naked-eye alterations despite the presence of severe acute rejection histologically.

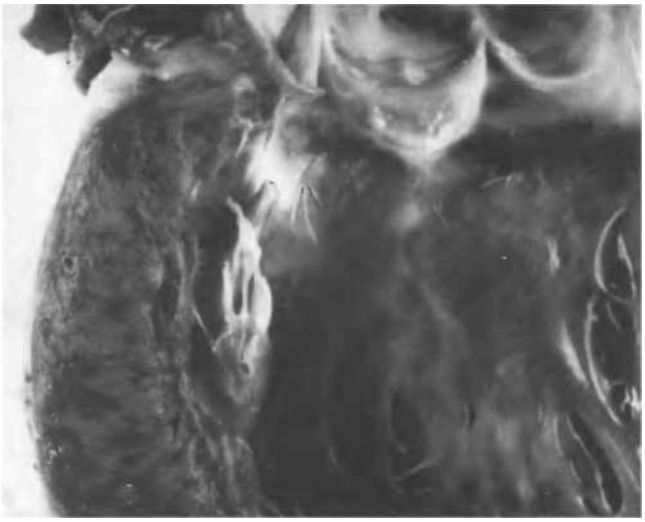


Figure 2 Close-up view of donor heart left ventricular outflow tract. Severe acute rejection has produced diffuse intramyocardial hemorrhage and the myocardial cut surface shows pale areas of necrosis which contrast with the hemorrhagic background

ROLE OF ENDOMYOCARDIAL BIOPSY IN THE DIAGNOSIS OF ACUTE REJECTION

Endomyocardial biopsy histology with grading of the severity of rejection continues to be the so-called gold standard for the diagnosis of acute rejection. Usually biopsies are performed weekly during the first 6 weeks post-transplantation and then fortnightly for the next few months. Gradually therafter the intervals between the biopsies are increased until a stage is reached at which the biopsies are done approximately every 3 months.

Non-invasive methods for diagnosing acute rejection, such as magnetic resonance imaging, assessment of peripheral blood lymphocytic activation and soluble interleukin-2 receptor levels, have failed to live up to expectations and have not superseded graft histology. It remains to be seen whether other non-histologic modalities, e.g. radioimmunoassay assessment of vascular adhesion molecules[12] in endomyocardial biopsies, may play a role in the routine management of cardiac transplant patients. The latter invasive procedure is still dependent on the taking of endomyocardial biopsies.

Earlier criticism of endomyocardial biopsy has focused primarily on the possibility of sampling error and the subtlety of the histologic changes in diagnosing rejection[13,14]. One study[15] attempted to validate the technique by examining 'biopsy' samples taken with a bioptome from formalin-fixed explanted human donor hearts, and comparing these in a blind fashion with standard histologic sections taken from the same hearts. Agreement of results between the bioptome samples and the routine sections was found in 86% of cases. False-negative results were less than 1%. Acute rejection involved both ventricles equally.

Due to the rigidity of the bioptome catheter, endomyocardial biopsy usually only samples the septal wall of the right ventricle towards the apex. Four or five tissue samples are regarded as adequate[16]. In practice most pathologists usually receive three or four endomyocardial samples per biopsy procedure. In the study referred to earlier, it was found that even as few as two endomyocardial samples revealed the presence of acute rejection[15]. If fewer samples are received this should be noted in the pathologic report, since it holds the implication that significant rejection may be missed (false-negative biopsy). In the light of other clinical and laboratory parameters, the cardiac surgeon has to decide whether an immediate or earlier than usual repeat biopsy is indicated. The size of the biopsy specimen varies according to the bioptome used. If the biopsy specimens are small, then a suboptimal number of samples has more severe implications than would be the case if the samples were large.

At some centers the endomyocardial biopsies are submitted to the laboratory in 5% buffered glutaraldehyde to facilitate subsequent ultrastructural examination of one of the fragments, if this is deemed necessary. Selected fragments for light microscopic assessment of rejection are transferred into 5% buffered formaldehyde and processed in a hypercenter tissue processor for expedited handling.

Paraffin-embedded sections are stained by the hematoxylin–eosin, Masson's trichrome, elastic van Gieson, and Unna-Pappenheim methods. One or two biopsies may also be submitted unfixed for immediate frozen section. This gives immediate information regarding the presence of rejection, and additional sections may be cut for the determination of lymphocyte subsets. The latter is performed more for research purposes than for influencing management of the patient. Electron microscopy and immunofluorescence microscopy play only a small role in the routine diagnosis of acute rejection.

HISTOPATHOLOGY OF ACUTE REJECTION

One of the earliest changes observed in acute rejection is the development of interstitial edema (Figure 3), which is most prominent perivascularly and less evident in the endocardium, which has a denser connective tissue component. The edema is probably a result of microvascular damage. Interstitial edema is less severe in patients receiving cyclosporin compared to the earlier, steroid-based immunosuppression. The vascular endothelium is that portion of the graft which first encounters the host lymphocytes which are attracted into the graft, since these cells reach the graft via the bloodstream.

In the early stages of acute rejection the small blood vessels within the graft contain increased numbers of mononuclear cells (Figure 4), which may also be seen to be passing through the vessels' walls into the surrounding myocardium. The early

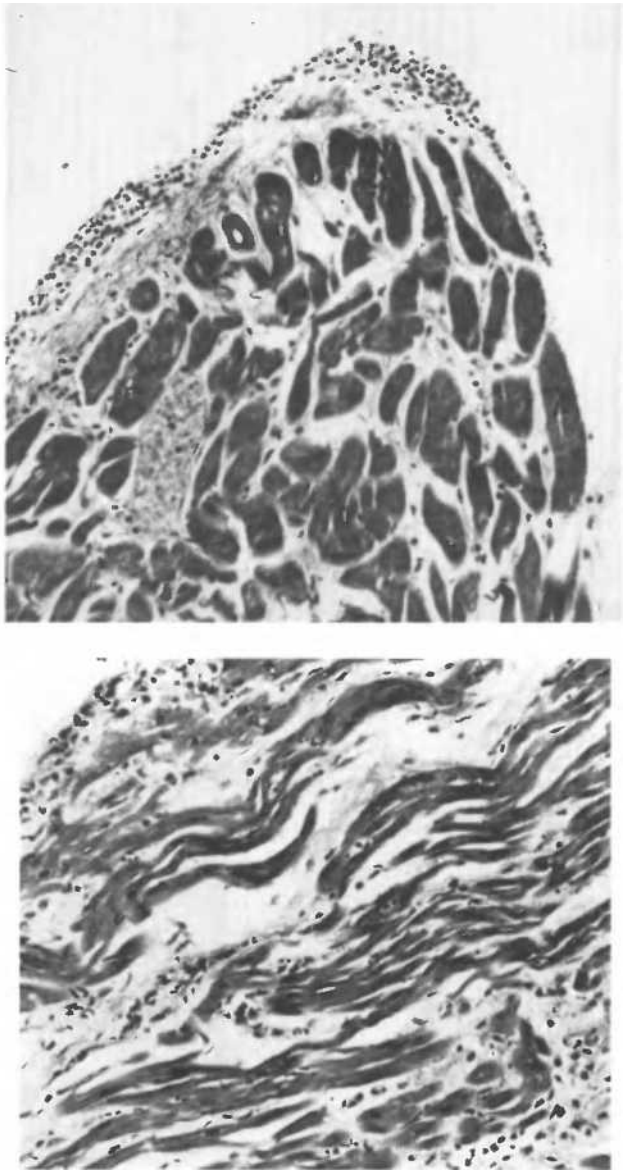


Figure 3 **A**: Early acute rejection (grade 1A of ISHLT). Mild interstitial edema spares the endocardium, which contains a more florid lymphocytic infiltration than is seen in the myocardial interstitium (H&E, × 135). **B**: Scanty interstitial lymphocytic infiltrate of mild acute (grade 1B of ISHLT) rejection (H&E, × 60)

infiltrating cells (Figure 5) consist mainly of non-activated lymphocytes and small unidentified mononuclear cells of lymphoid type, together with histiocytes and scanty neutrophils plus eosinophils. The cellular infiltration of mild acute rejection (Figure 6) has a focal, mainly perivascular distribution. The lymphocytes soon develop a prominent cytoplasmic pyroninophilia (activated or aggressive lymphocytes), as do the endothelial cells of the small blood vessels. Focal infiltration by similar cells is also noted in the endomyocardium. Cardiac histiocytes (Anitschkow myocytes), presumably of donor heart origin, also appear activated and prominent.

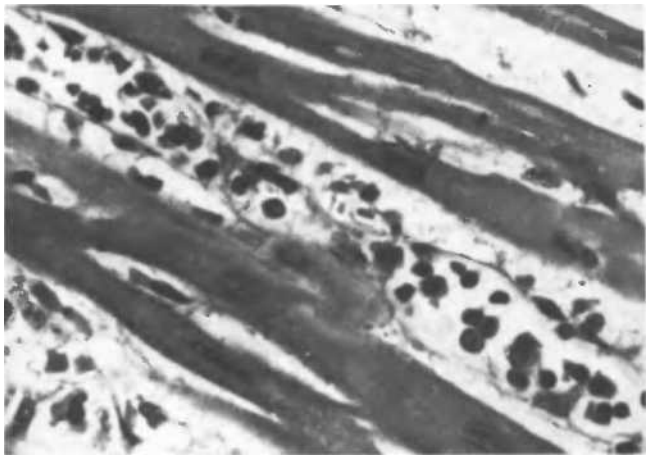


Figure 4 A capillary contains numerous mononuclear cells. Similar cells are present in the edematous interstitium of this rejecting graft (H&E, × 420)

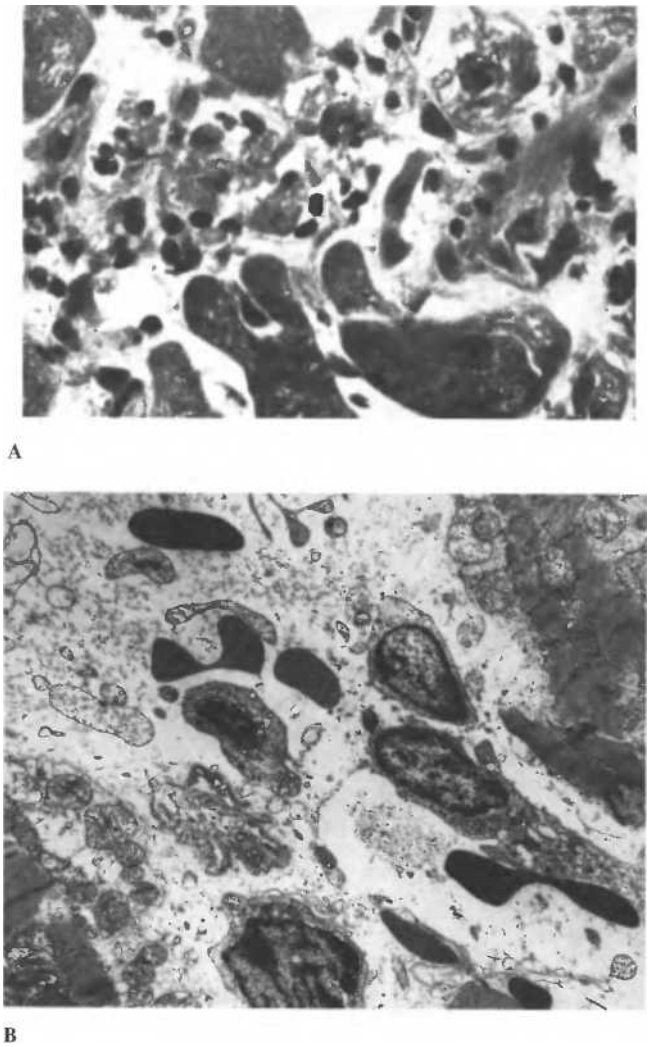


Figure 5 **A**: Donor heart biopsy shows numerous lymphocytes in relation to necrotic myocytes (H&E, × 420). **B**: The edematous interstitium between two myocytes (top right and bottom left) contains activated lymphocytes and some free-lying erythrocytes. (Lead citrate and uranyl acetate, × 2400)

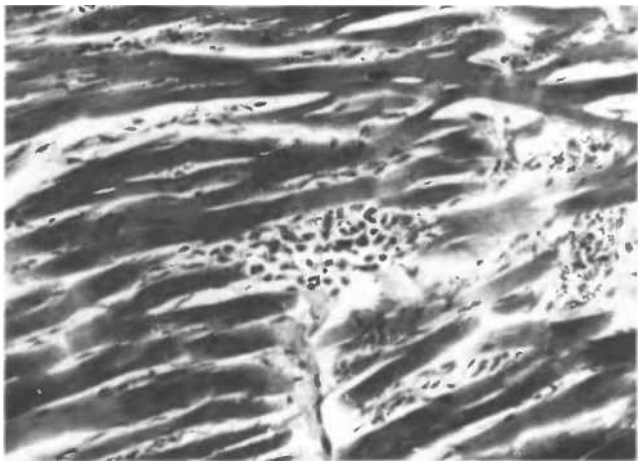


Figure 6 Mild acute rejection (grade 1A of ISHLT); focal lymphocytic aggregation without myocyte damage

If untreated, one may have the development of moderate (Figures 7 and 8) and severe (Figure 9) acute rejection. In such a case all of the above changes will progress and increase in intensity, and will be accompanied by damage to the myocytes and blood vessels. Thus, interstitial edema increases and interstitial fibrin deposition may also occur. Fibrinolysis may render the latter inconspicuous microscopically. The most reliable indication of progression in severity of acute rejection is more intense and widespread lymphocytic infiltration of the graft. With time there is enlargement of the nuclei and cytoplasm of the lymphocytes, which now have the appearance of immunoblasts (Figure 10). Similar lymphoid cells infiltrate the intima of intramyocardial blood vessels where they are admixed with proliferating endothelial and intimal cells. Fewer lymphocytes may also be seen within the media and the adventitia (Figure 11).

Herskowitz *et al.*[17] identified interstitial edema, perivascular keryorrhexis, and perivascular lymphocytic infiltration with intermyocyte extension as three histologic abnormalities that help predict the future development of more severe acute rejection associated with myocyte necrosis.

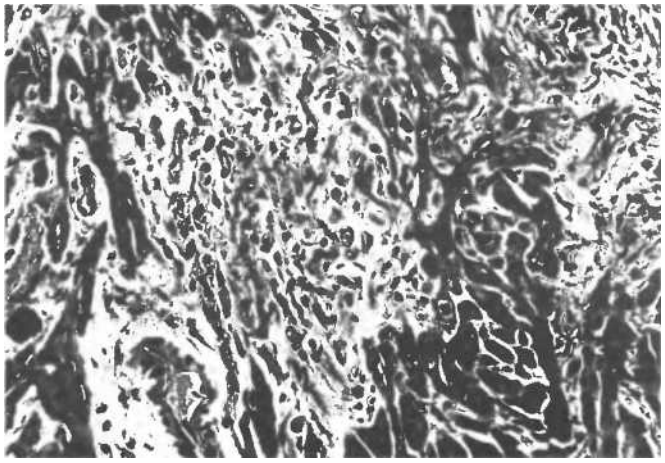


Figure 8 Moderate acute rejection (grade 3B of ISHLT). A diffuse lymphocytic infiltration is present together with focal myocyte necrosis (H&E, × 84)

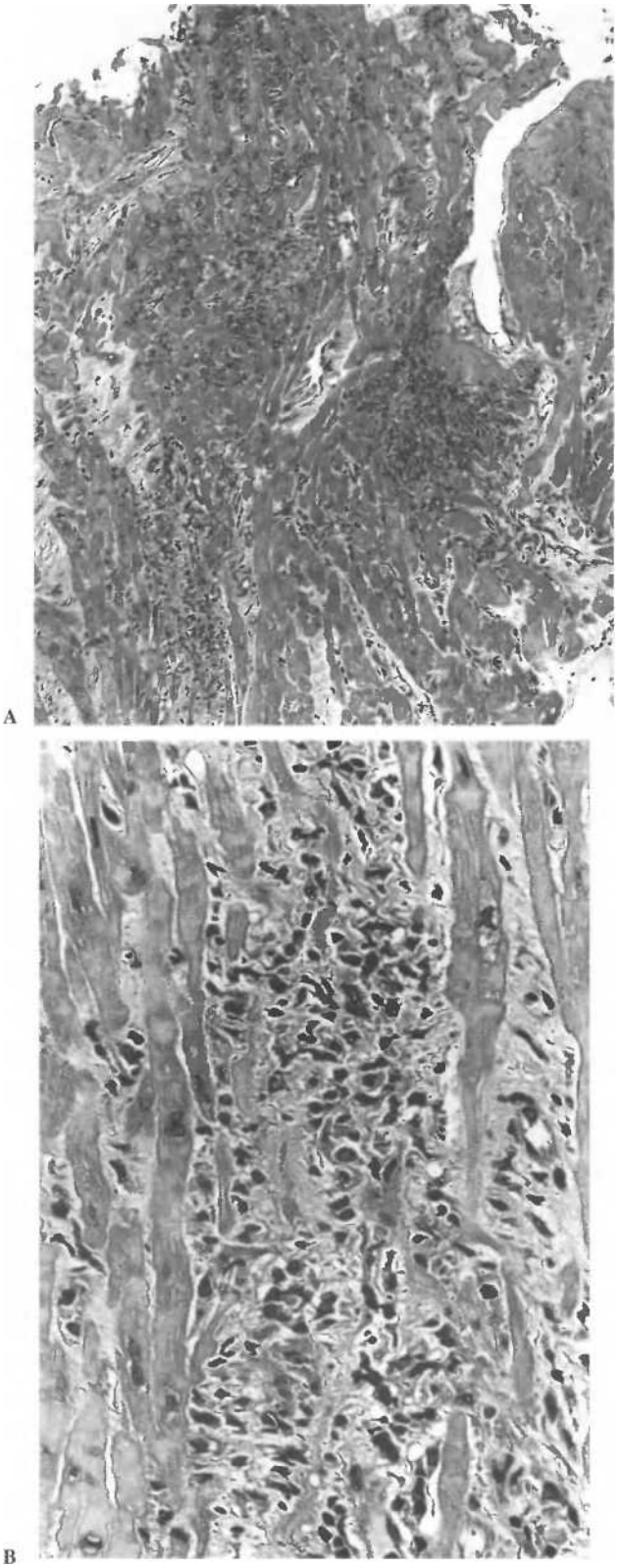


Figure 7 **A**: Grade 3A (ISHLT) acute rejection is characterized by multifocal lymphocytic infiltration of the myocardium (H&E, × 40). **B**: Higher-power view of same biopsy shows myocyte necrosis in relation to an intense mononuclear cellular infiltration. The presence of necrosis steers the grading towards grade 3B (H&E, × 280)

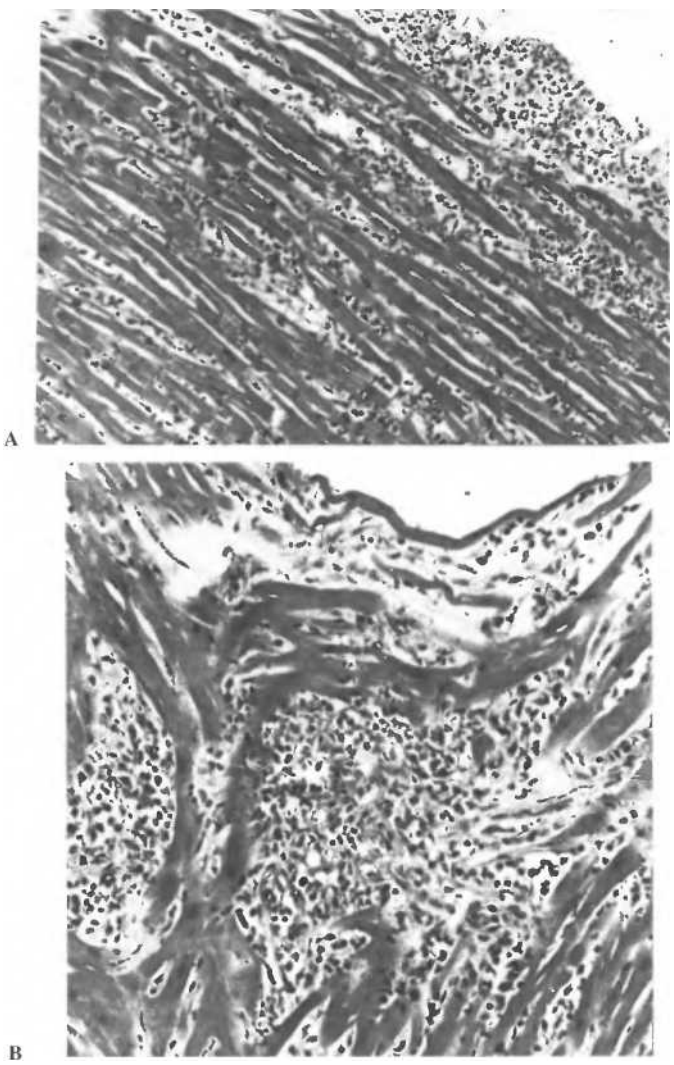


Figure 9 A: Severe acute rejection (grade 4 of ISHLT) exhibits severe interstitial lymphocytic infiltration and focal myocyte necrosis (H&E, × 100). B: Different area of the same biopsy shows focal loss of myocytes and masses of lymphocytes plus scanty haemorrhage (H&E, × 200)

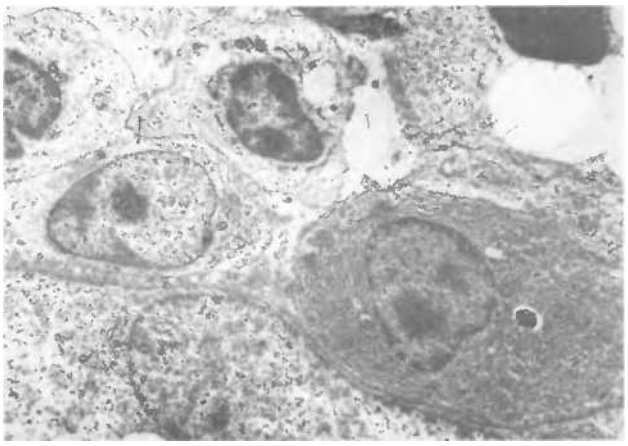


Figure 10 Electron micrograph in acute rejection. A group of activated lymphocytes occupies the myocardial interstitium. A plasma cell is present (lower right) (Lead citrate and uranyl acetate, × 1350)

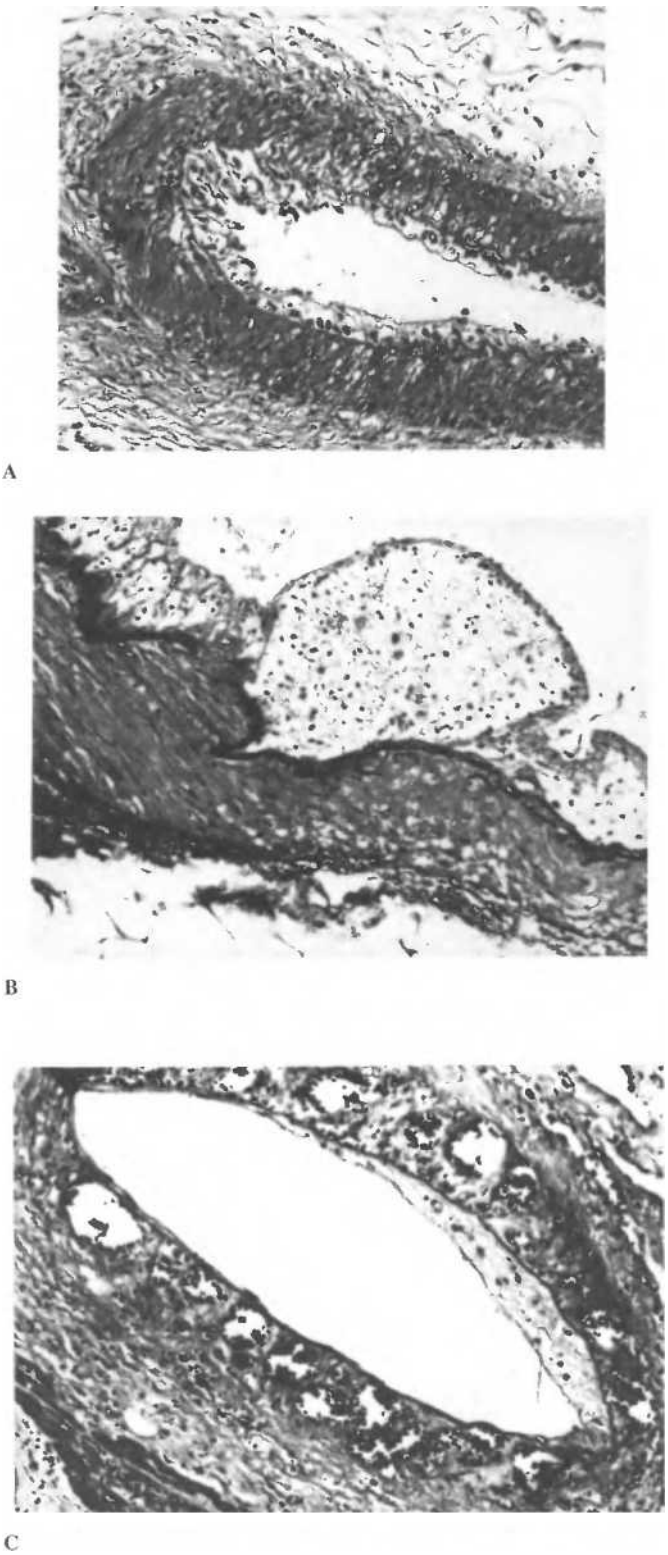
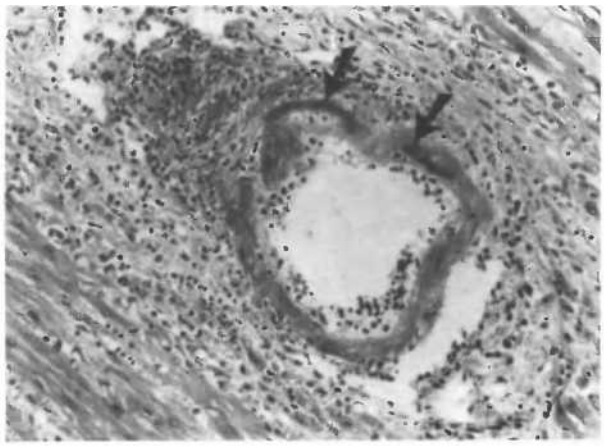


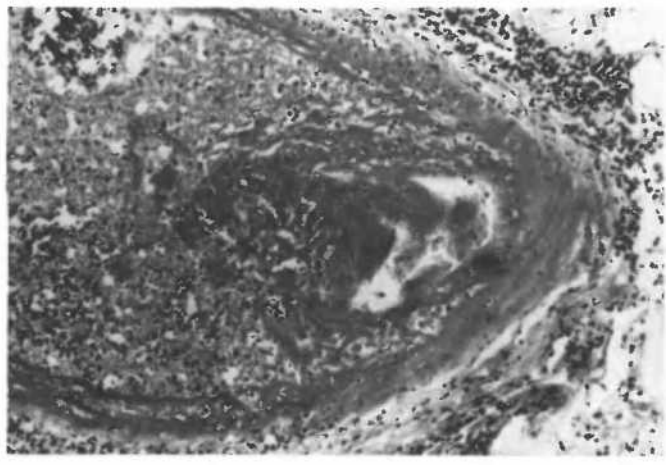
Figure 11 Coronary arterial alterations in severe acute rejection. A: Acute rejection has caused subendothelial intimal edema in a small epicardial coronary artery. B: At times the edema can be quite striking. C: Small coronary artery shows outer medial defects of the kind associated with an immune-mediated arteritis (All H&E, × 100)

Small-vessel thrombi have been an inconspicuous feature of mild and moderate acute rejection in human cardiac transplants, but they have been encountered by others in canine cardiac allografts[18]. The occurrence of such thrombi in severe acute rejection in humans is not surprising, since there is evidence that rejection may activate the coagulation mechanism[19,20]. The cardiac veins and venules are seldom affected by florid acute rejection changes[7].

The above-described microvascular changes, together with the cytotoxic effects of the infiltrating immunoblasts, combine to produce deleterious effects on the myocardium. Thus, the myocytes may show a range of appearances from normal through cytoplasmic swelling, lipid vacuolation to focal necrosis. The presence of zonal myocytolysis or coagulative necrosis usually indicates graft arteriopathy (chronic rejection) rather than acute rejection. Rarely, fibrinoid necrosis of coronary arteries (Figure 12) associated with very severe acute rejection may lead to zones of myocytolysis or coagulative necrosis in the donor ventricles. Associated focal interstitial hemorrhages may also occur in such cases. It will be necessary to avoid confusion with delayed vascular rejection, but lymphocytic infiltration is not a feature of the latter process in its pure form.



A



B

Figure 12 Severe acute rejection. **A**: Early fibrinoid necrosis (arrows) of wall of an intramyocardial coronary artery. **B**: Advanced fibrinoid necrosis of a small coronary artery with superimposed thrombosis (Both H&E, × 100)

Focal myocyte necrosis is not a uniform finding in severe acute rejection. Lesser changes indicative of myocyte damage are often noted, e.g. hypereosinophilia of individual myocytes, blurring of the edges of myocytes, attenuation of myocytes, reduction in visibility of cross-striations and vacuolization of the cytoplasm. Damaged myocytes often have lymphocytes closely applied to their sarcolemmal sheaths. It has been claimed that the myocyte injury in acute rejection is reversible[21] and that the injured myocytes in acute rejection are capable of reconstitution[22].

HISTOLOGIC GRADING OF ACUTE REJECTION

A variety of histologic grading systems for acute rejection have evolved at various centers worldwide[23–27]. No matter which grading system has been used the essence of its successful application has been frequent and clear dialogue between the pathologist and the clinicians looking after the recipient. The pathologist must clearly indicate the degree of rejection present. The use of a grading system has the advantage that the severity of rejection can be swiftly communicated without going into descriptive histologic details which may confuse the clinician. The pathologist examining the biopsy should be experienced in the interpretation of endomyocardial biopsies, since the unwary may read too much into biopsy-induced artefacts with possibly untoward results for the patient if such artefacts are interpreted as being due to rejection.

STANDARDIZED GRADING SYSTEM FOR HISTOLOGIC ASSESSMENT OF DONOR HEART ENDOMYOCARDIAL BIOPSIES

A standardized grading system was established by the International Society for Heart and Lung Transplantation (ISHLT)[28] in order to facilitate multicenter trials, and for uniformity in publications, so that results from different centers may be compared effectively. The Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart Rejection[28] acts as a Rosetta Stone by which gradings in one scheme can be compared with the gradings of another. Whilst the aim was not to replace the pre-existing individual grading schemes, many centers have adopted the proposed Working Formulation in their day-to-day practice.

The various categories within the standardized cardiac biopsy grading system are given in Table 1. This grading system for acute rejection may be paraphrased as follows.

Grade 0 (no acute rejection): this indicates the absence of acute rejection or of any feature of myocyte damage in the sample. Equivocal findings of rejection are similarly graded as 0.

Grade 1A (focal, mild acute rejection): one or more samples may show focal, interstitial or perivascular infiltrates of lymphocytes unassociated with myocyte damage (Figures 3A and 6).

Grade 1B (diffuse, mild acute rejection): a more diffuse infiltration (Figure 3B) of a similar intensity to that seen in 1A is present. Not all samples need to show features of rejection.

Table 1 Standardized cardiac biopsy grading

Grade	*'New' nomenclature*	*'Old' nomenclature*
0	No rejection	No rejection
1	A = Focal (perivascular or interstitial) infiltrate without necrosis B = Diffuse but sparse infiltrate without necrosis	Mild rejection
2	One focus only with aggressive infiltration and/or focal myocyte damage	'Focal' moderate rejection
3	A = Multifocal aggressive infiltrates and/or myocyte damage B = Diffuse inflammatory process with necrosis	'Low' moderate rejection 'Borderline/severe'
4	Diffuse aggressive polymorphous ± infiltrate ± edema, ± hemorrhage, ± vasculitis, with necrosis	'Severe acute' rejection

'Resolving' rejection is denoted by a lesser grade. 'Resolved' rejection is denoted by grade 0.

Grade 2 (focal, moderate acute rejection): this is indicated by the presence of only one focus of lymphocytic infiltration which is sharply circumscribed. Architectural distortion with myocyte damage within the focus is listed as an additional prerequisite.

Grade 3A (multifocal moderate acute rejection): multifocal inflammatory infiltrates (Figure 7) made up of large, aggressive-looking lymphocytes with or without eosinophils involve one or more of the endomyocardial samples.

Grade 3B (diffuse, borderline, severe, acute rejection): diffuse lymphocytic infiltration (Figure 8) is observed within several of the biopsy samples. Myocyte damage is noted. Scanty eosinophils and the occasional neutrophil may be seen. Hemorrhage is usually absent.

Grade 4 (severe acute rejection): the hallmarks of this grade (Figure 9) are a diffuse, polymorphous inflammatory infiltration composed of 'aggressive' lymphocytes, eosinophils and neutrophils. Myocyte damage or even necrosis is 'always' seen. Edema, hemorrhage and vasculitis are often present too.

It is recommended that 'resolving' acute rejection (Figures 13 and 14) should be indicated by denoting a lesser grade than that given in the previous biopsy. (The term 'resolving' may also be used in parenthesis after the given numerical grade). Similarly, 'resolved' acute rejection is diagnosed as grade 0. ('Resolved' may also be given in parenthesis after the grade).

Additional information that should be included in the pathologic report includes the following: (a) the number of endomyocardial samples supplied for the biopsy (four or more are considered adequate); (b) presence of humoral (antibody mediated) rejection; (c) presence of a Quilty effect (endocardial lymphocytic infiltration) ± myocyte encroachment; (d) ischemic changes (early on related to donor heart procurement and late due to graft arteriopathy), early on catecholamine-induced brain death effects may also be encountered; (e) infection present – biopsy cannot be interpreted for rejection; (f) lymphoproliferative disorder; (g) other (to be specified).

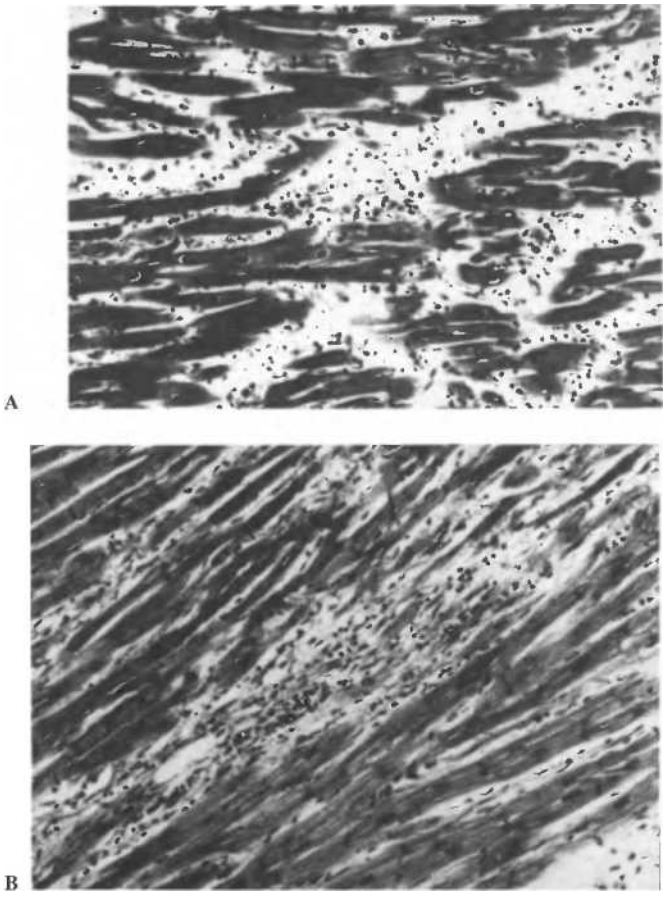


Figure 13 **A**: Prominent interstitial edema accompanies a moderate lymphocytic cellular infiltration of early-resolving acute rejection (H&E, × 120). **B**: Late-resolving acute rejection shows few mononuclear cells and early stromal collapse fibrosis where some myocytes have been lost (H&E, × 110)

PROPOSED REVISION OF THE ISHLT GRADING SYSTEM

As may be expected, practical use of the ISHLT grading system has highlighted its inherent deficiencies. The following revision to the ISHLT grading system (Table 2) was proposed at the 15th Annual Meeting of the International Society for Heart and Lung Transplantation held in San Francisco, 5–8 April 1995. *Grades 0 and 1 (1A and 1B)* remain as before. *Grade 2* has been abolished. *Grade 3* is divided into *Grade 3A* (two or more multifocal, discrete lymphocytic infiltrates with focal, occasional eosinophils) and *Grade 3B* (diffuse mononuclear cellular infiltration in several or all of the biopsy fragments; at least two samples show evidence of myocyte damage). *Grade 4* (all of the biopsy samples show edema, hemorrhage and vasculitis with rare areas of normality).

Footnotes to this revised grading system include the following:

(1) An inadequate sample is less than four pieces. Four to six samples are the optimum to be aimed for. If less than three samples are received, the pathologist cannot rule out the presence of rejection if appearances are negative. If less than

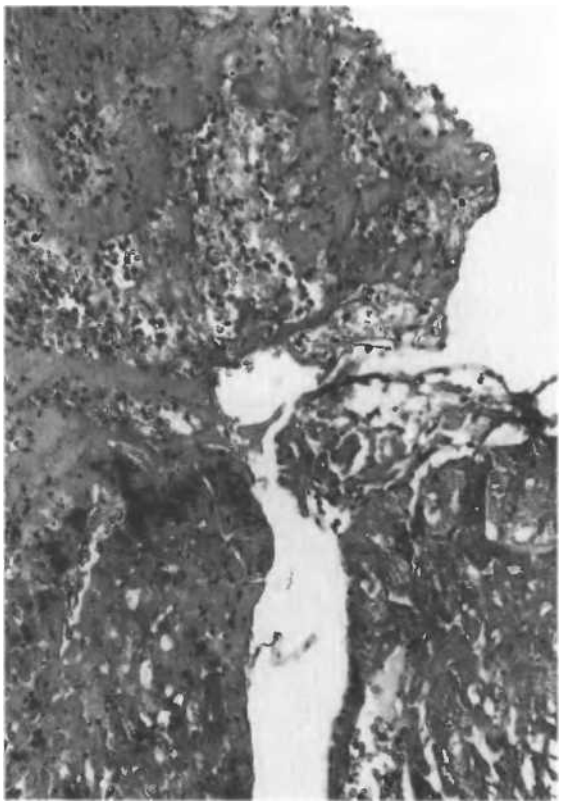


Figure 14 Early-organizing fibrin thrombus (top) covers the surface of a previous right ventricular endomyocardial biopsy site (H&E, × 150)

two samples are positive for rejection, one cannot rule out a higher grade of rejection.

(2) No immunofluorescence is needed.

(3) Quilty change is noted if present, but is not characterized further.

(4) Ischemic changes, both early (<3 months post-transplantation) and late (>3 months) are looked for and noted.

(5) The grading should not be applied to autopsy material.

(6) The worst area in the sampled myocardium should be used to choose the grading number.

GENERAL COMMENTS ON THE PATHOLOGIC DIAGNOSIS OF ACUTE CARDIAC REJECTION

Myocyte necrosis in acute rejection

The presence of myocyte necrosis is taken as a firm indicator of clinically significant acute cardiac rejection in most grading systems used for assessing endomyocardial biopsies[24,29]. Multiple foci of necrosis have more significance than only a single such focus. Such necrosis is rare in my experience, even in patients with florid lymphocytic infiltrates.

Ratliff *et al.*[21,22,30] have performed extensive electron microscopic studies of myocytes that appear to be necrotic by light microscopy, and they report that such myoctes show a unique form of reversible myocyte injury. Centralization of the myofilaments occurs and the latter are surrounded by a radially orientated bundle of myofilaments. They suggest that the myocyte injury of transplant rejection is generally reversible and is similar to apoptosis.

Resolving acute rejection

Augmented immunosuppresion usually leads to abolition of an acute rejection episode. This process, which may take days to weeks to reach completion, is termed resolving acute rejection (Figure 13). Since the clinical concern is whether rejection has been controlled, this is a period in which further biopsies are often taken.

In patients receiving cyclosporin-based immunosuppression less rapid dissolution of the lymphocytic cellular infiltration occurs when pulsed doses of corticosteroids are given to overcome acute rejection, compared to patients receiving the previous steroid-based immunosuppressive regimen[31]. Thus, acute rejection in patients treated with cyclosporin resolves slowly. Myocyte damage may persist for about 2 weeks despite increased immunosuppression[32]. With resolution of acute rejection the remaining lymphoid cells show minimal pyroninophilia, and the removal of dead myocytes leads to early replacement fibrosis.

Table 2 Revised standardized cardiac biopsy grading

0	No rejection
1	A = Focal (perivascular or interstitial) infiltrate without necrosis B = Diffuse, but sparse infiltrate without necrosis
2	Has been abolished/no longer exists
3	A = Multifocal, discrete lesions, focal occasional hypereosinophilia B = Diffuse mononuclear cells in several or all fragments. Two samples show myocyte damage
4	All biopsy samples show edema, hemorrhage, vasculitis with rare areas of normality in the sample

Less than four pieces is an inadequate biopsy. Three pieces cannot rule out rejection. If two pieces are positive rejection can be diagnosed, but cannot rule out a higher grade. At least three levels should be cut on each block.
The worst area is the keynote area for grading.
No immunofluorescence is needed.
Quilty (not Quilty A or B).
Ischemic changes may be noted early (< 3/12) or late (> 3/12). The grading is not for use in autopsy or explanted material.

Detection of possible over-immunosuppression

If repeated biopsies are totally negative for the presence of any lymphocytes within the myocardium, this should be brought to the attention of the clinician caring for the recipient, as there is the possibility of over-immunosuppression and an attendant danger of infection. This dictum is especially important in patients receiving cyclosporin, since endocardial lymphocytic infiltrations are commonly seen.

Immunofluorescent studies

Immunofluorescent studies for the immunoglobulins IgG, IgM, and IgA, and complement (C3) on biopsy and autopsy material from human and animal donor hearts yielded unhelpful results by both direct immunofluorescence and immunoperoxidase methods in acute rejection. Moderate amounts of fibrinogen and C3 may be found within the walls of some intramyocardial blood vessels. Immunofluorescence may be useful in some circumstances[33] and it has also been used for the detection of microvascular rejection[34].

Lymphocyte subpopulations in acute cardiac rejection

T lymphocytes are the predominant cell type in acute cardiac rejection. The ratio of helper to suppressor T lymphocytes in the cardiac biopsy does not correlate with rejection. However, the greater the number of lymphocytes in the biopsy, the greater is the likelihood of significant acute rejection. B lymphocytes are seldom present in endomyocardial biopsies and, if present, are seen in very scanty numbers only. Macrophages are more prominent in resolving acute rejection.

Cyclosporine suppresses the generation of inducer T cells, but allows the generation of suppressor cells. Monoclonal antibodies, which destroy the T3 cell subset, are playing a small, but possibly increasing, role in the treatment of acute ejection.

Electron microscopy

In the early stages of acute rejection there is a preponderance of mononuclear cells of undistinguished appearance. In biopsies of cardiac allografts implanted in baboons, unidentified mononuclear cells composed 53% of the interstitial cellular infiltrate overall in acute rejection[10]. Later activated lymphocytes predominate (Figure 10); occasional histiocytes, neutrophils and eosinophils may also be seen. In resolving acute rejection a few plasma cells may also be observed. The latter cells are characterized by the presence of numerous polyribosomes and cisternae of rough-surfaced endoplasmic reticulum. Such cells stain weakly with the Unna-Pappenheim stain.

The myocytes of donor heart biopsies may show a variable loss of myofilaments leaving free-lying Z-bands within the sarcoplasm. Some Z-bands have a widened, smudgy, ill-defined appearance. As detailed above, Ratliff *et al.*[30] report an unusual, reversible form of myocyte damage, characterized by radially arranged myofilaments. Severe acute rejection associated with vasculitis may cause complete myocyte destruction. Other ultrastructural features of note include swollen mitochondria, dilated T tubules, cytoplasmic lipid vacuoles and swelling or necrosis of capillary endothelial cells. Severe acute rejection associated with vasculitis may cause complete myocyte destruction.

SPECIAL PROBLEMS REGARDING THE LIGHT MICROSCOPIC DIAGNOSIS OF ACUTE REJECTION

Certain special problems may be encountered in interpreting donor heart endomyocardial biopsies[27,35,36].

Inadequate biopsy

The rejection process in cyclosporin-treated patients is more focal than that seen in steroid–azathioprine-treated patients[37]. At least three to five tissue samples from different areas of right ventricular endomyocardium should be obtained at each biopsy procedure, in order to accurately assess the degree of rejection present. Personal experience with steroid–azathioprine immunosuppression is that the rejection changes are not uniformly distributed throughout the myocardium, but endomyocardial sampling is representative of the overall situation[15]. In the Standardized Grading System of the ISHLT four or more samples of endomyocardium are considered adequate (see above).

Thrombus obtained on biopsy

Occasionally, one or more endomyocardial samples are found to consist solely of fibrin thrombus. The source of the latter is not always clear. Possible sites of origin include the biopsy catheter itself, endocardial thrombus, thrombus at the vein entry site, or even a previous biopsy site.

Previous biopsy site

Since right ventricular endomyocardial biopsy samples a limited area of the apical portion of the interventricular septum, there is a possibility that the thrombus may even be derived from a previous biopsy site if serial biopsies have been taken[35,36]. A localized lymphocytic response and/or even myocyte necrosis may also be evoked by the biopsy procedure, and this may lead the unwary to consider the presence of rejection[27,36]. The presence of organizing thrombus (Figure 14) or hemosiderin deposits should provide a clue as to the correct diagnosis. Myocytes running vertically into an organizing thrombus are also characteristic of a previous biopsy site.

Presence of fibrous tissue

Another problem is the sample that is composed solely of fibrous tissue. While such a finding raises the possibility of chronic rejection, it should be borne in mind that chronic rejection commonly spares the myocytes that lie immediately below the subendocardium[23]. If the donor heart has a greatly reduced ejection fraction, and biopsy reveals no evidence of acute rejection, then chronic rejection is high on the list of possibilities.

Sometimes the fibrous tissue is easily identifiable as a portion of the tricuspid valvular chordae tendineae. Usually such removal of tricuspid valvular tissue does not result in significant valvular dysfunction. Occasionally the bioptome may penetrate the right ventricle and sample the fibrosed epicardium, with or without small epicardial blood vessels. A healed previous biopsy site may also show abundant fibrosis.

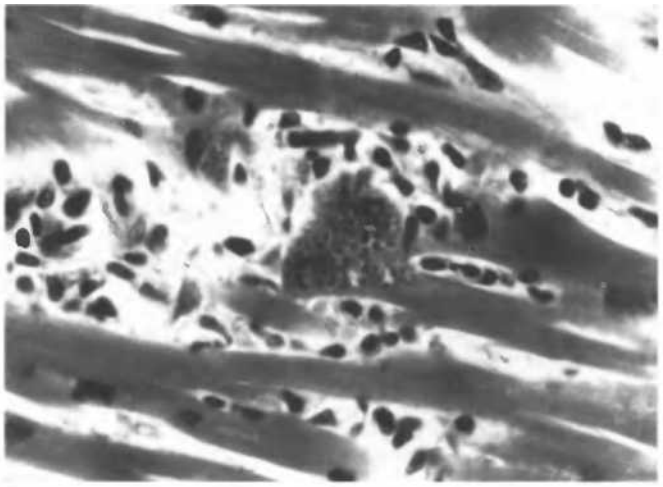
Infection of the donor heart

Although infection of the donor heart is very rare, the pathologist should be constantly on the alert for such a possibility. Infections include toxoplasmosis, coccidioidomycosis, cytomegalic inclusion disease, and Chagas disease. *Sarcocystis* species, which may occasionally infect humans, can produce a similar appearance to that of toxoplasmosis in the heart. These myocardial infections may elicit a mononuclear cellular infiltration that may be confused with acute rejection.

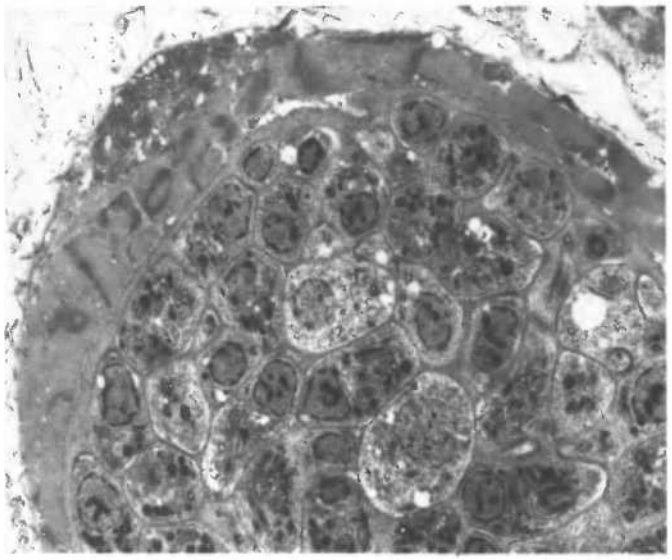
I have encountered two cardiac transplant patients with toxoplasmosis. In one patient with a heterotopic allograft a diagnosis of infection of both the donor and the recipient hearts by *Toxoplasma gondii* was made by endomyocardial biopsy (Figure 15A). Electron microscopy (Figure 15B) and serology served to confirm the diagnosis[38]. The infection had been transmitted to this recipient via the donor heart. Toxoplasmosis has also been reported from several other centers[39,40].

The interstitial mononuclear cellular infiltration that follows release of the *Toxoplasma* organisms from the cysts within myocytes is similar to that seen in acute rejection despite its supposedly more mixed nature (presence of plasma cells, as well as histiocytes and scanty eosinophils). In acute rejection the cellular infiltrate consists almost entirely of activated lymphocytes[33].

Despite these theoretical differences there is no certain way of diagnosing acute rejection in the presence of an active cardiac infection by *Toxoplasma*. Since our first patient with toxoplasmosis, referred to above, had a heterotopic transplant, the recipient heart served as a control for chemotherapy and for deciding whether a mononuclear cellular infiltration of the donor heart was likely to be due to acute rejection or toxoplasmosis.



A



B

Figure 15 **A**: Partially ruptured intramyocyte cyst of *Toxoplasma gondii* is evoking an early, mixed chronic inflammatory cellular response (H&E, × 250). **B**: Electron micrograph of donor heart biopsy shows numerous *Toxoplasma* within a myocytic cyst (Lead citrate and uranyl acetate, × 5180)

Myocardial ischemia

Early ischemia

Early myocardial ischemia may be encountered if the donor heart has not been adequately protected prior to implantation. The paucity of human donor hearts available for transplantation has led to distant heart procurement, whereby the excised donor heart may be stored and transported in a cardioplegic solution in ice, or by using a portable hypothermic perfusion system. A prolonged transplantation operation itself may cause myocardial damage. If preservation is unsatisfactory, various forms of myocardial necrosis (coagulative, myocytolytic, and contraction band) may be observed. Widespread interstitial hemorrhage indicative of a reperfusion-type infarction may also be observed. Subsequent stromal collapse fibrosis may be evident in 1–2 weeks.

Late ischemia

Late ischemic changes in the myocardium may be related to chronic rejection. The presence of a band of extensive coagulative necrosis or myocytolysis (Figure 16) in an endomyocardial biopsy in a patient who has survived longer than 3 months after transplantation may indicate graft arteriopathy (chronic rejection).

Similarly, healed infarction of the right ventricle may yield an endomyocardial sample which consists almost entirely of fibrous tissue with a band of surviving myocytes three to five myocytes thick immediately deep to the endocardium.

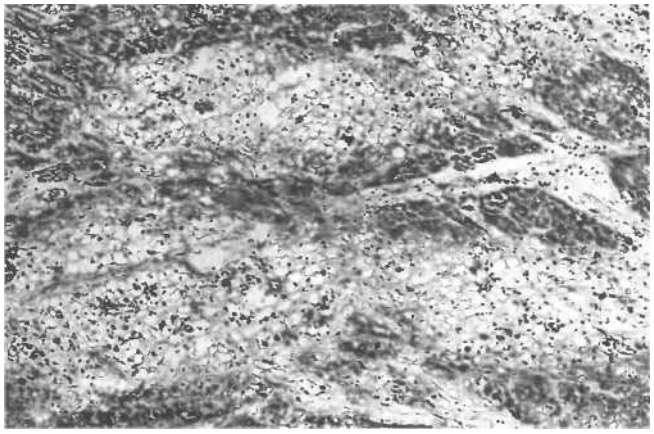


Figure 16 Late ischemic necrosis of right ventricle due to graft arteriopathy. Pale areas show myocytolytic necrosis and darker staining myocytes show coagulative necrosis (H&E, × 45)

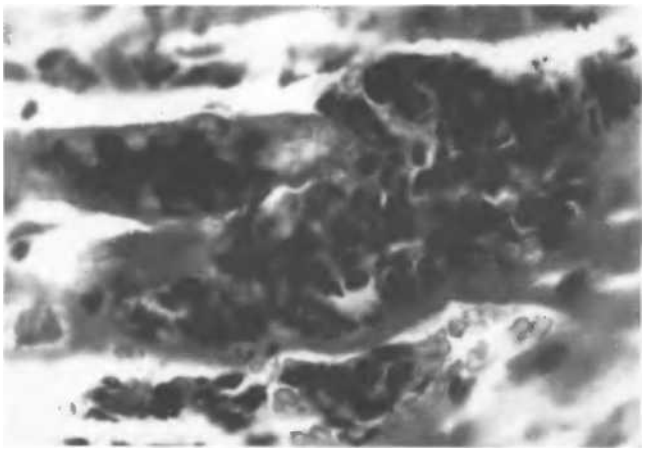


Figure 18 Numerous lymphocytes are applied to the surface of a necrotic myocyte in severe acute cardiac rejection (H&E, × 480)

Effects of donor brain death

Donor hearts are seldom biopsied in the first week following transplantation. Contraction banding of myocytes is a frequent biopsy-induced artefact, particularly at the margins of the biopsy. The presence of very numerous contraction bands has been taken as a sign of rejection by Kemnitz *et al.*[25]. Catecholamine overproduction associated with brain death produces myocyte injury[41–43], which consists of heightened cosinophilia of myocytes, contraction banding, focal coagulative necrosis, and application of mononuclear cells to the surface of damaged myocytes (Figure 17). The appearances can be similar to myocyte necrosis induced by acute rejection (Figure 18).

Localized endocardial lymphocytic infiltration (Quilty lesion)

Focal collections of lymphocytes which have been attracted to the endocardium are not an unusual finding in patients treated with

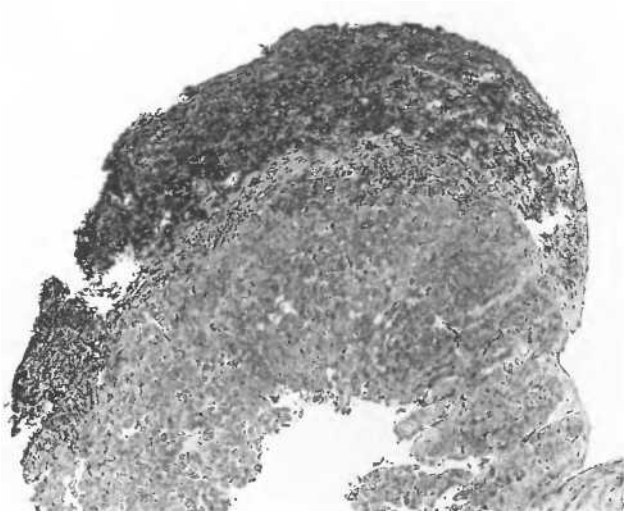


Figure 19 Endocardial lymphocytic infiltration with extension into the underlying myocardium (so-called Quilty B lesion). (Immunoperoxidase stain for T lymphocytes, × 20)

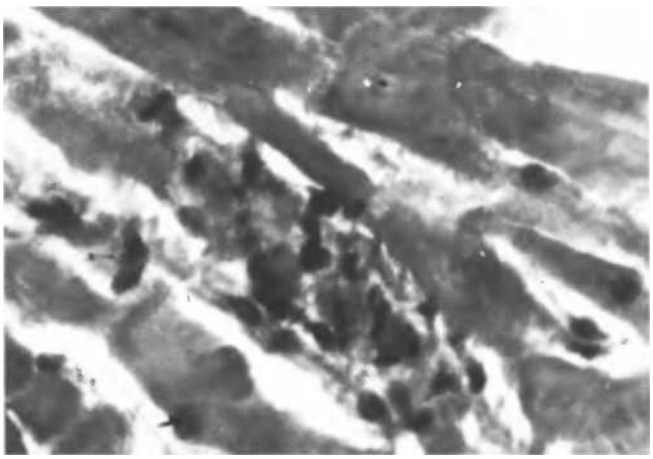


Figure 17 Mononuclear cellular response to necrotic myocyte in human donor heart damaged by brain-death-induced catecholamine excess (H&E, × 490)

cyclosporin. If the lymphocytic infiltration becomes florid it may lead to exophytic thickening of the endocardium, or it may extend into the underlying adjacent myocardium (previously termed an aggressive Quilty effect (Quilty B). The Quilty lesion (Figure 19) does not represent significant acute rejection, and augmented immunosuppression is not warranted. The response is more frequent in the paediatric age group.

Vascular (humoral/antibody-mediated) rejection

Vascular rejection may take one of two forms[44,45]: hyperacute rejection which occurs within 24 hours following transplantation, or delayed vascular rejection which is a similar process occurring later due to immunosuppression. The earliest features consist of swelling of capillary endothelial cells, venous thrombosis (seldom observed in endomyocardial biopsy), capillary con-

gestion, interfascicular followed by interstitial edema, and interstitial hemorrhage.

Lack of correlation with hemodynamic data

Hemodynamic function and inflammation or fibrosis detected on biopsy show a poor correlation. Greenberg *et al.*[46] found no significant difference in mean ejection fraction and left ventricular end-diastolic pressure between patients with and without fibrosis on biopsy, or between those with and without inflammation. At Groote Schuur Hospital, Cape Town, a good correlation was noted between changes in left ventricular volumes and histologic semiquantitative scores for acute rejection[47].

ALTERNATIVES TO ENDOMYOCARDIAL BIOPSY

As indicated earlier, several methods are being explored with the purpose of finding an acceptable substitute for endomyocardial biopsy in the diagnosis of acute cardiac rejection (Chapter 30). These include magnetic resonance imaging, assessment of peripheral blood lymphocytic activation, soluble interleukin-2 receptor levels[48] and induction of vascular adhesion molecules[49]. For the foreseeable future, graft histology will remain the gold standard for the early diagnosis of acute rejection.

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30
Clinical Diagnosis of Acute Rejection

M.R. COSTANZO

INTRODUCTION

The alloimmune response of the recipient against the transplanted heart can injure the constituent cells of the graft, impairing their function[1]. The potential target cells for injury include not only the myocytes, whose destruction forms the basis for rejection surveillance and treatment, but also the cells of the vasculature, which provide the interface between donor and recipient. The alloimmune response involves the presentation of donor antigen, by vascular endothelial cells and/or by passenger leukocytes, leading to the activation and clonal proliferation of T cells that specifically recognize alloantigens[1] (Chapter 7). These events, as well as recruitment of macrophages and the up-regulation of cell surface antigens for adhesion of inflammatory cells, lead to the release of cytokines and the up-regulation of their receptors. The consequence of these events is the release of powerful biologic effectors of cell injury[1]. Myocyte injury and necrosis occurring during this process are readily recognized by histologic examination, and form the cornerstone of rejection surveillance in heart transplant (HTx) recipients.

Acute rejection is not a steady phenomenon, but occurs in sporadic waves, extending over a few days or a week or two. Since the introduction of cyclosporin (CsA) as an immunosuppressive agent, severe acute rejection episodes have become relatively rare. Mild acute rejection (cellular infiltration) is seen fairly frequently, but many groups would not increase therapy in such cases. It is impossible to predict whether or not any individual patient will experience episodes of rejection and, when it occurs, it may be impossible to make the diagnosis on clinical evidence until it is extremely advanced.

The frequency and severity of acute rejection episodes tend to diminish with time, the recipient's immune system appearing to adapt to the presence of the donor organ and its histocompatibility antigens. A state of relative unresponsiveness is frequently achieved, and maintenance immunosuppressive therapy may be progressively reduced. A few patients have been weaned from immunosuppressive therapy entirely, usually in the presence of lymphoproliferative disease or severe infection, and there are anecdotal reports of patients surviving months or years when not receiving immunosuppressive drugs. However, it would appear that the possibility of an acute rejection episode is almost always present, even some years after HTx, particularly if a patient fails to take his/her medication regularly.

DIAGNOSIS OF ACUTE REJECTION

The patient may feel completely well until the rejection episode has progressed for some days and donor heart function has deteriorated (sometimes irreversibly) to the point that cardiac failure occurs. (Identification of features of cardiac failure from acute rejection may prove more difficult in a patient with a heterotopic HTx, in whom the recipient heart may assist the cardiac output for a considerable period of time, delaying the onset of symptoms and signs of cardiac failure.) Irreversible damage of the myocardium may occasionally occur before clinical features become manifest. For successful therapy to be initiated at an early stage, the diagnosis must therefore be made before clinical features of cardiac failure occur.

Endomyocardial biopsy remains the most reliable method of confirming rejection. The search for a simple, noninvasive method of detecting acute rejection in its early stages (or even of predicting rejection in advance) has continued for a number of years. Such methods are reviewed below. There are, however, some clinical features and simple investigations that may make the attending physician suspicious that a rejection episode is developing.

Clinical features

Acute rejection is frequently totally asymptomatic, particularly in its early stages. In a patient with an orthotopic allograft the clinical diagnosis of rejection relies mainly on symptoms and signs indicating cardiac failure, particularly of right ventricular failure due to the decreased compliance associated with cellular infiltration and edema of the graft. In the early stages following HTx, however, several other factors may affect the performance of the right ventricle, e.g. inadequate preservation of the heart

during transportation and transplantation, an increased pulmonary vascular resistance, and fluid overload (secondary either to steroid therapy or to impairment of renal function from CsA therapy). These factors may make a clinical diagnosis of rejection uncertain.

In the heterotopic HTx, however, due to the support given by the patient's own right ventricle, evidence of right ventricular failure may not occur during rejection and, therefore, such symptoms and signs cannot be relied upon in the diagnosis of this complication.

The onset of features of cardiac failure, however, should always be considered to be due to acute rejection until proved otherwise. If acute rejection is confirmed, the treatment is primarily increased immunosuppression rather than anti-failure therapy, though this may be indicated also.

Clinical features suggesting a reduction in cardiac output (e.g. weight gain, diminished pulse volume, cold extremities), muffled or reduced-amplitude heart sounds, the development of a pericardial friction rub, tachycardia or gallop rhythm, or a dysrhythmia (in the absence of electrolyte or acid–base disturbance) should be viewed suspiciously until acute rejection has been excluded. Very occasionally, patients complain of vague chest discomfort or are feverish during an acute rejection episode.

Radiographic appearances

Radiographic evidence of rejection consists of progressive cardiomegaly, increasing pulmonary plethora and, rarely, pulmonary edema. An increase in cardiac volume of more than 10% or 100 ml compared with the previous measurement, and a simultaneous increase of the cardiothoracic ratio, have been suggested as confirmation of acute rejection[2]. Using these criteria, sensitivity and specificity were 76% and 97%, respectively. Predictive values of a positive or negative test for the presence or absence of rejection were 82% and 96%, respectively. Such radiographic changes may well make the physician suspicious that rejection is occurring, but should not be relied upon exclusively.

Occasionally, the appearances are those of an exudate from the epicardium. Following orthotopic HTx, fluid exuding from the epicardium may show up as a pericardial effusion (and may be associated with clinical features suggestive of subacute tamponade); after heterotopic HTx, it may present as a right-sided pleural effusion. The presence of a pericardial or pleural effusion should be considered suggestive of acute rejection until proved otherwise. A pleural effusion may, of course, suggest an underlying infective condition of the lung, which should also be aggressively sought. With satisfactory treatment of the acute rejection episode, these effusions will regress and disappear.

Effusions, however, may be absent in patients with severe acute rejection, or may appear very late in the episode. Their development cannot be awaited, therefore, as a reliable diagnostic aid in the recognition of early rejection.

Endomyocardial biopsy (EMB)

Survival after HTx has improved steadily over the past decade, with mortality at 1 year falling to as low as 10% in many centers[3]. This improvement in survival is owed in part to better, more specific immunosuppression, but also, to a large extent, to surveillance endomyocardial biopsy (EMB), which allows diagnosis of rejection to be made, in most instances, before the development of allograft dysfunction[4]. Early diagnosis is exceedingly important due to the high mortality in patients with acute rejection associated with consequent allograft dysfunction[5]. The histologic diagnosis of cardiac allograft rejection is obtained by light microscopy examination of the specimens obtained by transvenous EMB, which is performed at regular intervals after HTx. In many centers, EMB are performed weekly for the first 4–6 weeks, biweekly for the subsequent 2 months, then at progressively longer intervals until a frequency of ≤ 3 EMB per year is reached[6].

EMB can be performed with a variety of bioptomes which are inserted through the internal jugular, subclavian or femoral veins, and advanced, under radiologic or echocardiographic control, to the apical portion of the right ventricular septum[7]. Adequate sampling and proper handling of EMB specimens are critical to obtain accurate diagnoses. Because rejection is often a focal process, three to five specimens are necessary to achieve a sensitivity ranging between 75% and 98%[8,9]. Once six specimens are examined, obtaining additional tissue does not significantly increase the diagnostic yield[9]. The specimens should be fixed in 10% formalin for light microscopy (Chapter 29). The tissue is processed for paraffin embedding, serially sectioned at 4 μm, and stained with hematoxylin and eosin and Masson's trichrome[6]. Electronmicroscopy, immunohistochemistry and immunofluorescent studies are performed at some centers as part of research protocols (Chapter 28), but are not mandatory for the routine diagnosis of acute rejection[6].

Since the Stanford original histopathologic classification of acute rejection[10], many modifications have been proposed (Table 1). The feature common to all classifications is that severity of rejection is graded according to increasing degrees of inflammatory infiltrates and increasing damage to the myocardium. The categories of mild and moderate rejection are often subdivided into focal and diffuse, in an attempt to predict which subset of patients within each category is most likely to progress to the next

Table 1 Comparison of individual centre grading systems for acute rejection

Stanford	None	Mild	Moderate	Severe
Texas	0 1 2 3 4		5 6 7 8 9	10
Hannover	A 0	A 1 A 2	A 3	A 4
Boston	0	1 2	3 4	5
Loyola	None	FM Mild	FMod Mod	Severe
Pittsburgh	0 1	2	3	4
Utah	1 2	2.5 3	4	5

From Ref. 6.

Table 2 International Society for Heart and Lung Transplantation (ISHLT) standardized cardiac biopsy grading

Grade	*Description*
0	No rejection
1A	Focal perivascular or interstitial infiltrate without myocyte damage
1B	Diffuse but sparse perivascular and/or interstitial infiltrate without myocyte damage
2	One focus only with aggressive infiltration and/or focal myocyte damage
3A	Multifocal aggressive infiltrates and/or myocyte damage
3B	Diffuse inflammatory process with myocyte damage
4	Diffuse aggressive polymorphous infiltrate ± edema ± hemorrhage ± vasculitis, with necrosis

From Ref. 13.

higher grade and should, therefore, be monitored more closely and/or receive increased immunosuppression[11,12]. The key histologic feature which differentiates 'moderate' from 'mild' rejection is the presence of myocyte damage since, in general, the presence of this finding warrants augmentation of immunosuppression. At present the rejection grading system introduced by the International Society for Heart and Lung Transplantation (ISHLT)[13] is the most widely accepted among transplant centers (Table 2).

Undoubtedly, standardization of cardiac allograft rejection criteria has greatly improved the ability of HTx centers to compare rejection rates, severity and thresholds for treatment. Accurate comparison of EMB results between institutions is also essential to the conduct of multicenter immunosuppressive trials. However, even the ISHLT grading system is not exempt from criticism. It has been pointed out that the ISHLT classification of rejection does not immediately convey information on whether the histologic changes detected in an individual EMB represent ongoing or resolving rejection. Criteria for the diagnosis of chronic and 'humoral' rejection are classified as 'additional information' rather than separate entities. The ISHLT classification does not account for the fact that the significance of a given histologic finding may change over time. For example, grade 2 detected early after transplantation may forecast impending rejection of greater severity, whereas the same rejection grade detected beyond the third postoperative month may be an entirely benign, self-limiting finding[12]. The relative severity of grades 1B and 2 remains unclear; recent studies on the evolution of untreated rejection have shown that *diffuse* cellular infiltrates *without* myocyte damage may progress more often than an *isolated* cellular infiltration associated *with* myocyte damage[12].

In addition to the specific limitations of the ISHLT rejection classification, other factors can complicate the histopathologic interpretation of EMB specimens (Table 3). Some of these are related to the procedure and tissue processing, some to tissue sampling, and others to transplantation-related effects[6]. Since the average HTx recipient undergoes 15–20 EMB in the first postoperative year, and the bioptome tends to follow the same path due to the structure of the instrument and the configuration of the right ventricular trabeculae, it is common to take an EMB specimen from a previous EMB site. EMB site changes, which can be present in 16–69% of surveillance EMB specimens, range from a fresh thrombus overlying an area of myocyte injury and hemorrhage in a recent EMB site, to granulation tissue, often containing mononuclear inflammatory cells and myocyte disarray at the periphery of the EMB site, to dense fibrous tissue in a fully healed EMB site[6,14].

It remains unclear whether endocardial infiltrates (Quilty effect), which are found in 5–10% of adult recipients and up to 50% of pediatric recipients receiving cyclosporin, forecast impending rejection, or are benign collections of lymphocytes[15,16]. In some cases it is difficult to distinguish Quilty lesions from rejection, particularly when the lesion extends into the subadjacent myocardium and encroaches upon, surrounds, and damages myocytes. Contiguity of the endocardial and myocardial components is the key histologic feature which confirms the presence of a Quilty lesion and excludes acute rejection[15].

Table 3 Diagnostic difficulties encountered in the histopathologic interpretation of endomyocardial biopsies

Procedural/processing
Forceps artifacts
Edema
Hemorrhage
Contraction bands
Sampling
Previous biopsy site
Endomyocardial fibrous tissue
Adipose tissue
Extracardiac tissue
Transplant-related
Ischemic injury
Endocardial infiltrates (Quilty effect)
Opportunistic infections
Post-transplantation lymphoproliferative disorder

Adapted from Ref. 6.

Histologic evidence of ischemia detected in the first few postoperative weeks may result from the use of pressor agents in donor and/or recipient, preservation injury, or microinfarcts due to air bubbles entrapped in the coronary circulation during the operation[17]. In ischemic injury the degree of myocyte damage appears to be disproportionate to the sparse infiltrate, which is composed of neutrophils in the early stages and of macrophages, histiocytes and granulation tissue in the organizing phase[6,18]. Differentiation of ischemia from acute rejection during the early postoperative period can avoid unnecessary intensification of immunosuppression. However, ischemia, when present later after HTx, may unveil cardiac allograft vasculopathy[19].

Other potential pitfalls of EMB include sampling error, which leads to a falsely negative diagnosis of rejection in 15% of cases, recovery of insufficient tissue in 6% of cases, and incorrect interpretation of entities that may mimic rejection, such as infections and post-transplantation lymphoproliferative disorder (PTLD)[6]. Careful search for infectious organisms is warranted. The two organisms most likely to be encountered in EMB are *Toxoplasma gondii* and cytomegalovirus. When the myocardium is involved with PTLD, the distinction between this infiltrate and reactive T lymphocytes of acute rejection can prove very difficult[6]. The presence of atypical lymphocytes, lymphoplasmacytoid or immunoblastic mononuclear infiltrates, necrosis and frequent mitotic figures should suggest the possibility of PTLD.

Immunohistochemical studies for light chain restriction, gene rearrangement for clonality and *in-situ* hybridization for EBV are useful diagnostic studies[20].

Recently, investigators have observed clinical examples wherein patients with hemodynamic and echocardiographic evidence of allograft dysfunction lack the classic histopathologic findings of cellular infiltrates and myocyte injury on EMB specimens[21]. The EMB specimens instead display evidence of endothelial activation and injury in the capillaries, venules, and arterioles of the myocardium[22]. The earliest findings include enlarged, prominent, swollen endothelial cells and interstitial edema. In the later phases vasculitis is observed with infiltration of the vessel wall by lymphocytes, macrophages and neutrophils. This has been designated as acute vascular or humoral rejection[22] (Chapter 28). In addition to these microscopic findings, immunofluorescent studies are required to establish the diagnosis. Vascular injury is characterized by deposition of immunoglobulin (IgG or IgM), complement (C1q or C3) and fibrinogen in linear or circular patterns indicative of deposition within the vessels[22]. Accurate diagnosis of humoral rejection is important, since this entity may require, in addition to high-dose pulsed corticosteriod therapy, the use of plasmapheresis and cyclophosphamide. Furthermore, patients with humoral rejection may be at higher risk of developing cardiac allograft vasculopathy[23].

The contribution of surveillance EMB to the improved outcome of HTx recipients is undeniable. However, some limitations of EMB cannot be ignored. The EMB is an invasive procedure which is associated with a rate of complication ranging between 0.3% and 1.3%[5,6]. Processing and interpretation of EMB specimens may delay diagnosis of rejection for 8–24 hours. EMB is performed at progressively longer intervals after HTx. Thus, since the detection of rejection is highly dependent upon the frequency of EMB, it is very difficult to estimate the duration of a rejection episode. Indeed, the EMB provides only 'snapshots' of the rejection process, while the immune response of the recipient against the allograft and the resulting effects on allograft function are continuously ongoing. Another important limitation of EMB is its expense, an increasing concern in this era of health-care rationing and cost containment. The limitations of EMB summarized above have spurred the search for reliable, safe and less expensive noninvasive methods for the diagnosis of rejection.

Electrocardiographic methods

During the initial decade of HTx the results of several studies suggested that changes in the amplitude of the QRS complex measured from the standard 12-lead electrocardiogram (ECG) could be used to detect rejection[24]. However, since the introduction of CsA, QRS voltage reductions on the standard 12-lead ECG are typically absent[25]. Possible reasons for this include minimal interstitial edema, earlier detection by surveillance EMB of rejection before it can cause hemodynamic and ECG changes, and the occurrence, in CsA-treated patients, of rejection that progresses more slowly and is more easily treated. Several investigators have therefore studied the potential of ECG methods, other than the standard 12-lead ECG, for the early noninvasive diagnosis of rejection (Table 4).

Table 4 Electrocardiographic methods evaluated for the diagnosis of cardiac allograft rejection

Authors/year (ref.)	*Echocardiographic method*	*Echocardiographic rejection criteria*	*Sensitivity (%)*	*Specificity (%)*
Keren *et al.* 1984[26]	SAECG	Decreased total QRS vector voltage amplitude	82	81
Lacroix *et al.* 1992[27]	SAECG	11% decrease of QRS voltage between two consecutive recordings	88	78
Haberl *et al.* 1987[28]	SAECG (FFT)	Increased frequency content of QRS complex 70–110 Hz	90	–
Warnecke *et al.* 1992[29]	Intramyocardial ECG transmitted by implanted telemetric pacemaker	15% decrease of QRS voltage from control	88	96
Grace *et al.* 1991[30]	Epicardial paced evoked response	Decrease in evoked T wave amplitude	92	100
Sands *et al.* 1989[31]	Power spectral analysis of heart variability	Increased heart rate variability	–	–
Picano *et al.* 1990[32]	Dipyridamole ECG	> 0.1 mV ST segment depression	72	94

High-frequency signal-averaged ECG (SAECG) is a technique that enhances the signal-to-noise ratio and excludes the low-frequency signals of the standard ECG by electronic filtering methods. With this technique the averaged QRS complex can be analyzed: (a) in the time domain, using a high-pass filtering technique, and (b) in the frequency domain, using fast Fourier transformation (FFT) techniques.

In one study of 20 CsA-treated HTx patients a decrease in the total high-frequency voltage amplitude of the QRS predicted EMB histologic findings consistent with definite rejection, with a sensitivity and a specificity of 82% and 81%, respectively[26]. The method, however, was inadequate in monitoring patients during the early postoperative period and in detecting mild forms of rejection in the late postoperative phase. In another study an 11% decrease in the QRS voltage between two consecutive recordings predicted rejection with a sensitivity and a specificity of 88% and 78%, respectively[27]. In contrast, in other studies, frequency domain analysis, but not time domain analysis, of the QRS complex provided useful information for the noninvasive diagnosis of rejection. A single-beat analysis of the QRS complex by FFT revealed a progressive change of the spectral morphology (increase in the frequency content between 70 and 110 Hz) on the days of rejection in 19 of 20 patients[28]. A decrease in the amplitude of the QRS complex was not a reliable predictor of rejection[28].

In a further effort to identify an ECG correlate to rejection, intramyocardial electrograms have also been compared with EMB. In a preliminary report using intramyocardial electrocardiography transmitted by an implanted telemetric pacemaker, a voltage reduction >15% of control had 88% sensitivity and 96% specificity[29]. Unfortunately, the value of this approach has been tested only in small series, and has not been confirmed by other investigators.

An alternative ECG technique is to measure the evoked T wave amplitude using an externalized QT-driven rate adaptive pacemaker[30]. This technique uses direct measurements from the heart rather than body surface recordings, and assesses the repolarization rather than the depolarization phase of the cardiac cycle. A significant fall in the mean evoked T wave amplitude from 1.3 mV to 0.6 mV ($p<0.005$) preceded by an average of 2 days the EMB diagnosis of rejection in 11 of 17 patients[30]. Since evoked T wave amplitude did not fall in the absence of rejection, and remained unchanged in only one rejecting patient, the sensitivity and specificity of this noninvasive method for the diagnosis of rejection were 92% and 100%, respectively[30]. The above observations, however, were limited to the first 20 postoperative days, and whether a fall in the evoked T wave amplitude predicts rejection late after HTx remains unknown[30].

Since denervation of the heart reduces heart rate variability, some investigators have studied beat-to-beat heart rate variability by power spectral analysis to determine if changes in this parameter might be a noninvasive marker of rejection. One study showed that the development of rejection was associated with a significant increase in heart rate variability[31].

Since characteristic ECG changes occur in response to dipyridamole infusion in conditions associated with a decreased coronary reserve, one study sought to establish whether acute cardiac allograft rejection might induce ECG alterations during dipyridamole infusion[32]. A dipyridamole-induced ST segment depression >0.1 mV detected EMB-proven acute rejection with a sensitivity and a specificity of 72% and 94%, respectively[32]. As with other ECG markers of cardiac allograft rejection, dipyridamole-elicited ST segment changes were studied only in the first few postoperative weeks, and it is not yet known whether this technique would continue to predict rejection later after HTx[31].

Echocardiographic methods

A variety of echocardiographic techniques have been used to characterize the transplanted heart from both an anatomical and a functional standpoint. Anatomic changes, such as the presence of a pericardial effusion, and quantitative changes, such as an increase in left ventricular (LV) mass, can be evaluated by echocardiographic techniques[33]. The EMB histologic abnormalities typical of acute rejection which produce qualitative and quantitative echocardiographic changes include edema, cellular infiltrates, and myocyte damage. In addition, since cells of the microvasculature express foreign antigens and produce cytokines, they may become the target of alloimmune responses. This microvascular damage, in turn, may produce acute diastolic dysfunction, a process that would go unrecognized by conventional histologic evaluation, but could be detected by echocardiography[34]. The cardiac allograft anatomic and functional variables and the echocardiographic techniques that detect them are summarized in Table 5.

Table 5 Echo cardiographic methods evaluated for the diagnosis of cardiac allograft rejection

Authors/year (ref.)	*Echocardiographic method*	*Echocardiographic rejection criteria*	*Sensitivity (%)*	*Specificity (%)*
Dawkins *et al.* 1984[37]	M-mode and phonocardiography	10% decrease of IVRT	87	90
Valantine *et al.* 1991[40]	Doppler	Decreased IVRT and PHT	82	79
Dodd *et al.* 1993[42]	M-mode and Doppler	Echocardiographic score ≥ 4 (LVEEDV, filling velocity LVPW, velocity of LVPW thinning)	100	75
Park *et al.* 1992[46]	Digitized M-mode	20% increase of time interval (Te) over prerejection values	80	94
Masuyama *et al.* 1990[43]	Ultrasonic backscatter instrumentation	Decreased cyclic variation of integrated ultrasonic backscatter	86	85
Lieback *et al.* 1994[44]	Texture analysis	Change in three texture parameters	89	84

In patients who underwent HTx before the introduction of CsA, increases in wall thickness and LV mass measured by M-mode echocardiography were reported to occur during episodes of acute rejection[35]. Subsequently, a decreased LV lengthening rate and posterior wall thinning rate consistent with significant LV dysfunction were reported in association with episodes of acute rejection[36]. In a prospective study of 20 patients, using M-mode echocardiography and phonocardiography, a 10% decrease in isovolumic relaxation time was 87% sensitive and 90% specific for the diagnosis of acute rejection[37]. After the introduction of Doppler echocardiography, Doppler indices of diastolic function have been applied to the diagnosis of acute rejection, since this process appears to be characterized by the onset of 'restrictive physiology'[38].

This has been defined as a decrease in either isovolumic relaxation time (IVRT) or pressure half-time (PHT), as determined by the analysis of the Doppler mitral flow velocity curve[39]. The discrete signal of aortic valve closure may be recorded with the transducer oriented to encounter both mitral and aortic flow velocities. IVRT is measured from aortic valve closure to the onset of mitral flow[39]. Peak early mitral valve flow velocity (M1) is measured vertically from the baseline to the maximum recorded velocity. The maximum velocity is related to the rate of decrease of pressure difference across the mitral valve[39]. The PHT is the time taken by the peak pressure difference to decline by one half[39]. In some studies a 15% decrease in either IVRT or PHT had a sensitivity in excess of 80% and a specificity of 70% in the diagnosis of acute rejection[39]. Analysis of the factors that contribute to an incorrect Doppler echocardiographic diagnosis of rejection revealed that while false-negative studies occur most frequently when rejection is already being treated with intensified immunosuppression, restrictive physiology preceding biopsy evidence of rejection accounts for a large proportion of Doppler false-positive studies. These observations suggest that positive Doppler echocardiography in the absence of concurrent rejection by EMB is a strong indication for an earlier follow-up EMB[40].

Other investigators have challenged the value of Doppler echocardiography for the diagnosis of acute rejection due to a wide overlap of measurements in individual recipients with or without rejection[41]. Factors which may be responsible for this overlap include a variety of hemodynamic factors (pulmonary hypertension, perioperative ischemia, hypertension) which affect diastolic function and are superimposed on the restrictive left ventricular filling pattern caused by rejection and LV hypertrophy[41]. Differences in patient selection, duration of follow-up, antihypertensive therapy, and immunosuppression may also influence the outcome of Doppler echocardiographic studies[33].

To accommodate differences between patients and between serial studies in the same patient, some investigators have combined multiple echocardiographic parameters into a scoring algorithm[42]. Such an algorithm is based on echocardiographic indices of LV chamber size, diastolic function and LV mass obtained by computer-assisted digital analysis of two-dimensional-guided M-mode echocardiograms. When applied prospectively to 49 studies, an echocardiographic score ≥ 4 detected rejection with a sensitivity of 100%, a specificity of 75%, and a negative predictive value of 100%[42].

Based on the assumption that abnormalities in microscopic tissue structures are identified by their acoustic properties, some investigators have evaluated the ability of texture analysis of echocardiograms to detect morphologic changes in the myocardium caused by rejection. In some studies abnormal patterns of echocardiographic myocardial texture detected rejection with a sensitivity and specificity of approximately 85%[43,44].

In summary, the main advantages of echocardiography in the diagnosis of rejection are the noninvasive nature of the technique, the ability to obtain results immediately, and its value as an adjunct to EMB. Echocardiographic changes consistent with rejection may prompt an earlier EMB, leading to an earlier diagnosis of rejection. Conversely, absence of echocardiographic changes consistent with rejection may reduce the frequency of EMB[45–48]. However, the ability of echocardiography to detect rejection is limited by: (a) insufficient sensitivity, (b) effect of image quality, (c) need for serial studies, (d) inter-observer variability, and (e) occurrence of echocardiographic changes resulting from conditions distinct from rejection[33].

Radionuclide methods

Different radionuclide techniques have been evaluated to determine their usefulness for the noninvasive diagnosis of cardiac allograft rejection[49]. These include myocardial function studies, imaging with radioisotopes which assess myocardial perfusion or detect inflammation, and imaging with radiolabeled blood cellular elements or radiolabeled monoclonal antibodies. Functional studies are not sensitive enough unless further investigation on quantitative ventricular volume changes shows a consistent correlation with acute rejection[49]. Routine myocardial imaging agents such as ^{67}Ga, ^{99}TcPP, or the perfusion agent ^{201}Th are clearly not specific enough to detect rejection until the allografts are nearly lost[49]. Use of radiolabeled lymphocytes may hold some promise. However, this technique is limited by an unacceptably long radiation exposure, and by the inability to discriminate between rejection episodes that require intensified immunosuppression versus those that do not[49].

^{111}Indium-radiolabeled Fab fragments of monoclonal antimyosin antibodies have been shown to specifically detect myocyte necrosis that occurs at the microscopic level. In one study the sensitivity, specificity, and overall accuracy of this technique in detecting cardiac allograft rejection were 80%[50]. In another study in which ^{111}indium antimyosin imaging had a sensitivity of 100% and a specificity of 67% in detecting cardiac allograft rejection, falsely positive images were obtained in patients that had myocyte necrosis attributable to ischemic rather than rejection-induced myocyte injury[51]. However, because of the high sensitivity of antimyosin scintigraphy, EMB can be avoided in the presence of a negative scan. Using the heart/lung (H/L) ratio as a measure of ^{111}indium antimyosin antibody uptake, a H/L ratio ≥ 1.6 was 93% sensitive and 98% specific in detecting rejection[52]. The results of more recent studies suggest that ^{111}indium antimyosin antibody scintigraphy predicts the occurrence of rejection-related complications in the early postoperative period and of subsequent rejection episodes ≥ 12 months after HTx[53,54]. Based on the latter finding, the authors recommend that, if the antimyosin scan is negative ≥ 1 year after HTx, rejection surveillance be carried out noninvasively; in contrast, if the antimyosin scan performed ≥ 1 year postoperatively is positive (H/L ≥ 1.55), EMB should be performed at 4-month intervals[54].

Despite these encouraging results the limitations of ^{111}indium should not be underestimated. These include: (a) the slow blood clearance of antimyosin antibodies which delays scanning and diagnosis; (b) the long half-life of ^{111}indium which limits the ability to repeat injections at clinically required intervals; (c) the hepatic uptake of ^{111}indium which may obscure the uptake in the adjacent myocardium; (d) the production of human anti-mouse antibodies; and (e) the binding of antimyosin antibodies to dying myocytes regardless of the etiology of myocyte injury[49].

Based on the knowledge that expression of class II major histocompatibility complex (MHC) antigens increases in rejecting organs, experimental work in a rat heterotopic HTx model has evaluated the ability of ^{111}indium-labeled anti-MHC class II monoclonal antibodies to detect *in-vivo* induction of MHC class II antigens and, therefore, rejection[55]. Radiotracer uptake in the grafts was significantly correlated with the presence and severity as defined by histologic abnormalities. Furthermore, increased expression of MHC class II antigens was detected in the presence of cellular infiltrates *before* the occurrence of myocyte necrosis[55]. These experimental results are encouraging, but scintigraphy with ^{111}indium-labeled monoclonal antibodies directed against the constant region of MHC class II antigens still awaits clinical application.

Magnetic resonance imaging

The possibility that magnetic resonance imaging (MRI) might be a useful tool for the noninvasive diagnosis of rejection is based on the assumption that cellular infiltrates, hemorrhage, and myocyte damage may alter MRI relaxation times[56]. Prolongation of T2 has been reported in rejecting heterotopic canine heart allografts[57]. To date only a few clinical studies conducted in a very small number of patients have been published on rejection surveillance with MRI[56,58]. The results of these studies suggest that MRI is of limited utility for the diagnosis of rejection in human HTx recipients. Limitations of MRI include: (a) a lack of diagnostic value in the early postoperative period when T2 values are uniformly increased by myocardial edema[57]; (b) a large degree of overlap in T2 values when T2 measurements are sorted according to EMB grade; (c) the difficulty in monitoring critically ill patients in a high magnetic field; and (d) the limited availability of MRI equipment[56–58].

Cytoimmunologic monitoring

A steady and progressive rise in the white blood cell (WBC) count, or in the total lymphocyte count, or, particularly, in the T-11 lymphocyte subset, may, on occasion, indicate that a rejection episode is occurring, but is unreliable[59]. A rise in the T cell fraction has been considered helpful, and there is some evidence that fluctuations in the number of circulating T cells may reflect an earlier phase of the host immune response to the cardiac allograft than that provided by endomyocardial biopsy[60,61]. This Stanford experience, however, was documented before the introduction of CsA, at a time when antithymocyte globulin (ATG) was administered regularly for several weeks post-HTx. A rapid expansion of the circulating T-lymphocyte population, after discontinuation of ATG, was shown to correlate closely with the development of acute rejection. As CsA does not depress the number of circulating T lymphocytes, monitoring of the T cell fraction has not been put forward as an indicator of acute rejection in patients receiving this drug. A persistent rise in the T cell fraction in the early post-transplant period, however, reflects the fact that the host's immune system is responding to the presence of the foreign tissue, and may therefore suggest that acute rejection is occurring, but is not reliable.

Numerous cytoimmunologic variables have been measured in an attempt to identify a highly sensitive, easy-to-administer repetitively, and inexpensive noninvasive index of cardiac allograft rejection (Table 6). The earliest studies involved the measurement of urinary polyamines that reflected increased cellular proliferation or degeneration[62,63]. Later, changes in prolactin levels were measured as an indicator of immune activation[64]. The sensitivity of these measurements never exceeded 80%. With improved understanding of the immune response, lymphocyte subsets, and activation markers, along with the development of flow cytometry techniques, several groups have investigated the possibility that assessment of activation antigens on circulating lymphocytes, cytokines[65] (plasma tumor necrosis factor alpha levels)[66–68], cytokine receptors (serum interleukin-2 receptor levels)[69–74], and components of MHC class I antigens (serum β-2 microglobulin levels)[75,76] might predict rejection. Although one group reported a sensitivity of cytoimmunologic monitoring of 94% in the detection of rejection[77], these striking results have not yet been reproduced by others[69,78].

Important limitations of cytoimmunologic monitoring include: (a) the possibility that events occurring in the periphery may not reflect immunologic activity in the allograft; (b) the observation

Table 6 Cytoimmunologic monitoring methods evaluated for the noninvasive diagnosis of cardiac allograft rejection

1. Lymphocyte subsets in the peripheral blood
2. Urinary polyamines
3. Serum prolactin levels
4. Plasma tumor necrosis factor alpha
5. Serum interleukin-2 receptor levels
6. Serum β-2 microglobulin levels
7. Interleukin-2-driven growth of graft infiltrating cells
8. Cytotoxicity of graft infiltrating cells
9. Expression of adhesion molecules (VCAM-1, E-selection, ICAM-1) in the graft
10. Frequency of alloreactive cytolytic T lymphocytes in the peripheral blood
11. T cell receptor subsets
12. Phosphotyrosine levels in peripheral blood mononuclear cells

that single determinations correlate poorly with EMB findings; and that (c) values are not reliable in the early postoperative period because abnormalities may be due to preoperative illness, surgical trauma and ischemic injury; and (d) abnormalities induced by rejection may be indistinguishable from those occurring as a result of infection[5,69,78].

Other immunologic approaches have recently been proposed either as adjuncts to the EMB histologic diagnosis of cardiac allograft rejection, or as noninvasive diagnostic alternatives to EMB. A correlation has been found between the frequency of IL-2-driven propagation of graft-infiltrating lymphocytes and the degree of cellular infiltration in EMB specimens. Furthermore, a positive correlation was found between the growth of alloreactive lymphocytes from histologically negative EMB and the incidence of subsequent histologic rejection[79].

In another study in which lymphocyte cultures established from EMB (taken before, during, and after rejection episodes of ISHLT grade ≥ 1) were assayed for cytotoxicity, the patients whose cultures remained cytotoxic after a rejection episode went on to develop further rejection episodes[80]. These results suggest that both the growth of alloreactive T cells and persistent cytotoxicity of IL-2-responsive T lymphocytes in EMB may be useful prognostic indicators of future rejection episodes[80].

Other studies have shown that increased expression of adhesion molecules in EMB specimens may be a marker of rejection, necessitating augmentation of immunosuppression, and that expression of inducible endothelial cell adhesion molecules may predict impending clinically significant rejection[81–84]. In one study a percentage of ICAM-1-positive vessels $\geq 80\%$ predicted the presence of rejection necessitating treatment, with a sensitivity and specificity of 85% and 95%, respectively[81]. In another study, increases in E-selectin and ICAM-1 were most likely to occur in EMB just prior to rejection episodes[82].

Some investigators have used limiting dilution analysis (LDA) technology to monitor the frequency of donor-reactive cytolytic T lymphocytes in the peripheral blood of cardiac allograft recipients. In one study the frequency of donor-reactive cytolytic T lymphocytes was significantly higher ($p<0.05$) in blood samples from patients whose simultaneous EMB showed histologic evidence of cardiac allograft rejection than in patients showing no evidence of rejection[85]. Although this method is noninvasive, and the results do not seem to be influenced by infection, the 10-day culture period required for LDA makes it impractical when a rapid diagnosis of rejection is needed.

Based on the hypothesis that quantitative changes in T cell subsets can be detected in the peripheral blood, and that these changes correlate with rejection, some investigators performed T cell subset analysis by flow cytometry using monoclonal antibodies recognizing six isotypic epitopes of the T cell receptor β-chain variable (V) region. In this study, regression analysis showed a significant ($p<0.001$, $R=0.91$) temporal association between cellular rejection and abnormal subset fluctuations[86]. Although this approach holds potential for the noninvasive diagnosis of rejection, much remains to be learned about the physiological mechanisms underlying changes in T cell receptor subset expression in the peripheral blood. Furthermore, it remains to be established whether there is a relation between receptor subset expression in the peripheral blood and T cell subsets that infiltrate the blood.

Tyrosine phosphorylation is an early critical event in lymphocyte signal transduction and activation. In a porcine HTx model, levels of phosphotyrosine in peripheral blood mononuclear cells were increased 2–5 days before histologic signs of allograft rejection[87].

Thus, future methods for the noninvasive diagnosis of rejection may focus on the detection of molecular events participating in T cell activation.

COMMENT

There are some clinical, electrocardiographic and radiographic features that suggest that an acute rejection episode may be occurring, but EMB represents the only generally accepted reliable method of confirming the presence or absence of rejection. Even EMB can be misleading on occasions, however, and if biopsy proves negative and yet clinical suspicion of rejection is high, then EMB must be repeated as a matter of urgency. There are occasions when the result of the EMB, if negative, should be ignored and, if the physician's suspicions are high, the patient should be treated for rejection even in the absence of confirmation.

The procedure of EMB is unpopular with the majority of heart transplant recipients, and is not without risk. The search for noninvasive techniques for diagnosing the presence of early rejection (or preferably for predicting the onset of rejection) should therefore continue. Further investigations and trials are required, however, before any noninvasive technique can be accepted as being totally reliable; only then will EMB prove unnecessary.

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Treatment of Cardiac Allograft Rejection

L.W. MILLER

INTRODUCTION

The development of the endomyocardial biopsy by Caves *et al.*[1] was one of the most important developments in the management of heart transplant recipients, and remains the gold standard today for the diagnosis of allograft rejection. Subsequent investigation has shown that a minimum of four and preferably five adequate pieces of endomyocardium are required to achieve satisfactory diagnostic accuracy[2]. The samples should be taken from as many different locations as possible for maximum sensitivity, a task perhaps made easier by use of echocardiography (versus fluoroscopy) to guide the bioptome and allow safe sampling from the anterior free wall and apex[3].

The decision or threshold to initiate bolus therapy for treatment of cardiac allograft rejection has evolved over time. In the mid-1980s low-grade rejection (grades 1B and 2) was routinely treated in many programs[4]. However, a number of investigators have shown that as many as 80–85% of both diffuse mild and focal moderate rejections resolve spontaneously, or with a simple increase in maintenance immunosuppression[5–8]. Although there is increasing evidence of a strong correlation between the occurrence of even one rejection episode and the development of chronic rejection in kidney transplant recipients[9,10], there are few data demonstrating a correlation between the incidence, severity, or time to first rejection and the development of chronic rejection or allograft coronary disease in heart transplant recipients[11–13]. As a result the pendulum has swung to a more conservative approach or threshold to treat cardiac allograft rejection over the past 5 years.

Most recently, however, data from Kobashigawa and the CVIS investigators[14], involving over 250 patients, demonstrated a correlation between the average biopsy score (ABS), which takes into account all low-grade untreated as well as treated rejection during the first 3 months post-transplant, and the development of coronary intimal thickening (chronic rejection) as measured by intravascular ultrasound. Of note, the incidence of treated rejection episodes did not correlate with intimal thickening. In addition, Anguita *et al.*[15] have recently reported that repetitive episodes of untreated grade 1B or 2 rejection impair long-term cardiac graft function. These important data suggest a need to re-evaluate the threshold for initiating treatment of even mild rejection, and studies are needed to compare the morbidity of a more aggressive approach.

The risk factors for early and late cellular rejection have been reviewed[16–18], and a number of factors have been identified which additionally impact on the decision to treat a given biopsy, including: (a) grade of the previous biopsy; (b) time post-transplant – data from a large review from Stanford[19] have shown that rejection is much more likely to progress to a higher grade if untreated early (<9 months) post-transplant than later (>9 months); (c) level of other maintenance immunosuppressive medications; and (d) clinical signs or symptoms.

Many patients are treated for rejection with <grades 1B or 2 histologic criteria on biopsy. The CTRD database on over 900 patients transplanted between January 1990 and January 1993 indicates that 12% of treated rejection episodes had grade 0 or 1A biopsy score[17]. Often the most compelling reason to treat low-grade evidence of rejection is hemodynamic compromise. Possible explanations for the disparity between histologic findings and clinical signs or symptoms include sampling error on biopsy, passage of cytotoxic lymphocytes out of the allograft after initiating damage, or the possibility that the primary mechanism associated with acute allograft dysfunction is not cellular but humoral[19–21]. Many centers give a course of corticosteroids for patients with acute graft dysfunction, even with limited evidence of cellular rejection[19], followed by more aggressive therapies if no response is evident[20] (see discussion on humoral rejection below).

The following is a review of the numerous options for the treatment of cardiac allograft rejection, including doses recommended and associated side-effects. Comments regarding treatment of rejection in pediatric patients are combined with discussion of adult treatment. This review will not deal with strategies or agents used as prophylaxis against rejection.

TREATMENT OF ACUTE CELLULAR REJECTION

Corticosteroids

Acute cellular rejection is the most common form of cardiac allograft rejection, and corticosteroids have been the most commonly employed treatment[22]. There is, however, no uniform agreement on the amount or route of administration for this agent in the treatment of acute cellular rejection. The most common route has been intravenous, utilizing doses of 1 g of methylprednisolone per day for three consecutive days[22]. However, the dose of 3 g in 3 days is frequently suprapharmacologic, especially for patients of small body size. Heublein *et al.*[23], in fact, performed a randomized prospective/retrospective study of 512 biopsies in 128 patients, comparing the 3 g dose to a dose reduced by 50% (i.e. 0.5 g/day for 3 days) and found no increased efficacy in reversing rejection or the rate of subsequent rejection with the higher dose.

Perhaps the most reasonable approach is to individualize the dose by kilogram body weight in order to minimize unnecessary steroid side-effects in smaller patients, and provide equivalent dosing to all patients. The dose of i.v. methylprednisolone used at St Louis University Health Sciences Center for the past 5 years has been 10 mg/kg per day for 3 days. Miska *et al.*[24] have shown the feasibility and practical advantage of giving the intravenous steroids as an outpatient. While there are clear side-effects of high-dose i.v. steroids[25], especially if given too rapidly, most patients can be treated safely as outpatients.

In contrast, many centers now use oral steroids to treat all rejections not associated with hemodynamic compromise that occur more than 3 months post-transplant[26,27]. Kobashigawa *et al.*[26] performed a randomized study comparing an oral dose of 100 mg/day for 3 days (plus an oral taper over 2 weeks) to a conventional dose of 1 g/day × 3 days of intravenous methylprednisolone in 41 asymptomatic patients with grade 3A rejection. They showed no difference in rate of resolution of the biopsy (95% with i.v. vs 91% oral) at 4 weeks post-treatment, and no difference in infection. However, cost was significantly lower with oral therapy ($6.30 vs $180). Unfortunately, there are no prospective trials comparing the dosage required for treatment of rejection with oral steroids, but the most common dose reported has been 100 mg/day for 3 days[26,27].

The use of a 'taper' of the steroid dose after the bolus dose is also controversial. Lonquist *et al.*[28] showed no statistically significant difference in the incidence of rejection over the next 3 months or the number of rejections requiring additional therapy to achieve resolution in patients returned directly to pre-bolus steroid dose (no taper) versus the group that had the steroid dose tapered from 200 mg/day to 30 mg/day over 2 weeks. These data raise questions about the need for further steroid exposure by routine use of a taper over 1–4 weeks. Oral steroids have become the standard therapy for uncomplicated rejection in many programs, but generally only after the first 3 months, during which period i.v. steroids remain the more common practice.

The response rate to a course of steroids for the treatment of grade 3A rejection may be as high as 85% with one course[26,29]. Patients who fail to demonstrate an improvement on biopsy after a course of steroids generally receive a second course of steroid therapy, typically i.v., if there is no evidence of graft dysfunction. Patients who fail to improve with the first course of corticosteroids have approximately an 80% chance of improving with a second course[29]. Most programs have evolved to waiting a minimum of 7 days, and most commonly 14 days, before repeating the biopsy in patients with no evidence of hemodynamic compromise, to allow adequate time for histologic evidence of improvement in biopsy grade. Biopsies performed at <1 week may give a false impression of persisting rejection where, without further treatment, the biopsy may be substantially improved after another 7–10 days. However, patients whose grade 3A biopsy progresses to a grade 3B or greater are at higher risk of not responding to a second course of steroids[29]. In these cases consideration should be given to cytolytic therapy with agents such as OKT3, particularly if there are any signs of hemodynamic compromise and the doses of cyclosporin and azathioprine are considered to be at the maximum tolerated. The response rate with OKT3 in this setting may be as high as 80%[30].

Many programs administer prophylactic antimicrobial therapy during or following a course of rejection therapy, as a course of antirejection therapy has been shown to significantly increase the risk of infection[31]. Common strategies include Mycelex (one tab. b.i.d.) for prophylaxis against *Candida* infection, and acyclovir (400 mg t.i.d.) to prevent exacerbations of herpes simplex virus during and/or for 1 week after a course of steroid therapy for rejection. Following antirejection therapy, patients should be carefully monitored for signs or symptoms of infection, as high-dose steroids may significantly inhibit typical signs of inflammation and pyrexia.

Cyclosporine

Cyclosporine (CsA) is not typically considered an agent for the treatment of acute cellular rejection, but several investigators[32,34] have documented successful use of high-dose oral or i.v. CsA for the treatment of mild to moderate acute allograft rejection. This approach would seem counterintuitive to our understanding of T cell activation which has progressed to acute cellular rejection in terms of the ability of CsA to turn off IL-2 generation and allow clearing of rejection.

However, Kobashigawa *et al.*[33] have shown that only one of 21 patients (5%) with mild rejection who had at least a 50% increase in CsA level (169–413 ng/ml) progressed to moderate rejection. Radovancevic and Frazier[34] reported an 81% response rate in a series of 24 patients with moderate rejection treated with high-dose oral and i.v. CsA (14 mg/kg per day of oral CsA plus 1–3 mg/kg i.v.). These patients were compared to a group treated with i.v. steroids over a similar time, and no significant difference was found in resolution of rejection, but there was a significantly lower incidence of infection with the high-dose CsA. Surprisingly, there was no irreversible liver or kidney damage with use of these high doses.

FK506 (tacrolimus)

Recently, the new immunosuppressant drug FK506, now known as tacrolimus, has been investigated in cardiac transplant recipients as a primary therapy for refractory cellular rejection[35], as well as maintenance immunosuppression[36]. Despite a nearly identical mechanism of action as CsA (inhibition of IL-2 syn-

thesis), in current doses this drug has been shown to be effective in reversing rejection in patients previously receiving CsA. The dose of tacrolimus for treatment of rejection is 0.15–0.30 mg/kg per/day p.o. in two divided doses. CsA should be held for 24 h before initiating tacrolimus due to the strong overlap of mechanism and side-effects. The target level for tacrolimus by the commercial kits now available is 15–20 ng/ml. The main side-effects are neurologic and renal toxicity.

Methotrexate

The folic acid analogue, methotrexate (MTX), has been utilized for reversal of refractory[37,38] or recurrent acute cellular rejection with reasonable success[37–43], including pediatric patients[38,44]. By blocking the conversion of dihydrofolic acid to tetrafolic acid this agent inhibits purine synthesis which is essential to DNA synthesis and cell division, especially in rapidly dividing cells. MTX is an antiproliferative that affects both cellular and humoral immunity.

Reports by Costanzo-Nordin *et al.*[37] and Bourge *et al.*[39] demonstrated resolution of acute rejection in nearly 90% of patients treated with high doses of MTX (15–50 mg/week i.v. or 10–30 mg/week p.o.) over 1–3 days/week for 3–6 weeks. However, nearly half of these patients had recurrent rejection after MTX was discontinued, but again responded to a second course of the drug. Given the efficacy of a short course of MTX, but the high incidence of rebound rejection, Jennison *et al.*[44] reported safe administration in 27 patients who received the drug for an average of 16 months (range 1–43 months). There was no significantly increased risk of infection with MTX, and the incidence of rejection was significantly reduced during the period of administration (compared to the pre-MTX incidence). Currently its most common usage is in patients with persisting grade 1B or grade 2 rejection[40,43,44], particularly without hemodynamic compromise.

MTX is usually given 2 days per week, either on 2 consecutive days (e.g. Saturday and Sunday) or split (e.g. Monday and Thursday) with doses ranging from 2.5 to 7.5 mg/day based on white blood cell count. Side-effects include gastritis and neutropenia, and occasionally patients describe a fatigue syndrome which may improve by separating the dose by a number of days. Bourge *et al.*[39] noted that the risk of neutropenia with MTX correlates with: (a) the dose of MTX, (b) the WBC before starting MTX, and (c) the WBC 1 month after therapy. Protocols vary with respect to whether the dose of azathioprine is reduced or discontinued entirely during MTX therapy.

Mycophenolate mofetil

The newest immunosuppressive agent which has been used for the treatment of recurrent or persistent cardiac rejection is mycophenolate mofetil[45]. This drug is a derivative of mycophenolic acid which inhibits the *de-novo* pathway for purine synthesis of activated lymphocytes[46]. Its ability to reverse recurrent cardiac rejection was evaluated in a multicenter study of 17 patients[45]. Rejection was controlled in all 14 patients who were able to receive the drug long-term at a dose of 3 g/day p.o. The incidence of rejection decreased from 0.67 to 0.27 episodes/month ($p<0.0001$), with no increase in the incidence of infection. The major side-effects are neutropenia and gastrointestinal symptoms. This drug is designed primarily as a maintenance immunosuppressive medication, but it may be effective as therapy for acute rejection, especially in patients not currently receiving it.

Total lymphoid irradiation

Total lymphoid irradiation (TLI) has been used as a form of immunosuppression since the 1960s, but largely as prophylaxis. Salter *et al.*[47] recently reported on the use of TLI as a primary therapy for the treatment of recurrent or persisting early severe cardiac allograft rejection. This therapy was able to reverse acute rejection in 18 of 19 patients. The authors also demonstrated a highly significant reduction in the incidence of subsequent rejection, from 1.3 to 0.07 episodes/month ($p<0.0001$). The usual dose is 800 cGy in divided treatments 2 days/week for 5 weeks. The major side-effects are neutropenia, which is correlated with a lower tolerated dose of azathioprine pre-TLI, and infection. The course of treatment may require several interruptions, each for 5–7 days, if neutropenia (WBC<3000 cells/mm^3) develops. Reversal of rejection is not very rapid with TLI, and it is therefore not designed for the treatment of severe or symptomatic rejection, but may be very effective in the long-term control of recurrent or persisting rejection, even if refractory to cytolytic agents[48,49]. TLI may also be effective in pediatric patients[50].

Photochemotherapy

Recently, a new form of therapy for the treatment of acute rejection has been reported which is termed photopheresis (PH) or photochemotherapy[51–53]. This technique requires patients to receive pretreatment with a photoactivating agent, 8-methoxypsoralen, either p.o. or i.v. The patient's mononuclear cells are then pheresed and extracorporeally exposed to UV-B irradiation in a flat field, which alters the cytotoxicity of the photomodulated cells. One treatment requires 3 hours to complete, which usually involves 1×10^6 cells. The exact mechanism of this modulation is unclear. Results of a randomized prospective study of 12 patients with 16 episodes of grade 2 or 3A rejection showed PH to be as effective as oral or i.v. steroids for reversing rejection, and there was minimal or no toxicity with PH. This therapy may have more of an application for the prevention of rejection than acute treatment[54].

RESCUE THERAPY FOR ACUTE CELLULAR REJECTION

OKT3

The use of cytolytic therapy with agents such as OKT3 is generally reserved for patients with allograft rejection that is refractory to multiple courses of conventional agents, or is associated with hemodynamic compromise[30,55–58].

OKT3 is a murine monoclonal antibody directed against the CD3 receptor which is intimately linked to the T cell recognition complex[45] on the surface of most circulating lymphocytes. The binding of OKT3 to the surface receptor causes loss and/or internalization of the T cell receptor–CD3 complex, thereby

rendering the T cell incapable of recognizing foreign antigen. In addition, there is loss of usual T cell functions, such as CD4 proliferation, CD8 cytotoxicity, and B cell proliferation, essentially inhibiting the entire immune response to antigen recognition. Coating or complexing of the CD3 complex with OKT3 leads to opsonization of the $CD3^+$ cells from the circulation by the reticuloendothelial system[59]. The count of $CD3^+$ cells falls dramatically within 12–24 hours of administration (usually to <100 cells), and biopsies have also demonstrated rapid clearance of lymphocytes from the myocardium.

The standard dose is 5 mg given over 15 seconds once a day. Smaller doses (2.5 mg/day) have been found to be effective in reversing rejection[60], especially in children, and there are anecdotal reports that the CD3 count may in fact remain <100 cells with only one or two doses. Additional empiric doses of OKT3 may only increase morbidity without enhancing clinical efficacy. However, the major concern of abbreviated, interrupted, or reduced dose therapy is enhanced sensitivity to the agent (development of human anti-mouse antibodies) or further immune activation[59–62].

The binding of the OKT3 to the surface receptor triggers a release of cytokines (particularly TNF and IFN-gamma) and, potentially, prostaglandins, which result in a clinical entity called cytokine release syndrome[63–65]. This syndrome, which may be manifest by fever, chills or even rigors, vasodilatation, hypotension, and a capillary leak syndrome with a pulmonary edema picture, particularly if the patient is volume-overloaded, can be avoided by pretreatment with a combination of 1 g of intravenous methylprednisolone, acetaminophen (650 mg p.o.), diphenhydramine (50 mg p.o.), and a dose of a non-steroidal anti-inflammatory agent such as indomethacin (50–75 mg p.o.) 60 minutes *prior* to giving the first dose of OKT3. This pretreatment should be continued for at least the first 3 days, although the dose of steroids can be reduced to 500–750 mg on day 2, and 250–500 mg on day 3. The steroid dose should then be converted to a dose of 0.3 mg/kg/per day p.o. from day 4 to completion of the course of OKT3, when the dose is again increased to 1 mg/kg/per day and tapered by 5 mg/day to the usual level at the time post-transplant. This post-OKT3 steroid bolus and taper are to prevent 'rebound' rejection with cessation of OKT3. Other side-effects include: (a) secondary infection, especially CMV, pneumocystis, and other opportunistic infections; (b) aseptic meningitis; and (c) seizures.

The overall success of OKT3 therapy to reverse allograft rejection varies from 70% to 95%[55–58]. Lower success[66] seems to be related to lack of the use of a pulse and taper of oral steroids at the completion of the 7–10-day course of OKT3, to prevent the rebound rejection which may occur due in part to the inherent property of OKT3 to activate the immune system[62]. This drug may allow substantial improvement in hemodynamic function within 1–2 days of initiating therapy.

Therapy with OKT3 is monitored by either serum levels of OKT3 or counts of $CD3^+$ cells[67]. Levels should be >800 ng/ml and CD3 counts should drop <100 cells with effective therapy. Prior use of this agent, such as perioperative 'induction' therapy to prevent rejection, may result in development of human anti-mouse antibodies (HAMA) which can inactivate OKT3 and reduce the response to treatment of rejection[57,61]. These antibodies can be screened for: (a) prior to initiating a second course or (b) in patients whose OKT3 levels fall, or CD3 counts rise, during therapy. Demonstration of rising CD3 counts during therapy requires use of an alternative therapy.

Antithymocyte globulin

The first cytolytic agent used for rejection rescue therapy was the polyclonal preparation antithymocyte globulin (ATG). This is currently used more for prophylaxis than rescue therapy. This agent is made by injecting human thymocytes into the animal host (a horse or rabbit), and then harvesting, pooling, and purifying the host plasma, which is rich in antithymocyte immunoglobulin. Side-effects of ATG include pyrexia and even serum sickness reaction, variable efficacy between lots, and contamination with other immunoglobulins (such as antiplatelet antibodies)[68]. Numerous studies have compared the efficacy of ATG and OKT3[69–73], but primarily as prophylactic (induction) therapy. The dose of ATG for rescue therapy is usually 10–15 mg/kg/per day i.v. or i.m. (in one or divided doses) for 7–10 days, with smaller doses used in pediatric patients[69]. Pretreatment with steroids, acetaminophen, diphenhydramine, and non-steroid anti-inflammatory agents (see above *re* OKT3) is recommended. Blood level details are not available.

TREATMENT OF HUMORAL (VASCULAR) REJECTION

The role of the humoral immune system in cardiac allograft rejection has been recognized only recently[21,74–76]. This type of rejection is relatively uncommon, but may be the cause of much of the acute hemodynamic compromise that may occur with cellular rejection. It is very uncommon for vascular rejection to occur alone (i.e. without evidence of cellular rejection) with the exception of hyperacute rejection, which usually presents in the operating room at the time of transplant and is due to circulating antibodies against one or more antigens on the donor endothelium. The treatment of hyperacute rejection is discussed below.

The graft dysfunction that may occur with humoral rejection is largely a result of thrombosis and/or vasospasm of the coronary arteries due to an antibody-mediated arteritis. Vascular rejection usually occurs within the first month post-transplant and should be anticipated in patients with a positive donor-specific cross-match[76]. The diagnosis is confirmed by histologic evidence of arteritis on routine endomyocardial biopsy and immunofluorescent staining of immunoglobulin deposition on the coronary endothelium[74]. The immunoglobulin(s) or antibody is (are) directed against foreign donor antigens located on the surface of the coronary arteries[77,78].

The treatment of this form of rejection is focused on: (a) removing circulating antibody and (b) suppressing new antibody formation. The recommendations that follow are based on clinical experience in a number of centers, but there are no prospective studies comparing various forms of therapy in heart transplant recipients.

The most rapid and effective therapy for humoral rejection is plasmapheresis[76,79–81]. This requires placement of a percutaneous,

dialysis-type central venous catheter to allow pheresis and exchange of the patient's plasma. The patient's weight is used to calculate the total plasma volume, and a single plamsa volume exchange is performed. The replacement fluid is usually a mix of 50% type-specific fresh-frozen plasma and 50% albumin. The exchange requires approximately 3 hours to perform, but rarely results in hypotension or any hemodynamic deterioration. Ionized calcium is monitored and replaced as needed. Due to the documented alteration in local plasminogen activators and significant coronary endothelial inflammation, patients are usually placed on full heparinization to minimize coronary thrombosis. This procedure should be repeated daily for a minimum of 3 days and can be done for 5–7 days or until the circulating antibodies (usually multiple) are reduced to very low titer, as demonstrated by repeated donor-specific crossmatch or panel-reactive antibody (PRA) assay. This therapy may be lifesaving, has very little associated morbidity and should be initiated as soon as possible when this diagnosis is confirmed.

A second alternative method for removing circulating antibody is with use of an immunoadsorption column (protein A column, Prosorba, IMRE Corp.). As with plasmapheresis, plasma is pheresed from the patient, but is then passed over the column which is covered with immunoglobulin that absorbs the circulating antibodies from the plasma, which is then reinfused to the patient. Olivari *et al.*[82] reported successful reversal of acute vascular rejection in three patients with significant hemodynamic compromise that was unresponsive to high-dose i.v. steroids and cyclophosphamide. After several courses of immunoadsorption the left ventricular ejection fraction improved from 23% to 56%. The PRA titer fell to <5%. The patients had no further vascular rejection. There are no data comparing the efficacy of the two forms of antibody removal.

The second goal of treating vascular rejection is to suppress antibody production. A combination of lympholytic agents is typically used, including high-dose i.v. methylprednisolone (1–1.5 g/day × 3–4 days), and substitution of cyclophosphamide[79] for azathioprine. The dose of cyclophosphamide ranges from 1 mg/kg per/day to 1000 mg/day, and several centers have noted improved efficacy with the higher dose for the first several days. Finally, most centers also use OKT3 to help resolve any component of cellular rejection and help turn off B cell proliferation[79–83]. There are a few anecdotal reports of additional improvement with conversion from CsA to FK506. Antimicrobial prophylaxis, as described above, is also recommended during and after this aggressive therapy.

The prognosis with vascular rejection (especially the hyperacute form) is worse than with cellular rejection.

COMMENT

There are new monoclonal antibodies directed against a variety of cytokines and surface receptors, and other agents such as rapamycin and leflunomide, that may offer improved or additional strategies to manage acute cellular and/or vascular rejection in the future. Progress is still needed to help manage an entity which continues to be one of the leading causes of death in heart transplantation.

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Infection in Relation to Thoracic Transplantation

K. LOVE

INTRODUCTION

Although the advent of focused and therefore superior immunosuppressive regimens has greatly decreased the incidence of infection in the thoracic transplant recipient, infection remains the leading cause of death in many centers[1–5]. Infection is acquired exogenously through usual nosocomial and community modes of transmission, and through transfusion, or is donor-transmitted. It can also result from endogenous reactivation of latent organisms within the recipient (Table 1).

Table 1 Organisms causing infections after thoracic organ transplantation

Bacteria	
Community-acquired bacteria	Various infections
Nosocomial bacteria	Nosocomial infections
Donor trachea flora*	Anastomotic infection in LTR
Legionella species	Pneumonia
Listeria	Meningoencephalitis
*Mycobacteria**†	Pulmonary/extrapulmonary disease
Mycoplasma hominis	Sternal wound infection
Nocardia species	Pulmonary/extrapulmonary disease
Rhodococcus equi	Pneumonia
Miscellaneous unusual	*Bartonella* (*Rochalimea*)
Fungi	
Candida species	Mucocutaneous candidiasis Mediastinitis, sternal infection
*Cryptococcus neoformans**†	Meningitis, extraneural disease
*Coccidioides immitis**†	Meningitis, extraneural disease
*Histoplasma capsulatum**†	Pulmonary, disseminated disease
Viruses	
Herpes simplex 1, 2†	Mucocutaneous vesicles
Varicella-zoster†	Chickenpox, shingles
Epstein–Barr virus*†	Lymphoproliferative disorders
Cytomegalovirus*†	CMV syndrome, enteritis, pneumonitis
Human herpesvirus 6†	? lymphoma, ? other
Hepatitis B, C*†	Liver disease, hepatoma
Retroviruses*†	AIDS, leukemia, lymphoma, paraparesis
Polyomaviruses†	JC Virus progressive multifocal leukoencephalopathy
Papillomaviruses*†	Warts, ? malignancy
Respiratory viruses	Respiratory syncytial virus, adenovirus, parainfluenza virus, influenza A, B
Parasites	
Pneumocystis carinii†	Pneumonia
*Toxoplasma gondii**	Myocarditis, encephalitis, pneumonia
*Trypanosoma cruzi**†	Myocarditis, disseminated disease
Strongyloides stercoralis†	Disseminated disease
Cryptosporidium	Diarrhea

* Potentially donor-transmitted.
† Reactivated latent infection in the SPR.
All infections can be exogenously acquired.
LTR = lung transplant recipients; JC = Jacob Creutzfeldt virus.

The type of infecting organism and severity of disease produced is determined by factors such as the age of the patient, the intensity and duration of immunosuppression, pre-existing diseases such as bronchiectasis, timing after transplant (Table 2), and whether disease is due to primary infection or reinfection with a donor strain, or reactivation of latent infection in the recipient.

The net state of immunosuppression

Rubin has aptly pointed out that it is the 'net state of immunosuppression' that determines the risk of infectious disease in the transplant recipient[6]. This concept takes into account the duration of immunosuppression and the contribution from immunomodulating viruses such as cytomegalovirus (CMV), Epstein–Barr virus (EBV), hepatitis viruses, and retroviruses. The intensity of pharmacological immunosuppression, especially the contribution from corticosteroids, is greatest during the first 3–6 months after transplant and after treatment for rejection. These are the periods in which most acute infections are seen.

Timing of infection after transplantation

First month

In the first month after transplant, nosocomial bacterial and fungal infections (pneumonia, intravascular device-related sepsis, urinary tract infection, wound infection, and *Candida difficile* diarrhea) predominate and are directly related to breeches of integumentary and mucosal integrity by invasive devices and wounds, as in any other ICU patient. Handwashing with an antibacterial soap and other fundamental infection control measures are indicated. There is no literature to support the use of barriers, such as masks and gowns that were a part of traditional 'protective isolation'. This has been studied in both the neutropenic[7,8] and the transplant patient[9]. Special air handling and procedures designed to minimize exposure to fungal spores are warranted (refer to the section on *Aspergillus*). Herpes simplex virus reactivation occurs in this time frame as well but, with the widespread use of acyclovir prophylaxis, is either delayed or suppressed.

Sternal wound infection and mediastinitis are particular problems early after thoracic transplantation; unusual causative organ-

Table 2 Timing of infection after transplant by type of disease

Site/type of disease	*Early (within 3 months)*	*Late (after 3 months)*
Pneumonia	Nosocomial pathogens Cytomegalovirus (CMV) *Legionella* *Aspergillus* *Toxoplasma gondii*	*Pneumocystis carinii* *Nocardia asteroides* CMV Community-acquired bacterial, viral, fungal pneumonia
Sternal and mediastinal infection, empyema	Staphylococci, GNB Donor trachea flora *Candida* *Mycoplasma* Mycobacteria	
Myocarditis	*Toxoplasma gondii* CMV *Trypanosoma cruzi*	
Central nervous system	*Aspergillus* *Toxoplasma gondii*	*Listeria monocytogenes* *Cryptococcus neoformans* *Nocardia asteroides* *Toxoplasma gondii* PML
Gastrointestinal tract	CMV, HSV esophagitis CMV enteritis Hepatitis *Strongyloides*	CMV enteritis Cholecystitis *Strongyloides*
Mucocutaneous	*Candida* species HSV Disseminated fungal infection Disseminated mycobacteriosis	*Candida* VZV Disseminated fungal infection
Malignancy	EBV-LPS	EBV-LPS Accelerated course of solid tumors. Hepatoma. Cervical cancer (? papillomavirus) EBV or retrovirus associated T-cell
Mononucleosis syndrome	CMV EBV HBV, HCV Retroviruses *Toxoplasma gondii*	EBV Community acquired virus

Disseminated infection can occur with most of these organisms

isms such as *Candida* species and mycoplasma have been reported[10]. At the Minneapolis Heart Institute the overall deep sternal wound infection/mediastinitis rate in 189 thoracic transplant-related sternotomies as of December 1994 was 3.2%, representing 4/177 (2.3%) heart transplant recipients, 1/6 heart–lung and 1/6 double lung transplant recipients, respectively. Literature rates with which to compare are not readily available. Usual perioperative antibiotic prophylaxis regimens are used (Tables 3 and 4).

In the lung transplant recipient, mediastinitis and pleural space infection can also result from contamination of the bronchial anastomosis by recipient and donor trachea flora, and can be prevented by pre-emptively treating the recipient with antibiotics directed against organisms grown in tracheal cultures, and monitoring and treating organisms cultured from chest tubes.

Despite the multiple suture lines involved and the immunosuppression, there are only two cases of endocarditis reported in heart transplant recipients[11,12], one at day 8 and the other at 7 months after transplant. Given the usual time to endothelialization of these anastomoses, it seems logical to provide antibacterial prophylaxis for dental, genito-urinary and gastro-intestinal

Table 3 Prophylaxis and prevention of infection

Organism/infection	*Prevention/prophylaxis*
Sternal wound infection/mediastinitis	Perioperative prophylaxis with cephalosporin or vancomycin to PCN allergic patients
Mediastinitis/anastomotic infection in LTR	Perioperative prophylaxis with Imipenem or Clindamycin/Aztreonam for PCN allergic patients, pre-emptive treatment of donor trachea isolates post-transplant
Legionella species	Water treatment for control of nosocomial transmission
Listeria monocytogenes	Recommendations in the text, TMP–SMZ prophylaxis
Mycobacteria	Testing and treatment for TB pretransplant Avoid exposure to aquaria (*M. marinum*)
Nocardia species	TMP-SMZ prophylaxis
Candida species	Clotrimazole troches or mycostatin S&S q.i.d. × 1 month Fluconazole 200–400 mg/day × 14 days for + donor trachea culture Daily ingestion of 8 oz of *L. acidophilus* + yogurt for females with recurrent vaginitis
Aspergillus	See Table 4
Herpes simplex 1, 2	Acyclovir 400 mg b.i.d.–t.i.d. for frequent recurrences
Varicella–zoster	Active immunization of SNR Post-exposure VZIG for exposed susceptibles
Cytomegalovirus	CMV-negative blood products, high dose p.o. ACV or GCV ± CMVIG for SNR of SPD and SPR × 3 months
Epstein–Barr virus	Optimal immunosuppression. ? ACV or GCV, CMVIG
Hepatitis B	HBV vaccine + hepatitis immune globulin (HBIG)
HIV	AZT as soon as possible
Pneumocystis carinii	TMP–SMZ × 1 year
Toxoplasma gondii	TMP–SMZ or pyrimethamine 25 mg/day × 6 weeks for mismatch
Trypanosoma cruzi	Benznidazole or nifurtimox probably indefinitely
Strongyloides stercoralis	Thiabendazole

SNR = seronegative recipient; VZIG = varicella zoster immune globulin; PCN = penicillin.
LTR = lung transplant recipient

Table 4 *Aspergillus* precautions

The patient is in a private room with positive ventilation and at least 10 air exchanges per hour.
At least 90% of particles over 0.5 μm in size are filtered from air entering the room.
Not allowed: plush toys, balloons, pepper for food, artificial or real flowers or plants.
All horizontal and other surfaces capable of gathering dust are damp-cleaned daily.
The patient wears an 8710 dust–mist respirator mask (3M) when out of the room and for 30 minutes after cleaning of the room.
All construction projects are reviewed in advance by the Infection Control Department and suitable barriers and removal systems implemented; some projects are postponed until there are no transplant patients in the area.
These precautions are in effect until the prednisone dose is down to 30 mg/day or, if the patient is discharged before that target is reached, wearing of the mask is encouraged for coming to the hospital because of constant renovation, and on windy days and being near excavation or construction sites for 3 months post-transplant.

procedures for only 3 months after transplant. The American Heart Association recommendations for antibiotic prophylaxis to prevent infective endocarditis are appropriate for this indication[13].

One to six months

In the 1–6 months after transplantation, cytomegalovirus is far and away the most common and important of the immunomodulating viruses which become active during this period and pave the way for other opportunists such as *Aspergillus*, *Nocardia*, *Pneumocystis*, and *Listeria*.

After six months

According to Rubin[6], after 6 months 75% of patients are doing well, and 10% have chronic infection with immunomodulating viruses, which eventually leads to destruction of the infected organ or to malignancy. Fifteen percent have poor allograft function due to both acute and chronic rejection, and have received repeated and prolonged intensive immunosuppression, complicated by chronic viral infection, making them prone to opportunistic infection with *Listeria*, *Cryptococcus*, *Pneumocystis*, and *Nocardia*. Varicella–zoster reactivation is also a late infection.

Primary infection, reinfection, reactivation

Primary infections occur with varying frequency in previously uninfected persons who receive the blood or organ of a previously infected donor, and tend to cause disease more often and with greater severity than reinfection or reactivation. Primary infections are usually diagnosed as such by serological testing, whereby the seronegative recipient (SNR) becomes seropositive after exposure to the seropositive donor (SPD). Primary infections are more common in pediatric transplantation.

Reinfection can occur in the seropositive recipient (SPR) who acquires a donor strain of an organism with the donated organ or transfusion that is different from the recipient strain. Disease following reinfection is usually less severe than that following primary infection. Molecular techniques are required to prove reinfection.

Endogenous reactivation of a SPR's latent organisms, such as herpesviruses, causes milder disease or none at all.

SPECIFIC INFECTIONS

Where cell-mediated immunity is impaired, infections with organisms which are engulfed but not killed by leukocytes ('intracellular parasites') may either occur more frequently or, though not more common in the immunocompromised host, be particularly invasive, disseminated, and severe ('hyperinfection').

BACTERIAL INFECTION

Legionella

Legionella is a Gram-negative rod aerosolized in a variety of hot and cold water sources, including evaporative condensers and cooling towers[14–18], showers[19], whirlpool spas[20], respiratory therapy equipment[21], and grocery store mist machines[22]. Microaspiration of contaminated water via nasogastric tubes has also been implicated in some outbreaks. *Legionella* has been found in 1–30% of home water heaters, especially electric units.

Legionella species cause <1–5% of cases of pneumonia in adults, but there is a marked geographic variation in the incidence[23,24]. In a 1988 survey of 28 centers with 2274 heart transplants, representing 73% of the cardiac transplants done in the USA as of 1986, the incidence of legionellosis was 2% (0–>5%)[23].

The 'classic' syndrome consists of lobar pneumonia with nonproductive cough, pulse–temperature dissociation, rigors, diarrhea, myalgia, confusion, abnormal liver function tests, hyponatremia and hypomagnesemia, but this presentation represents only a small subset of patients with the disease. Prospective studies have shown that the usual case consists of non-specific clinical, roentgenological, and laboratory manifestations of lower respiratory tract infection[24,25]. Rarely, *Legionella* can cause extrapulmonary infection[26,27].

The diagnosis is confirmed by culture of sputum, blood, or other clinical material; unfortunately, many otherwise sophisticated laboratories are not able to culture *Legionella* as they do not routinely use the selective media and techniques required. It is therefore necessary to obtain cultures before institution of antimicrobial therapy, and to alert the laboratory to the possibility of the diagnosis.

Antigenuria can be assayed with a very quick and sensitive commercially available radioimmunoassay that detects only *L. pneumophila* serogroup 1, but as 70–90% of all cases of Legionnaire's disease are probably caused by this serotype, it is still a useful test. In many laboratories it has replaced direct fluorescent antibody (DFA) testing of clinical specimens, which is technically difficult and suffers from a lack of specificity and sensitivity. DNA probe methods are about as accurate as DFA tests, are easier to perform, and detect all species of *Legionella*; however, they have no practical advantages over the antigenuria test. Polymerase chain reaction techniques hold great promise, but more experience is needed.

Serology is widely used for diagnosis, but the specificity of seroconversion may be unreliable for species other than *L. pneumophilia* serogroup 1, and false-positives occur due to cross-reactivity with other organisms, such as *Escherichia coli*. Also, seroconversion may take as long as 9 weeks.

In-vitro sensitivity testing is not relevant, as activity of an antibiotic against *Legionella* depends on its ability to enter the infected cell. In the past the preferred treatment was with erythromycin 1 g intravenously every 6 hours with rifampicin added for severe cases. Both of these drugs have major interactions with cyclosporin (CsA) which can result in renal failure. For this reason (and because in *in-vitro* models the new macrolides, azithromycin and clarithromycin, are much more active) these, and the fluoroquinolones, ciprofloxacin and pefloxacin, are probably the drugs of choice. They, too, have some interaction with CsA, but it is not so pronounced as with erythromycin, and careful monitoring of CsA levels will avoid toxicity. With the exception of azithromycin, which is given in a dose of 500 mg on day 1, followed by 4 days of 250 mg/day, antibiotic therapy should be given for 3 weeks.

Though conventional wisdom dictates that imipenem–cilastatin should not be effective, we have had good experience with three cases of culture-proven *Legionella* pneumonia treated empirically with this drug before the diagnosis was confirmed; after initial improvement these patients were sent home on oral ciprofloxacin, with complete resolution.

Prevention of infection with *Legionella* consists of monitoring water sources in areas where it is a prevalent pathogen in the transplant population; superheating water to 55–60°C is required to eradicate it. Sterile water should be used to irrigate nasogastric tubes, and for any respiratory therapy activities such as washing nebulizers, tubing or humidifiers, unless this equipment is to undergo subsequent sterilization. Samples from home water heaters could be cultured and, if positive, the transplant recipient could be advised either to replace an electric heater with a gas-fired one or to increase the temperature of the water heater, taking care to avoid scalding injuries.

Listeria

Listeria monocytogenes is a Gram-positive bacillus found in dairy products, raw vegetables, and undercooked meat and poultry[28], which colonizes the gastrointestinal tract in 5–10% of people and causes systemic infection in an estimated 7.0 per million population in the USA per year[29].

In a recent review of 74 Finnish cases, between 1971 and 1989, Skogberg *et al.* found that around 50% of all patients had primary bacteremia[30]. In an exhaustive analysis of 178 cases in the English literature between 1968 and 1978, Nieman and Lorber[31] recorded primary bacteremia in ~25% of patients. Acute, or rarely subacute or chronic[32] meningitis or meningoencephalitis occurred in 55% and encephalitis without meningitis in another 6.5%. Other sites of infection were described in 12 patients, and 14 patients were diagnosed as having endocarditis. Prominent CNS features consisted of nuchal rigidity (85% of meningitic cases), ataxia, tremors, seizures, and fluctuating consciousness, with positive blood cultures in 75% of cases. The cerebrospinal fluid profile was variable; the average leukocyte count was >1000 with 66% polymorphonuclear cells and counts did not correlate with outcome. The glucose was normal to depressed (average 60 mg/dl) and low levels (<40 mg/dl) seemed to predict a poor prognosis. The average protein level was around 200 mg/dl and values >300 mg/dl also appeared to portend a poor result. The Gram stain was positive in ~40%, and was misleading in some cases, as has been previously described by Buchner and Schneierson[33], because the organism is often mistaken for a diphtheroid contaminant or a streptococcus. Over-decolorization of the Gram stain can lead to the erroneous identification of Gram-negative bacilli. It is therefore necessary to have a high clinical suspicion for this organism in patients with compromised cellular immunity, including alcoholics, pregnant females, and neonates.

On CT scanning, localized abscesses of cortex, spinal cord or brainstem, and hydrocephalus can be seen, and generalized encephalitis may be diagnosed on biopsy or at autopsy.

Treatment of choice consists of ampicillin 2 g every 4 hours i.v., or penicillin G 3–4 million units every 4 hours i.v., with or without an aminoglycoside, for 14–21 days. Cephalosporins are ineffective. Patients with a history of life-threatening penicillin reactions can be treated with trimethoprim-sulfamethoxazole, 10–15 mg/kg every 8 hours, i.v. or p.o.[34,35] or, if also intolerant of sulfonamides, desensitized to penicillin and then treated as above[36]. Vancomycin is an alternative, but CSF levels should be monitored, as the drug has variable blood–brain barrier penetration.

Efforts at prevention should focus on educating transplant recipients as to avoidance of high-risk foods. Schuchat *et al.*[37] make the following dietary recommendations for persons immunocompromised by disease or medication, pregnant women, and the elderly (adapted):

(1) Avoid eating raw or partially cooked foods of animal origin.
(2) Avoid cross-contamination between raw and cooked foods during food preparation and storage.
(3) Reheat leftovers until too hot to touch.
(4) Avoid soft cheeses such as feta and Mexican-style cheeses; eat hard cheeses, cottage cheese, and cream cheese.
(5) Wash raw vegetables thoroughly before eating.

Trimethoprim-sulfamethoxazole is used as prophylaxis against *Pneumocystis* in most transplant programs today. It is likely that this drug also offers protection against *Listeria*.

Mycobacteria

As of 1993, only nine cases of mycobacteriosis complicating lung transplantation had been reported[38–42,49]. At least seven articles report on experience in heart transplant recipients[43–49]. Tuberculosis (TB) can be donor-transmitted, reactivated in the previously infected recipient, or acquired through exposure to persons with pulmonary TB. Non-TB mycobacteria are acquired from environmental sources, such as soil and water, and are not transmitted between humans.

In a world-wide literature review of renal transplant recipients before 1983, only 47 mycobacterial infections (23 cases of tuberculosis) were identified[50]. The overall incidence in western countries is between 0.3% and 2.3%, but up to 9.5% in high-prevalence areas[50–53].

Mycobacterium tuberculosis

With the recent resurgence of tuberculosis (TB) in North America, it seems likely that the incidence of this disease, and of drug resistance, will increase in transplant recipients. Most of the clinical experience has been with renal transplant recipients in whom the full gamut of pulmonary, extrapulmonary, and disseminated disease has been seen; the only exceptional feature seems to be a propensity for bone and joint involvement[54].

Diagnosis is made by isolating *Mycobacterium tuberculosis* from clinical material, but as the sensitivity is variable depending on the source, it is important to look for acid-fast bacilli on special stains and for granulomata on histology. With the advent of newer methodologies, mycobacteria will grow within 10–14 days and *M. tuberculosis* can be identified promptly by DNA probe.

There are no definitive studies on the optimal treatment of TB in the transplant recipient. Rubin[6] advocates a 12-month course of treatment with isoniazid (INH) and rifampicin (RFM) or pyrazinamide (PZA), which would suffice for patients with a low likelihood of having INH-resistant, or multidrug-resistant TB (MDRTB, resistant to at least INH and rifampicin), later confirmed

with sensitivities. This approach may minimize the toxicity of short-course regimens which require initial treatment with INH, RFM, and PZA for 2 months, followed by 4 months of INH and RFM. Both INH and RFM induce hepatic microsomal enzymes which increase steroid catabolism. RFM, and possibly also INH, competes with CsA for the cytochrome P450 enzyme system, thus decreasing CsA levels. Rejection has been reported in this circumstance[38,55–59] as even massive increases in doses of CsA may not overcome the RFM effect.

However, the short-term regimens are attractive because of their duration, and it may seem reasonable to try them. If RFM cannot be used, the patient should receive 12 months of INH and PZA[6] or 18 months of INH and ethambutol (EMB).

In communities or areas of the world where there is >4% primary resistance to INH, EMB or streptomycin (SM) must be added to the initial regimen, pending results of sensitivity tests. If the organism turns out to be resistant to INH, this drug should be discontinued and 6 months of therapy should be completed with RFM, PZA, and EMB or SM, or 12 months of RFM and EMB. Where MDRTB is prevalent, or where the patient with active TB has been exposed to someone with MDRTB, therapy with five or six antituberculous drugs must be initiated, and when sensitivities are available, at least three new drugs to which the organism is susceptible should be started. This polypharmacy must be continued until culture conversion occurs, after which 12–24 months of two-drug therapy is indicated. Pyridoxine should be given to prevent INH-induced peripheral neuropathy in this high-risk population. Other aspects of treatment and management are extensively covered in the Center for Disease Control's Core Curriculum on Tuberculosis[60].

The issue of whether or not to give Mantoux-positive transplant candidates INH chemoprophylaxis before transplant is controversial. The official recommendations of the Centers for Disease Control (CDC) state that, assuming active TB has been ruled out, tuberculin-positive persons on, or about to go on, prolonged therapy with immunosuppressive medications receive INH prophylaxis, preferably for 12 months, though 6–9 months' treatment is often used[61].

Rubin[6] maintains that prophylaxis should be reserved for Mantoux-positive transplant candidates who have other risk factors such as: (a) recent tuberculin conversion, (b) Asian, African, or Native American heritage, (c) other immunosuppressing conditions, (d) a history of inadequately treated active TB, and/or (e) significant abnormalities on chest radiograph. This is based on the fact that TB reactivated in only one of 73 Mantoux-positive renal transplant recipients, none of whom received pretransplant INH prophylaxis, and that patient was easily treated. This approach seems logical in low-prevalence areas of the world, though geographic risk also should include Mexico, Central and South America, Mediterranean countries, and central and eastern Europe. As the risk of INH hepatitis may be higher than the risk of TB in transplant recipients[62,63], every effort should be made to complete the prophylaxis before transplantation.

Non-TB mycobacteria

In a review of 17 years' experience with heart transplant patients, the Stanford program[49] reported on 14 patients with atypical mycobacterial disease, 11/200 maintained on azathioprine and prednisone and 3/302 on CsA-containing regimens ($p = 0.004$), who presented at 86 days to 11.5 years after transplant. Twelve patients had lung and/or skin involvement, and two developed lesions at the site of prosthetic devices, with *M. kansasii* in six patients, *M. avium-intracellulare* (MAI) in five, and *M. fortuitum* and *M. thermoresistibile* in one each. The fourteenth isolate was an unidentified Runyan group II scotochromagen. In recent years MAI has become the most common isolate. A fifteenth patient, with mycobacterial coronary arteritis, was reported elsewhere[48]. Other reports describe pulmonary and/or skin infections with *M. chelonae*[41] and *M. scrofulaceum*[44].

Treatment of these infections is variable, depending on sensitivities[64].

Nocardia

Nocardia is a partially acid-fast filamentous bacillus; the species *N. asteroides* and *N. brasiliensis* most commonly cause disease, usually late after transplant[65–67]. Transplant recipients accounted for 13% of the 500–1000 cases reported annually in one report from 1976[68]. The reported incidence among renal transplant recipients varies from 0% to 20%[66,69,70] and in one older study of heart transplant recipients was 21%[67]. The incidence has decreased in the CsA era independently of the use of routine trimethoprim-sulfamethoxazole prophylaxis for *Pneumocystis carinii* pneumonia (PCP)[66], though this might be as expected, as many cases of nocardiosis occur after prophylaxis has been discontinued.

Though some patients can present with dry cough and fever, pulmonary nocardiosis in transplant recipients is often asymptomatic and detected only on incidental chest X-rays showing single or multiple nodules with or without cavitation. Diffuse pulmonary infiltrates can also occur. Dissemination from the lung produces abscesses in skin, brain, and other tissue[71]. Primary skin inoculation can also occur[64].

Sulfonamides, either alone or in combination with trimethoprim, are the drugs of choice for treatment of nocardial infections[72]. For the allergic or intolerant patient, amoxicillin with or without clavulanate, and minocycline are good oral alternatives for susceptible strains[73]. Where parenteral therapy is indicated, imipenem–cilastatin, ceftriaxone, cefotaxime, and amikacin are effective[73–75]. Because of the propensity for secondary CNS involvement, CT or MRI scans should be done in any case of nocardiosis before starting therapy, as a positive result has several implications for management:

(1) The duration of therapy should be extended from 6 months to 12 months.
(2) Brain abscesses, which are large and accessible or do not respond to, or progress in spite of, medical therapy, should be evacuated.
(3) CNS lesions may develop on therapy without adversely affecting the prognosis or dictating a change in antibiotics, therefore it is useful to have baseline CT scan[76].

Miscellaneous bacterial infections

Rhodococcus equi

The first case of infection in a heart transplant recipient has been reported[77]. This aerobic, Gram-positive, weakly acid-fast cocco-

bacillus is a soil actinomycete which causes pleural-based, cavitating pulmonary nodules or, less often, lobar pneumonia, in immunosuppressed hosts. Half of the patients not infected with the human immune deficiency virus have a history of exposure to horses, swine, or cows or their products[78,79]. Fever, cough, with or without hemoptysis, dyspnea, pleuritic pain, and wasting come on insidiously in days to months. Recommended treatment includes up to 6 months of at least two lipophilic bactericidal antibiotics, the choice based on sensitivity testing. Vancomycin is widely used, with a quinolone or amoxicillin–clavulanate, or a macrolide.

Treponema pallidum

Reports of syphilis in modern transplantation literature are rare[80]. Clinical manifestations of this disease are extremely variable and diagnosis can be difficult. Testing of donors for syphilis is routine, but there is no consistent practice for recipient candidates. Storing pretransplant recipient serum for future testing can be very useful in making this and other obscure diagnoses, and it is more cost-effective than obtaining all possible serologies. Many cases of latent syphilis may well be treated by virtue of the frequent contact most people, especially transplant recipients, have with antibiotics. Donor seropositivity need not be a contraindication to transplantation, as good results have been obtained with antibiotic prophylaxis[81].

FUNGAL INFECTION

In a recent review by Paya[82], the incidence of fungal infection in the CsA era is cited as 0–21% for heart transplants[83–85] with *Aspergillus* predominating[84,86] and 15–35% for heart–lung and lung transplants[87,88], with *Candida* as the most common fungal pathogen[85,87,88]. Infections usually occur early after transplantation, with a median of 23 days for *Aspergillus* and 44 days for *Candida*[84], and a resultant mortality of between 40% and 70% for heart–lung and lung transplant recipients[85,87,88] and an almost universally fatal outcome for heart transplant recipients with aspergillosis despite amphotericin B treatment[84,89], though other reviews quote a better survival rate for isolated pulmonary aspergillosis (see below).

Several factors may contribute to the high mortality of these infections: (a) difficulty in making an early diagnosis and unavailability of reliable non-invasive tests such as serology[90]; (b) lack of effective therapy for *Aspergillus*, (c) toxicity of amphotericin B and the interaction of antifungal drugs with CsA, making treatment an ordeal, and (d) limited data on the efficacy of available antifungal prophylaxis[81].

Candida

Conditions which facilitate *Candida* colonization of the skin and gastrointestinal tract, such as antibiotics, intravascular and urinary catheters, malnutrition, and breaches of mucosal integrity, conspire with impaired neutrophil and macrophage number and function to establish *Candida* as an invasive pathogen. Suppressed cell-mediated immunity tends to be complicated by the mucocutaneous forms of the disease.

Candidiasis is usually caused by *C. albicans* and can be classified as mucocutaneous (thrush, vaginal candidiasis, intertrigo, and esophagitis), urinary, invasive (sternal osteomyelitis, localized abscesses) and disseminated (candidemia with or without organ involvement, endophthalmitis). Dissemination, with the potential for visceral involvement, results from transient candidemia in >50% of immunosuppressed patients as compared with 5% in immunocompetent hosts[91]. Pulmonary involvement is rare, and then occurs only in the context of disseminated disease. Culture of *Candida* from multiple sites usually accompanies disseminated disease, but is not specific for it.

Unique to thoracic transplantation is mediastinal abscess complicating dehiscence of the infected bronchial anastomosis[88] and fatal rupture of mycotic aneurysms of the aortic anastomosis[91–93]. These latter two disasters can be prevented by pre-emptively treating recipient and/or donor trachea cultures positive for *Candida* with fluconazole; there are no data as to the appropriate duration.

Until further data are available, invasive and disseminated disease in this population should be treated with amphotericin, not fluconazole, though after an initial course of 300–500 mg as 'induction therapy', the responding patient can receive 'consolidation therapy' with fluconazole if the organism is not *C. krusei* or *C. glabrata*, as these species cannot be assumed to be sensitive to fluconazole. Candidal sternal wound infections must be aggressively debrided and treated with amphotericin. Fluconazole does not achieve good levels in bone[94]. Candiduria can be treated with daily or continuous amphotericin bladder instillations or fluconazole, but none of these works if the catheter is not removed. Fluconazole is effective treatment for esophagitis and for other forms of mucocutaneous candidiasis that are not responding to topical antifungals, such as clotrimazole troches.

Increasing resistance is a concern; *C. glabrata* develops resistance on therapy and *C. krusei* is minimally susceptible[95,96]. Itraconazole is often effective in this circumstance. Antifungal therapy is discussed in more detail below.

Prophylaxis with topical antifungals has produced inconsistent results in other populations[97] but is standard in most transplant programs; 1–3 months seems long enough. While a limited number of studies suggest that fluconazole prevents both colonization and disease in neutropenic bone marrow transplant recipients and leukemics[98,99], routine prophylaxis in thoracic transplant recipients would be expensive, and carries the potential to induce more resistance and to increase the incidence of infection with *C. krusei* and *C. glabrata*[100]. Daily ingestion of 8 ounces of yogurt containing *Lactobacillus acidophilus* decreased the incidence of both colonization and candidal vaginitis in a population of women with recurrent vaginal candidiasis[101]. Surveillance cultures have a good negative predictive value but a poor positive predictive value, as it is often difficult to differentiate colonization from invasive disease and, as such, are not cost-effective.

Aspergillus

Spores of this ubiquitous mold are inhaled, and can colonize the respiratory tract or cause invasive pulmonary aspergillosis with the potential for extrapulmonary dissemination. For aspergillosis confined to the lung, survival rates are 28–82%[102–104]. The organ-

ism is highly angioinvasive. Clinical presentation can be insidious or acute and consists of:

(1) a pulmonary embolus-like syndrome with pleuritic chest pain, dyspnea, fever, pleural rub and peripheral cavitating nodules on chest X-ray;
(2) diffuse pulmonary infiltrates;
(3) pulmonary aspergilloma: a focal area of *Aspergillus* necrotizing pneumonia with cavitation in which saprophytic fungal growth occurs ('fungus ball');
(4) single or multiple brain abscesses;
(5) oculo- or otomycosis;
(6) disseminated disease.

It has been suggested that transplant recipients with *Aspergillus* infection can be treated with lower doses than the 1.0–1.5 mg/kg per day recommended for neutropenic patients. However, a significant number of the treatment failures in this review occurred in the patients receiving lower doses (<30 mg/day). Recommendations as to total doses of amphotericin B range from 1.5 to 4.0 g depending on the rate of radiologic resolution of lesions, and bone marrow recovery in neutropenics[105]. The role of surgical resection of isolated aspergillomas, pulmonary nodules and infiltrates is crucial and underemphasized in the literature[102,106,107]. Four heart transplant recipients in the Minneapolis Heart Institute program have been diagnosed with invasive aspergillosis (2.2%): two died with CNS dissemination and concomitant CMV infection, one did well after resection of a right upper lobe aspergilloma and a short course of amphotericin, and one with multiple pulmonary nodules is doing well on amphotericin, after at least 2 g of which he will be maintained on itraconazole.

Nosocomial outbreaks of aspergillosis in solid-organ transplant recipients have been related to local construction work and to contaminated ventilation systems[84,108–112]. Special air-handling systems have been shown to reduce the incidence of colonization and disease with *Aspergillus* in bone marrow transplant recipients[108,113–115] and in renal transplant patients[108]. Preventive measures at our institution consist of special ventilation with HEPA-filtered air under positive pressure, exchanged at a rate of at least 10 times per hour. Additional '*Aspergillus* precautions' are detailed in Table 4. Systems are monitored on a regular basis, but positive pressure can be easily checked with a smoke stick.

Cryptococcus neoformans

Cryptococcosis occurs infrequently in thoracic transplant recipients. It constitutes 20% of fungal infections seen in this population, but is the commonest cause of CNS infection in transplant recipients overall[6]. Although the potential for *C. neoformans* to be passed on in donor lungs is real, the few infections reported in heart–lung and lung transplant recipients are thought to have been acquired from environmental exposure[82].

C. neoformans is a yeast carried in bird feces, and spores are inhaled. It causes pulmonary infiltrates or nodules and, from there, disseminates to the CNS, skin, and urinary tract. All skin lesions and areas of cellulitis should be considered for biopsy, and sterile pyuria investigated. The presence of cryptococcal antigen in the blood is very helpful[6]. All patients with extraneural cryptococcosis should undergo lumbar puncture before starting therapy, as the likelihood of CNS involvement is high, even in the absence of clinical signs and symptoms. The typical CSF profile consists of <100 WBC, mostly mononuclear cells, glucose between 20 and 40 mg/dl, and a positive cryptococcal antigen.

Treatment of meningitis has traditionally consisted of 6–10 weeks of amphotericin B, 0.3 mg/kg per day, and 5-flucytosine[116], and that remains the therapy of choice for the acutely and severely ill; once stabilized, treatment can be completed with fluconazole. Ketoconazole and itraconazole do not cross the blood–brain barrier. Given the toxicity of amphotericin in the transplant population, many are using fluconazole as primary therapy in the subacutely ill patient[117]. In both cases the drug is given for 2–4 weeks after the last positive culture and cryptococcal antigen test. Sequential measurement of the cryptococcal antigen titer is helpful in monitoring the efficacy of therapy[6]. Extraneural cryptococcosis can be treated with fluconazole, 400 mg/day or an equivalent dose adjusted for renal function, using the same parameters for duration.

The dimorphic fungi (*Coccidioides, Histoplasma,* and *Blastomyces*)

Common to all of these organisms is a defined geographic distribution and the fact that, although infection is probably no more common than in normal hosts, immunocompromised persons are more likely to develop disease, and disease is more often disseminated and severe (hyperinfection). They exist in nature in mycelial forms and in the body as yeasts; hence the classification as dimorphic.

Coccidioides immitis

This fungus exists only in the Sonoran desert ecosystem in seven southwestern American states and in the Mexican northwest. Spores are inhaled and, 1–3 weeks later, cause symptomatic pneumonitis, often with pleuritis, in 40%, accompanied by a nonspecific febrile illness with arthralgias; associated erythema nodosum or multiforme is 2–10 times as common in women. Five percent have residual pulmonary nodules or cavities which can calcify. Chronic cavitary disease occurs more commonly in immunocompromised patients. Dissemination to musculoskeletal and central nervous systems and to skin occurs in 0.5% of all infected persons, this being much more likely to complicate immunosuppression, particularly when lymphopenia is present. Although reactivation disease can occur, primary infection is more likely to cause dissemination in transplant recipients[118].

Serum IgM precipitins can be detected in 75% of patients 1–3 weeks after the onset of symptoms of primary coccidioidomycosis and disappear within 4 months; persistence is a marker of progressive disease. They can also be measured in some cases of reactivation. At 3 months, 90% of patients are positive for IgG antibodies either by tube precipitin or complement fixation (CF) tests, and they may persist for 6–8 months. With the exception of meningitis, 61% of patients with disseminated disease have CF antibody titers of >1 : 32 and 41% have levels of at least 1 : 64[118]. The serologic response in im-

munocompromised patients appears to be at least qualitatively intact.

Coccidioidomycosis in heart transplant recipients has been sporadically reported before the CsA era[119,120]. Copeland's group in Tucson, Arizona, reported on their experience in the triple-drug immunosuppression era[121]. Six patients had a history of infection prior to transplant and were put on ketoconazole afterwards; none had recurrences. The rate of presumed coccidioidomycosis occurring after transplant was 4.5% or 1.5 episodes per patient-year, a rate that was half that seen in the pre-CsA era. Onset of disease occurred at a mean of 174 days (range 19–410 days) after transplant. Seven of the nine patients had only pulmonary disease, and dissemination from the lungs to blood, genito-urinary tract, joints, and skin occurred in the other two. Interestingly, no central nervous system disease was found. All patients were treated with 1 g of amphotericin B and then maintained on ketoconazole, 200 mg p.o. twice a day. Two of the nine had recurrent disease under circumstances which required the discontinuation of ketoconazole, a phenomenon that was also reported in previous studies[120,121]. Given the overall prevalence of coccidioidomycosis in that part of the continent, their practice is to screen transplant candidates, giving prophylaxis indefinitely to those with either a positive history or serology.

All transplant recipients with acute coccidioidomycosis should be treated with fluconazole, to prevent dissemination. Neither ketoconazole nor itraconazole crosses the blood–brain barrier, and meningitis has occurred in patients on ketoconazole. Although there is little specific data in the thoracic transplant literature on the preferred treatment of disseminated disease, patients with meningitis can probably be effectively treated with fluconazole. Extraneural disease can be treated with either fluconazole or itraconazole[122]. All patients should receive lifelong suppressive therapy. Doses of 400 mg/day are appropriate. Critically ill patients may need initial therapy with 0.5–1 g of amphotericin B followed by azole therapy and suppression. Intrathecal drug may be required to treat meningitis in such patients.

Where disease is confined to the lung, immunosuppressed patients do as well as their immunocompetent counterparts. Surgery may be indicated for some patients with cavitary disease[123].

Histoplasma capsulatum

This fungus exists in mycelial form in the soil of the east central United States, especially where concentrations of avian or bat feces are heavy. As in most other clinically significant fungal infections, the organism is inhaled and, in immunocompromised hosts, causes pulmonary disease with or without dissemination to skin, bone marrow, liver, and other organs. Presentation as acute (APDH), subacute, or chronic progressive disseminated histoplasmosis (CPDH) is dependent on the degree of parasitemia of the mononuclear phagocyte system, with high fevers, pancytopenia, visceral lymphadenopathy, pulmonary/gastrointestinal disease and hepatosplenomegaly characterizing APDH[124], and chronic wasting with focal disease the chronic form. Subacute disease is intermediate between the two. In all forms a careful search for skin or oral mucous membrane lesions is crucial as the diagnostic yield on biopsy of such lesions is higher than for any other site. Bilateral adrenal gland enlargement in the appropriate clinical setting should strongly indicate the diagnosis of disseminated histoplasmosis, and patients can become Addisonian.

Wright stain of peripheral blood is positive in 30–45% of AIDS patients with acute presentations[125]. Silver stain of the blood buffy coat and bone marrow biopsy specimens increases that yield, and is always positive on biopsies of oropharyngeal lesions. Rates of isolation from blood culture have been increased by lysis-centrifugation techniques[126]. Yield from cultures of clinical material depends on the presentation and the experience of the laboratory. Sputum culture is positive in only 10–15% of cases of acute pulmonary histoplasmosis[127]. Detection of a polysaccharide antigen in urine and blood is sensitive and specific for the diagnosis of APDH in AIDS patients, but less so in other hosts[125,128]; data for transplant recipients are needed. Complement-fixing antibodies (CFA) appear 3 weeks after exposure in 5–15%, and at 6 weeks in 75–95%. A titer of >1:32 is highly indicative of active histoplasmosis, titers of >1:8–1:16 are not as specific but are considered positive, and are more in the range of the level of response expected in the immunosuppressed[125,129]. Precipitin to the 150 kDa M antigen can be detected by immunodiffusion 2–4 weeks after CFA in 50–80% of patients but persists for years, thus losing its sensitivity as a predictor of active disease. It is present in 50% of chronic pulmonary or disseminated disease. Precipitin to a 120 kDa H antigen is highly specific and persists for only 6 months, but is present in <20% of patients[125,130]. Amphotericin B, 35 mg/kg or 2.0–2.5 g total, at a dose of 0.7–1.0 mg/kg per day, is indicated for the treatment of patients who are immunosuppressed, or either not responding to azoles or relapsing off them. Another approach is to give an induction course of 20–25 mg/kg of amphotericin B followed by itraconazole. It is not clear whether transplant recipients, like AIDS patients, require lifelong azole suppression[126,131]. Itraconazole has been effective as primary therapy in patients who are not intensely immunosuppressed, in a dose of 200–400 mg/day for 6–12 months[126,132–134].

Work continues on the development of a vaccine[135,136].

Blastomyces dermatitidis

Geographic distribution for this fungus includes the east central USA with extension along the St Lawrence River and into the territory around the Great Lakes. Most cases of blastomycosis are not associated with immunosuppression. In a case report of recurrent blastomycosis in a heart transplant recipient and a review of the literature[137], Serody *et al.* found only four, or possibly five, additional cases of blastomycosis in transplant recipients[138–142], one of which was a heart transplant recipient. Onset was from 3 weeks to 4 years after transplant, and disease was not associated with any particular immunosuppressive regimen. Pulmonary disease was active in all, with skin involvement in two, and eye lesions in one. Diagnosis was made by skin biopsy or bronchoscopy. Their patient responded to 1 g of amphotericin B, followed by 9 months of ketoconazole, but relapsed and was retreated with 1 g of amphotericin B with partial response, and worsened on ketoconazole.

Itraconazole is now the drug of choice for treatment of blastomycosis in the non-critically ill patient, but data on cures in transplant recipients remain to be accumulated.

Paracoccidioides brasiliensis

Dynamic population shifts can be expected to introduce cases of South American blastomycosis, the disease caused by this agent, into North American medical experience. As reactivation of dormant infection can occur in immunosuppressed hosts, this entity should be kept in mind in patients who have resided in endemic areas. Brazil is the focus of the geographic distribution, with some spillover into Colombia, Venezuela, and Argentina, and infrequently in the rest of Latin America from latitude 23 degrees north to 34 degrees south. The lungs are the site of primary infection, but dissemination to skin, mucous membranes, reticuloendothelial system, and adrenals is frequent[143,144].

Miscellaneous unusual fungi

Pseudallescheria boydii infection is often clinically and morphologically mistaken for aspergillosis, and includes otomycosis, oculomycosis, sinusitis, fungus balls, and angioinvasive pulmonary disease with dissemination. It has an array of clinical presentations according to sites of trauma and is a cause of eumycetoma. It is important to differentiate this organism from *Aspergillus* by culture, however, as it is resistant to amphotericin B, though responsive to azoles. Concomitant surgical management is necessary[145–148].

Mucormycosis, with rhinocerebral, pulmonary, cutaneous, gastrointestinal and central nervous system presentations, is very rare in transplant recipients[149–154], and there are no data for thoracic transplant recipients.

Phaeohyphomycosis is a term that applies to infections other than chromoblastomycosis and eumycotic mycetomas, caused by dematiaceous fungi present as saprophytes in nature, and seen with increasing frequency as the prevalence of immunosuppression rises. McGinnis has categorized these infections into superficial, cutaneous/corneal, and subcutaneous cystic forms initiated by direct inoculation, and systemic disease where widespread dissemination, often from a lung source, occurs[155]. Sudduth *et al.* reported the first case in a cardiac transplant recipient who had a subcutaneous abscess on the arm, from which *Exophilia jeanselmei* was isolated[156]. An excellent review of the *Exophilia* infections is presented. Other rare fungal agents causing infection in transplant recipients include *Trichosporon beigelii*[157,158] and *Fusarium*[146].

Antifungal therapy

Amphotericin B is the tried and true gold standard for the treatment of most serious fungal infections, despite its legendary toxicities. Management of amphotericin B therapy in patients on nephrotoxic immunosuppressive medications such as CsA and FK506 is particularly arduous, as serum creatinine may rise precipitously[159,160], even after just one dose. Salt loading and good hydration may help[161]. Starting with lower doses, and increasing the dose more gradually than usual, do seem to prevent big jumps in creatinine, but theoretically delay the response to treatment for seriously ill patients with virulent infections such as invasive/disseminated aspergillosis. The ability to change to the less toxic azoles after an initial induction period with amphotericin B helps to minimize the cumulative toxicity.

Liposomal encapsulated amphotericin B appears to be less nephrotoxic; thus higher doses can be given[162,163]. However, although data from open trials indicate equal or superior efficacy and less toxicity in the treatment of *Candida* and *Aspergillus* infections in neutropenic and bone marrow transplant patients[163–170], the safety profile remains to be established in solid organ transplantation, and the benefits may not extend to patients on CsA. Preliminary results indicate the potential in some situations[171].

Ketoconazole has no place in the treatment of mycoses in the transplant population, but itraconazole and fluconazole have added greatly to the management of fungal infection, either as primary therapy or after an induction phase of amphotericin B. Itraconazole down-regulates the cytochrome P450 hepatic enzyme system and raises CsA levels, but to a lesser extent than ketoconazole[159]. Prompt halving of the CsA dose can offset this effect. Fluconazole elevates CsA levels only at doses in excess of 400 mg/day[172]. Itraconazole cannot be given down a nasogastric tube and only fluconazole comes in an intravenous formulation. Itraconazole is useful for the continuation of treatment of aspergillosis after amphotericin B, and for primary or subsequent treatment for histoplasmosis, blastomycosis, and pulmonary coccidioidomycosis. It does not cross the blood–brain barrier, so is not useful for meningeal disease. Fluconazole does penetrate the cerebrospinal fluid and is useful for the primary treatment of the non-emergently ill patient with cryptococcosis, coccidioidomycosis, oral/esophageal candidiasis, and candiduria, and for the subsequent treatment of invasive/disseminated candidiasis with sensitive strains[82,117,173–178]. Saperconazole is a new azole which has excellent *in-vitro* activity against *Aspergillus*[177,178] but is still in trials.

VIRAL INFECTION

Human herpesviruses

Cytomegalovirus

Cytomegalovirus (CMV), or human herpesvirus 5, is a DNA virus that causes the most morbidity and mortality in transplant recipients[185]. The Stanford group has shown that CMV infections in heart transplant recipients constitute a risk factor for more frequent episodes of rejection, more frequent and severe graft atherosclerosis, and a greater risk of death (see below). Some of the reasons for this association include the fact that CMV is ubiquitous; seropositivity rates in Western European and North American populations are 15% by age 2, 30% in young adults, and 50–60% over age 50. Higher rates are seen in low socioeconomic groups, gay males, sexually promiscuous heterosexuals, and recipients of blood transfusions[179]. Day-care-age children and their parents are also at higher risk[180]. In addition to directly causing clinical disease, CMV infection has a number of indirect consequences: it is associated with, and may cause, rejection; it is immunosuppressive, thereby adding to pharmacologic immunosuppression and facilitating superinfection by other opportunists; it may be oncogenic.

Ninety percent of primary CMV infections in transplant recipients are due to reactivation of latent virus in the cells of the donor organ in a SNR, and 10% to transfusion of CMV-positive blood products. SPR can be primarily reinfected with donor strains different from their own, and subsequent disease is due to the donor

strain 50% of the time (as demonstrated by DNA-restricted enzyme analysis). Reactivation of recipient strains accounts for the balance of the 90% of CMV infections that occur in the first 1–4 months after transplant. Late-onset primary infections are community-acquired. Primary infection can be documented by seroconversion, and all forms by detection of virus in oropharyngeal secretions, urine, and blood. Disease results from 25–60% of the primary infections and in at least 20% of SPR[6,84,87,179,181–191], but there appears to be no difference in mortality.

Viral pathogenesis

CMV replicates best in fibroblasts and less well in epithelial and other cells. In the first 4 hours after cellular penetration (the immediate–early phase), proteins which regulate expression of viral genes are synthesized. The next 8 hours (the early phase) result in the production of DNA polymerase. In the final 6 hours of the 18–24-hour replicative cycle (the late phase) whole viral particles are assembled for release[192], which may not occur for 4 days[179]. The fact that multiple replicative cycles are required to detect the typical cytopathic effect on fibroblast monolayer cultures 3 weeks later (range 1–6 weeks) may explain the long incubation period until infection and disease can be detected after transplant.

The end-result is cytolytic or productive infection where new virus particles released from the lysed host cell are capable of infecting new host cells, or persistent or non-productive infection where, in the case of CMV, virus persists in circulating monocytes and polymorphonuclear leukocytes in a non- or intermittently replicative, latent state[193–195]. Viral persistence may also be manifested by chronic, replicative, cytolytic infections that are subclinical and do not impair organ function[196] and by slowly progressive, destructive infections such as progressive multifocal leukoencephalopathy caused by papovaviruses[197] (see below).

Immunosuppressive regimens interfere with the cell-mediated mechanisms that check the activation and expression of, and later destroy, virus-infected cells. Recovery from CMV disease is dependent upon the host's ability to elaborate MHC-restricted CMV-specific cytotoxic T8 cells and natural and antibody-dependent killer cells[198,199]. Specifically, the effects of antilymphocytic and cytotoxic agents facilitate reactivation of latent virus, while those of CsA, rapamycin, and FK506 allow the dissemination and amplification of actively replicating virus. Though the frequency of CMV infections has decreased in the cyclosporin era, particularly with low-dose triple regimens[200,201], spontaneous recovery is no longer seen, and relapsing CMV infections are now a problem in some transplant populations. Rubin[6] states that the addition of any antilymphocyte therapy to an immunosuppressive regimen causes an increased incidence of CMV disease and a decreased effect of preventive measures against it, such as interferon, acyclovir, or CMV immune globulin[182,188,201–209]. This conclusion comes mainly from the experience in renal transplant recipients; in thoracic transplantation there is some conflicting evidence that antithymocyte globulin or induction OKT3 results in a higher incidence of CMV[210–214].

Clinical syndromes

The mean time of onset of CMV disease after transplant is 5.5 weeks[215]. In a recent multi-institutional study[214] of 1553 heart transplant recipients at 26 centers, there were 230 episodes of CMV disease in 200 patients for an incidence of around 13%, with a 12% rate of recurring disease. Ninety-nine percent of the episodes were treated with ganciclovir, with a 93% resolution rate. Blood cultures were positive in 43%. Disease involved the lung in 30% of episodes, 13% of which were fatal, and the gastrointestinal tract in 23%, for a 6% mortality rate. Donor–recipient serostatus and cytolytic induction therapy were risk factors for disease occurring earlier in the post-transplant period, but fatal outcome was influenced only by preceding or concomitant infections with other organisms.

CMV infection in lung transplants is more problematic as it usually involves the lungs. Experience varies widely[216–218], with the Toronto group[216] reporting an incidence of CMV pneumonitis of 7.7% (fatal in 2%) in 57 single lung transplants (SLTx) and double lung transplants (DLTx) performed between 1983 and 1990, and the Pittsburgh group citing a 27.6% incidence (13.8% fatal) in 59 heart–lung transplants (HLTx), DLTx and SLTx transplanted between 1982 and 1989[217]. In the latter study, although 19/20 (95%) SPR showed evidence of active infection, with a 5% mortality rate, only six developed disease. Primary infection and disease developed in 13/38 SNR (38%) and was fatal in 7/13 (54%). Sixteen of the 19 patients with CMV disease had pneumonitis: 11 SNR (85%) and five SPR (26%). More SNR than SPR had severe pneumonitis (8/11 vs 1/5), and pulmonary superinfections with other organisms occurred at a significantly higher rate in the first year post-transplantation in SNR. Actuarial survival was also worse. Only 6/16 patients with pneumonitis were treated with ganciclovir. The higher incidence of CMV pneumonitis in LTx than in heart transplants (HTx) may be attributable to the younger age, and therefore greater proportion of seronegativity, of the recipient population, and the much larger inoculum of latent virus associated with the large amount of tissue transferred with a heart–lung block.

Singh *et al.*[184] have provided a standardized classification of CMV disease:

(1) CMV syndrome: Fever of 38 degrees C for a least 1 week without any other source. Laboratory evidence of CMV infection (see Diagnosis). One or more of the following:
   (a) white blood cell count $\leq 4000/mm^3$;
   (b) platelets $\leq 100\,000/mm^3$;
   (c) atypical lymphocytes $\geq 3\%$.
   Myalgias, arthralgias, and headache are non-specific but often present, as in anemia.
(2) Localized CMV disease: Tissue invasion of a single organ, proven histologically; and/or by positive tissue culture.
(3) Disseminated CMV disease: Tissue involvement of two or more non-contiguous sites.

There is likely much overlap, and empiric esophagogastroduodenoscopy (EGD) will often demonstrate the characteristic findings of CMV enteritis in patients with no localizing symptoms[219]. This can be a useful finding in confusing situations, so the threshold for doing endoscopy should be low[217].

CMV enteritis is a common manifestation of CMV infection in thoracic transplantation; pneumonitis is seen almost exclusively in lung and heart–lung, and myocarditis in heart/heart–lung, recipients. CMV retinitis is rare and denotes chronic CMV viremia[220,221].

Enteritis

Cytomegalovirus can cause mucosal disease anywhere along the gastrointestinal tract[222], but the stomach, duodenum, and right colon are most frequently involved. Esophagitis presents as dysphagia and gastroduodenitis with epigastic pain, often exacerbated by movement, nausea, vomiting, early satiety[223], and anorexia[224]. Diarrhea, sometimes with blood, and lower abdominal pain are characteristic of colitis[225–228], and perforation can be a serious complication. CsA levels tend to decrease, reflecting absorption abnormalities. Fever is not necessarily present. Diagnostic endoscopy reveals non-specific inflammation with erosions, and aphthous ulcerations and biopsy show characteristic perinuclear inclusion bodies.

Pneumonitis

Patients with pneumonitis present with constitutional illness, dyspnea, tachypnea, dry cough, and bilateral peribronchial interstitial and alveolar infiltrates of the lower lobes on chest roentgenogram in 70%. Focal infiltrates, nodules, and lobar consolidation occur less frequently. Although the diagnosis of CMV pneumonitis should be based on demonstration of typical histology and detection of CMV in lung tissue, seeing inclusions and/or detecting CMV on bronchoalveolar lavage is cited as sufficient indication for treatment[6,229].

Disseminated infection

CMV can cause disease of almost any organ, and viral septic shock is associated with an increased cardiac index, decreased systemic vascular resistance, and elevated oxygen delivery during both early and late phases. In contrast to bacterial sepsis and endotoxemia, pulmonary resistance is normal[230].

Miscellaneous sites

CMV has been identified as causing endometritis[231], encephalitis[232], transverse myelitis[233], Guillain-Barré-type polyneuropathy[234], cutaneous vasculitis[235], hemorrhoiditis[236], hepatitis[237], nephritis[238], epididymitis[239] and coronary thrombosis[240].

Diagnosis

Pathology

Positive biopsies are those which show CMV inclusions and focal inflammation and/or antigens of CMV by monoclonal antibody staining or CMV DNA by *in situ* hybridization[241].

Serology

CMV IgG antibody tests are useful only to establish the serostatus of the recipient and the donor, and to identify candidates for prophylaxis (Table 5). For that purpose, complement-fixation tests using a glycine-extracted antigen are sensitive and specific at >1:8[188]. Newer immunofluorescent, ELISA, and latex agglutination techniques are being used by many laboratories as they are faster, less labor-intensive, and cheaper. However, there is no standardization, except at a local experiential level, as to what constitutes a positive test, and low-level positives can be falsely so. Chou has reviewed the advantages and problems of these tests[241]. IgM antibody, an indicator of active infection, has poor sensitivity and specificity, especially in immunosuppressed patients[241]. Measurement of anti-CMV neutralizing antibody is cumbersome and unhelpful[242].

Viral isolation

Detection of CMV in blood, body fluids, and tissue offers the best sensitivity for diagnosis of disease, when matched with clinical assessment. Viremia is the best overall marker for both acute and chronic infection[182,188,206]. The 'gold standard' test is the demonstration of the typical cytopathic effect on a fibroblast culture 1–6 weeks after inoculation. Rapid diagnostic techniques have evolved, and have contributed greatly to improved outcomes in patients with CMV disease. Using the shell vial technique, the 72 KDa immediate–early antigen can be detected 24–48 hours after inoculation of fibroblast monolayers, using monoclonal antibody and immunofluorescence[243]. The major drawback is that, due to technical difficulties, false-negative blood buffy coat cultures can occur in up to 50% of patients[244].

The antigenemia assay of The *et al.*[245] involves direct immunoperoxidase staining of buffy coat preparations using a monoclonal antibody to the 65 kDa lower matrix phosphoprotein, a late antigen, and yields a diagnostic sensitivity and specificity of 95%. As it is a semi-quantitative technique it can be used to determine when pre-emptive therapy might be indicated, as rising titers and sustained antigenemia have been shown to herald the onset of disease. It is therefore also of value in monitoring the effects of therapy. Other antigenemia assays based on different antigens and monoclonal antibodies have proven to be less sensitive and specific[246–255]. A similar technique can be used on bronchoalveolar lavage (BAL) material[254,256].

In-situ DNA hybridization can detect CMV genome copies in clinical material[257]. Polymerase chain reaction (PCR) allows selected amplification of specific nucleic acid sequences, and is as good as the antigenemia assay but technically more difficult[258–260].

Table 5 Pretransplant work-up: donor

Infection risk	*Specifics*	*Action*
Viral	CMV IgG SPD	Prophylaxis of SNR
	EBV VCA IgG SPD	May dictate matching to SPR
	HBV surface antigen SPD	Exclude
	HCV 2nd or 3rd generation ELISA and immunoblot test SPD	May need to exclude or to use only in SPR
	Retrovirus SPD or SND at high risk	Exclude
Parasites	*Toxoplasma gondii* IgG SPD	Treat SNR
	Trypanosoma cruzi risk or SPD	Exclude

A newer technique is reverse transcription PCR, that can detect viral transcripts which code for different CMV proteins[261]. This could improve the sensitivity of diagnosis as existing techniques rely on single antigens, whereas CMV has genomic and antigenic heterogeneity.

Treatment

The advent of ganciclovir (GCV) has had a great impact on the previously poor outcome of CMV disease in transplant recipients. The 7% mortality rate from GCV-treated CMV disease reported by the Cardiac Transplant Research Database Center[214] compares favorably to the 25–70% mortality rates quoted before the drug became readily available[210,229,262]. Other groups have confirmed this experience[262–270].

CMV enteritis is usually successfully treated with 5 mg/kg every 12 hours (adjust for renal dysfunction) for 2 weeks.

Optimum therapy of CMV pneumonitis consists of GCV plus intravenous immune globulin (IVIG) or hyperimmune CMV immune globulin (CMVIG)[271–274] for 4 weeks. A beneficial effect of humoral responses on symptoms not attributable to a major reduction in viral replication has been noted in the mouse model[275]. CMV hyperimmunoglobulin (CMVIG) alone has not proved beneficial in treating CMV disease[271].

Concomitant decrease in doses of immunosuppressive drugs, particularly azathioprine, may be advisable and necessary, as leukopenia is the commonest side-effect of GCV. Care must be taken to reinstitute higher doses when the infection and its immunosuppressive effects are resolving, as rejection can occur.

Although not much resistance to GCV has been experienced, the increase in the use of this useful drug for prophylaxis and pre-emptive therapy can be expected to result in more. Erice *et al.*[275] reported three patients (two with AIDS, one with chronic lymphocytic leukemia) in whom GCV-resistant CMV was isolated; in one, resistance emerged on therapy. GCV was ineffective in eradicating CMV from the blood and all three patients died.

Trisodium phosphonoformate (Foscarnet, Astra), a virostatic agent, has been used with some success in the treatment of CMV disease in immunocompromised hosts, including a heart transplant recipient[276,277]. This drug could serve as an alternative in the patient with GCV-resistant or GCV-unresponsive CMV.

Prevention

A number of measures to prevent CMV infection and disease have been tried for many years, with mixed results:

(1) Matching D : R serostatus.
(2) CMV-negative blood products or leukocyte filters.
(3) Passive immunoprophylaxis.
(4) Active immunoprophylaxis.
(5) Chemoprophylaxis.
(6) Combinations of the above.
(7) Pre-emptive therapy.

1. Matching serostatus. Matching donor and recipient by CMV serostatus (SNR/SND) significantly reduces the incidence of CMV disease[216,278,279]. Even in the absence of documented CMV infection, survival and functional status are better than in seromismatched pairs[216]. The shortage of donor hearts and our ability to maintain a relatively low morbidity and mortality from CMV disease in HTx have made this an unattractive strategy, though evidence is mounting that the greatest toll of CMV infection may be its contribution to chronic rejection as manifested by accelerated atherosclerosis. The stakes are higher in LTx and HLTx however, as CMV pneumonitis develops in at least 75% of SNR of SPD lungs, and preventive tactics tried so far have been relatively ineffective. Some lung transplant programs are therefore trying seromatching[280].

2. CMV-negative blood products. Approximately 20% of SNR of SND hearts develop CMV infection, presumed to come mainly from blood transfusion[181,281]. About 10% of seropositive blood donors transmit infection[282,283] and positive IgM antibody is a marker of transmissibility[284]. The estimated risk, based on older studies, is 2.7% per unit of blood[285]; in more recent studies the numbers are much lower[286]. It is more cost-effective to screen for CMV antibody than to administer all blood products with high-efficiency leukocyte filters[6], though these work well and offer the additional advantage of possibly also removing Epstein–Barr virus (EBV) and human herpesvirus 6 (HHV-6) infected cells[287,288]. Exclusion of CMV IgM-positive (6%) blood products reduced the incidence of transmission-associated disease in one study[289].

3. Passive immunoprophylaxis. There is evidence from bone marrow (BMTx) and renal transplantation after that although various hyperimmune (CMVIG) and unselected CMV globulins do not prevent infection, they significantly modify the severity of CMV disease and prevent the complicating superinfections[281,290–295]. For example, in one multicenter trial using CMVIG in SNR of SPD kidneys, only 21% of CMVIG-treated patients developed CMV disease as opposed to 60% in controls; 4% of CMVIG recipients had CMV pneumonitis vs 17% of placebo controls. Fungal and parasitic infections were seen in 20% of controls but in none of the CMVIG group. Results in thoracic and liver transplant recipients are mixed[216,218,296–298].

The definition and delivery of protective titers of antibody are complicated by the heterogeneity of CMV, the antibody titer variation between and within IG preparations[299–301], the fact that antibody titers measured by ELISA do not correlate with *in vivo* neutralizing titers[300], and, finally, the fact that it is not known which antibody response is protective against CMV[196]. Prophylactic and therapeutic trials of human monoclonal CMV antibodies with high levels of neutralizing activity have been proposed[301,302], but neutralizing antibodies do not correlate with recovery from CMV disease, nor do they confer protection[303].

There is no evidence that CMVIG is superior to IVIG for this purpose, and various unselected globulin preparations have been shown to have equal neutralizing activity[300,301]. The advantages of CMVIG, now marketed as Cytogam (MedImmune, Inc., Gaithersburg, MD), are that there is lot-to-lot consistency in antibody titers and that, because titers are high, less infusion volume is required so that cost is also less. Nonetheless, the cost in one Snydman study was $4800 per patient, $29 800 per life saved for those at risk of primary disease[304], or $13 000 per case of CMV disease prevented following lung transplantation[297]. Also, at least one preparation of IVIG has been associated with the trans-

mission of hepatitis C virus as far back as 1992, and has been taken off the market[305].

It has also been shown that CsA and, more so, antilymphocyte rejection therapy attenuate the beneficial effects of CMVIG[295,297].

In HTx, the role of CMVIG prophylaxis is definitely not clear, but in HLTx/LTx the combination of this and chemoprophylaxis needs careful study.

4. Active immunization. Plotkin's work with a live human CMV (Towne strain) vaccine[306-309] has shown that the incidence of CMV infection and disease was not altered, but the frequency of severe disease was markedly decreased in vaccinated vs. placebo-treated SNR of SPD kidneys ($p<0.05$) in one study[306]. Both groups received similar regimens to prevent and treat rejection, and had the same average number of rejection episodes. One-year and 5-year actuarial survival rates for cadaver renal allografts were 73% and 62% for vaccinees vs 40% and 25% for placebo patients. A cell-mediated immune response demonstrated in vaccinees pretransplant disappeared with the introduction of immunosuppressive therapy after transplant. Vaccine strain did not reactivate. These observations were not corroborated by a Minnesota study using the same vaccine[310].

A subunit glycoprotein B vaccine has been developed; it produces neutralizing antibodies and the CMV-specific cytotoxic T cell proliferation, though this seems to wane in the face of immunosuppressive therapy[311].

It may prove difficult to develop a vaccine that accounts for the heterogeneity of CMV. There is also concern over the potential oncogenicity of CMV and over the possibility of chronic disease related to its latency.

5. Chemoprophylaxis

(a) *Acyclovir (ACV).* Balfour *et al.* reported a significant decrease in the incidence of CMV disease, particularly primary disease, in renal transplant patients taking ACV 800 mg q.i.d. for 3 months post-transplant[312]. Dummer *et al.* noted no benefit in HTx and three of 10 patients succumbed to CMV pneumonia after the drug was discontinued[210]. In our own experience with HTx randomized to this protocol, or to a no-prophylaxis group, there has been no difference in the incidence or severity of CMV disease regardless of donor– recipient serostatus.

(b) *Ganciclovir (GCV).* This highly active nucleoside analog is more toxic than ACV and therefore is less appropriate for prophylaxis. It has been shown to be effective in reducing the incidence and severity of CMV disease in BMTx, though neutropenia was a troublesome side-effect in this population[313,314]. Merigan *et al.* reported success in preventing disease in SPR but not primary disease in SNR using GCV for 1 month post-heart transplant[315]. Bailey *et al.* documented failure of prophylactic GCV in LTx[316]. Martin *et al.* showed that 2 weeks of GCV followed by high-dose ACV was superior to ACV alone in liver recipients[317]. An oral preparation is now available; it is not well absorbed and in half of the patients in whom we have used it to suppress disease, clinical and histologic evidence of CMV enteritis was still present after 1 month of therapy.

6. Pre-emptive therapy. Once-daily dosing with GCV when preclinical CMV infection is demonstrated by virological monitoring techniques has been shown to be effective in preventing CMV pneumonia in BMTx[318,319]. The rate of CMV disease seen after treatment of rejection with antilymphocyte agents in renal transplant was reduced in GCV recipients[6,320-322]. LTx may well benefit from this approach given the inadequacy of other preventive modalities. Each center should assess its own experience with CMV disease in HTx, as there is so much variation in immunosuppressive regimens. Rubin predicts that, in the future, patients at risk for primary disease will receive prophylaxis, and pre-emptive therapy will be given after treatment of rejection and for patients with presymptomatic viremia detected by periodic monitoring[6].

CMV and rejection

Simmons was the first to recognize a possible association between CMV infection and rejection in 1970[323] and since then a relationship has been demonstrated in LTx[324], liver transplantation[325,326], and renal transplantation[327,328], but the most compelling evidence comes from HTx, where CMV infection has been linked to both acute and chronic rejection[329-332].

Accelerated atherosclerosis is the hallmark of chronic rejection in HTx and severely limits long-term survival[329]. Koskinen *et al.* documented arteriolar endothelial cell accumulation and increased intimal thickness of intramyocardial vessels significantly more frequently in the 1-year endomyocardial biopsy (EMB) specimens of CMV-infected HTx, indicating a CMV-associated acceleration of allograft vasculopathy. At 2 years the endothelial cell response had subsided, but the intimal thickening had progressed. Thereafter there was no difference in histologic findings between CMV-infected and non-infected[333]. Other organisms have been associated with atherosclerosis[344] and human herpesviruses have been detected in atheromatous lesions in major arteries by DNA hybridization and immunoperoxidase staining for specific viral antigens[345].

In LTx, increased donor alloreactivity and obliterative bronchiolitis is seen in CMV-infected patients, but the process is thought to be multifactorial and is not statistically significantly CMV-associated[217,334-336].

The most detailed studies are in renal transplant patients, where a characteristic glomerulopathy distinct from classical rejection is associated with CMV infection. The lesion consists of increased numbers of $CD8^+$ cells and activated mononuclear phagocytes, and the glomeruli stain more intensely for MHC class-I antigen than do the tubules which are maximally involved in classic rejection. The lesion is less likely to respond to antirejection therapy, and GCV appears to decrease its incidence.

Proposed mechanisms could be direct and/or indirect[329,333]:

1. Direct

(a) CMV infection of vascular endothelial and smooth muscle cells[337] and the resultant inflammatory and immunologic responses render allograft blood vessels vulnerable to chronic injury[343].

(b) Virus may transform cells by incorporating into the cell genome and inducing local proliferation and changes in cellular metabolism of these cells, e.g. growth factors[338,339], thus causing endothelial and medial cell hyperplasia.

The hypothesis that latent CMV infection of the coronary arteries is associated with graft atherosclerosis has been refuted by Skowronski *et al.*, who found CMV by PCR in only two coronary artery specimens from one HTx with no post-mortem evidence of graft atherosclerosis and a history of CMV infection. They found CMV in stomach, kidney, and lung of only one other patient in the study, despite the presence of both clinical CMV infection and graft atherosclerosis[355].

2. *Indirect*

(a) There appears to be down-regulation of MHC class I antigens in CMV-infected cells, whereas in the surrounding uninfected cells there is up-regulation of these antigens and of the adhesion molecules ICAM-1 and LFA-3; together, they modulate a local immune attack on infected allograft endothelium.

(b) Immediate–early antigens have sequence homology and immunologic cross-reactivity with HLA-DR beta-chain[340], and CMV encodes a glycoprotein homologous with the heavy chain of MHC class I molecule[341,342]. This 'molecular mimicry' could provoke immune-mediated damage to cells displaying these antigens[341–348]. DR-matched transplants may result in more CMV disease[349,350] as studies in the mouse model suggest that the MHC locus equivalent may represent a receptor for CMV.

(c) CMV infection is associated with the activation of T cells and the release of interferons (especially gamma) which up-regulate the display of MHC class I and II antigens on the allograft[351–354]; this provides another opportunity for immune-mediated attack on the graft[348]. In the mouse model, CMV infection exacerbates graft vs host disease and increases the cytolytic T cell response to alloantigens[346,347].

CMV infection is immunosuppressive

Another by-product of the down-regulation of class I MHC antigens in CMV-infected cells is impairment of the presentation of viral antigen to the T lymphocyte so that cytotoxic T cell killing of virus-infected cells is also impeded. The function of both monocyte and natural killer (NK) cells is impaired in CMV infection, and the end-result is monocyte-induced suppression of lymphocyte function[347,356–360]. As with HIV infection, both CMV and EBV cause reversal of the CD-4 (T-helper cells) and CD-8 (T-cytotoxic/suppressor cells) ratio. Clinically this translates into 'superinfection' with other organisms, including opportunists such as *Aspergillus, Pneumocystis, Listeria*, and *Toxoplasma*[6,214,329,361,362].

CMV oncogenesis

Portions of the CMV genome are homologous to the myc oncogene[363]. Transformation in certain cell lines does occur, and malignancies have been produced in animal models[188]. In humans, CMV has been most closely linked to Kaposi's sarcoma[188,364].

Epstein–Barr virus (EBV)

EBV causes post-transplantation lymphoproliferative disorders (PTLD) or lymphoproliferative syndromes (LPS), ranging from active viremic disease to lymphomas[365–368] caused by oncogenic transformation of virus-infected cells. Primary infection in the SNR results from intimate contact with infected respiratory secretions or transfusion or transplantation from SPD, and is responsible for 50% of the EBV infection in pediatric transplant recipients and 80% in SNR overall. As >90% of adults are seropositive, reactivation, which is estimated to occur in 40% of SPR, is the most common precursor of disease in adult transplant populations, but reinfection of the SPR is also possible since there are two strains of EBV (1 and 2). Primary infection causes 70% of PTLD[368].

Stanford University reported a 7% incidence of lymphoma in HTx in the pre-CsA era[369]; this increased to 13% in the early years of CsA use[370], but familiarity with the drug and the ability to monitor blood levels resulted in a drop in this rate[371]. Ten years of experience with cyclosporin-based immunosuppression at the University of Pittsburgh yielded a rate of 4% for all thoracic transplant recipients surviving more than 30 days; 3.4% in HTx and 7.9% in HLTx/LTx. Peak occurrence was at 3–4 months post-transplant. Early-onset PTLD (<1 year) was characterized by presentation with disseminated disease in 23%, an 89% response to reduction in immunosuppression, and a 36% mortality rate from lymphoma. Late disease was associated with disseminated infection (86%), no response to reduced immunosuppression, and a mortality rate from lymphoma of 70%. Most infections were primary[375]. It has been stated that there is a substantial increase in PTLD in those receiving antilymphocyte therapy in addition to triple-drug immunosuppression[6,372–374,401]. This has not been our experience, nor that of others[375–378].

Viral pathogenesis

The virus binds to the complement receptor C3d (the CD21 molecule) on B lymphocytes, developing T lymphocytes, non-T, non-B lymphocytes, and epithelial cells[379,380]. Productive infection takes place in the epithelial cells of the upper respiratory tract. Non-productive, latent infection in B lymphocytes predominates in normal hosts[371,372,382] and is characterized by the persistence, in circular episomal form, of EBV genomes. The best marker of latency is the detection of small mRNA encoded by the EBER-1 gene[387].

Infected cells undergo polyclonal or monoclonal transformation into lymphoblastoid cell lines (LCL) in which viral genomes are replaced in constant balance with host cell DNA[371,372,381–384]. EBV-1 appears to be a more potent transformer than EBV-2 and has been associated more strongly with lymphoproliferative disease[384–386]. These LCL produce their own B cell growth factors, including interleukin-6 and lactic acid[372], and grow indefinitely (immortalization); they not only perpetually produce viral antigens but also secrete immunoglobulins and may facilitate augmented transcription of cellular oncogenes. Other processes associated with the elaboration of cytokines and growth factors, such as rejection and CMV infection, may enhance the lymphoproliferative response[388]. Recent evidence has emerged that a protein product of the BCRF-1 region of the EBV genome has extensive homology with interleukin-10, and these molecules appear to inhibit IFN-γ secretion, which suppresses T cell proliferation and may interfere with the elimination of EBV-infected cells[6,372,389]. Recent evidence suggests that elevated levels of

interleukin-6 play an important role in the pathogenesis of PTLD[372].

Viral expression in transformed cells is continually checked by MHC-restricted, EBV-specific, cytotoxic T cells, which suppress the outgrowth of LCL and virus-associated immunoglobulin synthesis. CsA interferes with interleukin 1 and 2 production, which precludes this response to EBV[390]. OKT3 abrogates cytotoxic T cell effector functions by modulating the T3–Ti complex on all mature T lymphocytes until the receptor complex reappears[391]. In addition, maintenance immunosuppression increases the incidence of viral shedding to 30%, and the addition of anti-lymphocyte therapy raises this to 80%. Higher rates of viral shedding are associated with primary disease and with the development of PTLD[384,392].

EBV has also been linked to T cell lymphomas[393,394].

EBV is immunosuppressing[6] and may contribute to chronic allograft dysfunction[395]. Co-infection with CMV may cause additive effects[388,404].

Clinical syndromes

Hanto *et al.*[396] provide a clinical and pathologic approach to classification of EBV LPS (Table 5), but the clinical presentation can be protean and non-specific including fever of unknown origin, a mononucleosis-like illness sometimes with sepsis syndrome, enteritis/hepatitis, central nervous system dysfunction and focal disease, and focal tumors in various extranodal locations[365,373].

Group I patients (mean age 23 years)[371] present within the first year post-transplant, may have acyclovir-responsive disease and may not require reduction in immunosuppression. *Group II* patients are similar to group I but pathologically show evidence of malignant transformation. The B cell proliferation is still considered to be acyclovir-responsive, but concomitant reduction in immunosuppression is needed, and serial biopsies are indicated to monitor for monoclonal transformation. Patients in this group often succumb to aggressive disseminated disease with lactic acidosis and intravascular coagulopathy. *Group III* patients tend to be older (mean age 48 years) and present longer after transplantation (average 6 years) with acyclovir-insensitive solid tumors, presumably composed of latently infected B cells of monoclonal origin. The prognosis is poor, with a mortality rate of >80%[396].

Patients in all groups may be asymptomatic at presentation. Ho *et al.* found no difference in the clinical course according to age, time of onset, or clonality[367]; however, their patients fit well into Hanto's clinicopathologic groups[368].

Diagnosis

Serology

The normal host generates high levels of IgG and IgM antibody to viral capsid antigen (VCA-IgG, VCA-IgM) during acute infection along with transient levels of antibody to the diffuse component of early antigen (EA-d). Weeks to months later, antibody to EBV nuclear antigen (EBNA) develops, and is a marker for EBV immortalization. In some patients with protracted illness, EA-r (restricted component) antibody may emerge when EA-d lapses. The only reliable marker of infection in transplant recipients is seroconversion or a four-fold rise in VCA IgG. Some patients may generate an IgM response and some may also develop persistent EA-r antibody. None manifests heterophile antibodies[203].

Other studies

Lymphoproliferative lesions should be examined histologically and surface markers of lymphoid cells studied to determine clonality. EBNA staining of touch imprints and frozen sections using appropriate controls and DNA hybridization studies on fresh tissue frozen at –70°C to detect EBV genomes, help to establish EBV causality[366]. Detection of EBER-1 RNA in tissue is confirmatory[372,384,387]. Karyotypic analysis also helps to detect the presence of cytogenetic abnormalities such as oncogenic translocations.

Treatment

Reducing immunosuppression

In 1984 Starzl *et al.* described 17 recipients of various organs who subsequently developed LPS while on regimens containing CsA. In patients in whom LPS was diagnosed pre-mortem, immunosuppression was decreased in all but one. Eight patients had combinations of surgical excision, irradiation, chemotherapy, and acyclovir[397]. Overall, tumor-free survival occurred in 11, six of whom had reduction of immunosuppression as the only treatment. At the University of Pittsburgh the usual approach to patients with EBV-LPS is to decrease CsA to a dose that provides serum levels of 50–100 ng/ml by high-density liquid chromatography (HDLC). If an organ is expendable, immunosuppression may be stopped altogether. Acyclovir is reserved for unresponsive lesions.

Acyclovir

The University of Minnesota has been the main proponent of acyclovir therapy (500 mg/m^2 every 8 hours) for patients with group I or group II LPS[390,398]. Other investigators have reported no benefit[399,400]. However, although acyclovir inhibits EBV replication, reduces the number of VCA-positive cells, decreases the number of viral genomes per cell *in vitro*, and suppresses oral EBV shedding *in vivo*, the effects persist only during the course of therapy. Return of viral replication and clinical LPS occurs when acyclovir is stopped. Of particular concern is the possibility that acyclovir therapy may facilitate the transition from polyclonal to monoclonal B cell lymphoma; such tumors contain latently infected LCL which are acyclovir-insensitive because there is no active production of DNA polymerase for acyclovir to inhibit[397].

Ganciclovir

Ganciclovir is more potent than acyclovir *in vitro* against EBV. Pirsch *et al.*[400] reported two patients with severe polyclonal LPS, unresponsive to reduction of immunosuppression plus acyclovir, who were treated with ganciclovir at a dose of 3 mg/kg every 12 hours. One patient survived and is free of evidence of LPS

6 months after transplant nephrectomy. More data are clearly needed.

Monoclonal antibodies

Blanche *et al.* reported success with the treatment of two children with EBV polyclonal LPS following HLA-mismatched bone marrow transplantation with T cell depletion. Two antibodies were used: (a) CD24, which binds B cells at all steps of differentiation; and (b) CD21, an anti-C3d (anti-CR2) receptor antibody. Both patients had failed cessation of CsA, but had complete resolution of abnormalities over 2–3 weeks after antibody infusions, and remain well after 1 year of follow-up[401]. The authors reported further experience with 26 BMTx and solid-organ transplant recipients; the treatment was ineffective in patients with monoclonal B cell proliferation, but was successful in all patients with oligiclonal B cell proliferation who did not have CNS involvement[402].

Other modalities

Success has been reported in treating polyclonal and monoclonal PTLD with IFN-α and IVIG[403]. Monoclonal lymphomas do not respond well, though surgery, chemotherapy and/or radiation are indicated[373,375].

Prevention

The Mayo Clinic has established that EBV SNR in a study of 381 non-renal solid-organ transplant recipients have a risk of developing PTLD that is 24 times that of SPR, and that this risk is further amplified 4–6-fold by concurrent CMV mismatch and/or antilymphocyte therapy. Together, all three risk factors acted synergistically to increase the incidence rate of fatal or CNS PTLD by a factor of 654 over that of patients with none of these risk factors, regardless of the aggressive chemoprophylactic and immunoprophylactic regimens they used. The EBV SNR is being rejected for lung transplantation at their institution, and they have suggested that dual EBV/CMV seronegativity be considered a potential contraindication to transplant[404].

Antiviral chemoprophylaxis or pre-emptive therapy with ACV or GCV may be of some use to suppress low levels of viral replication such as exist in early reactivation during time of more intense immunosuppression. CMVIG also contains high levels of anti-EBV antibodies (Specialty Laboratories, Santa Monica, California) and needs to be looked at as an immunoprophylactic approach, either alone or in combination with chemoprophylaxis.

Given that the transplanted organ is often the focus of viral replication and disease, one approach would be to monitor the number of EBER-1 RNA-positive cells in routine post-transplant biopsies and begin immunosuppression reduction and/or pre-emptive therapy before malignant transformation occurs[387].

Herpes simplex virus (HSV)

HSV1 and 2 infections are not problematic in HTx and are easily treated with oral ACV. There have, however, been several reports of HSV pneumonitis, with some fatal outcomes, in early publications on HLTx/LTx[87,405]. In a study of 51 HLTx, Smyth *et al.*[406] reported six episodes of pneumonitis in five of nine HLTx with clinical HSV out of 23 SPR. Four patients were co-infected with CMV. No disease developed in SNR. Prophylactic ACV and GCV, as given to prevent other herpesvirus infections, are highly effective in suppressing HSV activity[407], and have been recommended for the HSV SPR in lung transplantation[406]. One case report of primary HSV infection in two recipients of kidneys from the same donor suggests that donor-transmitted disease can also rarely occur[408].

Varicella–zoster virus (VZV)

By the age of 15 years >85% of the population has had varicella, so that this infection is mainly a problem of pediatric transplantation, especially BMTx[409]. A live, attenuated varicella vaccine is now available[410], and SNR should receive it prior to transplantation. Vaccinees shed virus for approximately 30 days, which poses a potential risk for transplantation occurring in that time period; as yet the risk is unknown, but pre-emptive ACV, ± varicella–zoster immune globulin (VZIG), should be effective. The problem of exposure of a susceptible (SNR) immunosuppressed patient to a recently vaccinated household contact has not been addressed, but options include forgoing varicella vaccination, avoiding contact for 30 days, or giving chemoprophylaxis with or without VZIG. Post-exposure prophylaxis with VZIG is indicated for the SNR with significant exposure to a diagnosed case of varicella and should be given within 72 hours[411]. Assuming that pretransplant immune status has been established by ELISA, fluorescent antibody to membrane antigen (FAMA), or immune adherence hemagglutination[412], SPR do not have to avoid contact with either varicella or zoster.

Untreated clinical disease in this population is often complicated by hemorrhagic pneumonia, hepatitis, encephalitis, and death[413], and there is some evidence for allograft injury similar to that caused by CMV[414]. Intravenous ACV, 10 mg/kg every 8 hours, or 500 mg/m^2 for children, should be started within 24 hours of onset[415].

Reactivation of varicella in the SPR occurs late after transplantation and causes zoster (shingles). While immunosuppressed patients can have unilateral pain and no skin findings, the usual presentation with dermatomal lesions with or without pain is most common. The process can take significantly longer to resolve, even with treatment. Neither the duration of acute pain nor the occurrence of post-herpetic neuralgia is affected by ACV, but an abbreviated evolution of cutaneous lesions results from starting ACV (in a dose of 800 mg four times daily for children and five times for adults) within 4 days of onset, and treating for 10–14 days. Patients should therefore be educated to report radicular pain and/or lesions immediately. Treatment is particularly indicated for ophthalmic, sacral, and multidermatomal zoster. Oral ACV does not achieve blood levels that correlate with inhibition of viral replication *in vitro* and immunosuppressed patients with VZV infections should be watched closely for signs of visceral dissemination that would dictate prompt initiation of intravenous ACV at a dose of 10 mg/kg every 8 hours[415–420].

Human herpesvirus-6 (HHV-6)

HHV-6, tropic for B lymphocytes, normal CD4+ T lymphocytes, and T-cell-derived lines[421], has no defined role in transplant patients, though both primary and reactivation infections have been documented in recipients by serology, positive blood cultures, and evidence of viral replication in circulating mononuclear cells[422]. Seroprevalence nears 100% by age 2–3 years[423]. There is significant sequence homology with CMV[424], which causes serologic cross-reactivity, and co-infection is common. HHV-6 has been linked to non-specific febrile illnesses after transplant[425], to exanthem subitum (roseola infantum) in children[426], and to lymphoproliferative processes[427,428].

Recently HHV-7 has been described; its clinical significance remains to be elucidated.

Hepatitis viruses

Hepatitis viruses A–E have now been described. Only hepatitis B (HBV) and hepatitis C (HCV) have an impact on thoracic transplantation; delta virus (HDV) replication requires co-infection with HBV, and results in fulminant hepatitis in countries such as Italy, where seroprevalence is high[429]. Primary infection in transplant recipients results from transfusion and transplantation from SPD to SNR, and through intimate contact with infected secretions, in which case HBV is a much more efficient infective agent than HCV. Immunosuppression of SPR causes active viral replication; reinfection with donor strains also occurs. All infected patients have the potential to proceed to chronic hepatitis, liver failure, and hepatocellular carcinoma[430–432].

The issues in transplantation are whether or not to transplant a SPR, and whether to use a SPD for either a SNR or a SPR. Protection of the SNR from exposure to contaminated blood products and sexual transmission is the same as for the population at large. Screening of blood donors and exclusive use of volunteered donations has reduced the incidence of post-transfusion HBV infection by 80%, so that >90% of transfusion-associated hepatitis is now caused by HCV or as yet unidentified agents[429]. At the present time the incidence of HBV infection after transfusion or transplant is approximately 0.002% per exposure[433].

HBV

HBV is a DNA virus which replicates by reverse transcription, a mechanism which is unusual for a DNA virus and is unique to the hepadnaviruses and a plant virus[429]. Hepatitis B infection results in persistent viral replication in 0.1–20% of infected patients[434], depending on geographic location and age; the average carrier rate in the USA is 10%. Persistent infection is characterized by detectable hepatitis B surface antigen (HBsAg), HBe antigen (25–50%), HBcAg (core antigen), HBV DNA, and/or DNA polymerase activity. HBsAg-negative persistent HBV infection attributable to levels of HBsAg lower than those measurable by the radioimmunoassay (RIA) test has been blamed for rare instances of transmission of HBV via seronegative blood products[435–437]. Although hepatitis B surface antibody (anti-HBs) confers protection against reinfection, renewed HBsAg positivity has been documented, both spontaneously and after immunosuppression in HBsAb+ patients[438,439].

Primary infection after transplantation causes fulminant hepatitis. It is not common practice to immunize thoracic transplant candidates with HBV vaccine, which would take 6 months to complete, or 2 months to achieve 90% immunogenicity. There are therefore no data on the efficacy of this plus hepatitis B immune globulin (HBIG) in preventing or attenuating hepatitis B in this situation; therefore, HBsAg positivity renders the donor unacceptable for the SNR.

Since a determinant of the HBsAg complex gives protective immunity against virus of any subtype[429], it seems feasible that the HBsAb+ recipient could accept an organ from a HBsAg+ donor, especially a HBsAg and HBV DNA negative one.

Initially, the HBsAg+ transplant recipient may actually seem to improve in terms of liver function tests and biopsy appearance, but after 2 years, liver damage usually proceeds relentlessly and/or hepatocellular carcinoma develops[430,440–443]. Corticosteroids particularly stimulate viral replication. The prognosis in renal transplant patients has improved with the advent of CsA-based regimens using reduced doses of prednisone but, prior to that, 38% had chronic progressive hepatitis, 38% had chronic active hepatitis, 42% had cirrhosis, and 54% died of liver failure in one study[440]. The subset of patients with evidence of liver disease, or with HBeAg and/or HBV DNA in the blood, has been shown to do much worse, so that HBsAg positivity itself may only be a relative contraindication to transplantation, and decisions as to candidacy should be individualized[6,440,444,445].

Treatment with IFN-α has not been as successful in transplant recipients as it has been in nonimmunosuppressed hosts[446]. Prolonged immunoprophylaxis with HBIG has been shown to be of some benefit in liver transplant patients, but is very costly[447].

HCV

HCV is an RNA virus of the flavivirus family that causes 80% of the progressive liver failure in both liver and non-liver transplant recipients[430] and has been linked to hepatocellular carcinoma as well[447,448,474]. The virus is acquired by parenteral contact with blood, by infusion of certain IVIG products[305], by transplantation[449–455], and inefficiently by sexual intercourse[432]. As with the herpesviruses, virus-specific cytotoxic CD8 T cells are responsible for lysing infected cells, a mechanism that is abrogated by immunosuppressive therapy, permitting persistent infection to go unchecked[461].

In one study, transmission by transplantation was infrequent and the resulting infection relatively inconsequential[451]. Diethelm *et al.*[452] and Vincenti *et al.*[451] have challenged the premise of Pereira *et al.*[449] that HCV is transmitted by this route. In Pereira's studies[449] of the outcome of 29 recipients of organs from 12 confirmed SPD (1.8% seroprevalence) of kidneys, hearts, and livers, 75% of SPD-transmitted HCV and liver disease was documented in 14 recipients (48%) by 6 months (mean 3.8± 1.5 months), with no difference in rates according to organ transplanted. Chronic liver disease developed in 12 (86%) and subfulminant liver failure in two (14%). Liver biopsies showed chronic active hepatitis in six and cirrhosis in two. Liver failure eventually occurred in four (29%) and contributed to death in two (14%). In two other studies of renal transplant patients[430,439], clinical liver disease was unusual in the first 2 years after transplant, and the main effect of the infection during this time period was to

add to overall immunosuppression. After 2 years, infected patients went on to progressive liver disease. HCV has also been linked to aplastic anemia in this population[456–459].

Viremia develops within a few days of exposure. Symptoms occur at 6–7 weeks and correlate with elevated transaminases. Antibodies to HCV can be detected by second-generation ELISA ~2 weeks after that[460–464]. HCV RNA can be detected earlier by PCR, but a simpler and cheaper antigenemia test is needed. Antibody tests have gone through an evolutionary process aimed at improving the sensitivity and specificity. Of patients with well-documented chronic hepatitis C, 75–90%, and almost 100%, have positive first- and second-generation ELISA tests (EIA-1 and EIA-2), respectively[461]. A recently developed multiantigen enzyme immunoassay is the most sensitive assay to date, and will detect antibodies in immunosuppressed patients for whom conventional assays have been suboptimal[462].

The specificity of current tests is still, however, problematic, with false-positives seen in regular blood donors and in patients with hyperglobulinemia. The positive predictive value for the viral carrier state is still only ~0.5 among blood donors screened with a third-generation ELISA[463]. Confirmatory recombinant immunoblot assays (RIBA), of which the best one available is RIBA-3, are therefore important, especially in low-seroprevalence populations, though they are subject to misinterpretation. Indeterminate results, as indicated by the manufacturer, may in fact be positive when cross-checked with PCR for HCV DNA. With the EIA-2 or EIA-3 and the RIBA-3, seroconversion can be detected in the majority of patients within 4 weeks of onset of clinical disease. A core IgM antibody test is being evaluated[464].

Treatment with IFN-α has resulted in a fall in transaminase and HCV RNA levels in 40–50% of immunocompetent patients[465,466], but this success has not been duplicated in transplant recipients[203,430]. Ribavirin may decrease viral replication, but the response is not sustained. Recent data suggest synergistic benefit from a combination of the two modalities[467–469].

There is no known effective prophylaxis against HCV. Antibody to HCV is not protective against reinfection with either homologous or heterologous strains[470,471]. This, plus the fact that HCV displays antigenic drift, raises serious concerns about the possibility of developing a vaccine[464]. The present recommendation of the US Public Health Service is to limit the use of organs from HCV-positive donors to 'life-saving' transplant procedures, which include thoracic transplants[432]. Others[6,449,472] advocate not using them at all, even for SPR, because reinfection of the SPR with a donor strain could occur. The experience of many centers does not seem to reflect the findings of Pereira *et al.*, and, given the shortage of donors, they continue to transplant SPD organs into SPR[451–455]. Reactivated or up-regulated infection in the SPR appears to cause mild disease, but the long-term sequelae are not yet known.

Policies regarding SPD and SPR of thoracic organs are inconsistent across a wide range of centers responding to a survey published by Milfred *et al.*[472], though a follow-up survey 2 years later showed a marked increase in the number of centers which would not transplant SPR and would use a SPD organ only for SPR or status I candidates[473]. The presence of HCV RNA in the serum of the donor is the best predictor of transmission, and there is a much lower prevalence of HCV infection and clinical liver disease in the recipient of an EIA2-positive/HCV RNA-negative donor liver[474], suggesting that such organs can be used for transplantation especially in the case of urgent need. Until such time as a rapid test for HCV RNA becomes available, it seems reasonable to forgo transplanting SPD organs into SNR, but to continue to transplant SPD organs into SPR, with ongoing study, preferably on a multicenter basis, to further characterize the outcome of all types of HCV infections.

Retroviruses

HIV

Since routine serological screening did not became available until 1985, there has been much experience with primary infection as acquired by SPD of blood and/or organs and by high-risk behaviors in the community, and also with the course of disease in SPR[476–483]. Inadvertent transplantation of infected organs has occurred, presumably during the 6–8-week 'window period' when newly infected persons are viremic and have not yet produced detectable antibodies[483–486]. In some individuals, especially if immunosuppressed, this may take as long as 1 year[480,484,487,488]. False-negative results are also possible after massive blood transfusion, which can dilute antibody, as reported by Bowen *et al.*[478].

In a case report and review of the literature, Erice *et al.* noted that 28% of 88 HIV-infected transplant recipients described between 1985 and 1990 developed acquired immune deficiency syndrome (AIDS) at a mean of 27.5 months, and 80% of these died within 23.9 months of transplantation. Another 10% had, at the time of the report, developed HIV disease[482]. Seroconversion occurred within 2 months of transplant (range 5 to >135 days) and was associated with a non-specific febrile mononucleosis-like illness in some. SPR developed AIDS within 17 months as opposed to 32 months after primary infection in SNR. AIDS-defining conditions were similar to those in non-transplant patients, though a lower incidence of *Pneumocystis carinii* pneumonitis was probably attributable to routine post-transplant TMP–SMZ prophylaxis. Graft survival was not affected and, in fact, due to the immunosuppressive effects of HIV infection, many patients could be maintained on low-dose immunosuppressive regimens. Overall, the clinical course of HIV infection in transplant recipients does not differ much from other patients with HIV, except for earlier onset of AIDS.

Prompt treatment with azidothymidine (AZT) did not prevent infection of the SNR[478]. Current recommendations are to start AZT at lower doses (500–600 mg/day) when $CD4^+$ lymphocyte counts drop below 500/mm^3. Concomitant reduction in dosages of immunosuppressive medication will help to avoid bone marrow toxicity[482]. Other aspects of management are the same as for non-transplant patients infected with HIV.

Since serological testing can still miss infected donors, it is important to take a good history for any risk factors for HIV infection, which, if present, are a contraindication to donation.

Other retroviruses

Human lymphotropic virus 1 and 2 (HTLV-1, HTLV-2) and HIV-2 have similar modes of transmission to HIV-1[489]. HTLV-1 is endemic in Japan, the Caribbean, Africa, the southeastern USA, and South America[490], and causes T cell lymphoma/leukemia and

spastic paraparesis in a small percentage of infected individuals[490–495]. A case of rapidly progressive myelopathy resulting from transmission of HTLV-I from a blood transfusion during cardiac transplantation has been reported[496]. HTLV-2 has been linked to hairy-cell leukemia[497] and HIV-2 causes AIDS[498]. Screening for these viruses needs to be part of the work-up of donors.

Papovaviruses

These include the papillomaviruses, which can cause disfiguring warts in transplant recipients, and the polyomaviruses BKV and JC virus which infect most people during childhood and rarely result in disease in normal hosts, but cause progressive multifocal leukoencephalopathy in immunosuppressed patients[500], many of whom shed these viruses in secretions and urine. BKV has been associated with urethral stricture in renal transplant patients. Both may be oncogenic[499].

Community-acquired respiratory viruses

Adenovirus, RSV, parainfluenzavirus (PIV), and *influenza viruses A and B* all seem to cause more severe diffuse interstitial pneumonitis, associated with complications, in the transplant recipient, though this has not been well studied[6]. Adenoviruses also cause hepatitis, enteritis, nephritis, encephalitis, and hemorrhagic cystitis mainly in BMTx recipients[501–504]. RSV should be especially considered in pediatric recipients and should be treated with ribavirin[505,506]. Pneumonia due to parvovirus B19 has recently been reported in a HTx patient[507]. Yearly influenza vaccination is indicated in this population. Nosocomial spread may play a prominent role in transmission.

PARASITIC INFECTION

Toxoplasma gondii

The life cycle of the protozoan *Toxoplasma gondii* is completed in the cat, the only animal known to excrete the infectious oocyst[508], which is ingested by a variety of intermediate hosts, including domestic animals, whose meat and organs contain encysted parasites[509]. Humans acquire toxoplasma by ingesting soil contaminated with oocysts while farming, gardening, or changing cat litter, or by eating undercooked meat or possibly by drinking unpasteurized milk[510].

Less commonly, the organism can be transmitted via blood transfusion. *Toxoplasma gondii* remains viable in banked blood for 50 days at 5°C and can be isolated from buffy coat leukocytes over long periods of time in asymptomatic patients[511]. Because cysts persist in muscle, heart, brain, leukocytes, and lymph nodes for years, the organism can also be transmitted by transplantation[512]. HTx recipients have a higher incidence of toxoplasmosis (4–12%) than do other transplant recipients[513–517].

Clinical syndromes

Prevalence of antibody to *Toxoplasma gondii* varies with age and geographical location. In the usual thoracic organ donor population in the USA, approximately 15–30% will have had toxoplasmic infection (data adapted from Feldman[518]), and the transplantation of a heart from a SPD into a SNR has been estimated to occur 10% of the time[519].

Clinical disease is most prominent in HTx recipients and results from primary infection; it is rarely due to reactivation[520]. Symptoms can coincide with seroconversion at 4–6 weeks post-transplant or can follow as much as 10 months later, and represent CNS involvement with encephalitis, meningoencephalitis, or mass lesions accompanied by cerebrospinal fluid mononuclear pleocytosis with high protein concentration, or myocarditis simulating graft rejection. Pneumonitis can occur concomitantly[519–521].

Diagnosis

Strict pathologic and serologic criteria for the diagnosis of active infection and disease have been advocated by Remington and colleagues[520,521] and, as applied to thoracic transplantation, include:

(1) Compatible clinical manifestations.
(2) Histologic demonstration of toxoplasma tachyzoites in body fluid tissue or in association with numerous cysts in a localized area of tissue.
(3) Isolation of *Toxoplasma gondii* from blood or body fluids.
(4) Conversion of toxoplasma IgG antibody from negative to positive.
(5) High toxoplasma IgM titers with a single high-titer IgG antibody test.

Tests in common usage include the indirect fluorescent antibody test (IFA) which measures IgG antibodies, and the IgM immunofluorescent antibody test (IgM-IFA). Significant (≥4-fold) rises in antibody titer can occur without symptoms in transplant patients, and many will not generate an IgM response. Empiric endomyocardial biopsy may yield the diagnosis even in the absence of clinical myocarditis, but the pathologist must be alerted to look for toxoplasmosis[522].

Treatment

Pyrimethamine and sulfadiazine or triple-sulfa constitute successful therapy in most cases. Complete blood and platelet counts must be followed closely; hematologic side-effects can be countered with oral folinic acid (leukovorin). The regimen is given for 4–6 weeks after the resolution of symptoms, often resulting in therapy for 6 months or more. Approximately 80% of patients improve[519,521].

Prevention

In low-prevalence areas it is cost-effective to test frozen recipient serum only if donor serology is positive, and then to give prophylaxis to mismatches. Patients already on prophylaxis with TMP-SMZ are very likely protected against toxoplasmosis[515,523], but pyrimethamine prophylaxis (25 mg/day for 6 weeks) is what is recommended[6,516,517]. Monitoring toxoplasma IgG and IgM antibody tests with pre-emptive pyrimethamine and sulfa treatment given to seroconverters for 6 weeks is another approach. SNR of

SND organs should be taught to avoid activities that would expose them to *Toxoplasma*: (a) use gloves when handling raw meat, (b) eat well-cooked meat, (c) delegate litter-box duty to another, and (d) avoid cat feces while gardening or in children's sandboxes.

Pneumocystis carinii

Whether a fungus or a protozoan, this organism causes pneumonitis (PCP) and, rarely, disseminated infection in hosts with impaired cell-mediated immunity. Before the implementation of routine prophylaxis with TMP-SMZ, attack rates were 5–41% for HTx recipients[84,524–526] and 16–43% for HLTx/LTx recipients[85,527,528]. Hughes *et al.* definitively established low-dose TMP-SMZ as effective for the treatment and prevention of PCP[529–531]. The recommended duration of prophylaxis varies from at least 4–6 months post-transplant[6,532]. In those patients who require more prolonged or repeated immunosuppression to treat rejection, it should be continued for 12–18 months[532]. Dose recommendations vary widely[527,532,536]. Alternatives include dapsone and aerosolized pentamidine[536].

PCP without HIV infection has been particularly related to corticosteroid therapy[533,534]. In one retrospective study of 142 cases of PCP[534], the median time on steroids was 2 months (at a maximum dose of 40 mg/dl) and the risk appeared to be dose-related[534,535].

PCP in transplant patients presents more acutely than it does in AIDS patients, with fever, non-productive cough, dyspnea, interstitial infiltrates on chest radiograph, and hypoxemia out of proportion to the auscultatory or radiological findings. Diagnosis is confirmed with induced sputum[537] or bronchoalveolar lavage (BAL); biopsy should be done if the BAL is negative. Sensitivity has been improved with the use of monoclonal antibodies[538]. Co-infection with CMV is not uncommon and may facilitate PCP. Treatment with 15–20 mg/kg of the TMP component can lead to CsA interaction and renal failure[6].

Strongyloides stercoralis

Residence in or extended travel to endemic parts of the world (Latin America, Mexico, southern USA, and all countries outside of northwestern Europe) may expose patients to this nematode which can remain in the gastrointestinal tract for years without symptoms. With immunosuppression, fever, diarrhea, and disseminated disease can occur. Transplant candidates with a significant travel history or geographic origin should be screened with three stools for ova and parasites or empirically given a 2-day course of thiabendazole[539,540].

Trypanosoma cruzi

This protozoan, transmitted by the bite of the reduvid bug, causes American trypanosomiasis, or Chagas' disease, which is a lifelong infection. It is endemic in all Latin American countries, including Mexico, and it is estimated that 16–18 million persons are chronically infected[541]. Transmission by transfusion is a major problem in endemic areas[542,543], with a risk of 13–23% per unit of blood transfused in Brazil[544]. Acquisition through kidney transplantation has also been reported[545–547]. Seroprevalence in Santa Cruz, Bolivia, approaches 50%[548].

In the USA vector transmission occurs rarely, despite the fact that many insects and wild mammals of the south and southwest are infected. With the continued influx of Mexican and Central American immigrants, acquisition by blood transfusion[549] and transplantation is likely to be more common. Transfusion-related trypanosomiasis has occurred in two Americans and one Canadian[550–552]. The seroprevalence in a group of Salvadoran and Nicaraguan immigrants living in the Washington, DC area was 5%, and extrapolation of these data suggests that there are 50 000–100 000 persons with chronic infection in the USA today[544].

Cardiac transplantation for end-stage Chagas' cardiomyopathy has been reported in 31 Brazilian[553,554] and six American patients[555], as of the time of publication of Kirchhoff's excellent review article[544], and results are summarized there. Outcome data on 12 of these patients indicate a high incidence of reactivation of disease (8/12) which responds to benznidazole or nifurtimox, but relapses off treatment. Lifelong suppressive therapy after transplantation may be effective. The toxicity of this is not known, except that both drugs cause lymphomas in rabbits[556]. Kirchhoff advocates avoiding transplantation for end-stage Chagas' cardiomyopathy.

Available tests have lacked sensitivity and specificity, but ELISA tests seem to be best[544,548]. Presence of IgM antibody may correlate with the infectivity of a unit of blood. With the low seroprevalence in the USA at this time there is no prospect for routine screening. However, Kirchhoff and co-workers found that eliminating high-risk blood donors in the Los Angeles area by questionnaire reduced the blood supply by only 2.1%[544] and correlated well with seropositivity. The seroprevalence of donors at that blood bank is 0.1%.

Organs from donors deemed to be at risk should not be transplanted unless shown to be seronegative by a reliable test.

Cryptosporidium

In immunocompetent hosts, this protozoan causes a self-limited diarrheal illness, but in the immunosuppressed patient, chronic disease with voluminous, watery diarrhea can result in progressive wasting and death[557,558]. The organism may be acquired through contact with infected domestic animals and contaminated water. Person-to-person spread has been described in day-care centers[559] and within families[560], and by sexual transmission[561].

Although transplant recipients would seem to be at risk for severe disease, there are no reports in HTx or HLTx/LTx patients. Collier *et al.*[560] reported on one BMTx recipient who did well with spiramycin treatment. High-dose azithromycin is an alternative, but may interact with CsA. Transplant recipients should be instructed in infection control measures to prevent acquisition of enteric pathogens.

COMMENT

Great strides have been made in transplantation. Continued improvement in the outcomes of the patients we transplant will

Table 6 Pretransplant work-up: recipient

Infection risk	*Specifics*	*Action*
Tuberculosis	Immigration from a high-prevalence country. Ethnic origin associated with high-prevalence, high-risk lifestyles/behaviors. Exposure to persons TB. Hx of a positive Mantoux	Place Mantoux skin test with controls and read at 48–72 h. Treat positives according to CDC guidelines[60]
Herpes simplex 1, 2	Hx of orolabial or genital lesions	Give prophylaxis only for frequent recurrences
Varicella–zoster	Hx of chickenpox or shingles	Varicella immune status by reliable test
Epstein–Barr virus	Hx is often negative though >90% of adults are VCA-IgG positive	Serology is not helpful unless donor : recipient matching or prophylaxis is planned. Testing stored sera may aid diagnosis later
Cytomegalovirus	Hx of blood transfusions	Positive CMV IgG may dictate post-transplant prophylaxis
Hepatitis	Hx of blood transfusions, high-risk behavior	HBV and HCV serology indicated in all candidates. Positive HBV serology is a contraindication to transplant. HCV ELISA+/RNA – organ may be safe to transplant, especially in a HCV+ recipient
Toxoplasmosis	Cats, undercooked meat ingestion	Do toxoplasma IgG on stored sera if donor is positive. Treat SNR with pyrimethamine or TMP/SMZ × 6 weeks.
Geographic exposure Hx	SW USA (coccidioides) Third world travel/residence	Work up any diarrheal or other illness that seems travel related. Stools × 3 for *Strongyloides* or empiric thiabendazole. HIV-2, HTLV-1 antibody
	Mexican, Central or South American	*Trypanosoma cruzi* serology × 2 transplant contraindicated for SPR
Hx of recurrent infection	UTI, upper and lower respiratory infection dermatitis, dental status	Assess for potentially corrective measures
Pets/hobbies/habits	Who changes the cat litter? Bird exposure? Spelunking? Aquarium cleaning? Eating habits: unpasteurized dairy products, undercooked meat?	Lifestyle changes, avoidance of high-risk exposure, barrier precautions
High-risk behaviors for HIV: male to male sexual intercourse, IV drug abuse, birth to an HIV+ mother, intercourse with an HIV+, blood transfusion before 1985, never tested	Candidate could be in the seronegative 'window' period Risk factors for other retroviruses include geographic exposure and drug abuse	HIV antibody indicated for all candidates; transplantation not advisable for SPR. Consider exclusion of the high risk candidate until repeat test at 3–6 is negative HIV–2, HTLV–1 and 2 antibody
Immunization Hx	All candidates: Tetanus–diphtheria booster in past 10 years. Pneumococcal 23 serotype vaccine Influenza vaccine this year	No? Update No? give Give if seen between October and February
	Children/young adults: Childhood immunizations MMR booster Varicella non-immune	Complete DPT, OPV, MMR, HBV No? Give ? Give varicella vaccine

Store a sample of serum to help with diagnosis and/or studies later.

depend on further innovations that decrease net immunosuppression[6] and prevent infection. An approach to screening donors (Table 5) and recipients (Table 6) and preventing infection and/or disease (Tables 3 and 4) is summarized above. Collaborative clinical research is desperately needed to arrive at standard approaches to these issues, given the sample sizes that exist in any one center.

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33
Pathology of Cardiac Allograft Vasculopathy (Chronic Rejection)

A.G. ROSE

INTRODUCTION

In the quarter-century since the first human heart transplant was performed by Barnard in 1967[1], this operation has become a well-established therapy for treating irremediable cardiac failure. Survival rates have progressively improved[2,3] (Chapters 43 and 44) and the 1-year survival following the operation is 80% or greater. Over 20 000 heart transplants have been performed worldwide. In patients who die in the first few months after cardiac transplantation when immunosuppression is maximal, infection is a major cause of death[4,5] (Chapter 32). Rejection is also an important factor which, if not primarily responsible for the patient's death, contributes significantly to it, even when overwhelming infection is present[6,7]. The clinical pattern of acute cardiac rejection, and the role of endomyocardial biopsy in its diagnosis, have been outlined in Chapter 30.

Acute rejection may be encountered beyond the first few weeks post-transplantation, and may be seen in patients who die years after transplantation. With longer survival one has the progressive development of a chronic rejection response that is dominated by obliterative lesions affecting both the large, epicardial coronary arteries and their intramyocardial branches.

In 1968 Lower and his colleagues were the first to draw attention to the proliferative and obliterative intimal changes of chronic vascular rejection in the epicardial coronary arteries of long-surviving canine transplants[8]. The first description of a human heart with the obliterative vascular changes of chronic rejection was in 1969, when Thomson described the donor heart of Barnard's second cardiac transplant patient who had survived almost 20 months after transplantation[9]. In 1970 Bieber *et al.*[10] described their experience with graft coronary disease. Since then, with longer survival times, this lesion has been recorded with increasing frequency[6,7,10–17]. Graft arteriopathy is currently the major factor limiting the long-term success of cardiac transplantation.

MACROSCOPIC APPEARANCES

The donor heart with chronic rejection may show a variable spectrum of macroscopic appearances (Figures 1 and 2). The anastomotic sites usually show signs of complete healing within a few weeks. The sutures are often buried in connective tissue with smooth endocardial surfaces (mostly comprising compacted fibrin) overlying the surface of the anastomoses. In none of our patients have these been the site of macroscopic thrombus formation or infection. Depending on the degree of chronic rejection, the ventricular myocardium in some appears near-normal, while in others a varying extent of scarring (Figure 1), similar to healed infarction, is noted. In yet others there is evidence of

Figure 1 Bisected, explanted cardiac transplant of 18 months duration shows white stasis thrombi filling the apices of the left ventricle (left) and the right ventricle (right). Ventricular dysfunction was due to graft arteriopathy which had led to extensive fibrosis of both ventricles

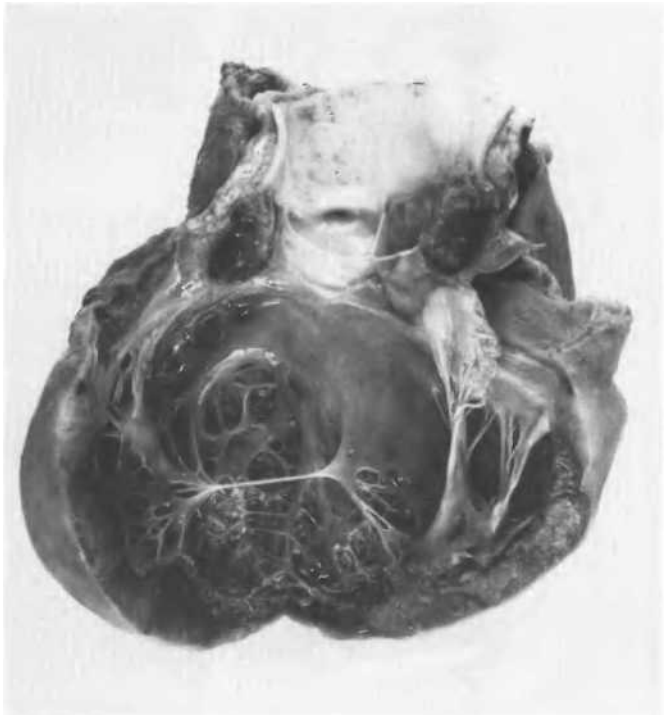


Figure 2 Stasis thrombus fills the left aortic valvular pocket. Extensive infarction of the left ventricular free wall is present. Prior organization of thrombus between the non-coronary and the right coronary leaflets has led to the development of an acquired bicuspid aortic valve

superimposed acute infarction caused by superimposed thrombotic obstruction of the coronary arteries (Figure 2). This is particularly prominent in heterotopic transplants which have ceased to function for some time before the patient's death, or before surgical extirpation of the heart.

In such hearts the coronary arteries appear macroscopically strikingly abnormal. The main coronary arteries and their epicardial branches are thickened, have a yellow-orange color due to lipid deposition in the vessel wall and, on transverse section, show a marked reduction in lumenal cross-sectional area, sometimes with accompanying thrombotic occlusion. In heterotopic transplants with chronic rejection examined at autopsy, mural stasis thrombus often occupies a large portion of both ventricular cavities of the donor heart. Stasis thrombi may also fill the valve pockets and occlude coronary ostia. The epicardial surface is bound by fibrous adhesions to adjacent structures, while the pulmonary arteries and aorta usually appear macroscopically unremarkable.

MICROSCOPIC APPEARANCES

Some microscopic changes of chronic rejection may be detectable as early as 1 month postoperatively. In one of our cases the intimal thickening of early chronic vascular rejection was observed as early as 20 days after transplantation. It should be borne in mind that a donor heart, even from a young person, may occasionally exhibit significant focal coronary atherosclerosis before transplantation.

A gradual development of the changes characterizing chronic rejection may be observed in the donor heart, depending upon the survival time of the recipient and whether death was primarily due to infection, late acute rejection, or chronic rejection. Even in patients who survive periods from 1 to 20 years, a proportion of the hearts examined at autopsy show evidence of coexistent acute rejection. These changes, similar to those already described (Chapter 29), take the form of infiltrates of lymphoid cells in the interstitium and subendocardial connective tissue. Many of these cells acquire abundant RNA-rich pyroninophilic cytoplasm. Such lymphocytic infiltrates may be difficult to distinguish from focal lymphocytic infiltration that may accompany previous zones of myocardial infarction on an ischemic basis.

Acute vascular rejection, presenting as fibrinoid necrosis of the walls of large and medium-sized coronary artery radicles, associated with an infiltrate of lymphoid cells, infrequently coexists. Evolving graft arteriopathy is often characterized by a lymphocytic infiltration in the subendocardial portion of a progressively thickening intima (Figure 3), as well as focally on the endothelial surface.

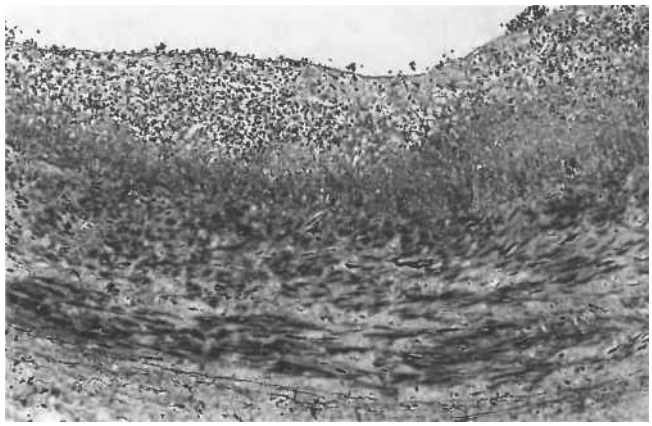


Figure 3 Evolving graft arteriopathy shows a lymphocytic infiltrate which is most marked within the upper portion of the intima (top). Lymphocytes are also seen within the adventitia (bottom) (H&E, × 24)

The most striking and significant component of chronic rejection is the process of graft arteriopathy which, due to continued proliferation of myointimal cells, leads to progressive obliteration of the lumena of the epicardial branches of the main coronary arteries (Figures 4–11), their penetrating branches and some intramyocardial, small coronary arteries. It should be noted that the latter (penetrating and smaller intramyocardial) arteries are usually unaffected by ordinary atherosclerosis. Lipid deposits are seldom seen in the thickened intimas of the intramyocardial vessels in chronic rejection. The intimal thickening in the epicardial coronary arteries may be concentric or eccentric (Figures 5 and 6), whereas the intramyocardial arteries usually show concentric intimal thickening.

Since the arterial changes in chronic rejection appear to be an accelerated form of atherosclerosis, understanding of the pathogenesis of atherosclerosis may be enhanced if the reason for this differential involvement of the penetrating branches of the epicardial coronary arteries could be elucidated.

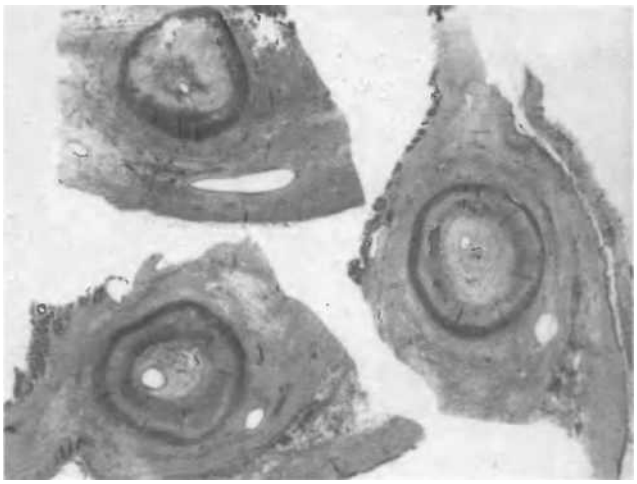


Figure 4 Chronic vascular rejection. These epicardial coronary arterial branches are almost totally occluded by concentric intimal thickening which mimics natural atherosclerosis. Graft survived 12.5 years (H&E, × 4)

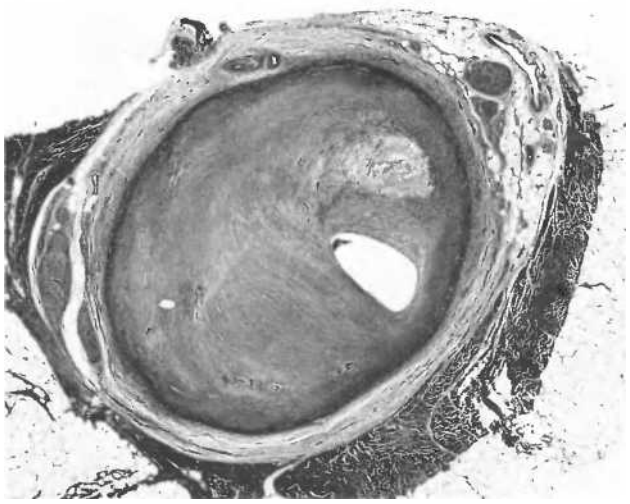


Figure 6 Eccentric intimal thickening in another coronary artery in the same donor heart illustrated in Figure 5 (H&E, × 8)

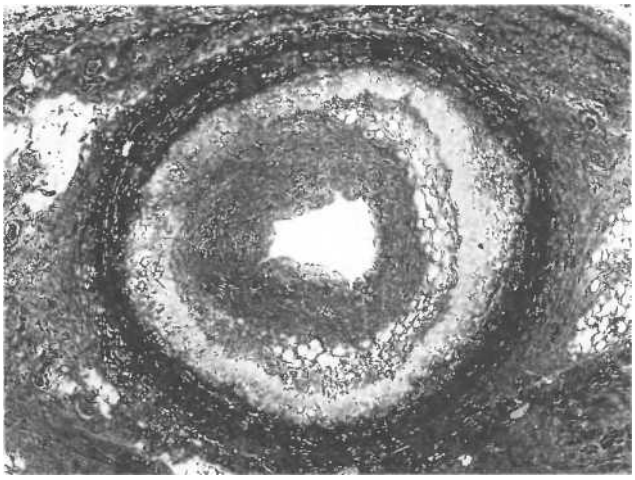
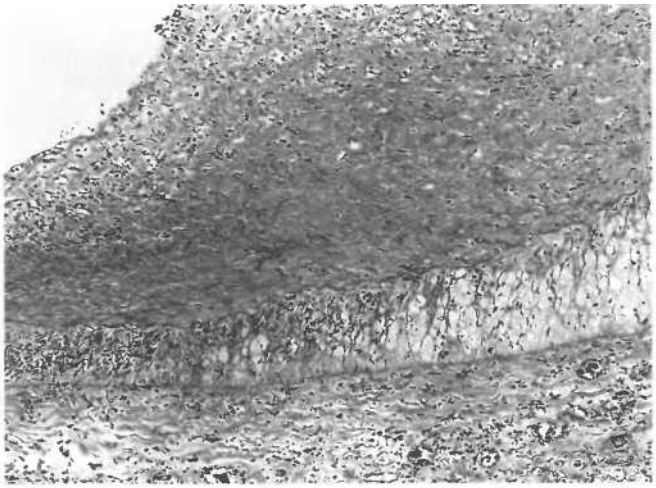
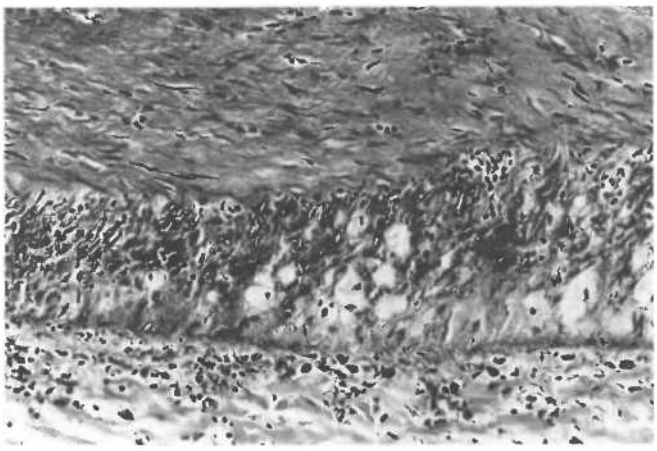


Figure 5 Concentric coronary arterial intimal thickening in a 537-day-old cardiac allograft. The intima has a lipid-rich deeper portion which is covered by a circumferential, thick fibrous cap (H&E, × 10)



A



B

Figure 7 Foam cell transformation of donor coronary arterial media in graft arteriopathy. The thickened intima (top), the media (middle) and the adventitia (bottom) contain variable numbers of lymphocytes (both H & E, A × 34 and B × 80)

Initially, the lipid deposits in the major epicardial coronary arteries are observed in myofibroblasts and macrophages. Later these cells disintegrate and release free-lying lipid. There is a variable lymphoid cellular infiltration of the walls of the affected vessels. Endothelialitis is a term currently in vogue, but its relationship to the subsequent development of graft arteriopathy is still an open question. The internal elastic lamina may be fragmented, and occasionally large gaps in both the elastic lamina and the media may result from healed arteritis.

These lesions of chronic rejection in the large coronary artery branches, particularly when associated with abundant lipid deposition in the thickened intima, bear a close resemblance to ordinary advanced atherosclerosis of native coronary arteries. The smaller coronary artery branches more often show reparative fibrosis of previous medial necrosis, giving the lesion the appearance of a healed arteritis. Mostly the intramyocardial coronary

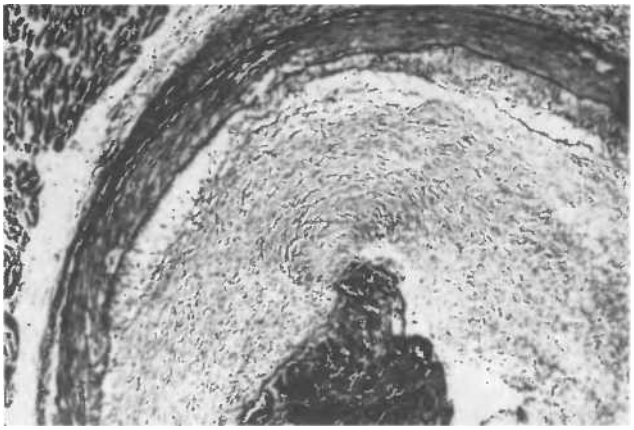


Figure 8 Graft arteriopathy (2.7 years post-transplantation) with superadded recent occlusive thrombus (H&E, × 40)

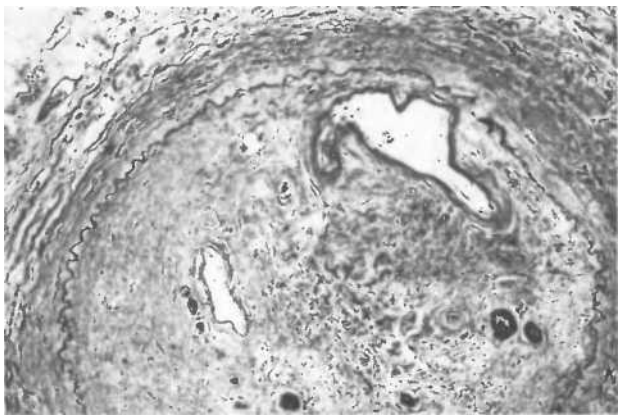


Figure 9 Advanced graft arteriopathy with organized, partially recanalized, occlusive thrombus. Graft survived 2.2 years (H&E, × 40)

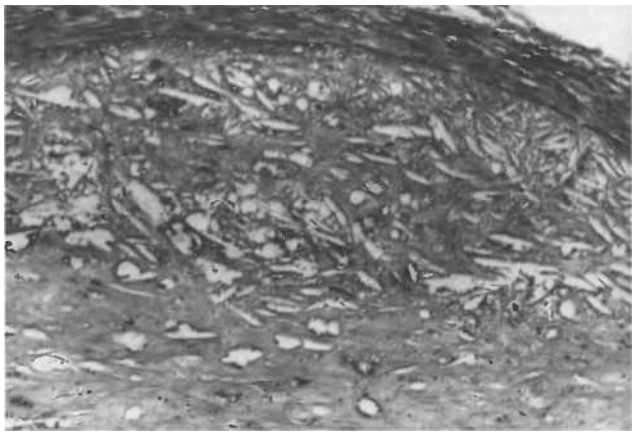


Figure 10 Natural atherosclerosis-like appearance of coronary artery in chronic rejection in a 4.2-year-old allograft (H&E, × 110)

arteries (Figures 12 and 13) show prominent, concentric intimal fibrosis of a marked degree.

Graft arteriopathy in the more severely affected large coronary arteries predisposes to superadded thrombosis, which appears as

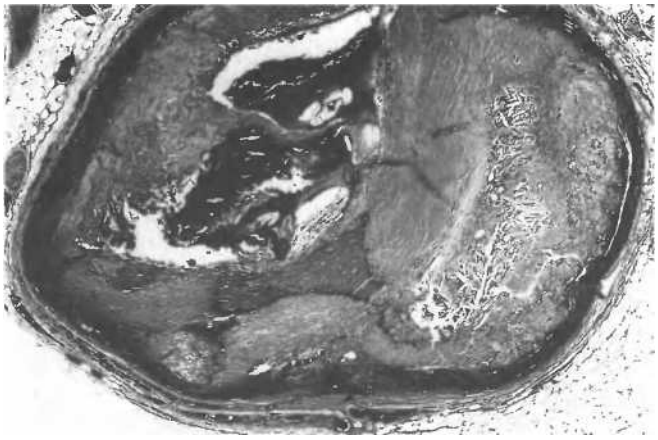


Figure 11 Calcification within accelerated atherosclerosis-like plaque in left coronary artery of a 2656-day-old allograft (EvG, × 9)

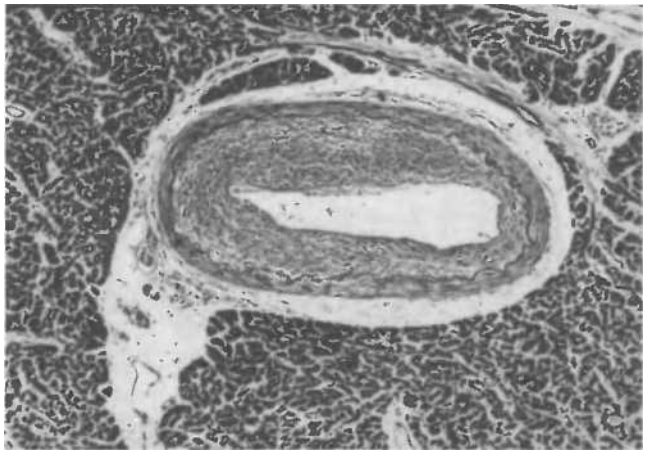


Figure 12 Small, intramyocardial coronary artery shows moderate lumenal stenosis due to almost circumferential intimal thickening caused by intimal smooth muscle cellular proliferation with fibrosis. Graft survived 12.5 days (H&E, × 60)

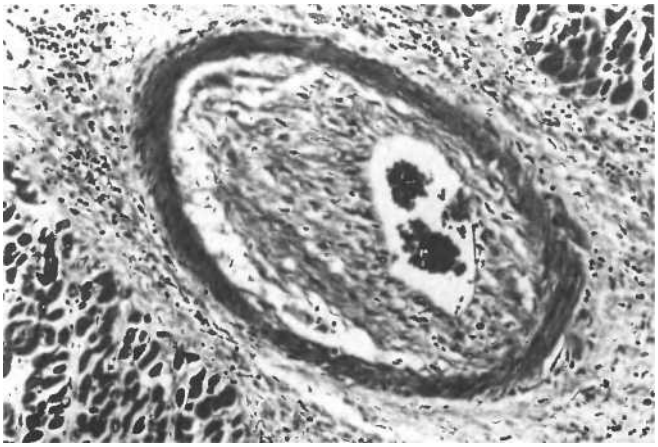
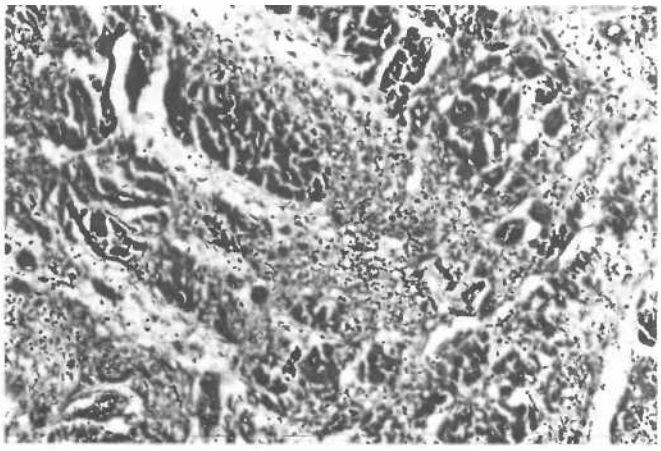


Figure 13 Intramyocardial coronary artery, which is slightly obliquely sectioned, shows severe lumenal stenosis due to intimal thickening. Scanty foam cells are present in the deeper portion of the thickened intima (H&E, × 75)

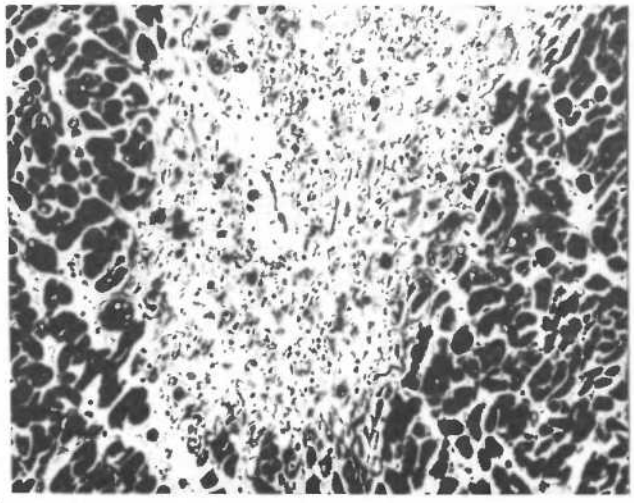
fresh thrombus in a few cases (Figure 8), but in the majority as old occlusive thrombus, which has undergone fibrous replacement and recanalization (Figure 9). Veins are seldom affected by this process and such involvement when present does not produce more than modest intimal fibrous thickening.

Frequent scars are noted in the myocardium. In some, the remnants of degenerate myocytes occurring in collapsed stroma in the presence of a lymphoid infiltrate suggest previous foci of myocytolysis (Figures 14 and 15). In others the appearance of large acellular scars is indicative of fibrous replacement of ischemic infarcts (Figure 16). Extensive acute infarcts may be observed in heterotopic transplants with thrombotic occlusion of severely narrowed coronary arteries.

The obliterative vascular changes may lead to extensive loss of ventricular myocardium, and the resultant massive fibrous replacement is often most evident in the right ventricle (Figure 17). Subendocardial myocytes (Figures 17 and 18) are kept alive by intra-cavity blood. This may be a cause of misleading results on endomyocardial biopsy, since the biopsy may show relatively normal myocytes at a time when much of the ventricle exhibits replacement fibrosis.



A



B

Figure 14 **A**: Multiple foci of myocytolysis caused by graft-arteriopathy-induced partial ischemia have evoked a lymphocytic response (H&E, × 105). **B**: Scar showing remnants of degenerate myocytes and persisting lymphoid infiltrate suggestive of previous myocytolysis. Graft survived 2.7 years (H&E, × 130)

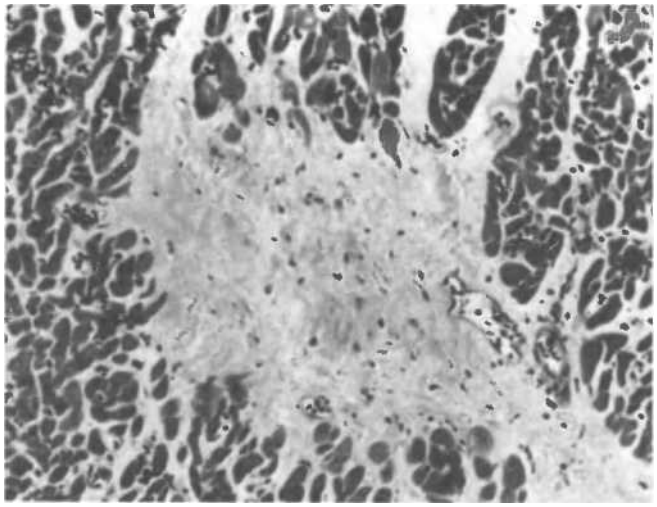


Figure 15 Small, dense, hypocellular scar, possibly due to previous myocytolysis with stromal collapse fibrosis due to ischemia caused by chronic rejection in a 1.6-year-old graft (H&E, × 170)

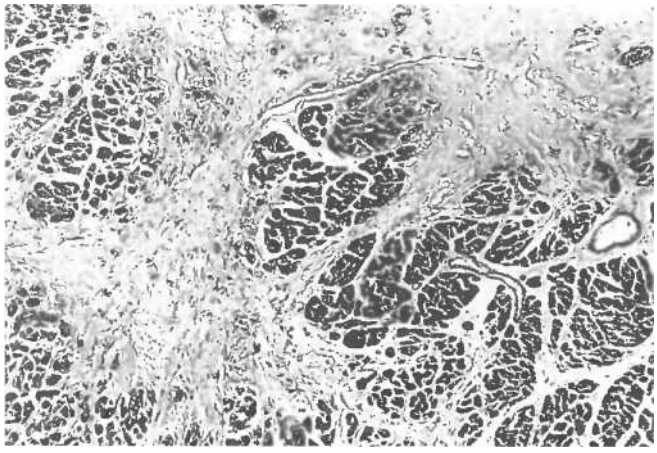


Figure 16 Large, acellular, fibrous scar of healed myocardial infarction. Graft survived 12.5 years (H&E, × 48)

Other cardiac structures are surprisingly little affected. Intimal fibrosis of modest degree with some loss of medial smooth muscle may be observed in both the aorta and the pulmonary arteries, with little accompanying cellular infiltration. Only in the presence of severe acute rejection are the valves affected by a focal lymphoid infiltrate. Chronic rejection does not appear to produce any specific changes in the heart valves.

INCIDENCE OF CHRONIC REJECTION

The frequency with which chronic rejection changes occur in long-surviving cardiac transplants is illustrated by a personal

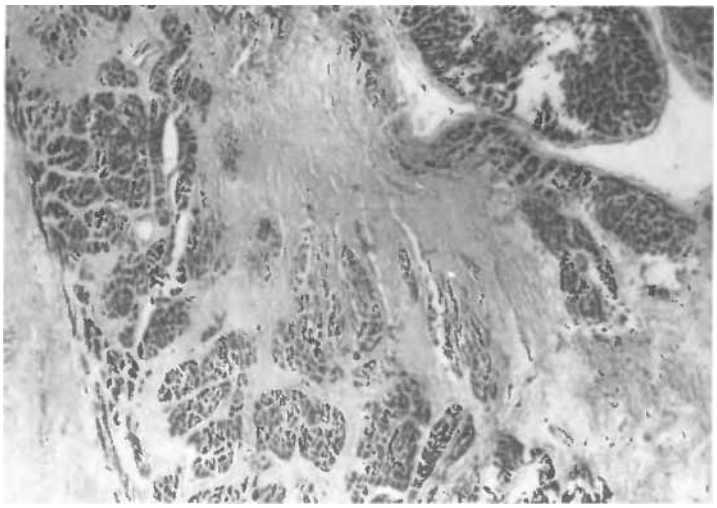


Figure 17 Massive replacement fibrosis of the right ventricle due to chronic rejection in a 12.5-year-old graft. Note the survival of subendocardial myocytes (H&E, × 3.4)

study of 12 patients with 14 cardiac transplants with survival times greater than 1 year[18]. These consisted of three orthotopic and nine heterotopic transplants examined at autopsy, and two surgical specimens from patients who underwent heterotopic retransplantation. The grafts survived 1.1–12.5 years with a mean of 2.7 years.

Histologically, 29% of hearts showed evidence of persisting acute rejection, and 93% showed signs of significant chronic rejection. In the response of chronic vascular rejection, severe myointimal proliferation was present in most cases (86%), while a cellular infiltration of the vessel wall was generally absent (72%).

The degree of vascular occlusion of chronic rejection bore no relationship to the frequency or severity of previous acute rejection episodes. Severe lumenal encroachment of between 51% and 100%, resulting from these changes, was frequently present (79%), while superadded thrombosis of the affected arteries was present in 72% of cases. Myocardial scars were present in 92% of hearts, while extensive acute infarction was present in 43% of heterotopic transplants. Further, it is striking that in all of the cases with chronic rejection there was an almost complete absence of infective lesions. A significant degree of rejection was responsible for the patient's death or graft failure in 79%.

FEATURES DISTINGUISHING GRAFT ARTERIOPATHY FROM ORDINARY ATHEROSCLEROSIS

In a later personal study of 43 human allografts with graft arteriopathy[19] the coronary arteries showed purely concentric narrowing in 56% of patients and eccentric narrowing in 44%. Others[20] stress the concentric nature of graft coronary disease compared to naturally occurring atherosclerosis, but this differs from our experience even in grafts with a mean duration of only 2 years.

Whilst many vessels affected by graft arteriopathy showed changes indistinguishable from ordinary atherosclerosis, certain pathologic features separate graft arteriopathy from usual atherosclerosis: (a) the presence of either active or healed vasculitis in some of the affected vessels, (b) a prominence of outer medial defects due to mediolysis and/or foam cell transformation in the major coronary arteries, (c) the diffuse nature of the atherosclerosis-like changes, (d) obliterative narrowing of the intramyocardial coronary arteries and (e) calcification being observed less frequently in graft arteriopathy compared to usual atherosclerosis.

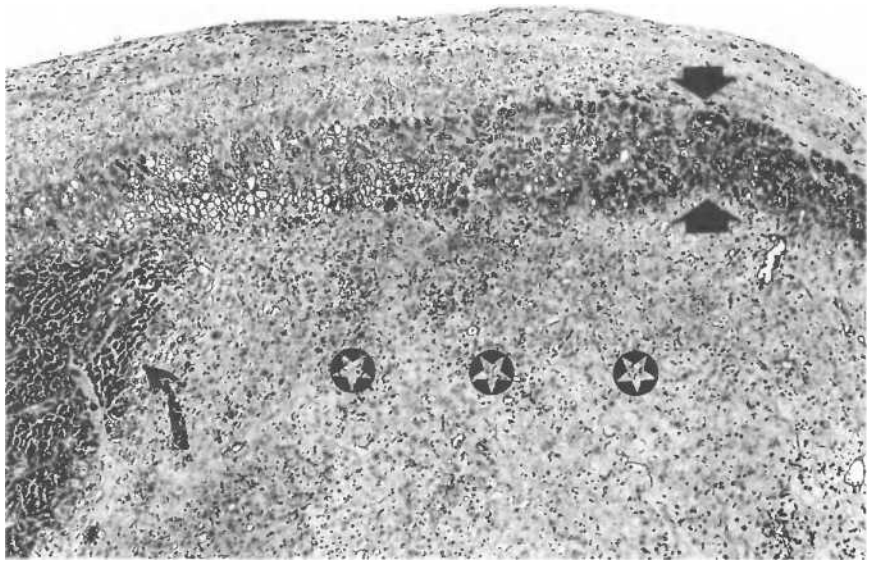


Figure 18 Graft arteriopathy has produced severe ischemic damage in the donor right ventricular subendocardium. A thin layer of surviving subendocardial myocytes (between the two arrows) contrasts with a paler, deeper zone of extensive myocytolysis (stars) and an adjacent zone of coagulative necrosis (curved

COMMENT

It is not safe to assume that the donor heart that is transplanted is always free of (significant) coronary atherosclerosis even with a young donor age. Pre-existent donor coronary arterial disease is easy to detect if the recipient dies (or if the graft is explanted) shortly after transplantation. If the graft lasts for a longer period, pre-existent natural atherosclerosis will be difficult to distinguish from graft arteriopathy despite the more often focal distribution of the naturally occurring disease. In a personally studied series[21] of 47 patients who died within 30 days following cardiac transplantation, pre-existent coronary atherosclerosis was found in 19% of the hearts (Figure 19).

The appearances of the vascular lesions of graft arteriopathy are those of a reparative process following immune-mediated intimal and medial arterial damage. The entire length of the coronary artery is affected by the immune insult, but the resultant lesions may show regional differences in severity.

The severity of these lesions bears no constant relationship to the survival time of the graft or patient. Graft arteriopathy may be detected as early as 3 months after transplantation. It is detected angiographically in over 50% of recipients 5 years postoperatively[22]. The incidence has even been put as high as 90% after 5 years[23]. It remains to be seen what effect on the prevention of allograft arteriopathy will be achieved by newer drug therapy with agents such as the calcium antagonist dilitiazem[24] and pravastatin[25,26] following grafting. Angiography tends to underestimate the severity of histologic determination of graft arteriopathic narrowing[27]. Myocardial bridges are also more frequently demonstrated in transplanted hearts angiographically[28], possibly due to increased myocardial stiffness and myocardial hypertrophy.

Von Scheidt and Erdman[29] reported a specific subtype of allograft coronary disease which they termed dilated angiopathy. This unique angiopathy, in which the dilated coronary arteries exceeded the diameter of adjacent vessels by at least 50%, was observed in 7.3% of their cardiac transplant patients. This angiopathy was as frequent as diffuse vessel obliteration in their patient population.

Inflammatory weakening of the media of the affected coronary arteries is the postulated etiology of dilated angiopathy. In this regard we found microscopic evidence of coronary vasculitis in 60% of our patients with chronic rejection[19]. Active vasculitis was evident in 21%, both active and healed vasculitis was present in 19%, and healed vasculitis was seen in 21%. As far as I am aware, no pathologic study has been performed to define the microscopic features of dilated angiopathy. Dilated angiopathy has a better prognosis than its obliterative counterpart[29].

Gravanis[30] considers cell-mediated immunity to be the basis for graft arteriopathy. This proliferative arterial lesion is not unique to cardiac allografts[31], but has been described in every organ grafted, including the kidney, liver and lung. Libby *et al.*[32] have advanced a unique hypothesis for cardiac allograft vascular disease. They observed a significant deposition of CD4 lymphocytes and macrophages plus smooth muscle cells just below an intact endothelial cell layer in patients with allograft coronary disease. These vascular wall cells have important immunologic capabilities not only in presenting antigen, but in stimulating the release of growth factors and cytokines, which might result in a chronic, local delayed-type hypersensitivity reaction. Others are paying attention to factors derived from endothelial cells (e.g. endothelin-1 mRNA and peptide expression) that are up-regulated in allografts with rejection and graft arteriopathy[33].

Since T-lymphocyte depletion has been used effectively to prevent graft-versus-host disease in bone marrow transplantation, it has been suggested that this therapy may be applied to cardiac transplantation in order to prolong graft survival[34].

Not all vessels in the donor heart show accelerated atherosclerosis, which is surprising in view of the suspected immunological etiology. The determining factor for graft survival, in the presence of chronic rejection, is myocardial ischemia, manifesting as extensive recent or old infarction. The fact that donor hearts are generally procured from young people further underlines the significance of this event. This situation is ironic, since ischemic heart disease is a frequent indication for cardiac transplantation.

While endomyocardial biopsy is an effective means of monitoring acute rejection episodes, it is of less use in chronic rejection. Experience has shown that extensive RV myocardial fibrosis may exist, which is not detected in a superficial endomyocardial biopsy, in which the subendocardial myocytes remain viable since they are nourished by the RV intra-cavitary blood. Similarly, the biopsy does not include coronary arteries, which are the vessels affected by the process of chronic rejection. Chronic rejection (graft arteriopathy) continues to be the major factor limiting the long-term success of cardiac transplantation.

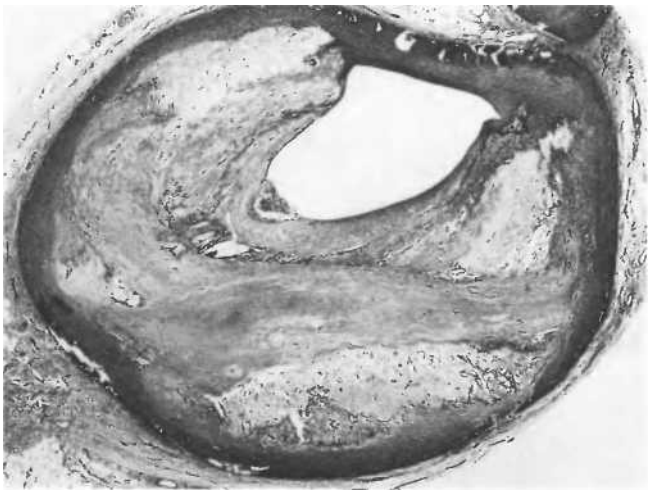


Figure 19 Pre-existing, natural atherosclerosis of a donor heart's left anterior descending coronary artery. The donor heart, which came from a 47-year-old female, had only been implanted for 7 days (H&E, × 12)

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34
Pathogenesis of Cardiac Allograft Vasculopathy (Chronic Rejection)

B. ARKONAC AND J.D. HOSENPUD

INTRODUCTION

Despite the dramatic improvement of early and 1-year survival following cardiac transplantation, long-term mortality has not been substantially impacted[1]. The major cause of late mortality is an accelerated obliterative coronary artery disease, presumably a manifestation of chronic rejection[2], which will be referred to in this review as cardiac allograft vasculopathy (CAV). The incidence of CAV is between 10% and 15% per year, with a prevalence of as high as 45% at 5 years based on angiographic diagnosis[3]. With the more sensitive intravascular ultrasound, some investigators have reported virtually 100% prevelance of the disease[4]. This review will focus on the pathophysiology and immunologic mechanisms proposed for CAV, as well as current diagnostic and potential therapeutic interventions for the disease.

PATHOLOGIC FINDINGS

The pathologic features of CAV (Chapter 33) differ from the native coronary artery arteriosclerosis in many aspects, especially early in the disease process (Table 1). In native coronary arteriosclerosis, typically the lesions are focal, predominate at the proximal bifurcations of the coronary system, and are eccentric in their distribution. The lesions frequently contain calcium, and the internal elastic lamina is disrupted. This process usually takes several years even in its most aggressive manifestations as seen in patients with multiple risk factors, including familial hyperlipidemia[5].

The pathologic findings of end-stage CAV may eventually match the above features, but the initial presentation differs. Typically, the endothelial layer is intact and, as demonstrated in Figure 1, there is a significant concentric intimal hyperplasia[6]. The internal elastic lamina is also intact until very late in the disease process, when it may be disrupted. Calcium deposits are rarely present. Although originally described as a bland proliferative lesion, more careful examination has demonstrated an inflammatory cell infiltrate, typically subendothelially[7]. Rarely, a frank vasculitic picture is observed[8,9].

The strongest evidence for this process being immune-related is its exclusive confinement to the allograft. In addition to the coronary tree, intimal thickening occurs in the coronary veins[10] and the great vessels up to, but not extending beyond, the suture line[11]. CAV is therefore a true vasculopathy, involving all of the vessels of the allograft, rather than solely an arteriopathy. Although this disease characteristically is diffuse, with greatest involvement of distal vessels[6], a more recent investigation has demonstrated that the appearance of increased severity in the distal vasculature is an anatomic phenomenon due to branching, rather than a physiologic difference[12]. Furthermore, nearly half of these patients have focal epicardial stenoses, more typical of the non-transplant-associated coronary artery disease[13,14]. In CAV, possibly as a result of the distal vessel obliteration, the coronary collateral formation develops in an atypical form, and has been described as a 'blush pattern'. This pattern may represent an angiogenic response to microvascular ischemia[15].

Table 1 Pathologic differences between CAV and CAD

Histologic attribute	*Cardiac allograft vasculopathy*	*Coronary artery disease*
Angiographic localization	Diffuse, distal	Focal, proximal
Intimal proliferation	Concentric	Eccentric
Calcium deposition	Rare	Frequent
Internal elastic lamina	Intact	Disrupted
Inflammation	Present	Rare

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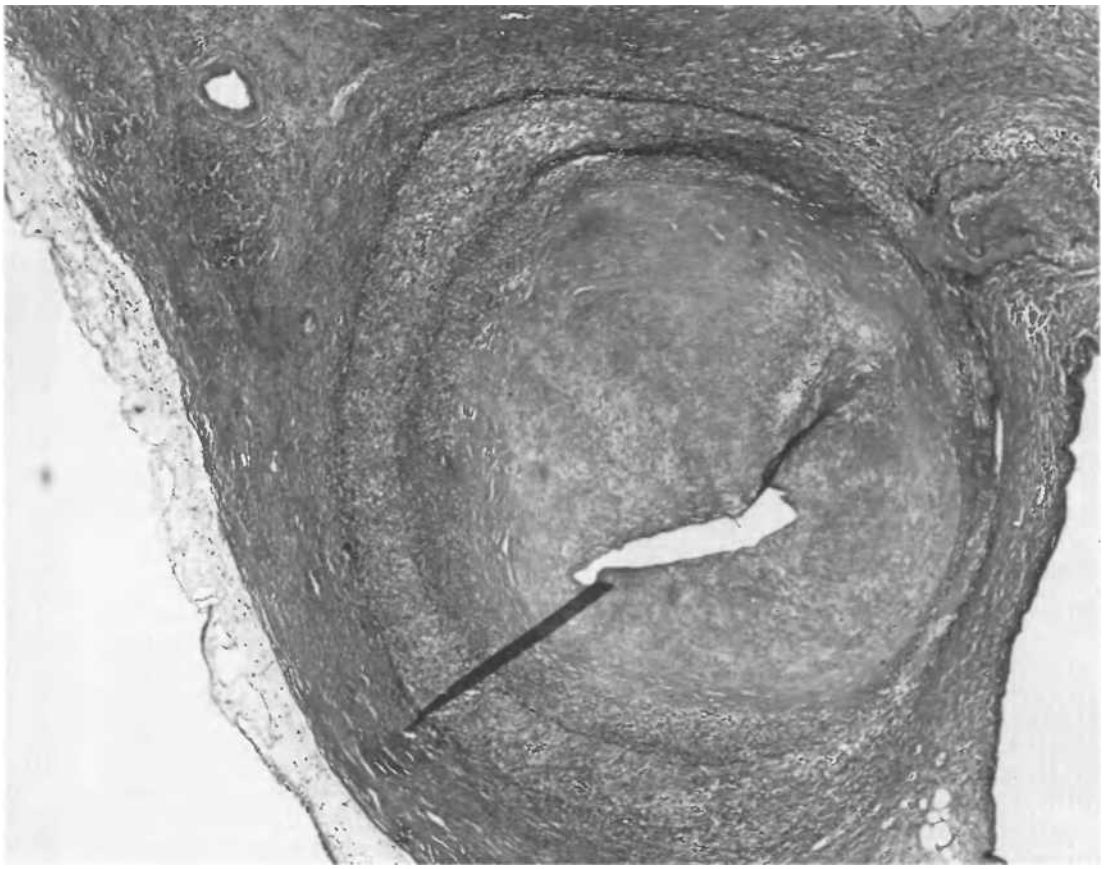

Figure 1 Cross-section of human allograft coronary artery at explantation showing severe myointimal proliferation consistent with CAV (×34)

CLINICAL FEATURES

In contrast to traditional atherosclerosis, the time-course for the development of CAV is measured in months rather than years. Due to initial cardiac denervation at the transplant operation, clinical signs and symptoms of ischemia may be minimal. Generally, recipients who lack afferent cardiac innervation will not experience typical angina pectoris. There are, however, reports of patients late following cardiac transplantation experiencing chest pain, which is presumably anginal[16] and, more recently, chemical and physiologic evidence of partial autonomic reinnervation has been reported[17]. The problem of inability to experience angina is most important in CAV, but increasingly relevant is the transmission of pre-existing coronary artery disease due to the use of older donors. Tuzcu *et al.* demonstrated a high frequency of donor atherosclerosis in their series of 50 cardiac allograft recipients studied early post-transplantation by angiography and intravascular ultrasound[18].

Depending on whether there is a discrete clinical event such as a myocardial infarction, or more commonly progressive global myocardial ischemic dysfunction, clinical presentation can be quite insidious. Symptoms range from those as nondescript as malaise or decreased exercise tolerance, to overt congestive heart failure. Alternatively, the initial presenting finding can be arrhythmia or sudden cardiac death. Thus, the practice of performing annual screening coronary angiography is routine in most cardiac transplant programs. Currently, given limited intervention for this disease, this screening is used for prognostic purposes and the timing of repeat transplantation if this procedure is considered.

DIAGNOSIS

As stated above, although coronary angiography is the most widely used surveillance method internationally, it has been criticized for greatly underestimating the prevalence of CAV[19,20]. Despite this criticism, Everett *et al.*, in a small series of 28 cardiac allograft recipients, demonstrated that at 1 year the specificity of normal qualitative angiography in predicting absence of cardiac allograft vasculopathy is 81%[21], based on a change of luminal diameter by quantitative angiography.

When a more sensitive technique such as intracoronary ultrasound is compared with qualitative coronary angiography, however, 70–90% of transplant recipients who had normal coronary angiograms had significant intimal thickening[22,23]. Ventura *et al.*[24] compared routine coronary angiography, intracoronary ultrasound and percutaneous coronary angioscopy. The sensitivity for detecting plaque was 79% for percutaneous coronary angioscopy and 10% for angiography, and for detecting stenosis was 24% and 3%, respectively. For intracoronary ultrasound versus

angiography for plaque and stenosis detection, the sensitivities were 76% vs 10% and 45% vs 3%, respectively. The authors concluded that intracoronary ultrasound, when compared with percutaneous coronary angioscopy, is more accurate in assessing the severity of stenosis (45% vs 24%), and that intracoronary ultrasound could better detect calcification and presence of intimal thickening[24].

It is, however, unclear what prognostic significance ultrasound-diagnosed intimal thickening has in the face of normal angiography. To address this issue, Anderson *et al.*[25] assessed the functional significance of intimal thickening detected by intravascular ultrasound by investigating endothelial-dependent and independent vasodilatation in 40 patients 1–8 years post-transplantation. Endothelial dysfunction occurs early, even before intimal thickening, yet may recover in recipients that survive more than 5 years even in the presence of moderate intimal thickening. Recently, Heroux *et al.*[26] investigated the coronary vasodilatory response to intracoronary papaverine, and demonstrated that all the patients with intimal thickening by intracoronary ultrasound had blunted response to intracoronary papaverine, suggesting intimal injury resulting in decreased coronary EDRF production.

IMMUNOLOGIC MECHANISMS

Cocanougher *et al.*[27], using death due to CAV proven by autopsy as an endpoint, demonstrated in 101 patients that worse HLA match was associated with severe CAV. The association with HLA matching has also been suggested by Costanzo-Nordin[28], who demonstrated that complete HLA-B and DR mismatch is associated with a higher incidence of CAV. The general assumption is that CAV represents a chronic alloimmune response to the vasculature. Given this assumption, the likely target for the immune response is the vascular endothelium, which is appropriately located at the blood vessel interface, expresses both MHC class I and II antigens *in vivo* and *in vitro*[29–33], and can also serve as antigen-presenting cells, capable of presenting either alloantigen or nominal antigens such as microbiological antigens[34–39]. Finally, endothelial cells either express constitutively or can be induced to express a variety of adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), necessary for the adherence of various classes of white blood cells to initiate the immune response[40,41].

Whether the vascular smooth muscle cells are capable of expressing appropriate antigens to augment an allogeneic response is still not clear. Salomon *et al.*[42] showed *in vitro* that there is no lymphocyte activation in a co-culture of interferon-pretreated arterial smooth muscle cells. In addition, Theobald *et al.*[43] demonstrated that, despite an increase in MHC class I and II antigen and an increase in ICAM-1 in response to IFN-γ, smooth muscle cells isolated from human umbilical veins were poor stimulators of lymphocyte proliferation. Contrasting these studies, Fabry *et al.*[44] demonstrated that both syngeneic and allogeneic rat splenocytes proliferated in response to brain microvascular smooth muscle cells, though the syngeneic response was greater than the allogeneic one.

Cellular versus humoral immunity

In general, most studies have been unable to find a correlation between typical cellular rejection and the ultimate development of CAV, with few exceptions[45,46]. Thus CAV was thought to be a manifestation of humoral immunity. This widely accepted opinion began to shift with the emergence of a number of animal studies using heterotopic models of heart and aortic transplantation. They all demonstrated the ability to reliably produce vasculopathic lesions which consisted of extensive mononuclear cell infiltration associated with smooth muscle cell proliferation and intimal thickening. A number of rat strain combinations have been investigated, which vary from mismatches at MHC class I, class II or minor histocompatibility antigens[47,48]. Recently, Adams *et al.*[49], using Lewis rats as donors and F344 rats as recipients (which match for MHC and differ for non-MHC), demonstrated diffuse arteriosclerotic lesions which were identical in appearance to those seen in human allografts. Their series demonstrated 20% acute rejection within 3 weeks, 50% surviving at least 3 weeks, and 25% surviving indefinitely. Rejected allografts demonstrated intense mononuclear cell infiltration and necrosis. Indefinitely surviving grafts demonstrated moderate mononuclear cell infiltration and myocyte destruction. More than 90% of all arteries present in allograft cross-sections demonstrated marked coronary intimal lesions. Early lesions consisted of subendothelial accumulations of mononuclear cells, while later lesions demonstrated diffuse fibrotic intimal thickening with occasional focal cellularity that was identical in appearance to CAV seen in humans[49].

Cramer *et al.*, using several rat strain combinations to select for mismatches in class I (RT1.A and C), class II (RT1.B and D), or only minor non-MHC antigens, demonstrated that CAV could be produced in a majority of combinations[50,51]. The severity of CAV was proportional to the duration of the allografting, suggesting the requirement of time (i.e. those allografts that rejected acutely showed little or no CAV). The severity of both the intimal proliferation and the intensity of lymphocytic infiltration could be enhanced by a single intraperitoneal injection of donor lymphocytes, and reduced by the administration of cyclosporin. The authors concluded justifiably that CAV in these models is not the result of an allogeneic response to a single specific vascular alloantigen, but may result from any number of different antigen mismatches. They also concluded that, based upon the ability to modify the lesions by immunization and T-cell-specific immunosuppression, CAV in these models is probably a chronic cell-mediated rejection response.

Using Lewis-to-F344 rat model, Cramer *et al.* studied the cellular composition of CAV serially from 7 to 90 days[52]. In these lesions the first cell line to appear was macrophages followed by T, natural killer and, to a lesser extent, B cells. The more bland lesions were made up primarily of smooth muscle cells.

Recently, several groups have begun using a rat heterotopic aorta allograft model. In this model a segment of descending thoracic aorta is looped and anastomosed end-to-end to the recipient abdominal aorta below the renal arteries and above the bifurcation[53–55]. Most recently, Shi *et al.* reported a new mouse model, in which the carotid arteries were transplanted between B.10A(2R) (H-2H2) donors and C57BL/6J (H-2b) recipients, and compared with arteries isografted between H-2b mice[56]. Both of these

models demonstrated similar histopathologic changes, which are basically seen in cardiac allograft models. The process starts with an inflammatory infiltrate containing activated T cells and macrophages followed by a progressive intimal thickening made up primarily of smooth muscle cells. The adventitia is infiltrated, with the external elastica lamina staying intact. The internal elastica lamina is also largely intact, but can show small areas of disruption. The advantages of these models are the large segment of vascular tissue that can be studied and the lack of the allograft being a vital organ. The difference between these models and the rat cardiac allograft model or the human disease is the extent of medial necrosis and complete replacement by fibrosis. It is unclear whether ischemia (the loss of the vasa vasorum with the transection of the vessel) plays a contributing role in this model. This important difference makes interpretation and the potential relevance of this model difficult to ascertain.

Evidence for cell-mediated immunity in human cardiac transplantation has been relatively rare. Although Uretsky *et al.* (1987) suggested that CAV is more common in patients with multiple episodes of cellular rejection[57], this has not been supported by most other series of cardiac transplant patients from individual centers. Multiple explant/autopsy series, although with small numbers of cases, supported the cell-mediated process with their histological studies. Liu and Butany[58] demonstrated cellular vascular lesions containing T cells and macrophages in both media and adventitia. Hruban *et al.*[59] showed marked lymphocytic endothelialitis in their cases with CAV. Salomon *et al.*[60] investigated seven freshly obtained allografts and demonstrated lymphocytes (both $CD4^+$ and $CD8^+$) and macrophages in coronary arteries in a ring distribution directly under the endothelial layer. They also noted an up-regulation of endothelial cell DR expression in these arteries.

We have investigated lymphocyte responses to donor-specific human aortic endothelial cells in 52 allograft recipients at three time-points following cardiac transplantation. First, as shown in Figures 2 and 3, despite a reduction in acute parenchymal rejection following transplantation, lymphocyte proliferation in response to donor-specific endothelial cells increases, as does the up-regulation of MHC class II antigen on the endothelium in response to the exposure to lymphocytes[61]. Furthermore, as shown in Figure 4, those patients who have developed CAV at 1 year post-transplantation have greater lymphocyte proliferation responses to their donor-specific endothelial cells at all time-points compared to those without CAV[62].

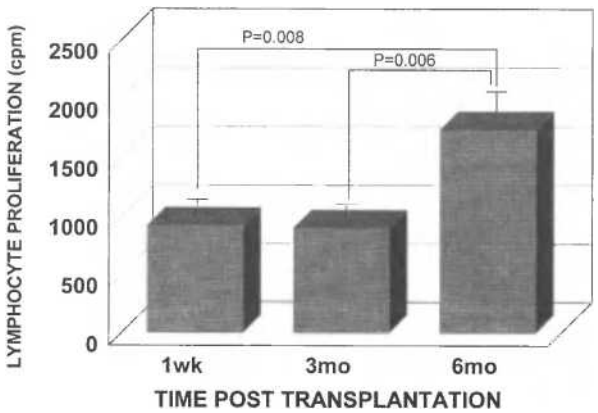

Figure 2 Lymphocyte proliferation to donor-specific aortic endothelial cells increases over time following cardiac transplantation (reprinted with permission, see ref. 61)

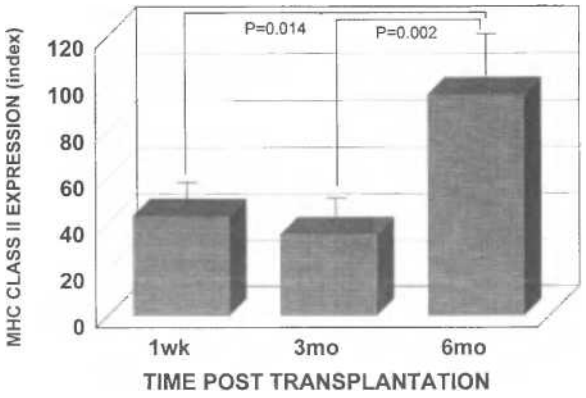

Figure 3 Endothelial cell expression of MHC class II antigen increases over time post-transplantation with exposure to recipient lymphocytes (reprinted with permission, see ref. 61)

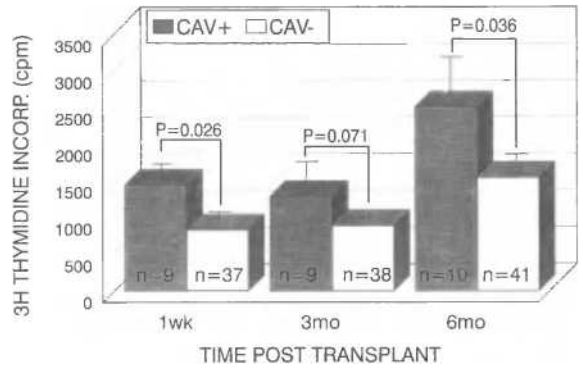

Figure 4 Lymphocytes obtained serially from patients who develop CAV 1 year following cardiac transplantation have a greater proliferative response to their donor-specific endothelium compared to those who have no coronary artery disease by angiography (reprinted with permission, see ref. 62)

The presence or development of antibodies to donor MHC antigens has long been associated with a reduction in graft survival, in the renal transplant literature[63]. In a recent study, Halloran *et al.*[64] reported on 13 patients who were prospectively identified as having antibody to donor class I antigens, and had higher frequency of acute graft rejection, endothelial abnormalities and ultimately a greater graft loss compared to 51 patients who did not have circulating anti-class-I antibody. Hammond *et al.*[65] demonstrated the association between antibody deposition in the cardiac allograft by immunofluorescence, acute rejection and reduced survival. They also presented data suggesting an increased incidence of CAV in those patients with positive immunofluorescence[66]. In a large retrospective study of 463 cardiac transplant recipients, Lavee *et al.*[67] demonstrated that the degree of panel-reactive antibody (PRA) positivity correlated both with acute cellular rejection within the first 3 months post-transplantation and with CAV. However, Smith *et al.*[68] demonstrated that although patients who

develop lymphocytotoxic antibodies directed against donor-specific HLA determinants had more cellular rejection, there was no increased incidence of CAV.

In the same study described above, we investigated the role of alloantibody to donor-specific endothelium using a flow cytometric assay for antibody binding. Of the 52 patients studied, two had positive antibody binding which was new, two additional patients had alloantibody detected which was known to be present prior to transplant (retrospective crossmatch), and four additional patients had borderline positive results. As shown in Figure 5, there was no relationship between the presence of alloantibody and the ultimate development of CAV on quantitative angiography[62]. Our negative findings have recently been confirmed by Mehra *et al.*[69] by comparing intravascular ultrasound to antibodies directed to umbilical vein endothelial cells and standard panel cytotoxicity assays.

A recent study by Rose and colleagues[70] showed that antibodies specific for the doublet of endothelial antigens (of polypeptides of approximately 60 and 62 kDa) are rarely produced by patients other than those with progressive CAV after transplantation. A follow-up of this study suggests that the target antigen is vimentin[71]. Given that vimentin is monomorphic, and does not vary within a species, it is unclear how an allogeneic response would develop to this protein. Alternatively, if this represents a sequestered (intracellular) antigen, its exposure by prior endothelial cell damage could elicit an autoimmune response.

Finally, Russell and colleagues[72] have demonstrated in a mouse model that, using the same degree of MHC mismatch, those strain combinations which result in alloantibody formation have a greater degree of intimal proliferation than those combinations where alloantibody is not produced. Furthermore, it appears that the intimal proliferative disease can be passively transferred in this model.

Based upon the available human evidence one must conclude that, although alloantibody may contribute to CAV, the data are insufficient to support a primary role. There are data, however, to support a complementary role for alloantibody if a cell-mediated response to vascular endothelium is important in the development of CAV. We have recently demonstrated that pooled serum containing high titers of alloantibody from patients awaiting renal transplantation increases the expression of ICAM-1 on vascular endothelial cells *in vitro*[73]. A typical experiment is shown in Figure 6. One could theorize that, once alloantibody develops, its presence could enhance T cell binding to the vascular endothelium. Alternatively, the development of alloantibody could solely

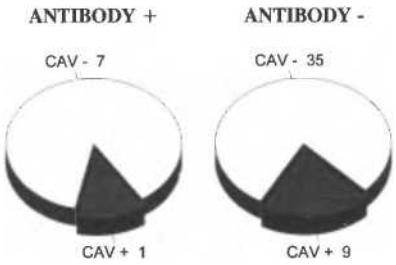

Figure 5 There is no correlation between antibodies in recipient serum directed to donor-specific endothelium and the development of CAV (reprinted with permission, see ref. 62)

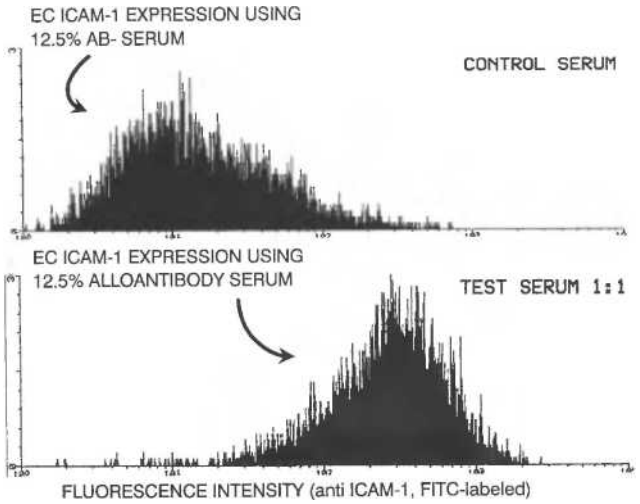

Figure 6 Serum containing high titers of anti-HLA antibodies stimulates endothelial cell up-regulation of ICAM-1: top panel, flow histogram using control serum; bottom panel, flow histogram using serum with anti-HLA antibodies (reprinted with permission, see ref. 73)

be an epiphenomenon of a more active cell-mediated response to vascular endothelium.

In preliminary studies[74] we have demonstrated that allogeneic lymphocytes can up-regulate endothelial IL-6, a cytokine which has, among other properties, the ability to recruit and expand B cell clones. The optimal production of endothelial IL-6 requires direct contact between the lymphocyte and endothelial cell. Although a variety of cytokines (IL-6, tumor necrosis factor-α (TNF-α)) are known to up-regulate endothelial cell IL-6, the regulation produced by allogeneic lymphocytes occurs extremely rapidly (peak mRNA response in 2 hours), suggesting that a second cytokine is not required.

Growth factors and cytokines

It has been well demonstrated that multiple polypeptide growth factors can act to modulate the proliferation rate of mesenchymal cells in either a promoting or a suppressing mode[75–77]. One family of growth factors that has been studied extensively is the heparin-binding growth factor/fibroblast growth factor (HBGF). Seven separate genes of the HBGF family have been identified: acidic and basic fibroblast growth factor (aFGF and bFGF), the int-2 locus, kFGF/hst, FGF-5, FGF-6, and keratinocyte growth factor (KGF). Platelet-derived growth factor (PDGF) is an another growth factor that is produced by a number of tissues, including endothelium, and is a potent smooth muscle cell mitogen as well as a chemoattractant. It is important to emphasize that smooth muscle cells likewise produce a number of growth factors and cytokines capable of amplifying smooth muscle cell proliferation (autocrine stimulation), including PDGF, bFGF, TGF-β, IL-1 and IL-6[78–81].

There is now emerging evidence that a variety of lymphokines and monokines can directly regulate the synthesis of growth factors, such as IL-1 stimulating the expression of PDGF-A gene in cultured fibroblasts, and TNF stimulating the endothelial cell release of PDGF[82–84]. Clausell *et al.* showed that, in the rabbit

heterotopic heart transplant model, *in vivo* blockade of TNF-α suppresses the acute development of neointimal formation by selectively reducing the vascular immunoinflammatory reaction and accumulation of fibronectin[85]. More recently, Russell *et al.*, in the Lewis-to-F344 rat transplantation model, have shown the allograft-specific up-regulation of IFN-α, IL-6, and monocyte chemoattractant protein-1 gene expression in cardiac allografts undergoing chronic rejection[86]. They also localized the gene products to infiltrating mononuclear cells in the interstitium and vasculature, supporting the cellular activation theory. Calderon *et al.* have demonstrated that IL-6 up-regulates PDGF B chain mRNA[87]. Ikeda *et al.* have shown that IL-6-dependent smooth muscle cell proliferation was blocked by monoclonal antibody directed against PDGF[88].

Recently, Oho *et al.*[89] reported a 10-fold increase in elastase activity associated with the development of post-cardiac transplant arteriopathy, and demonstrated a 5-fold increase in the breaks in the internal elastic lamina. They suggest that elastase may play a pathophysiological role in neointimal proliferation by potentially activating cytokines and growth factors, and by release of chemotactic peptides. Molossi *et al.*[90] demonstrated that in a piglet heterotopic cardiac transplant model there is increased fibronectin production by donor vascular endothelial cells, which is partly regulated by an autocrine mechanism involving IL-1β. They suggest that fibronectin may mediate lymphocyte trafficking and smooth-muscle-cell migration related to CAV. Recently, Zhao *et al.* have shown that the expression of aFGF and its receptor, along with PDGF A chain, is significantly increased in cardiac allografts during rejection, and that cardiac myocytes and vascular walls are the predominant sources for these factors[91,92].

We have previously reported that endothelial cells exposed to allogeneic lymphocytes up-regulate mRNA coding for a panel of mesenchymal growth factors, including bFGF, TGF-α, TGF-β, and PDGF A and B chains (Figure 7)[93]. We have now recently extended these findings to the cardiac transplant population. In those allograft recipients who ultimately develop CAV, their lymphocytes preferentially up-regulate mRNA coding for TGF-a, PDGF A chain and heparin-binding epidermal growth factor[94]. There is a direct link, therefore, between cell-mediated immunity to vascular endothelium, alterations in the levels of endothelial-cell-derived growth factors, and the ultimate development of CAV by angiography.

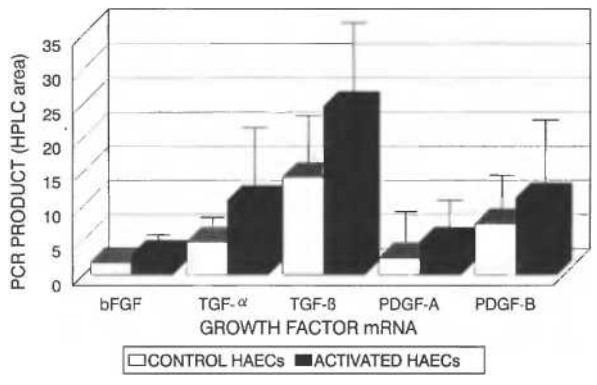

Figure 7 Endothelial cells exposed to allogeneic lymphocytes up-regulate mRNA levels coding for a panel of growth factors known to stimulate smooth muscle cell proliferation[93]

It is clear that the endothelial cell, and possibly the smooth muscle cell, can serve as targets for allogeneic immunity and be activated to produce a host of cytokines known to stimulate either paracrine or autocrine smooth muscle cell proliferation. In addition, several lymphokines and monokines directly stimulating these vascular cells can amplify this response.

NON-IMMUNOLOGIC PRECIPITATING FACTORS

Most transplant centers attempt to aggressively modify the usual risk factors for atherosclerosis, with the assumption that risk factors for native coronary artery disease may contribute to CAV. Although there are no controlled trials that have shown a decrease in the incidence of CAV with a decrease in serum lipids, several studies have documented the association between hyperlipidemia and CAV[95–101]. The development of hypercholesterolemia, an increase in low-density lipoprotein cholesterol, and hypertriglyceridemia have been uniformly shown to develop 3–18 months after heart transplantation in several studies[102–109].

It appears that multiple factors are in effect in causing post-transplantation hyperlipidemia, including (a) obesity that develops after transplantation[104]; (b) prednisone, by increasing hepatic apolipoprotein B production[103]; and (c) cyclosporin by inhibiting prednisolone clearance, either due to hepatotoxicity or interactions with the cytochrome P450 system[110]. Dietary changes frequently produce minimal lowering of cholesterol or triglyceride levels, leading to administration of lipid-lowering agents that may have troublesome side-effects, and that may interact with immunosuppressive agents. This makes their use difficult in the cardiac transplant population[108,111]. Of interest are the preliminary data from Kobashigawa *et al.*[112] suggesting that the early use of an HMG CoA reductase inhibitor (pravastatin) appears to reduce the development of severe CAV and the incidence of death in cardiac transplant patients on triple-drug immunosuppression.

Hypertension is common in post-transplant patients, especially in the cyclosporin era. However, studies have not found a relationship between hypertension and the development of CAV[95–99]. Likewise, smoking was not a factor in CAV development in these studies[95,100]. Recently, Munoz *et al.* confirmed previously published data on diabetic patients who underwent heart transplantation, showing that the long-term survival rate is similar to non-diabetic patients without an increased risk of CAV[113]. The role of gender was investigated by Sharples *et al.*[99] and compared with transplantations performed between donors and recipients of the same sex. Female recipients of a male donor heart had a relative risk of 1.56 of graft loss from CAV, and male recipients of a female donor heart had a relative risk of 3.33[99]. Other potential risk factors, such as ischemic time and donor age, have not been consistent with regard to the development of CAV, either in clinical or in experimental studies[114,115].

CYTOMEGALOVIRUS AND CARDIAC ALLOGRAFT VASCULOPATHY

The first suggestion that there was an association between cytomegalovirus (CMV) and CAV was from the Stanford group,

who reported their experience with 301 patients. Twenty-eight percent of CMV-infected patients developed 'severe' coronary obstructive lesions, whereas only 10% of patients not infected with CMV developed the same degree of CAV[116]. A similar association between CMV and CAV has been reported by others[117,118]. In contrast, Balk *et al.* failed to find a relation between CAV and the occurrence of CMV infection[119]. In their cohort of 100 patients who survived at least 1 year after cardiac transplantation, there was no significant difference in the incidence of CAV (any coronary disease) between CMV-seropositive and seronegative patients, between patients with and without CMV infection (culture positivity), or between patients with and without a clinical CMV syndrome.

Recently, Koskinen *et al.*[120] reported a cohort of 53 heart transplant patients, and carried out a correlation between their coronary angiograms, capillary and arteriolar changes in endomyocardial biopsy specimens and their CMV status. They documented that biopsy specimens showed significant change post-CMV infection (which was diagnosed on specific IgM, a positive viral culture from blood, urine or bronchoalveolar lavage fluid, together with a 4-fold IgG rise, or positive CMV antigenemia test). The significant changes in coronary angiograms followed 2 years post-transplantation[120].

In a study from Oregon (Table 2), using subset analysis, only those patients who had evidence of prolonged CMV infection (defined as persistently positive blood buffy coat cultures over a 4-month period) had a statistically significant increase in the incidence of CAV compared to the remainder of the study cohort[121]. Wu *et al.*[122] documented that allograft explants with CAV had a much higher incidence of positive *in-situ* hybridization for CMV nucleic acid in the vascular intima compared to explants without CAV. Finally, Lemstrom *et al.*[123], using the DA to WF (complete MHC mismatch) rat aorta transplant model, demonstrated that CMV infection enhanced the vascular inflammation and increased the degree of intimal proliferation by a factor of 2 compared to uninfected controls.

The mechanism by which CMV plays a role in the development of CAV is likely to be interaction between the virus and the allogeneic response, which to date has not been defined. The presumed initial response is an infection of one or more cell types within the vascular wall. Waldman *et al.*[124], using a strain of CMV propagated in endothelial cells, infected human umbilical vein endothelial cells and investigated lymphocyte proliferative responses using responder cells from CMV-seropositive individuals. They noted a substantial increase in proliferation of the $CD4^+$ lymphocyte population in response to CMV-infected human umbilical endothelial cells compared to uninfected controls. This increased $CD4^+$ lymphocyte proliferation occurred despite the lack of an increase in MHC class II antigen expression on the endothelial cells, suggesting a unique interaction between this strain of CMV, the endothelium and $CD4^+$ lymphocytes, as was shown in a previous study[125]. Van Dorp *et al.*[126] demonstrated that, in cultured endothelial cells, MHC class I antigen was induced by CMV infection, but likewise could not demonstrate a change in MHC class II antigen. This increase in endothelial cell MHC class I antigen has been confirmed by Tuder *et al.*[127].

In contrast, although we have demonstrated that human umbilical endothelial cells and human aortic endothelial cells are targets of CMV infection, only 5–10% of cells are infected, and we could document no alterations in MHC antigen expression. However, we have confirmed prior reports[128] that human aortic smooth muscle cells are readily infected by CMV. Furthermore, this infection of smooth muscle cells results in the increased expression of MHC class I antigen, hence potentially providing a mechanism for altering the allo-response[129].

PROGNOSIS

Keogh and colleagues demonstrated, in a cohort of over 350 patients, that those with proximal or midvessel discrete coronary stenoses had a 34% reduced survival compared to those without coronary stenosis[130]. Sharples *et al.*[131], using an analysis which factored in severity and progression, documented that if the initial angiogram was normal, progression to mild disease was low (12%/year) and progression to death was very low (3%/year). Once mild disease was detected, progression to severe disease was 47% per patient-year, and once severe disease occurred progression to death was 40% per patient-year[131].

Gao and colleagues analyzed their data specifically looking at the rate of development of CAV. Patients who had hemodynamically significant CAV by 1 year had a greater than 35% increased risk of an ischemic event at 1, 3 and 5 years post-initial detection of CAV, compared to those whose CAV developed later than 1 year post-transplantation[132]. Data from Oregon (Figure 8) also confirm the greater risk of mortality (in this case following their 1-year angiogram) for those with CAV, demonstrated or implied by the above studies from other centers.

MANAGEMENT

Although data from animal studies have suggested that several interventions, including antiplatelet agents[133], estrogens[134], androgens[135], somatostatin analogues[136], older[137] and newer[138] immunosuppressive agents, fish oil[139] and calcium antagonists[140], may be helpful in preventing or delaying the development of

Table 2 CMV infection subgroups and cardiac allograft vasculopathy (CAV)

Subgroup	*No. of patients*	*CAV incidence (%)*	*Probability*
CMVi+/–	89/40	20/23	n.s.
1°CMV+/–	20/109	25/20	n.s.
CMV4+/–	36/92	31/23	0.107
CMV6+/–	19/98	37/18	0.089
CMVv4+/–	17/111	47/18	0.012

CMVi = CMV infection, CMV4 = 4-month persistent CMV infection, CMV6 = 6-month persistent CMV infection, CMVv4 = 4-month persistent CMV viremia. From ref. 121.

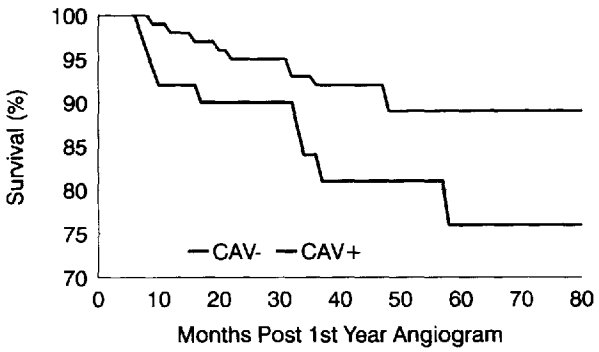

Figure 8 Patient survival following 1 year anniversary angiogram depending on presence or absence of CAV ($p = 0.044$)

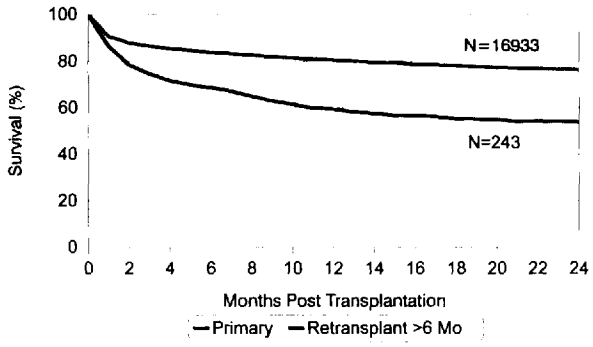

Figure 9 ISHLT/UNOS thoracic registry data comparing survival following first cardiac transplant to survival after retransplantation assuming retransplant occurred at least 6 months following first transplant operation

CAV, few data are confirmatory in humans. Most centers prescribe aspirin or other antiplatelet drugs, lower lipid levels and elevated blood pressures, and insist on no tobacco products, but there is little evidence to suggest that these measures have had any impact on CAV incidence or progression.

Only two studies have demonstrated any efficacy of medical therapy on the development or severity of CAV. Schroeder and colleagues from Stanford randomized patients to receive diltiazem or placebo[141]. They demonstrated a reduction in the severity of CAV and a lower incidence of ischemic events and mortality in the diltiazem group. As noted above, Kobashigawa *et al.*[112] in a preliminary presentation demonstrated a reduction in CAV and mortality in patients randomized to pravastatin.

The role of augmenting immunosuppression as a means of altering the progression of CAV would seem to be a promising strategy if, as assumed, CAV is a manifestation of chronic rejection. Addonizio *et al.*[142] compared double immunosuppression (cyclosporin and prednisone) with triple therapy (cyclosporin, prednisone and azathioprine) in 55 pediatric cardiac allograft recipients. The mean follow-up time was 36 months. They experienced a significant decrease in CAV with triple therapy, as diagnosed by coronary angiography. Also, most recently, Valantine *et al.*[143] documented that a lower dose of cyclosporin (defined as $\leq$ 3 mg/kg per day) is associated with an increased prevalence of death from CAV. Both of these studies suggest that inadequate immunosuppression represents a direct predisposition to CAV.

Revascularization procedures such as PTCA or directional atherectomy remain palliative measures. Although many centers report good initial success, ultimate outcome does not appear to be impacted; furthermore, most of the patients do not qualify for these procedures, due to the diffuseness of their disease[144–146]. Finally, retransplantation still remains the only clearly defined treatment for CAV, but with patient survival approximately 20% below first-time transplants (Figure 9)[1,147,148], along with a national shortage of acceptable organs for transplantation, this procedure is clearly not a long-term solution.

COMMENT

It is encouraging to see the proliferation of data on the basic mechanisms of CAV and trials of novel treatment modalities. However, CAV continues to be the major obstacle to the long-term survival of cardiac allograft recipients, and our knowledge base is far from complete. Solutions may come from better or more specific immunosuppression, or more probably from a more comprehensive understanding of the basic mechanisms resulting in the disease.

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35
Diagnosis and Management of Cardiac Allograft Vasculopathy (Chronic Rejection)

L.W. MILLER, T. DONAHUE, T. WOLFORD AND J. DRURY

INTRODUCTION

The greatest impediment to long-term survival for adult and pediatric heart transplant recipients is the development of an accelerated form of coronary artery disease[1–4]. This is variously termed chronic allograft vasculopathy, chronic allograft coronary artery disease, or chronic rejection. This disease was reported in the original preclinical animal experiments[5], and subsequently in the early human heart transplant recipients at Stanford, USA[6,7] and Cape Town, South Africa[8]. The incidence of this disease has been estimated at 10% per year post-transplant[4,9–11]. Although cyclosporin has been associated with an improvement in 1- and 5-year graft survival, as well as a decrease in death due to rejection, it has not had an impact on reducing the incidence of allograft coronary artery disease (ACAD)[12–15] in adults, but increased immunosuppression may decrease the incidence in pediatric patients[15].

Progression of this disease is variable[16], with a percentage of patients having a very accelerated course, particularly in the epicardial vessels, which may be evident within 1–3 years of transplant, while other patients may have an isolated single vessel stenosis which remains unchanged for years. However, it has become apparent that, once patients develop at least a 70% stenosis by angiography, in even one coronary vessel, they have a very poor prognosis (Figure 1), with approximately a 70% mortality at 1 year, and an even higher mortality with an increasing number of vessels involved[17].

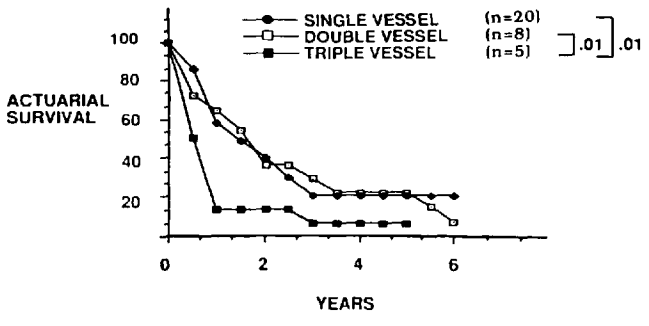


Figure 1 Survival over time post-heart-transplant once a 70% or greater stenosis is noted by angiography, by number of vessels diseased

Clinical presentation

The clinical presentation of ACAD may be acute, with new-onset heart failure, arrhythmia, or sudden death, often heralding an acute myocardial infarction[18,19] (which is typically not accompanied by anginal-like pain, due to the cardiac denervation that occurs at implantation). The inconsistent presence of chest pain with an acute MI often results in delay or misdiagnosis[19] (nearly 50% in the Stanford series) and, coupled with the paucity of collateral vessels, contributes to the reported mortality of >25%[19]. Recently, Wilson *et al.*[20–22] have shown that, with increasing time post-transplant, many heart transplant patients have detectable reinnervation, as confirmed by norepinephrine release in the coronary sinus in response to an intracoronary tyramine challenge. The extent of reinnervation is unpredictable but, if present, it would allow patients to have anginal symptoms. This makes it mandatory to investigate heart transplant patients, especially those more than 3–4 years post-transplant, who develop: (a) a change in functional status, or (b) EKG pattern, or (c) chest pain, which is consistent with angina pectoris.

Pathogenesis

The pathogenesis of ACAD has been the topic of extensive review[23–29] (Chapter 34). Most investigators describe the process as multifactorial, having both an immune and a non-immune basis.

Immune factors

Support for the primary role of an immune etiology includes: (a) concentric uniform involvement of the entire coronary vessel of the allograft (versus eccentric focal disease with typical atherosclerosis); (b) restriction of this rapidly proliferative disease to the allograft vascular bed; the development or occurrence in patients

of all ages, including neonates, or in patients with donor hearts under the age of 20 years; (c) the clear line of histologic demarcation between the donor and recipient aorta; and (d) reproducibility of the disease in numerous animal models of immune-mediated injury[30–33].

Debate continues on the relative contribution of the cellular versus humoral arms of the immune system to the pathogenesis of ACAD[34–37]. Although there are increasing data showing a high correlation between the occurrence of even one episode of acute rejection and the development of chronic rejection in kidney transplant recipients[38–40], there have been few data to support the correlation between the incidence, severity, or time to first cellular rejection and the development of allograft coronary disease in heart recipients[41–45] as measured by contrast angiography. This lack of supporting data, coupled with the reports of the high incidence of spontaneous resolution of diffuse mild or even focal moderate rejection[46–48], has caused most centers to become more and more conservative in initiating treatment of rejection.

Most recently, Kobashigawa[49] presented data from the CVIS investigators in which the ISHLT biopsy grading system was converted into a numeric scale. They noted that the average score of all biopsies during the first 3 months post-transplant, but not the incidence of treated rejections, correlated with the development of allograft coronary disease as defined by intimal thickening on intravascular ultrasound. These data raise the question of whether increased immunosuppression, or a lower threshold to treat rejection, will decrease the incidence or progression of ACAD, or, alternatively, result in significantly increased morbidity. This question remains to be answered.

The role of the humoral arm of the immune system in the pathogenesis of ACAD has been demonstrated. Hammond has described the association between vascular or antibody-mediated rejection and ACAD[50], and several centers have noted that the development of measurable circulating HLA antibodies post-transplant correlated with a poor prognosis and development of coronary disease[51–55]. The specificity of these antibodies has recently been shown to be directed against both HLA and non-HLA antigens on the surface of the endothelium[56–59].

HLA matching is not attempted in heart transplant recipients due to time constraints, but several series have shown an increase in rejection and decreased survival with increase in HLA mismatch[60–62], including a review by Costanzo[63]. There are increasing data demonstrating an inverse correlation between degree of match at the DR locus and the development of ACAD[63–65]. The presence of even one match at the DR locus reduces the likelihood of ACAD.

Non-immune factors

There are several non-immune factors which may correlate with or contribute to the pathogenesis or development of allograft coronary disease[66]. These include: (a) donor age, with a high direct correlation between risk and increasing donor age, particularly over the age of 50[67,68]. This is largely due to the presence of unsuspected coronary intimal irregularities and thickening in the general population by this age, a fact which may limit expansion of the donor pool; (b) ischemic heart disease pretransplant; (c) hyperlipidemia[69,70], in particular, both elevated triglycerides and total cholesterol. Recently, Valentine[71] reported that a low HDL was as important as an elevated LDL subfraction of total cholesterol. Hyperlipidemia plays an important, but secondary, role in the development of this disease. Animal data have shown that animals with immune-mediated intimal thickening have a marked potentiation of the development of this disease when fed a high-cholesterol diet, but a high-cholesterol diet alone could not alter the natural history of this disease[72,73];(d) cytomegalovirus (CMV) infection. The mechanisms of this correlation are at least two-fold, as CMV has been shown both to up-regulate expression of class 2 donor antigens on endothelium and to enhance lipid incorporation into the intima of the vessel[74–77]. Numerous clinical series have shown a correlation of CMV infection with the development of ACAD[78–82]; (e) obesity[69]. The mechanism by which obesity contributes to the pathogenesis is somewhat unclear, but may relate more to the associated finding of hyperlipidemia in these patients; (f) cryopreservation and reperfusion injury. Hypothermia can induce an injury to the coronary endothelium which may predispose the recipient to this disease[83]. In addition, cold preservation is a perfect model of reperfusion injury which may, by release of oxygen free radicals, result in early endothelial ischemic damage or dysfunction[84,85], that may play an important causal role in the subsequent development of ACAD. Blockade of free-radical-induced injury by superoxide dismutase significantly reduced the incidence of acute and chronic rejection in a prospective double-blind study in kidney transplant patients[86].

One new concept in the pathogenesis of allograft coronary disease is that not only is it a disease not limited to hearts or other vascularized allografts[87], but it may also represent an accelerated form of conventional atherosclerosis, with other examples being post-angioplasty restenosis and saphenous vein graft stenosis. The unifying theme of this paradigm of accelerated atherosclerosis is endothelial cell injury. Ip *et al.*[88] have described and graded endothelial injury from 1 to 4, with transplant coronary disease being a grade 3 and angioplasty restenosis representing grade 4 injury, not only involving the intima, but often extending into the media. The acute nature, deep extension, and severity of the PTCA lesion are perhaps the primary reasons for angioplasty restenosis being the most accelerated form, often evident within 2–6 months after angioplasty.

There has been a great deal of work in vascular biology[88–95] which has contributed greatly to our understanding of the factors involved at the molecular level in the pathogenesis of both ACAD and post-angioplasty restenosis. The most obvious pathologic feature of both allograft coronary disease and post-angioplasty restenosis is a significant smooth muscle cell (SMC) proliferation and subsequent migration across a relatively intact internal elastic membrane which results in a very expanded intima and progressive luminal compromise[96–98]. Central to the current understanding has been the identification of a number of cytokines, such as IL-2, IL-6, IL-10, and interferon-gamma[99,100], and growth factors[101], such as platelet-derived growth factor, insulin-like growth factor, and fibroblast growth factor, as well as other molecules that can induce or promote smooth muscle cell proliferation[102]. Equally important has been the demonstration of the capability of not only lymphocytes and macrophages, but endothelial cells to secrete or express a number of these molecules[99,103], largely in response to up-regulation of class II HLA antigens on the surface of endothelial cells. In addition, recent attention has been directed at the important role of matrix and

ground substance in plaque development[104]. This paradigm provides a unifying theory of host response to endothelial cell injury[88], whether immune-mediated, as with ACAD, or traumatic, as with post-PTCA restenosis[93,105], and provides new insights into the potential role of the immune system in conventional atherosclerosis[106].

Data supporting the concept of non-specific response to endothelial cell injury have been shown by Foegh *et al.*[107,108] in experiments with the somatostatin analogue, angiopeptin. This molecule, which inhibits insulin-like growth factor, has been shown to inhibit intimal proliferation in animal models of heterotopic heart transplantation as well as balloon-induced injury to a carotid artery. The importance of the understanding of these molecules and cofactors that inhibit smooth muscle cell proliferation[109,110] is that they also become potential targets for therapy to alter the natural history or prevent the development of this disease in the future (see discussion below on therapeutic agents).

DIAGNOSIS OF ALLOGRAFT CORONARY DISEASE

Non-invasive

Although non-invasive tests have become the mainstay of the diagnostic armamentarium for conventional atherosclerotic disease, these tests have unfortunately had far less sensitivity and specificity in heart transplant patients. Smart *et al.*[111] and others[112–114] have reviewed the diagnostic accuracy of these tests, using angiography as the gold standard. The original studies comparing nuclear thallium imaging involved very few patients, but suggested a sensitivity as low as 50%, which is below clinical applicability. Reasons for the lack of sensitivity with nuclear perfusion imaging have been thought to be due to the diffuse nature of the disease, particularly in small vessels. Use of SPECT, rather than conventional tomographic imaging, may improve the diagnostic accuracy, but use of newer agents, such as sestamibi, has not resulted in improved accuracy[114].

Recently, a good deal of interest has been focused on the use of dobutamine stress echocardiography to diagnose ACAD[115,116]. Unfortunately, despite its proven value with conventional atherosclerosis, this test also has been too insensitive to help manage transplant patients. However, Akosah *et al.*[117] recently found a high predictive accuracy between a positive test and the presence of angiographically significant stenosis. Other non-invasive tests being investigated include soluble IL-2 receptors as a marker of immune activation[118], as well as ultrafast CT and positron emission tomography[119], both of which are expensive and have limited availability.

Invasive

As a result of the poor sensitivity of non-invasive tests, the diagnosis of allograft coronary disease remains based on invasive techniques. Conventional contrast angiography remains the gold standard. Gao *et al.*[120] first described the angiographic appearance of this disease which includes epicardial stenoses very similar to conventional atherosclerosis, but the hallmark of ACAD is stellate tapering and/or abrupt cut-off of third- and fourth-order branch vessels (distal obliterative arteriopathy). Due to the very rapid development of this disease, collateral vessels are usually absent[121]. A number of investigators have used quantitative methods to caliper vessel diameter, quantitate percentage stenosis, and measure disease progression[122–125]. Although angiography is highly specific and predictive of a poor prognosis when significant narrowing or stenosis is evident[17,126–128], numerous patients have had an apparently normal angiogram only to die within months of the study, and have autopsy evidence of severe multivessel coronary disease[129].

The reasons for this disparity between pathology and angiography are numerous, and include use of neighboring vessels to judge luminal diameter of a vessel when, in fact, the vessel may be diseased from its origin and throughout the coronary vascular tree[130]. Perhaps even more important is the potential role of compensatory vessel dilatation in response to development of flow limitation as described by Glagov[131,132]. This phenomenon, which has been observed in carotid, cranial, and coronary vessels[133,134], as well as post-PTCA restenosis[135], allows a relatively normal luminogram and minimizes the flow limitations of progressive intimal thickening[136].

Historically, most centers performed the 'baseline' angiogram at the first-year anniversary of the transplant procedure. However, Gao *et al.*[137] have shown that, even using quantitative contrast angiography, there is a statistically significant decrease in luminal diameter between a study performed within the first 3–4 weeks post-transplant and a study at 1 year. The early study allows detection of disease transmitted in the donor that could be erroneously ascribed to a host response. As a result, many centers now perform the baseline angiogram within 1 month of transplant to detect unsuspected disease in the donor[138]. Nitroglycerin must be used to maximally dilate vessels, minimize spasm[139] which may occur, and maximize visualization of collateral or branch vessels.

There are several reports of making the diagnosis of ACAD by the finding of an obliterated intramural vessel on endomyocardial biopsy[140,141] or evidence of myocardial infarction or ischemic damage[84,142]. While these findings are very specific, they are extremely insensitive, especially for events involving the left coronary system and left ventricle, which is not sampled. The timing of catheterizations, and strategies to decide how often they should be repeated in the individual patient, are being evaluated by several groups[143,144].

Intravascular ultrasound (IVUS)

As a result of the lack of sensitivity of contrast angiography, a great deal of attention has been focused on the development of intravascular ultrasound (IVUS) to diagnose ACAD. This technique, which has recently been reviewed[145,146], employs the use of a catheter, with an ultrasound probe mounted on the tip, which can be advanced over a small guidewire down the coronary artery, providing a 360 degree real-time cross-sectional view of the vessel and the degree of intimal thickening. Research has resulted in miniaturization of the catheter to approximately a 3.0 French, or <1 mm diameter, thus allowing the catheter to be advanced nearly to the most distal portion of all three major coronary vessels. Recent reports have documented the safety of this procedure[147–149], as the operator can clearly detect prohibitive luminal narrowing on-line, particularly when unsuspected by an apparently normal angiogram.

One of the most important findings with IVUS is that it has been shown to be much more sensitive than angiography for the detection of ACAD[150–152]. A study from Stanford[152] demonstrated moderately severe intimal thickening with IVUS in over 50% of 42 patients with a normal coronary angiogram. Patients with abnormal angiograms also had moderate or severe intimal thickening, which was indistinguishable from the patients with normal angiograms. This significant increase in sensitivity with IVUS over angiography has also been reported in other series[145].

The appearance of a transplant coronary artery by IVUS has been reported by a number of investigators[151–154], and includes a line of demarcation for the intima, media, and surrounding tissue. The image can be planimetered to provide measurements of total area, luminal area and intimal area, and calculation of an intimal index, which is the percentage of luminal area occupied by plaque (Figure 2). The intima must be at least 150 μm, or 0.15 mm, before it is apparent on ultrasonography. Intimal thickness, as measured by ultrasound, has been validated by a number of autopsy studies and shown to be very accurate[145].

Intravascular ultrasound not only has provided a detailed look at the coronary intima and its composition, but has raised some fundamental questions about the pathogenesis of ACAD. Although the typical pathologic description of coronary arteries from heart transplant recipients with ACAD is of a uniform, concentric, doughnut-like appearance of the expanded intima, a

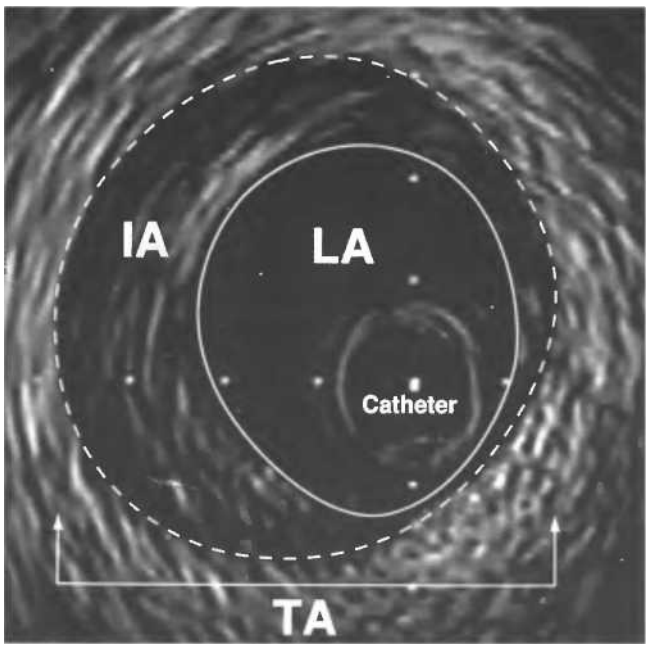


Figure 2 IVUS image of transplant coronary artery (dark area represents blood-filled lumen with small circle catheter; with planimetry of plaque showing TA = total area; LA = luminal area, IA = intimal area. Intimal index = TA–LA/TA

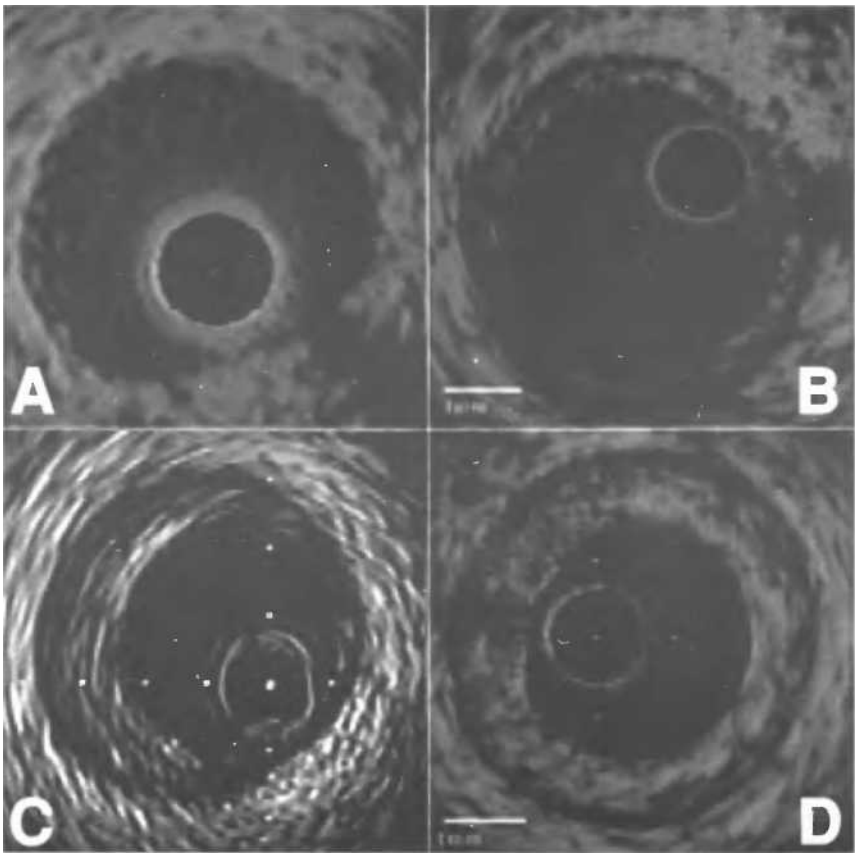


Figure 3 Patterns of intimal thickening by IVUS: **A:** no detectable intimal thickening; **B:** mild concentric intimal thickening; **C:** focal, eccentric plaque; **D:** severe concentric intimal thickening

variety of other patterns of involvement have been observed by IVUS (Figure 3). The most common finding by ultrasonography is a non-uniform eccentric lesion with the arc of involvement <180 degrees of the luminal circumference[151,155,156], although many patients have only the diffuse concentric pattern, or a combination of eccentric and concentric disease. Data from investigators at the Cleveland Clinic[155,156] have shown that the greatest percentage of eccentric intracoronary stenoses occur at vessel branch points, much like traditional atherosclerosis. These data suggest that non-immune factors, such as shear stress and flow dynamics[157], may be important in not only the pathogenesis, but also disease progression.

While the distal obliterative disease has been the hallmark of ACAD, the high proportion of focal epicardial stenoses has seemed inconsistent with the immune hypothesis. However, Botas[158] and investigators at Stanford have shown that most of the epicardial stenoses represent donor-transmitted disease which was evident at the early post-transplant study. Although these donor-transmitted lesions still represented the areas of maximal thickening at the 1-year follow-up, the greatest percentage change in intimal thickening occurred in *de-novo* sites rather than the pre-existing lesions. A multicenter observation study[159] has recently shown that the greatest percentage change in mean intimal thickness in 300 patients who underwent serial ultrasound studies from time of transplant occurred during the first year after transplant, but progression of mean intimal thickness occurred in each of the subsequent 2 years (group means). These studies[158,159], which examine patients serially to evaluate disease progression, will help to differentiate the risk factors that are directly related to host immune response from those related to pre-existing disease.

One of the commonly held beliefs about ACAD is that the development and progression of this disease is uniform throughout the coronary vessel[160]. Lin *et al.*[161] have recently reported a nearly uniform degree of intimal thickening in both proximal and distal vessels by careful autopsy analysis. However, this concept has not been supported by *in-vivo* ultrasound studies. Figure 4 shows a patient with all three forms of coronary disease, including focal eccentric epicardial disease in the most proximal segment, diffuse concentric disease in mid-vessel, and minimal if any disease in the distal vessel. In addition, there can be wide heterogeneity between vessels in the same patient (Figure 5). These findings are somewhat inconsistent with an immune-mediated injury, which should be uniform throughout the vessel.

Not only is the progression of this disease not uniform, but its development is also not inescapable. A significant number of patients have been studied with IVUS at nearly 10 years post-transplant[145], and were found to have minimal, if any, intimal thickening. There may be significant disparity between IVUS estimates of luminal compromise and that observed at autopsy, as can be seen in Figure 6 from a patient who died 2 weeks post-transplant. Pathologic specimens may potentially either *overestimate* the degree of luminal compromise by intimal thickening if they are not fixed under pressure, or *underestimate* the potential increase in total area (and secondarily luminal area) of the vessel

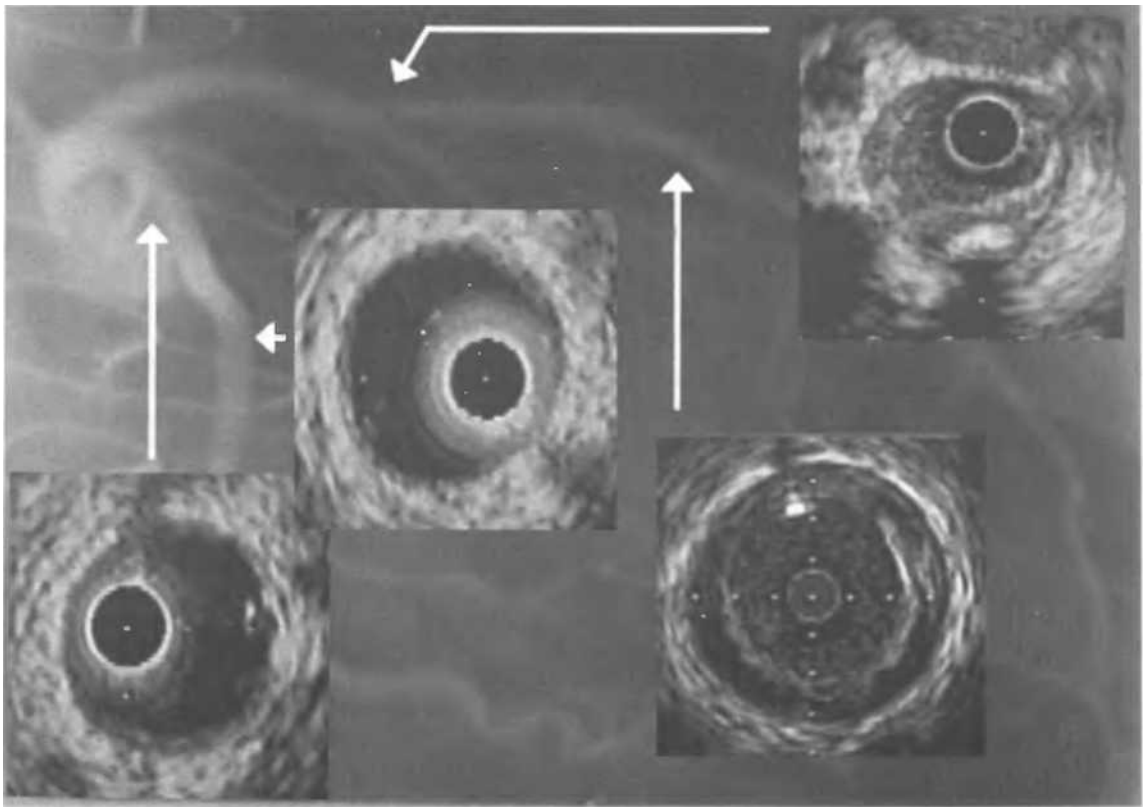


Figure 4 Heterogeneity of ACAD by angiography and IVUS between LAD and circumflex coronary arteries in the same patient

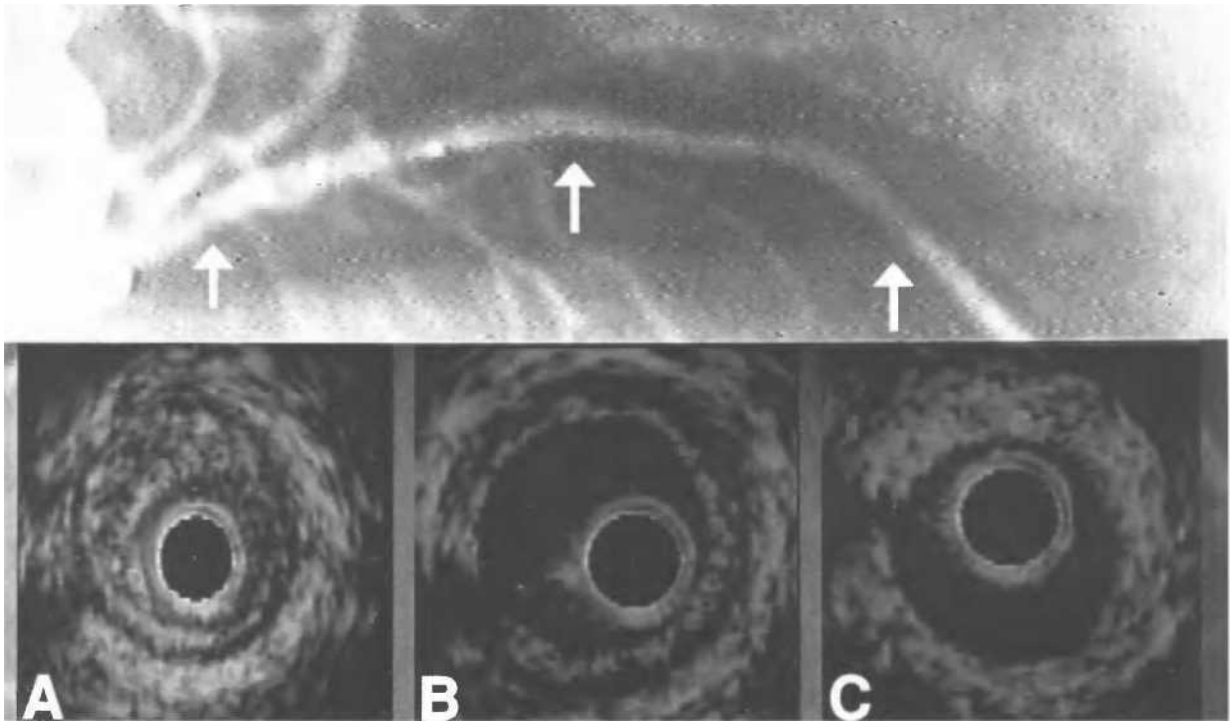
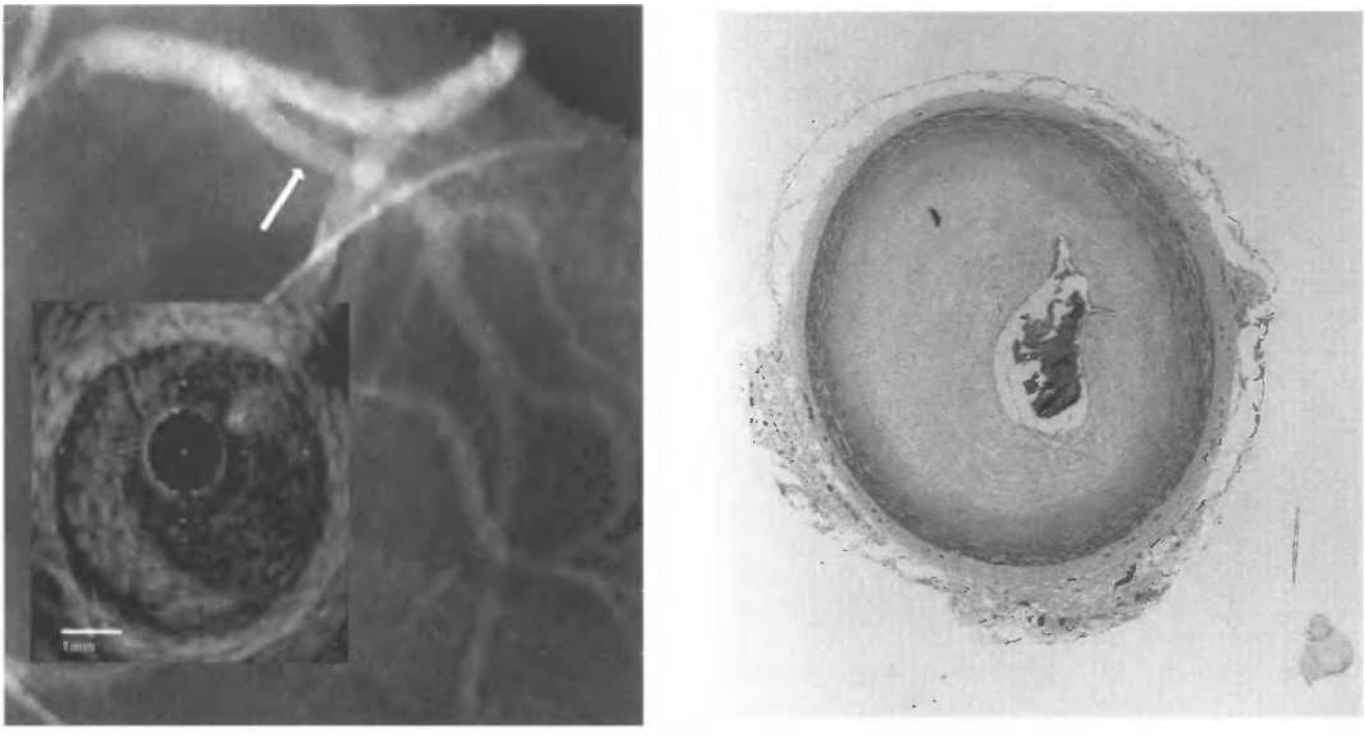


Figure 5 IVUS image showing non-uniform intimal thickening in a transplant coronary artery, **A:** proximal focal, eccentric plaque; **B:** mid-vessel concentric intimal thickening; **C:** distal vessel with minimal disease



A B

Figure 6 **A:** Coronary angiogram and corresponding intravascular ultrasound image of donor heart pre-harvest; **B:** coronary artery autopsy specimen 2 weeks post-transplant

achieved *in vivo* by remodeling and compensatory dilatation as described by Glagov[137].

Perhaps one of the most important contributions of intravascular ultrasound is the ability to detect intimal disease at an earlier stage and with greater sensitivity than by conventional contrast angiography. Recent studies have also confirmed the prognostic importance of intimal thickening detected by IVUS. Intimal thickness >500 μm has been shown to be highly correlated with the subsequent occurrence of significant clinical events (e.g. myocardial infarction or death)[162,163].

Johnson and Kobashigawa[164] recently described the use of morphometry, a mathematical analysis of the number of sites in the coronary tree needed to minimize sampling error and potential bias, maximize reproducibility of IVUS measurements, and best describe disease progression. This approach employs a withdrawal of the catheter from the distal coronary at a fixed rate with periodic random sampling, as opposed to the current standard approach, which attempts to remeasure points of maximal intimal thickening by using injections of contrast to visualize branch vessels to guide site selection and measurement[159]. The comparative advantages or accuracy of the two techniques remain to be defined.

Ultrasound will play a key role in the future as the new gold standard[165] for the assessment of the efficacy of a number of therapeutic strategies designed to alter the natural history of this disease (see below in Future Treatment). This technology will allow a new description of the true incidence of this disease, and allow a refocus on the risk factors that contribute to its progression.

Coronary flow reserve (CFR)

The presence or extent of allograft coronary disease in the distal branch vessels has been very difficult to determine. The conventional approach has been to perform side-by-side visualization of the current and baseline angiograms to detect loss of third- or fourth-order branch vessels. The development of coronary flow reserve (CFR) measurement has dramatically advanced our ability to measure the *functional* impairment in flow in the coronary resistance bed, which is the major determinant of overall coronary flow[145,166,167].

A 0.018-inch wire can be inserted to the most distal portion of each epicardial coronary artery. Figure 7 is an example of the online signal obtained through that wire that demonstrates the predominant diastolic phasic nature of coronary flow. The status of the resistance bed can be assessed by the ability to augment flow in response to a hyperemic challenge which can be induced by a number of pharmacologic agents. Our laboratory utilizes an intracoronary bolus of adenosine at a dose of 12–24 μg. Coronary flow reserve can then be calculated as the ratio of a maximum hyperemic flow to basal. Normal flow reserve has been determined in our laboratory in over 800 non-transplant and transplant patients for age, gender, and time post-transplant with normal CFR>3.0[167].

Coronary flow reserve measurements performed early post-transplant document a mild impairment which is consistent with endothelial and arteriolar dysfunction, perhaps mediated by ischemia and cold preservation as well as by immune injury, which then normalizes by the first-year anniversary study. Flow reserve appears to remain normal until at least 3–4 years post-transplant,

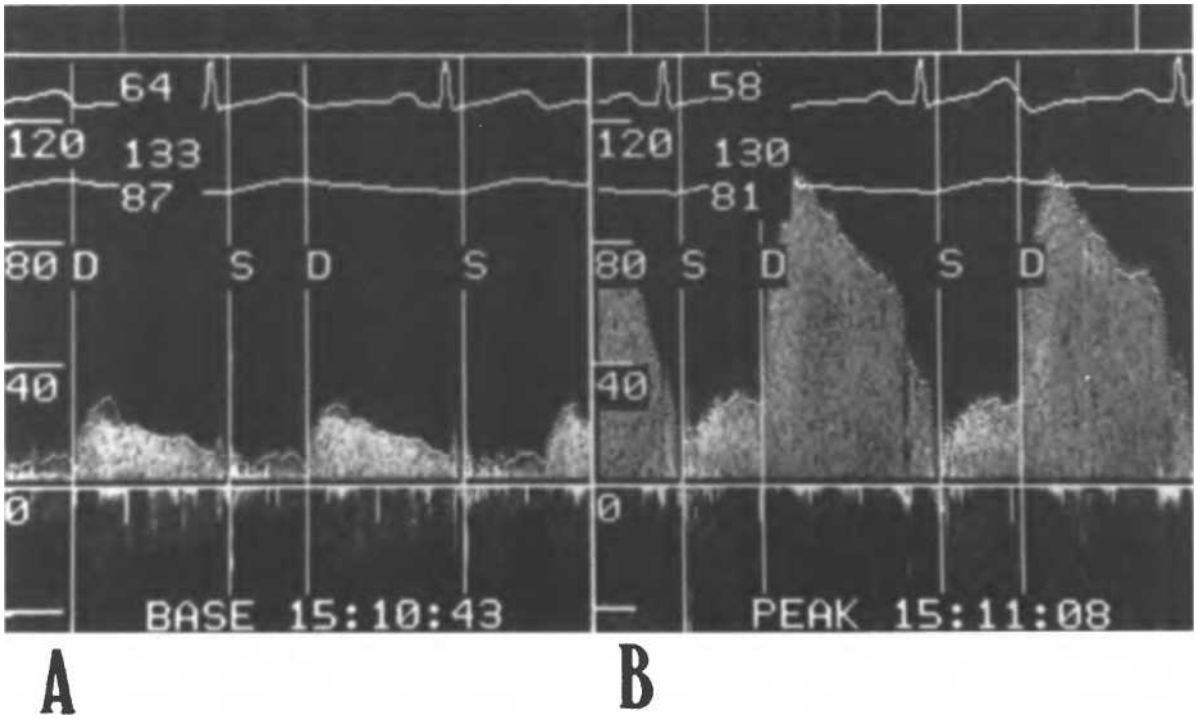


Figure 7 Real-time image from coronary Doppler flow probe in cardiac transplant patient: **A:** predominant diastolic flow, at rest; **B:** following hyperemic stimulus with adenosine. CFR = Hyperemic flow ÷ rest flow

when it begins to decline. Studies from our laboratory have demonstrated no correlation between CFR measurement with the extent of either focal or diffuse intimal thickening[168-170]. This again is in conflict with the concept of a uniform immune-mediated intimal injury and thickening.

One of the most important aspects of the measurement of CFR is the potential ability to utilize the documentation of relatively normal flow reserve in patients with severe epicardial stenoses to select ideal candidates for coronary bypass surgery as an alternative to retransplantation. Conventional bypass surgery has historically been considered not to be an option in transplant patients, because of the belief that the distal resistance 'run-off' vessels are always obliterated by the time epicardial disease is evident. Our preliminary data refute that premise. We have reported use of CFR to select a patient only 2.5 years post-transplant for coronary bypass surgery who had a normal CFR (3.2) and three-vessel epicardial ACAD. The revascularization surgery went uneventfully and resulted in an improved functional status and amelioration of nuclear scan evidence of ischemia.

Angioscopy

Ventura and colleagues[171] at the Ochsner Medical Foundation have reported use of intracoronary angioscopy to describe the topographic appearance of endothelial lesions in the coronary vessels of transplant patients. They are able to differentiate a white versus yellow plaque, with the yellow plaque consistent with the fatty streak of conventional atherosclerosis on pathologic examination, and the white plaque more consistent with immune-mediated diffuse thickening of the coronary vessel. Application of this technique is limited, but may potentially help elucidate the differential progression of pre-existing and *de-novo* disease.

Endothelial function

The endothelium represents the primary interface between the host and the allograft. As such, several investigators have examined measures of endothelial function, e.g. the response to endothelium-dependent vasodilators such as acetylcholine, as potentially the earliest manifestation of the development of ACAD[172-174]. Yeung *et al.*[175] originally reported a paradoxic vasoconstrictor response to high-dose acetylcholine in patients early post-transplant, that normalized by the 1-year anniversary study. Endothelial dysfunction has been shown to correlate with subsequent development of coronary artery disease, but there is growing controversy over the sensitivity and specificity of both endothelium-dependent and independent vasodilator responses in a given segment of a coronary artery. Other agents, such as Substance P, may be used to assess endothelial function, but their use remains a research application only.

TREATMENT OF ALLOGRAFT CORONARY DISEASE

The treatment of ACAD, once it is diagnosed, has been primarily preventive and based largely on extrapolation of the risk factor reduction approach used in patients with non-transplant atherosclerosis, specifically aspirin, exercise, lipid reduction, avoidance of smoking, and treatment of hypertension[176,177].

There is growing evidence to suggest that lipid reduction may also be one of the most important interventions in transplant recipients[178,179]. Kobashigawa[180] has shown that, if lipid therapy is not initiated until more than 1 year after transplant, it is unable to alter the subsequent progression of ACAD. These data suggest that lipid incorporation is an early post-transplant phenomenon, perhaps in association with the documented endothelial inflammation and injury that occur early in most patients. Kobashigawa *et al.*[181] have subsequently shown that use of pravachol from the time of transplant can, in fact, decrease the degree of intimal thickening, possibly due to an effect on natural killer cells as well as cholesterol levels. The specific lipid fractions to target also include serum triglycerides, which have correlated more strongly with the development of ACAD than have total, or subfractions of, cholesterol[182]. If the HMG-CoA reductase agent fails to normalize triglycerides effectively, specific therapy such as lopid or niacin may be warranted. Caution should be exercised with the combination of a 'statin' drug, most particularly mevacor, and lopid, due to the reported incidence of rhabdomyolysis with statin drugs alone, but particularly in combination with lopid.

Aspirin has been shown to reduce platelet aggregation and to be associated with a significant reduction in clinical events in non-transplant patients with coronary disease and unstable angina[183,184]. It has also become a mainstay for nearly all transplant patients, despite data indicating that it is of no benefit in transplant patients[185,186]. The dose of aspirin required remains controversial. French investigators[187] have shown that platelet aggregation is significantly abnormal in heart transplant patients, perhaps related to cyclosporin[188], and that the dose of aspirin required is at least twice that in non-transplant patients with atherosclerosis (500 mg vs 250 mg), a fact which may explain the negative results of previous studies of aspirin in heart transplant patients.

Recently, preliminary results of a trial at Stanford University have shown that the calcium-channel blocker, diltiazem, is associated with a significant decrease in intimal thickening compared to control patients[189]. Calcium-channel blockers have anti-atherosclerotic[190-192] and potentially immunosuppressive properties[193], and may be an ideal first-line therapy for the treatment of hypertension, as well as empiric preventive strategy for the development of coronary disease in high-risk patients. In addition, newer agents such as fish oil[194-196], antioxidants[197], ACE inhibitors[198,199] and, potentially, estrogen[200], which have been shown to reduce the development of atherosclerosis, may be future forms of preventive treatment for ACAD.

One of the most promising new strategies is the use of the drug angiopeptin[201], which has antiproliferative properties by virtue of its ability to inhibit insulin-like growth factor, a potent stimulant of smooth muscle cell growth. Preliminary results of a trial in Germany[202,203] have shown that it significantly inhibited intimal thickening in heart transplant patients as assessed by IVUS. Finally, almost all new immunosuppressive agents will have to demonstrate some ability to inhibit smooth muscle cell proliferation in addition to inhibiting rejection. Current examples of this combined ability included mycophenolate mofetil[204] and rapamycin[205,206].

Retransplantation

Traditionally, the only definitive form of therapy for allograft coronary disease has been retransplantation. However, the overall

results of retransplantation have been very discouraging due to poor overall survival and redevelopment of the disease in the second heart[207] (Chapter 43). Results, however, are apparently best for patients beyond 4 years post-transplant who undergo re-transplantation for ACAD[208]. In addition, the significant increase in the number of patients on active heart transplant waiting lists has further diminished the enthusiasm of most centers in offering retransplantation to many patients.

PTCA

Recently, a number of single[209] and multicenter[210,211] trials have been reported regarding the use of PTCA or arthrectomy in cardiac transplant patients with coronary disease. These studies suggest that the response rate in transplant patients is highly variable, but very similar to non-transplant patients with coronary disease, in which a restenosis rate of at least 30–35% can be anticipated for dilatation of a single lesion. However, in comparison to the Stanford data on patients with a luminal diameter stenosis of >70% without intervention[17], angioplasty would seem to potentially improve survival and outcome over the first year following angioplasty[212]. Clearly, a number of patients have had excellent long-term results from a single- or two-vessel dilatation, but the response is quite variable as the disease may be evident in another vessel on follow-up study, and all rates and types of progression have been described.

Coronary artery bypass surgery

Historically, conventional coronary artery bypass surgery has been considered to have no application in transplant patients because of the concept that patients had obliterative disease in the distal vessels once epicardial disease was evident[213–215]. This concept may no longer be valid and bypass surgery may become an alternative for patients, particularly with the very accelerated form of the disease. The use of coronary flow reserve (described above) may identify patients with a functionally intact distal vascular run-off bed who might do well with conventional bypass surgery. If so, coronary artery bypass would certainly be a better option for patients who are not amenable to angioplasty, either because of the high number of focal stenoses or where there is proximal severity (where bypass surgery would be a preferable option in non-transplant patients)[216].

Transmyocardial laser revascularization

The newest form of treatment for ACAD is the use of transmyocardial laser revascularization[217–219]. Although spurned by many skeptics, who do not support the basic premise of the possibility of the perfusion of the myocardium from the endocardium outward[218], this technique employs a limited anterior thoracotomy or median sternotomy to expose the heart without need for cardiopulmonary bypass support. A series of up to 40 holes is placed from the epicardium through into the left ventricular chamber by use of a CO_2 laser. This energy source can be controlled, and the blood in the chamber allows dispersion of the energy source, preventing injury on the contralateral side. The laser is able to achieve a burn that does not cause diffuse thermal injury to the myocardium, but allows enough scarring to keep the channels open.

Nuclear studies in animals, using separate isotopes to identify direct antegrade coronary perfusion from retrograde blood flow originating in the LV cavity, have, in fact, shown a greater degree of myocardial perfusion from endocardial to epicardial flow through these channels after laser revascularization. March[219] has recently reported on an experience in non-transplant patients (who were considered too ill for conventional revascularization) who have done well, with improved ventricular function and anginal control on follow-up. Clearly, this procedure, which causes limited morbidity, may be a superior option for a number of transplant patients with diffuse coronary disease, particularly if associated with ventricular dysfunction.

PROPHYLAXIS

Finally, strategies need to be evaluated for potential non-pharmacologic prophylaxis of ACAD. This may include such strategies as prospective HLA-DR matching, if preservation time can be extended, to allow better HLA-DR matching of donor and recipient, thereby potentially reducing the incidence of acute rejection. In addition, newer therapies, such as photochemotherapy[220,221], which has been shown in animal models to inhibit arterial smooth muscle proliferation as well as diminish development of donor-specific endothelial antibody formation in patients, may be particularly effective in high-risk patients, such as highly sensitized patients or those who develop vascular rejection following transplantation. Similarly, prospective matching by CMV serologies may potentially decrease the likelihood of infection with this agent, and acceleration of the development of this disease. The most futuristic approach is gene therapy[222], which is being explored as a means of deleting or inserting critical elements of the response to this disease.

COMMENT

There has been, and continues to be, a great deal of progress in the last several years, not only with our ability to diagnose ACAD and begin to understand its pathogenesis, but also to develop and begin trials of new therapies to alter the natural history of the disease, which remains the greatest obstacle to long-term survival following heart transplantation.

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Cardiac Retransplantation – Indications and Results

S. TANIGUCHI AND D.K.C. COOPER

INTRODUCTION

When allograft failure or severe dysfunction of an orthotopically or heterotopically transplanted heart occurs, replacement of the heart may be indicated and can substantially extend patient survival. There are surprisingly few reported data on the indications for retransplantation, or on the complications and results of this procedure[1–4].

Cardiac retransplantation in a patient with a previous orthotopic heart transplantation was first performed at Stanford Medical Center as early as 1968[1]. The first such intervention after heterotopic heart transplantation took place in Cape Town in 1980[5] (Chapter 37). This present chapter is confined to a discussion of the indications and results of retransplantation after orthotopic heart transplantation. A similar discussion in regard to retransplantation after heterotopic heart transplantation is included in Chapter 37.

The introduction of cyclosporin (CsA), though undoubtedly reducing the incidence of severe acute rejection episodes, does not as yet appear to have prevented the development of graft arteriosclerosis, which today is the main indication for retransplantation. The demands made by the need for retransplantation may significantly reduce the number of donor hearts available to newly selected potential recipients, providing the medical team with an ethical dilemma.

As patient mortality is significantly higher after retransplantation when compared with primary transplants, retransplantation must be considered particularly carefully in each individual case. Each donor heart, a valuable and scarce commodity, must be utilized as effectively as possible. Retransplantation should therefore be performed only in carefully chosen recipients where the prospect of a successful outcome is high.

INDICATIONS FOR RETRANSPLANTATION

Retransplantation should be considered in any patient in whom the cardiac allograft undergoes failure or severe dysfunction from acute and/or chronic rejection. Retransplantation has also been performed on occasion for intractable arrhythmias of the donor heart, and for acute donor right ventricular failure due to an excessive pulmonary vascular resistance following orthotopic transplantation[1]; refinements of criteria for selection of both recipients and donors, and the use of such agents as prostaglandin E1 (PGE1) to reduce pulmonary vascular resistance, have made these indications for retransplantation rare. Early donor heart failure (primary allograft dysfunction) from causes other than acute rejection accounts for a significant proportion of the deaths of patients undergoing transplantation (Chapter 43); if a second donor heart can be obtained in time, or the patient can be maintained by prolonged pump–oxygenator support[6–8] or by an artificial heart or a mechanical assist device (Chapter 21), retransplantation may be lifesaving. Jurmann *et al.*[8] reported a patient who underwent retransplantation after 'bridging' with an intra-aortic balloon pump and right ventricular assist device for 14 hours after early primary graft failure. Morris *et al.*[7] described successful support by extracorporeal membrane oxygenation (ECMO) for 22 hours between immediate graft failure of a primary transplant and successful retransplantation.

Intractable acute rejection of an orthotopic graft, though relatively rare today, is clearly an urgent indication for retransplantation. These patients, however, do particularly poorly after retransplantation, almost certainly because they have frequently been very heavily immunosuppressed for the previous few days, or even weeks, in an attempt to retain the initial allograft. They are therefore at high risk for infection in the early post-retransplant period. Furthermore, as retransplantation is frequently required urgently, there is probably a tendency to accept a less-than-perfect donor heart in such circumstances. The risk of early graft dysfunction and failure is therefore increased.

There remains some controversy over the decision to perform retransplantation as an emergency for primary graft failure or intractable acute rejection. In view of the reduced success of heart transplantation under such circumstances, there are those who believe that acute cardiac retransplantation is not justified while there remains a critical shortage of donor organs[9].

The decision to retransplant a patient undergoing chronic rejection (graft arteriosclerosis) and, in particular, the timing of the procedure, may be difficult, as the patient's general condition may

remain good, despite evidence of increasing coronary arteriosclerotic changes. Both surgeon and patient may be reluctant to undertake retransplantation whilst the patient remains asymptomatic; delay, however, may result in sudden death from major myocardial infarction or dysrhythmia. Alternatively, the patient's general condition may have deteriorated from chronic infection or other complications of long-term immunosuppression, creating doubt as to his or her suitability for retransplantation.

Even with regard to primary graft failure for graft arteriosclerosis, some physicians and surgeons believe that retransplantation should not be allowed in view of concerns regarding: (a) the lower survival rate when compared with primary heart transplantation, (b) the less impressive cost–benefit ratio, and (c) the equitable allocation of scarce donor hearts[10,11]. Our own policy has been to offer retransplantation only to those patients in whom we believe the likelihood of successful short- and long-term outcomes is high, and is approximately equivalent to that in a patient undergoing a primary transplant. We have therefore excluded patients in whom primary graft failure, intractable rejection, or graft arteriosclerosis have already resulted in significant complications, such as failure of other essential organs or life-threatening infection. In the small group of carefully selected patients who have undergone retransplantation at our center, for whatever reason, the results have been comparable to those following primary transplantation (unpublished data).

SELECTION OF PATIENTS FOR RETRANSPLANTATION

All of the criteria for transplantation should be reassessed before retransplantation is performed (Chapters 5 and 19), since significant changes may have occurred since the patient was assessed initially. In particular, the increased immunosuppression that is necessary during acute rejection episodes, or the prolonged immunosuppression in patients who have survived long enough to develop graft arteriosclerosis, may have resulted in foci of infection. If possible these infections must be eradicated or suppressed before retransplantation is undertaken. If it is not possible to eradicate a significant focus of infection, then the patient may not be suitable for retransplantation.

Some degree of renal dysfunction may well have developed from prolonged CsA therapy and/or acute or chronic cardiac rejection (leading to impaired cardiac function) by the time retransplantation is considered. Careful assessment is required to ensure at least that the patient's renal function or reserve is sufficient to get him or her through the difficult early post-retransplant period when CsA dosage will of necessity have to be higher.

Such decisions are sometimes difficult, as the relative contributions to renal dysfunction of CsA and cardiac insufficiency may be difficult to distinguish.

The presence of such complications of immunosuppression as lymphoproliferative disease, even if currently controlled by a reduced immunosuppressive therapeutic regimen, remains a contraindication to retransplantation as the disease is likely to recur and spread when high-dose immunosuppressive therapy is administered after the second transplant.

The patient should also be carefully reassessed from a psychological standpoint to ascertain whether he or she can cope with the stresses and strains of a further transplant procedure. Particular attention should be paid to the patient's compliance with medical guidance, and his or her adherence to therapy during the course of the first transplant. Retransplantation may be inadvisable if non-compliance had contributed toward failure of the initial allograft.

Meticulous testing of the recipient for the presence of lymphocytotoxic antibodies must be carried out, and antibodies against any new potential donor excluded. Antibody formation occurs to a greater or lesser degree in many patients who have received cardiac allografts with or without blood transfusions, and may preclude the use of certain donors for retransplantation[12] (Chapter 6).

According to an investigation reported by Ensley *et al.*[13], based on data from 449 recipients of second allografts reported to the Registry of the International Society for Heart and Lung Transplantation, together with a further 125 repeat transplants at 13 transplant centers in the United States, the 'ideal candidate' for a second transplant is a patient with: (a) a long interval since the first transplant, (b) accelerated coronary artery disease as the cause of allograft loss, and (c) an absence of current mechanical assistance.

TIMING OF RETRANSPLANTATION

In primary allograft dysfunction or intractable acute rejection

Primary allograft dysfunction or failure unfortunately remains a significant complication in a small percentage of the heart transplants performed today. In contrast, with the immunosuppressive agents currently available, intractable acute rejection is a relatively rare occurrence. When graft failure from either of these causes develops in a patient with an orthotopic transplant, this, of course, constitutes a surgical emergency. Either retransplantation must be carried out as an emergency, or the patient must be assisted by some form of mechanical assist device. Without such mechanical assistance a second donor must be found within hours or a day or two, or death of the patient will occur.

In advanced chronic rejection

As with acute rejection, complete failure of an orthotopic allograft from chronic rejection results in the death of the patient; it is usually clear, however, that chronic rejection is occurring, and time is available to plan the retransplant procedure.

The timing of retransplantation may prove difficult, therefore, in patients with orthotopic grafts in whom chronic rejection is occurring. The decision to retransplant must not be delayed until graft function becomes totally inadequate; on the other hand, retransplantation should not be undertaken until absolutely essential. The exact timing is influenced by many factors, notably the ease or difficulty with which a suitable donor will be obtained; for example, if the patient has a high level of circulating lymphocytotoxic antibodies, some considerable delay may occur in obtaining a suitable donor, and the search should begin earlier rather than later.

The policy of the Stanford group has been to offer retransplantation to patients with evidence on coronary arteriography of life-

threatening occlusive lesions in the major coronary arteries, irrespective of the patient's exercise tolerance[2]. This policy evolved following the sudden death of three long-term survivors with such lesions. Our own policy has been possibly rather less aggressive, as we have rarely seen sudden death in an otherwise asymptomatic patient. We have generally waited until exercise tolerance has significantly deteriorated, and coronary arteriography and/or thallium scanning has confirmed advanced disease.

The rate of development and progression of graft arteriosclerosis is extremely variable. In the pre-CsA era we have seen advanced disease as early as 3 months, yet no disease as late as 13 years, after transplantation. The development of even moderately advanced disease may be compatible in many cases with an acceptable quality of life for several further months or even years. In one of our patients a 50% stenosis of the right coronary artery was demonstrated 6 years before death; this progressed to complete occlusion of this vessel at its origin and widespread disease of the left coronary system over the next 4 years. Some 2 years later the patient finally succumbed to the disease, retransplantation having been contraindicated on other grounds[14,15].

RETRANSPLANTATION – OPERATIVE CONSIDERATIONS

It is our present policy in all cases of retransplantation to prepare the femoral artery and vein. This allows initiation of pump–oxygenator support through this route if cardiac function deteriorates before median sternotomy is performed. In all cases inotropic agents should be available during induction of anesthesia. Very rarely, in a patient with particularly poor cardiac function who is hemodynamically very unstable, initiation of cardiopulmonary bypass by the femoral route (or at least preparation of the femoral vessels) under local anesthesia may be advisable before induction of general anesthesia.

Following encouraging reports of reduced blood loss following the use of aprotinin[16,17], more recently it has been our policy to utilize aprotinin in all patients undergoing retransplantation (or initial transplantation after previous open-heart surgery). Although our experience to date is small, our impression is that the use of aprotinin during the operative procedure has been associated with reduced blood loss, which is in agreement with others.

The operation of retransplantation in a patient with an existing orthotopic allograft presents the same technical problems and risks of reoperation as in any patient who has previously undergone cardiac surgery. Adhesions have invariably developed between pericardium and heart, frequently making the initial dissection time-consuming. As myocardial function in these patients is poor, particular care must be taken not to handle or disturb the heart more than is absolutely essential before pump–oxygenator support has been initiated; for this reason it may be necessary to resort at some stage during the dissection to the use of the femoral vessels to commence cardiopulmonary bypass.

After excision of the first donor heart, which can usually be achieved through or close to the old suture lines, the second heart is inserted as described in Chapter 24.

POSTOPERATIVE CARE AND IMMUNOSUPPRESSION

The immediate postoperative care of patients who have undergone retransplantation does not differ significantly from that following the initial procedure (Chapter 26). If retransplantation is performed during or immediately following an irreversible acute rejection episode, care must be taken not to over-immunosuppress the patient, as he or she will almost certainly already have received a considerable amount of immunosuppressive therapy; preoperative 'loading' doses of the various drugs will probably be unnecessary.

RESULTS OF RETRANSPLANTATION

The overall results of retransplantation as reported through the Registry of the International Society for Heart and Lung Transplantation (Chapter 43) and the Collaborative Heart Transplant Study (Chapter 44) are significantly inferior to those following primary heart transplantation. One- and 5-year survivals are approximately 50% and 30% following retransplantation compared with approximately 80% and 70% after primary transplantation. Most of this increased mortality, however, is during the early part of the first post-transplant year. If the retransplant patient survives 3 months, he or she has a comparable survival to the patient undergoing heart transplantation for the first time.

There are relatively few reports of series of patients undergoing retransplantation[4,13,18–20].

Between January 1968 and March 1980, 202 hearts were transplanted in 185 patients at Stanford University Medical Center. Sixteen patients with orthotopic allografts received second transplants, eight for accelerated arteriosclerotic coronary disease, six for unrelenting acute rejection, and two for dysrhythmia or right ventricular failure[4]. One patient required a third transplant because of donor left ventricular ischemia. All sequential transplants were managed similarly to the primary transplant.

Of the 16 initial transplant hearts at risk, 60% functioned for more than 1 year, and 57% for more than 2 years; these results were similar at that time to heart survival in patients not requiring retransplantation. Of the secondary transplant hearts at risk, however, only 31% survived for more than 1 year and 29% for more than 2 years, survival of approximately only 50% when compared with the primary group. The mortality was due largely to severe infection and the development of malignant tumors.

Infection of the secondary transplant (retransplant) patient appeared to play a more dominant role in fatality. The patients in this group were suspected to be initially free of infection in spite of primary allograft immunotherapy. The number and type of infections, however, were not substantially different from those in the group undergoing a primary transplant. Prolonged periods of immunosuppression during the perioperative period of the secondary transplant exposed these patients to this complication.

The Stanford group concluded that sequential orthotopic cardiac transplantation offers an acceptable alternative to patients with allograft failure, though survival was not as favorable because of the prolonged immunosuppression required.

Between December 1980, when immunosuppression with CsA was introduced, and May 1988, 288 patients underwent primary heart transplantation at Stanford University Medical Center[20].

During this period, 23 patients with orthotopic allografts received second transplants, 14 for accelerated graft atherosclerosis and nine for intractable allograft rejection. Four patients had received conventional immunosuppressive therapy (consisting of azathioprine, corticosteroids, and antilymphocyte globulin) after the primary allograft procedure. All other patients received CsA as part of the immunosuppressive regimen after transplantation of both the first and second allografts. Actuarial survival following primary transplantation (81 ± 2% at 1 year and 58% ± 4% at 5 years) was significantly better than that following retransplantation for intractable rejection (44% ± 17% at 1 year and 44% ± 0% at 5 years) ($p<0.05$). Cardiac retransplantation for acute rejection was associated with rejection and infection rates similar to those following the primary procedure. However, patients who underwent retransplantation for rejection survived these complications significantly less often than did patients who received primary transplants. Patients who underwent retransplantation for accelerated graft atherosclerosis experienced a lower rate of early rejection and similar rates of infection and survival compared with patients who received primary transplants.

Ensley *et al.*[13,19] analyzed data from 449 recipients of second allografts reported to the Registry of the International Society for Heart and Lung Transplantation and a matched group of 421 primary transplant recipients. Survival was markedly decreased in repeat transplantation patients with a 1-year actuarial survival of only 48% compared to 79% in the primary transplant group ($p<0.001$). Univariate analysis showed no impact on survival of recipient age or gender, ischemic time, or transplant center experience. Accelerated coronary artery disease as the cause of allograft failure, a longer interval between transplants, lack of preoperative mechanical assistance, and a second transplant performed after 1985 were predictive of increased survival following retransplantation. However, an 'ideal candidate', defined by these predictive variables, still had a 1-year survival rate of only 64%, which was not as good as in the matched primary transplant group.

Furthermore, a multicenter database was developed using data from 125 repeat transplant patients and 1325 primary transplants at 13 centers in the USA. In this group of retransplant patients the 1-year survival rate was greater than that reported in the International Society for Heart and Lung Transplantation Registry (60% vs 48%), and the impact of the predictive variables listed previously was decreased. The incidences of rejection, infection, and accelerated coronary artery disease were not different between the secondary and primary allograft recipients. Malignancies (other than skin cancer) occurred more frequently in repeat transplant patients (8% vs 4%; $p<0.05$). Recipients of second allografts were more likely to have major surgical complications, had a higher level of sensitization to HLA antigens, and were more likely to have a positive donor-specific lymphocytotoxic crossmatch (17% vs 2%). A trend towards improved survival was noted in patients with repetition in the second donor of mismatched HLA antigens present in the first donor (1-year survival rate of 68% vs 47%; $p=0.06$).

Michler *et al.*[16] reviewed 13 patients who had undergone retransplantation, including one patient who had received a third graft. Immunosuppression and follow-up protocols used in this cohort were similar to those in the primary transplant population at that center. No significant difference was observed in actuarial survival between primary transplant recipients (75.1% ± 2.2% at 1 year and 71.3% ± 2.4% at 2 years) and patients who underwent retransplantation (71.4% ± 12.1% at 1 year and 59.5% ± 14.8% at 2 years). Similarly, no differences were observed with regard to age, sex, race, cause of end-stage heart disease, or early (<30 days) mortality. The cause of primary graft failure did not correlate with survival outcome in the retransplantation cohort. Approximately 50% of patients in both groups experienced at least one rejection episode by 3 months. Within the limited time period of this study only one patient developed transplant coronary artery disease (approximately 27 months after retransplantation for acute rejection). The authors concluded that the prognosis for patients undergoing cardiac retransplantation was good if the indication for retransplantation was identified >30 days after the initial transplant procedure.

COMMENT

In summary, retransplantation should be reserved for very carefully selected patients, particularly those with graft arteriosclerosis in whom assessment of the patient and the retransplant procedure can be carried out electively without undue haste. Although it should be considered as a therapeutic option, it is probably least successful when performed as an emergency in patients with primary allograft failure from donor heart dysfunction or intractable acute rejection (humoral or cellular). The decision to offer retransplantation should always be made with due consideration for the needs of other patients awaiting primary heart transplants in light of the increasing shortage of donor organs.

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Heterotopic Heart Transplantation – Indications, Surgical Techniques and Special Considerations

D.K.C. COOPER AND S. TANIGUCHI

INTRODUCTION

Heterotopic heart transplantation (HHT) has a long history in the experimental laboratory[1] (Chapter 18). Early experimental work was mainly by the Russian surgeon Demikhov[2], and subsequently by others[3–6], but it was Barnard and Losman's techniques of implanting an auxiliary heart in the chest in 1974[7–9] that led to the initiation of a clinical program. The surgical technique of biventricular assist (Figure 1) is described below. The technique of left ventricular assist alone (Figure 2) is now less commonly performed, but can be associated with excellent long-term survival[11].

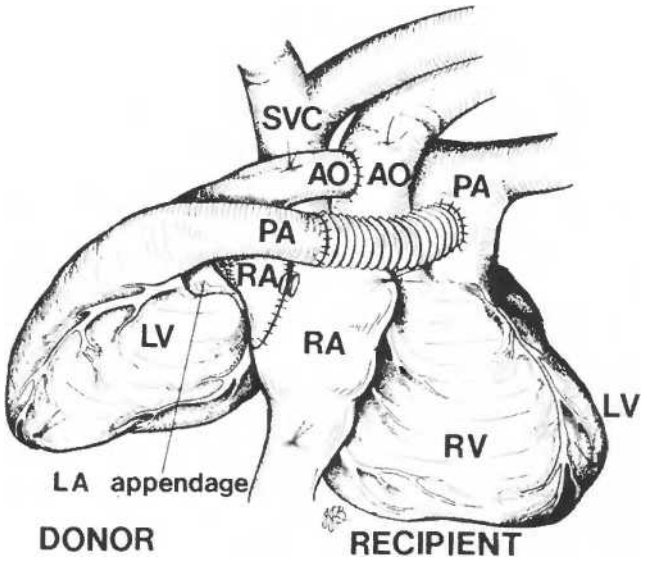

Figure 1 The completed operation of biventricular assist using a heterotopic heart transplant. (Arterial and venous cannulae have been removed.) (Abbreviations used in figures in this chapter are: LA = left atrium; RA = right atrium; SVC = superior vena cava; IVC = inferior vena cava; PV = pulmonary vein; RV = right ventricle; PA = pulmonary artery; AO = aorta; LV = left ventricle; CS = coronary sinus)

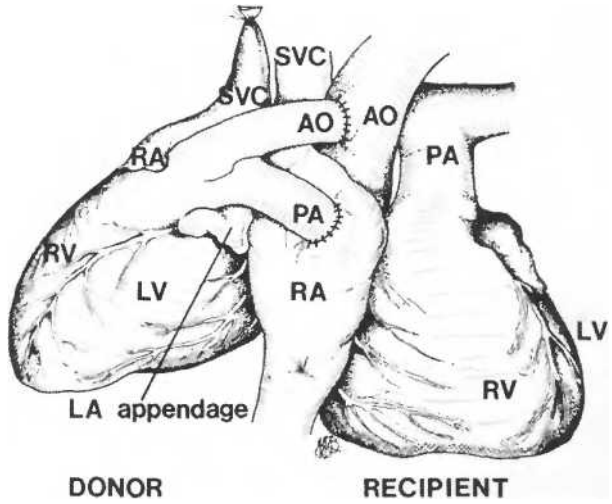

Figure 2 The completed operation of left ventricular assist using a heterotopic heart transplant. Anastomoses are performed between the donor and recipient left atria and aortae. Donor coronary venous return drains by the donor right atrium, right ventricle, and pulmonary artery into the recipient right atrium

Based on extensive experimental work by Losman and Barnard[8], a form of HHT was carried out in 1974 whereby the donor heart acted solely as a left ventricular assist device[7]. This operation (Figure 2) involved anastomoses between the donor and recipient left atria and aortae; donor coronary sinus venous return was drained via the donor right atrium and right ventricle into the recipient circulation by anastomosing the donor pulmonary artery to the recipient right atrium. Two such operations were performed. Both patients suffered recurrent attacks of native heart dysrhythmias, including ventricular fibrillation, during which

time the donor heart supported the circulation alone, though with significant loss of blood pressure[10]. Despite this, one patient survived for almost 10 years[11]. As a result of this experience, however, the technique was modified to allow bypass and support of both recipient ventricles[7,9]. Today HHT has relatively few specific indications, and is performed only under special circumstances[12].

The implantation of a natural auxiliary heart using the technique of HHT may be on a permanent basis, or may occasionally be temporary as a means of support while recovery of the patient's own heart is awaited, or a more definitive procedure is planned (as will be outlined later). Considerable clinical experience has now been amassed with auxiliary hearts implanted on a permanent basis; all such hearts have to date been allografts. Clinical experience of temporary support by an auxiliary heart has been small, and has largely been in the realm of xenotransplantation (Chapter 82). It is feasible that an animal heart may be utilized in the heterotopic position to provide a bridge for an infant or child in cardiac failure who is awaiting a suitable human heart, and for whom a suitable left ventricular assist device is not available.

(Techniques for utilizing the *left* ventricle of a heterotopically placed heart to support a failing *right* ventricle of the native heart (for example in certain complex congenital cardiac deformities[13] or in patients with pulmonary hypertension[14]) have been described but, to our knowledge, have not been utilized in clinical practice, and will not be discussed further here.)

HHT has both advantages and disadvantages when compared with orthotopic heart transplantation (OHT) (Table 1)[15,16], which have been documented fully previously[17–19]. In particular, the heterotopic procedure may allow recipient survival despite temporary or permanent loss of donor heart function following acute or chronic rejection (Figure 3); the circulation may be maintained by the recipient's own heart for at least a period of time. This advantage was of considerable practical importance in the precyclosporin era when irreversible rejection was not uncommon. In several patients the circulation was maintained by the recipient native heart until retransplantation could be performed. Indeed, this was one of the reasons that influenced Barnard to explore the technique. However, the efficiency of current immunosuppressive agents in preventing irreversible acute rejection has greatly diminished this advantage.

Nevertheless, HHT still has a role in certain specific conditions.

Indications

1. Whenever there is any possibility of recovery of the recipient's own myocardium, e.g. in acute myocarditis (of viral or rheumatic origin), then HHT should be considered. Unfortunately, the number of cases where recovery has been documented remains small[20,21].

2. When there is any possibility that initial donor heart function will be less than adequate to maintain the circulation alone, HHT may allow successful transplantation. This could be expected most commonly when there is a large discrepancy in body mass (>33%) between recipient and donor. Although a small donor heart will eventually hypertrophy to adapt to the demands made upon it, it might fail in the early post-transplant period unless the recipient heart remains *in situ* to lend some support. In this respect, however, it should be noted that a small child's heart may be technically impossible to insert heterotopically as a biventricular assist in an adult, as it may not be possible to join the two right atria; insertion of the heart solely as a left ventricular assist would, however, be possible.

The Baylor (Houston) group[22] utilized small donor hearts in HHT in a series of large patients (body surface area >1.95 m^2). Waiting times for large patients receiving a HHT (mean 67 days) were significantly shorter than those who received an OHT (mean 166 days). Waiting time mortality decreased from 24% to 9%. There was no difference between the two groups with regard to mid-term survival or early post-transplant functional status. This is one example of how HHT can increase the size of the donor pool for larger patients, who tend to wait much longer for a donor heart than do smaller patients.

Similarly, when a donor heart has undergone a particularly long ischemic period during transportation and transplantation, or for some other reason is deemed less than ideal (and yet the recipient's cardiovascular status is deteriorating so rapidly that survival (until the next donor becomes available) is not expected), then again HHT would seem to be advisable; the support given by the recipient heart in the early transplant period may allow time for recovery of the donor heart.

Table 1 Advantages and disadvantages of heterotopic over orthotopic heart transplantation

Advantages	*Disadvantages*
1. Recipient heart acts as a built-in cardiac assist device and may maintain circulation (a) During reversible loss of donor heart function during: (i) period of recovery of donor heart from ischemia sustained during transplantation; (ii) severe acute rejection episodes (b) Following irreversible loss of donor heart function from: (i) acute, or (ii) chronic rejection (while patient awaits retransplantation) (c) During adaptation of a very small donor heart to the demands of the circulation 2. Allows for possible recovery of recipient heart, e.g. after viral myocarditis 3. Can be performed even in the presence of a high pulmonary vascular resistance if the hypertrophied recipient right ventricle can continue to support the pulmonary circulation	1. Risk of systemic emboli from thrombus in poorly contracting recipient left ventricle 2. Requires long-term anticoagulation 3. Continuing angina related to ischemic recipient myocardium (rare) 4. Risk of infection and/or thrombus formation in relation to: (a) presence of valve prosthesis in recipient heart (this is a contraindication to HHT) (b) presence of Dacron graft between donor and recipient pulmonary arteries (c) compression of lower lobe of right lung due to presence of donor heart in right pleural cavity (rare) 5. Hemodynamically significant dysrhythmias of the recipient heart requiring high doses of antiarrhythmic agents

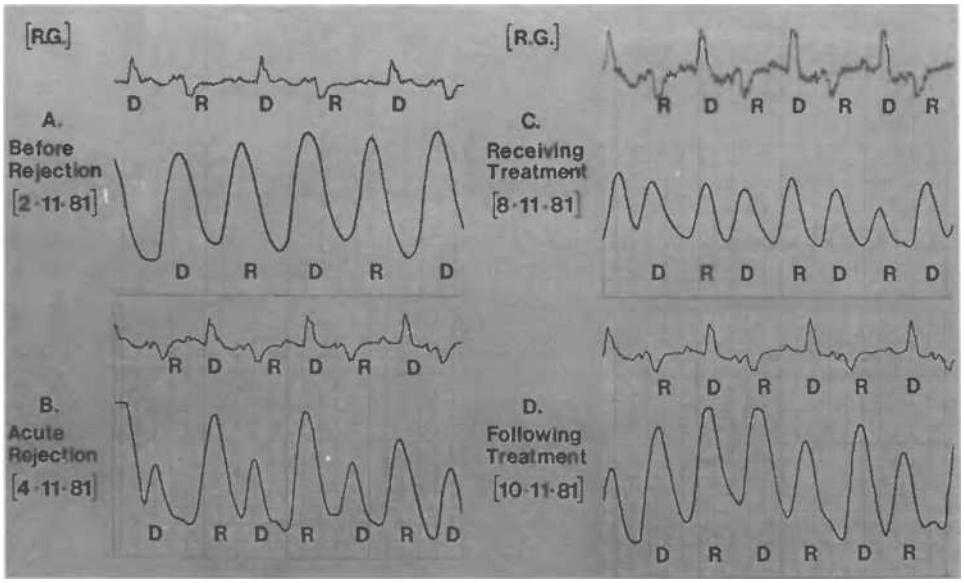

Figure 3 Diagram showing electrocardiogram (above) and femoral pulse trace (below) of donor (D) and recipient (R) hearts after heterotopic transplantation. Note the deterioration in the donor heart pulse in relation to the recipient heart pulse during an acute rejection episode, and the reversal of this trend following increased immunosuppressive therapy

Recovery of an ischemic donor heart was clearly demonstrated when two hearts in the Cape Town series were stored by hypothermic perfusion for periods of 7 and 13 h, respectively[23]. Diminished donor heart function was observed for 16–19 h until full recovery occurred; the recipient heart was invaluable in supporting the patient during this period.

3. When the patient is suffering severe anginal attacks unresponsive to full medical therapy and unrelieved (where possible) by myocardial revascularization procedures, and yet where left ventricular function continues to be good, it may on occasion be contraindicated to excise the recipient heart and perform OHT.

Disabling angina, although relatively rare today, might be considered by some to be a contraindication to HHT, as the risk of continuing angina post-transplant remains. Following HHT, however, a well-functioning transplant would greatly diminish the demands on the ischemic recipient heart, thus reducing the oxygen requirement (and the threshold of angina) and yet retaining the support given by the recipient left ventricle.

4. A fixed pulmonary vascular resistance (PVR) greater than approximately 5 Wood units (400 dyne/s per cm^{-5}) has long been considered by many a contraindication to OHT, as it was anticipated that early failure of the donor right ventricle would occur (Chapter 20). When the PVR is fixed >8 Wood units (640 dyne/s per cm^{-5}), it has generally been assumed that transplantation of the heart and both lungs should be carried out. When the PVR is fixed between 6 and 8 units, and where right ventricular failure is not severe, then HHT has been advocated. There have been few patients, however, in whom this theory has been put into practice and the results clearly documented. Furthermore, the extent of reversibility of the PVR may be difficult to ascertain absolutely before transplantation.

Reichenspurner *et al.* documented a fall in PVR from 4.9 to 2.4 Wood units in patients with HHT in Cape Town[24], and the La Pitié group in Paris confirmed a decreased PVR in the first post-transplant month in 42 patients undergoing HHT[25,26]. In the La Pitié series, however, although PVR fell significantly, post-transplant survival of patients with pretransplant pulmonary hypertension was poor, in part because of pulmonary complications and infection. A reduction in an elevated transpulmonary gradient has also been demonstrated in pediatric patients undergoing HHT using the left heart assist technique[27]. Even when a markedly elevated PVR does not reverse, the contribution of the heterotopic heart to left ventricular function can greatly improve the clinical status of the patient[28].

A statistical study by Kirklin and his colleagues from Alabama confirmed that elevated PVR is an incremental risk factor for premature death after heart transplantation[29]. The value of PVR used was that obtained closest to the time of transplantation, without specific attempts to modify it by vasodilator or inotropic agents. The rate of rise in risk of death corresponded with the progressive increase in PVR (particularly when expressed as Wood units × square meters = PVRI), rather than abruptly increasing at a certain point. Although this study indicated that there is no precise level of PVR beyond which OHT is contraindicated, there must come a point when the risk is so high that OHT is no longer advisable or, at least, an alternative surgical technique, e.g. HHT or heart–lung transplantation, is indicated. This topic is discussed fully in Chapter 20.

If significant right ventricular failure is present, secondary to irreversible pulmonary vascular disease (as opposed to being secondary to left ventricular failure alone), then right ventricular function will not improve after HHT. In doubtful cases we have found measurement of right ventricular ejection fraction by radionuclide scanning to be a valuable guide to the adequacy of right ventricular function. A right ventricular ejection fraction <20% (i.e. approximately 50% of normal) would discourage us from performing HHT in the presence of a fixed PVR >5 Wood

units and, instead, we would consider transplantation of the heart and both lungs.

The indication for HHT in relation to the PVR and/or transpulmonary gradient remains controversial, but it would seem reasonable to suggest that, in patients with left ventricular failure from ischemic or cardiomyopathic disease, HHT should be preferred whenever there is serious doubt that the right ventricle of the transplanted heart will be able to support the pulmonary circulation successfully. If the PVR were fixed >8 Wood units (or the transpulmonary gradient (TPG) fixed >15 mmHg) then heart–lung transplantation should be considered.

5. The temporary insertion of a human auxiliary heart as a 'bridge' to OHT would seem to have little place in modern cardiac transplantation. It has been clearly demonstrated that temporary support of a failing circulation can be satisfactorily provided by one of the several ventricular assist devices or artificial hearts which are currently available (Chapter 21). The use of an auxiliary (or orthotopic) non-human primate heart, however, as a bridge to transplantation would seem feasible, especially in infants and children for whom no mechanical device of suitable size is presently available. Current experimental data would suggest that a nonhuman primate heart would function satisfactorily in a human recipient for several days, and possibly weeks or months (Chapter 80). This would allow time for a suitable human heart to be located and inserted (with removal of both the non-human primate organ and the recipient's native heart, if it is still *in situ*).

Indeed, this was one possibility in two adult patients in whom auxiliary cardiac xenografts (one baboon and one chimpanzee) were implanted by Barnard and his colleagues in 1977[30]. The baboon heart proved of insufficient size to support the circulation, and, in this pre-CsA era, the chimpanzee heart was rejected on the fourth postoperative day before a suitable human donor could be found. No more recent attempts have been reported.

SURGICAL TECHNIQUES

Donor heart excision

Donor heart excision is very similar to that described previously for OHT (Chapter 24), but a greater length of superior vena cava (SVC) should be retained. The SVC is, therefore, mobilized along the whole of its length and two ligatures passed around it cranial to the azygos vein, which itself is doubly ligated and divided. Otherwise, excision is as described for OHT.

Preparation of donor heart

The heart is placed in a bowl containing saline or cardioplegic solution at 4°C, where it is prepared for implantation into the recipient (Figure 4).

The orifices of both right pulmonary veins and of the inferior vena cava (IVC) are closed with continuous, double-layered sutures of 5/0 polypropylene, care being taken to ensure that coronary sinus drainage is not obstructed during closure of the IVC.

The bridge of tissue between the left superior and inferior pulmonary veins is excised to make a single opening into the left atrium; this opening may need to be extended to achieve a diameter of approximately 3.5–4.0 cm or the equivalent of a normal mitral valve orifice. The midpoint of the posterior wall of this opening may be marked with a suture to act as a reference during subsequent implantation into the recipient.

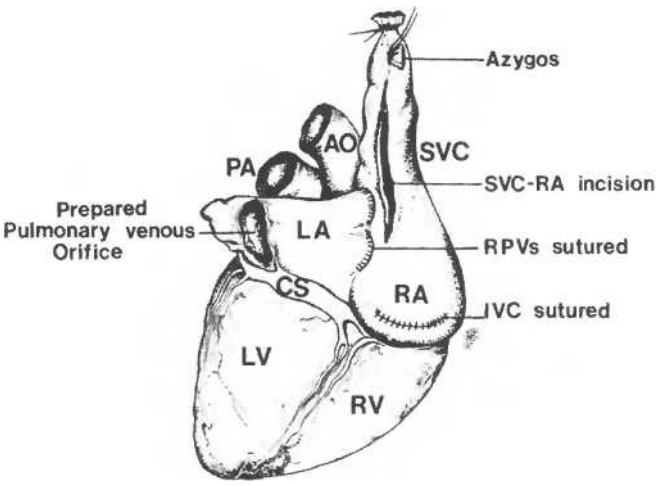

Figure 4 Donor heart (posterior view) prepared for implantation

A longitudinal incision is made in the posterior aspect of the SVC, beginning immediately caudal to the ligated azygos vein, and extended down just to the right of the interatrial septum into the right atrium. At least half the length of this 5 cm incision must involve the right atrial wall. It is essential to ensure that the incision is posteriorly sited in order to avoid injury to the sinoatrial node.

Approximately 10 min are required to prepare the heart in this way. The organ is then transferred to the recipient surgical team.

The recipient operation

With the patient supine, a midline sternotomy is performed and the pericardium opened longitudinally. A right-sided pleuropericardial flap is created (Figure 5), first by dividing the mediastinal

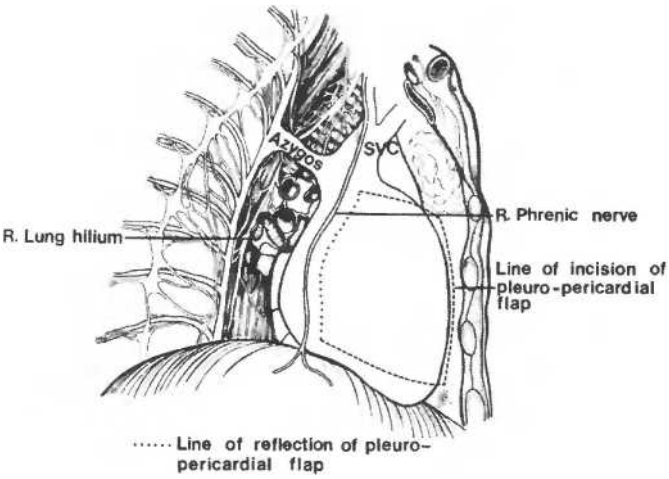

Figure 5 Recipient; right-sided view of mediastinum; the line of the pleuropericardial incision is indicated

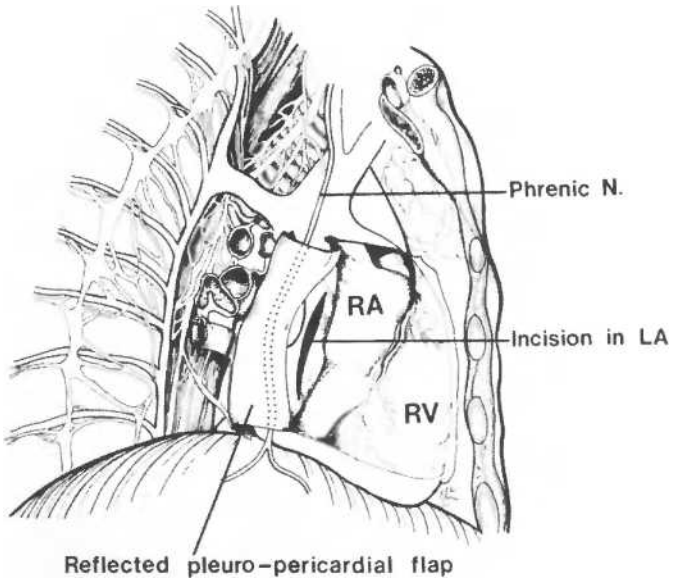

Figure 6 Recipient; reflection of pleuropericardial flap to lie anterior to the hilum of the right lung

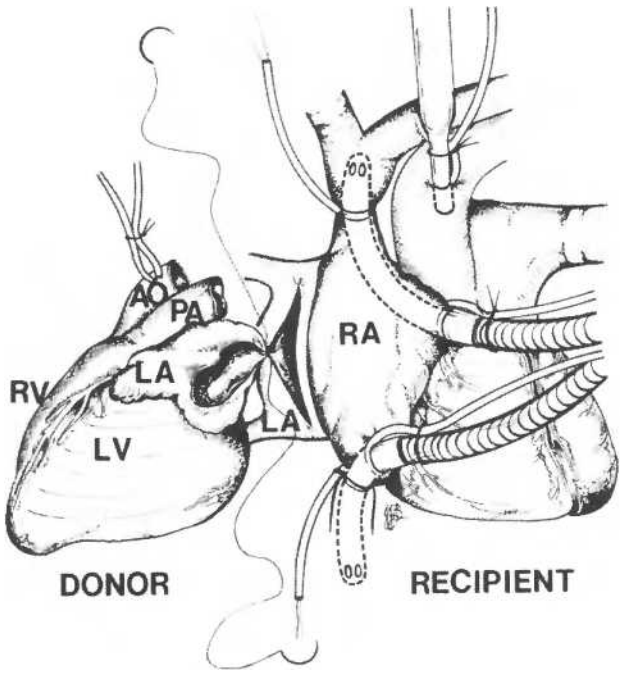

Figure 7 Donor and recipient hearts, showing the beginning of the posterior suture line of the left atrial anastomosis

pleura immediately posterior to the sternum and then by extending this incision posteriorly at the level of the diaphragm to a point 2 cm from the right phrenic nerve; a similar reflection of the pleuropericardium is made superiorly, extending the incision toward the SVC at the level of the azygos vein, again taking care to avoid the phrenic nerve. In this way a rectangular flap is created that comprises the parietal pericardium and mediastinal pleura. Hemostasis of the edges of this flap must be carried out carefully as no further opportunity to do this will arise. The flap is allowed to fall back over the hilum of the right lung (Figure 6) (or can be resected), creating a single large right pleuropericardial cavity.

Initiation of cardiopulmonary bypass

The patient is fully heparinized. An aortic cannula is inserted at least at the level of the origin of the brachiocephalic artery, and preferably higher, between the brachiocephalic and left common carotid arteries. Venous cannulae are placed in the SVC (either directly or through the right atrial appendage) and IVC (low in the lateral wall of the right atrium) (Figure 7). Cardiopulmonary bypass is initiated and the patient cooled.

For most open-heart procedures our cardiopulmonary bypass system includes two cardiotomy suction catheters which return blood to the pump–oxygenator; for heterotopic heart transplantation we have available three such suction catheters.

Myocardial protection of recipient and donor hearts

The recipient heart can be continuously perfused by the pump–oxygenator, and therefore allowed to beat throughout the period of insertion of the donor heart. If the recipient heart is to remain beating, however, the temperature of the circulating blood must not be lowered much below 32°C, or ventricular fibrillation is likely to occur, which may result in less satisfactory myocardial protection.

Alternatively, the recipient aorta can be crossclamped and the recipient heart protected by the infusion of cardioplegic agent and by the topical application of cold saline throughout the operation. Crossclamping of the recipient aorta facilitates the technical steps of the operation by preventing recipient coronary sinus blood return to the operating field, and also allows the blood temperature to be reduced to lower levels, thus facilitating the maintenance of a low donor myocardial temperature. Systemic hypothermia of 26–28°C is maintained, largely to diminish rewarming of the donor heart by its proximity to the recipient organs during its ischemic period. Our own preference is for cardioplegic arrest and hypothermic protection of the recipient heart, as this allows rather better myocardial protection of the donor heart, which we feel has a high priority.

The operation will therefore be described with the recipient heart arrested throughout (although for simplicity the aortic crossclamp is not indicated in the accompanying figures).

A catheter for cardioplegic solution infusion is placed high in the root of the recipient aorta, which is then crossclamped immediately proximal to the pump–oxygenator cannula at the level of the brachiocephalic artery. Cardioplegic solution is rapidly infused into the root of the aorta, and cold saline poured over the heart to irrigate the entire pleuropericardial cavity. Irrigation with cold saline of both the pericardial cavity (to cool the recipient heart) and the right pleural cavity (to cool the donor heart) must be carried out. Clamps or snuggers (snares) are placed around the SVC and IVC.

Until the donor heart is revascularized, between each anastomosis cold saline (4°C) is poured over both hearts to help main-

tain an adequate state of myocardial hypothermia. If there is evidence of ventricular activity in either heart, either mechanical or electrocardiographic, then further increments of cold cardioplegic solution should be infused into one or both recipient and donor ascending aortae as necessary. The cannula inserted into the donor aorta for the initial infusion of cardioplegic agent before excision can be used again for this purpose; once cardioplegic infusion has begun, and all air displaced from the donor aorta, a crossclamp is applied to occlude the distal end of this vessel, thus ensuring that the donor coronary arteries are adequately perfused.

Anastomosis of left atria

An incision, as for mitral valve surgery, is made into the recipient left atrium immediately posterior to the interatrial groove, extending from the superior to the inferior extremes of the groove (Figure 7).

The donor heart is then placed in the right thoracic cavity anterior to the collapsed right lung and lying alongside the recipient heart. It is frequently necessary to lay the heart on a sponge or swab, soaked in cold (4°C) saline, to support the donor organ and thus facilitate performance of the left atrial anastomosis. Using double-ended 4/0 polypropylene, the midpoint of the posterior lip of the incision in the recipient left atrium is sutured to the midpoint of the posterior lip of the opening in the donor left atrium. The two atria are anastomosed by a continuous suture, first along the posterior aspect and then along the anterior aspect. The completed anastomosis will be totally inaccessible at the end of the operation and therefore it is essential that it be hemostatic.

A wide communication between the two left atria has been created, forming a common atrium from which blood can enter either donor or recipient left ventricles.

Anastomosis of right atria

A 5 cm longitudinal incision is made into the lateral aspect of the recipient SVC and right atrium just anterior to the interatrial groove, beginning 2–3 cm above the vena caval–right atrial junction and continued 2–3 cm into the right atrium (Figure 8). It is essential that the SVC–right atrial incision should be at least 5 cm in length, and that it be made posteriorly to avoid the region of the sinoatrial node.

The donor SVC is extended alongside its counterpart. An eyelid retractor is used to retract the anterior lip of the incision in the recipient right atrium. The midpoint of the posterior lip of the incision in the recipient atrium is sutured to the *most caudal point of the incision in the donor atrium*, using a double-ended 4/0 polypropylene suture (Figure 9). The two right atria are then anastomosed by a continuous suture carried in each direction (superiorly and inferiorly), first to complete the posterior wall anastomosis (Figure 10), and then to complete the anterior wall anastomosis. At the completion of this anastomosis the ligated donor azygos vein remnant will lie anterior to the midpoint of the anterior suture line (Figure 10), over which a small metal ring is tied down as a fluoroscopic reference for the passage of endomyocardial biopsy forceps into the donor heart during the postoperative period.

The maneuver of suturing the midpoint of the posterior lip of the recipient atrial wall to the most inferior aspect of the incision

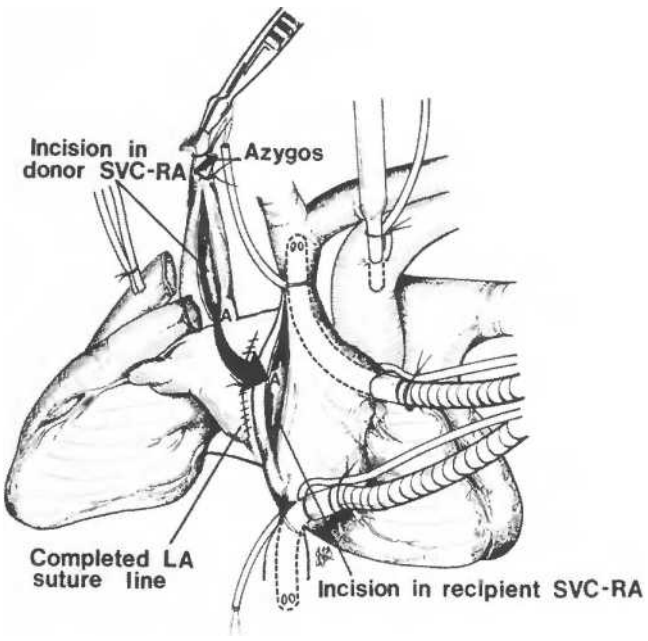

Figure 8 Completed anterior left atrial suture line. The SVC-RA incision in each heart is shown; note that the inferior point of the incision in the donor SVC-RA (A) will be sutured to the midpoint of the posterior lip of the incision in the recipient SVC-RA (A)

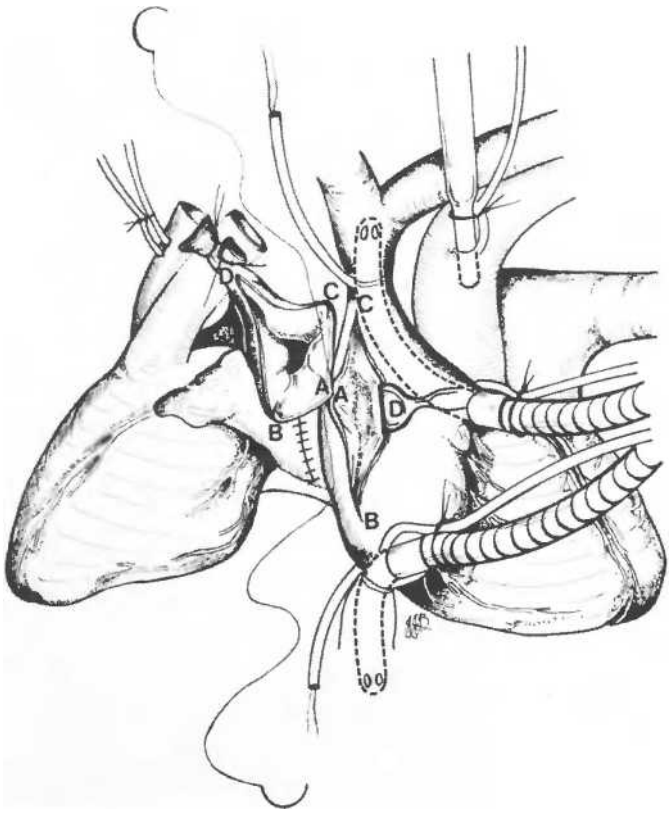

Figure 9 The first suture in the anastomosis between the donor and recipient SVC-RA has been inserted (A:A)

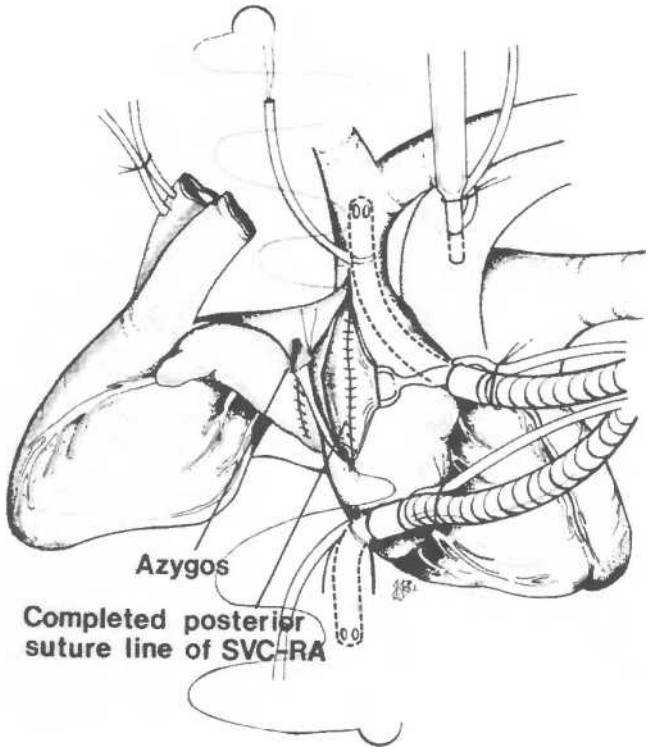

Figure 10 Completed posterior right atrial suture line

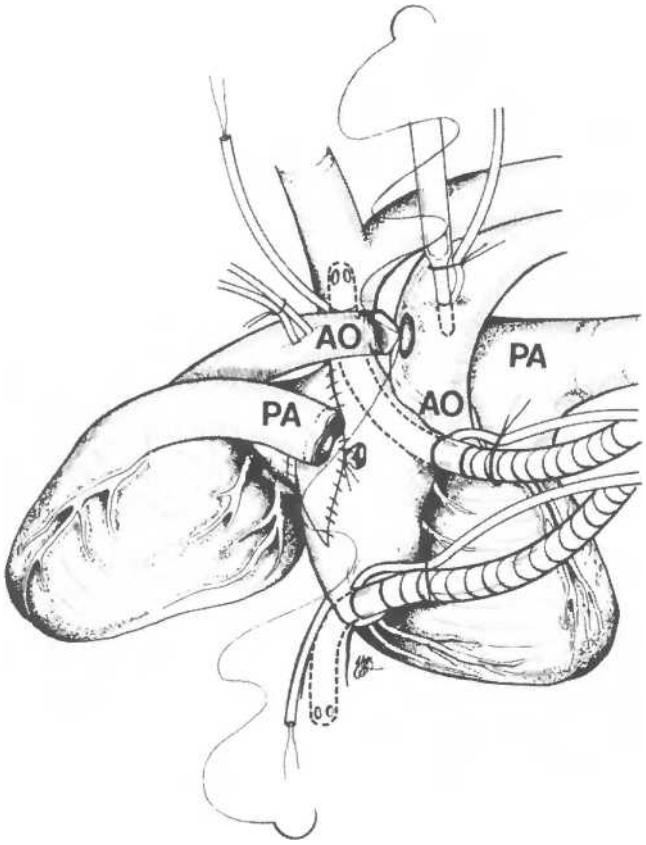

Figure 11 The SVC-RA anterior suture line has been completed; beginning of aortic anastomosis. (When the recipient heart is continuously perfused throughout the operation, a side-biting clamp is applied to the aorta for the performance of this anastomosis)

in the donor atrium, thus creating a diamond-shaped opening, ensures that this anastomosis will remain wide, allowing free flow of blood from one chamber to the other, and permitting the easy passage of endomyocardial biopsy forceps.

Anastomosis of aortae

The donor aorta is trimmed to the minimum length required to allow anastomosis to the recipient aorta and yet avoid distortion or kinking of the left or right atrial anastomoses. An unnecessarily long donor aorta will allow the donor heart to drop back into the right pleural cavity, compressing the right lung. A short donor aorta will lift the donor heart anteriorly and superiorly, and allow for maximal expansion of the right lung posterior to the transplanted organ. Temporary inflation of the lungs at this stage will help in estimating optimal length.

A longitudinal incision, equal in length to the diameter of the donor aorta, is made into the *right lateral wall* of the recipient ascending aorta. Correct siting of this incision is essential to ensure that the donor aorta lies satisfactorily without kinking, and is not compressed when the sternum is reunited. End-to-side anastomosis of donor to recipient aorta is made using a continuous suture of 4/0 polypropylene (Figure 11).

Anastomosis of pulmonary arteries

In our experience the donor pulmonary artery (PA) will not adequately reach to the recipient PA without undue tension or distortion of the other anastomoses; a conduit of preclotted woven Dacron is therefore inserted. The diameter of the conduit chosen will depend largely on the diameter of the donor PA; this is usually of the order of 20–22 mm. (With a similarly sized polytetrafluorethylene ('Gore-tex') graft, difficulty has been found in positioning it to configurate with the surrounding anatomy without kinking.)

A longitudinal incision of suitable length is made in the recipient main PA. This incision should be slightly shorter than the diameter of the Dacron conduit, as stretching will inevitably occur during anastomosis. The Dacron graft is anastomosed end-to-side to the recipient PA using continuous 4/0 polypropylene, the first stitch being placed at the distal end of the incision (Figure 12). The graft is tailored to the correct length to bridge the gap between the two pulmonary arteries. The end-to-end anastomosis to the donor PA is again performed using continuous 4/0 polypropylene (Figure 13). To ensure a bloodless field during this procedure it is sometimes necessary to insert a flexible cardiotomy sucker along the lumen of the Dacron graft into the recipient PA; both recipient and donor PA suckers are removed before completion of the final anastomosis. The Dacron conduit will be the most anteriorly placed structure in the pericardial cavity, crossing the recipient ascending aorta, and will lie immediately behind the sternum.

All anastomoses have now been completed. The cardioplegic catheters in both aortae are converted to use as air vents, and air

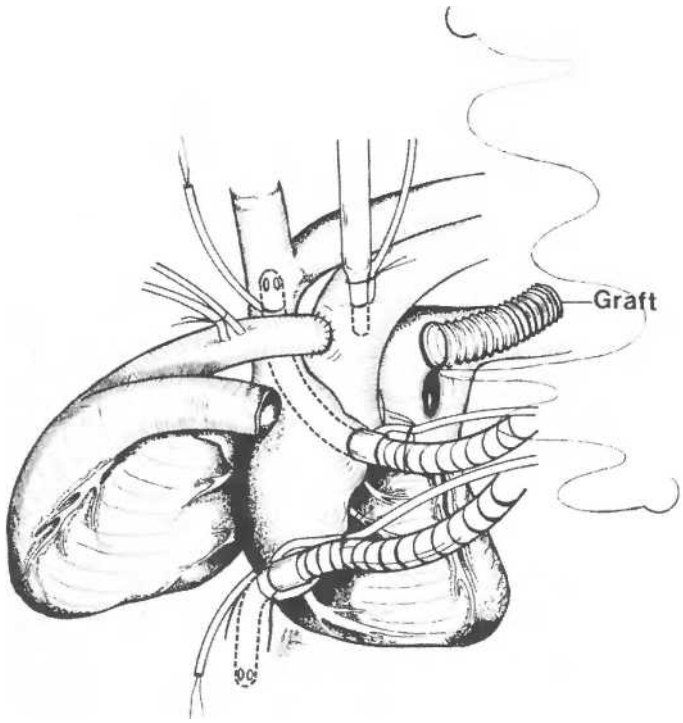

Figure 12 The aortic anastomosis has been completed; recipient pulmonary artery (PA) incision and beginning of anastomosis of Dacron graft

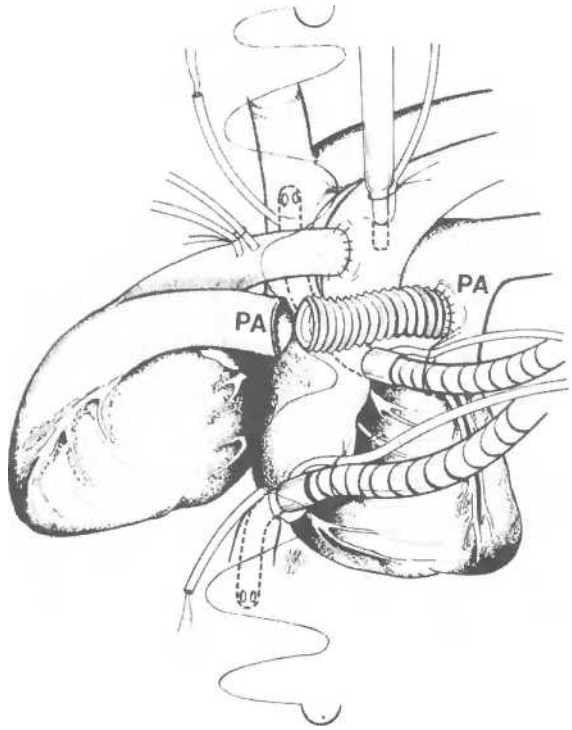

Figure 13 Completed distal (recipient) PA-graft anastomosis; beginning of proximal (donor) PA-graft anastomosis. A suction catheter is usually inserted through the pulmonary valve into the donor right ventricle to ensure a relatively bloodless field while this anastomosis is being performed; the catheter is removed before completion of the anastomosis

needles placed in both donor and recipient left and right ventricles and pulmonary arteries, the caval cannulae are unsnugged and each heart is gently massaged to expel air. The crossclamp is removed from the recipient aorta, and from this point, both donor and recipient myocardiums are continuously perfused with blood from the pump–oxygenator. Further efforts are made to remove air from the cavities of both hearts. The patient is rewarmed to 37°C.

As rewarming occurs, each heart will either begin spontaneous sinus rhythm or lapse into vigorous ventricular fibrillation, requiring electrical defibrillation. Both hearts must be gently manually compressed if they show any sign of overdistension. Inotropic and other vasoactive agents are given as necessary (as after orthotopic heart transplantation), though care must be taken not to cause dysrhythmia in either heart, each of which may respond differently to the various agents used. Further attempts to expel air from both hearts are made, and then all air needles are removed and their sites of insertion oversewn. Pacing wires should be placed in both hearts as there is a risk of atrioventricular dissociation of bradycardia in either or both hearts in the early post-transplant period.

Discontinuation of pump–oxygenator support

As with orthotopic transplantation it has been our policy to allow at least 30–60 min for donor heart recovery, especially if the ischemic time has been long. During this period a careful inspection is made of the accessible suture lines to confirm hemostasis; the PA and aortic anastomoses can usually be inspected satisfactorily, though the posterior aortic suture line may be difficult to see. The anterior right atrial suture line can usually be inspected without difficulty, but it is impossible to see the deeper suture lines (posterior right atrial and both left atrial). The venous cannula in the SVC is withdrawn into the right atrium, the IVC cannula removed and, if the hemodynamic status of the patient is stable, cardiopulmonary bypass is discontinued and the patient decannulated (Figure 1). (At this stage the recipient's own heart may provide considerable support for the circulation; the donor heart may take some hours for full recovery from the ischemic episode.) The heparin is neutralized with protamine sulfate. At least three drains are inserted: one into the pericardial cavity infero-posterior to the recipient heart, a second anterior to this heart, and a third (and possibly a fourth) inserted preferably directly through the right chest wall to ensure adequate drainage of the right pleural cavity basally (and apically).

Before closure of the chest the anesthesiologist is required to ventilate both lungs fully, to ensure expansion of the right lung, particularly of the lower lobe, which has been compressed by the donor heart throughout the procedure. The sternum is united with at least six wire or other strong sutures. ECG electrodes are positioned to allow clear monitoring of complexes from both hearts.

COMMENT

Although the technique of HHT involves the inclusion of a vascular prosthesis into a patient who will subsequently be heavily immunosuppressed, we have seen no infectious complications related to the presence of this graft, although two such cases have been reported in the literature[31,32].

On occasion the operation has been combined in Cape Town[20] and elsewhere[33] with other procedures to the recipient heart, such as resection of a left ventricular aneurysm, coronary artery bypass graft, or mitral annuloplasty. Free or friable thrombus within the recipient left ventricle can be sucked out through the left atrial incision before anastomosis of the donor heart.

Neither the left nor the right atrial anastomosis must be restrictive. If the right atrial anastomosis is confined to the SVC, then inadequate flow into the donor right atrium may result. Blood returning from the systemic circulation will be directed almost entirely through the recipient right heart; after passage through the lungs the blood will pass predominantly through the more compliant donor left ventricle. Though this circulation is unusual, the patient may remain asymptomatic. Any subsequent contraction at the SVC–right atrial suture line may lead to difficulty in manipulating biopsy forceps into the donor right ventricle; in such cases left ventricular biopsies must be obtained by the arterial route. The incision in each heart must, therefore, be extended well down into the atrial wall.

Poorly functioning ventricles are the sites of thrombus formation, and in patients with HHT it is the native ventricles which are usually involved. Thrombus may be present, or may form in the poorly functioning recipient left ventricle, and be ejected through the aortic value. Furthermore, left ventricular thrombus may spread retrogradely through an incompetent mitral valve into the common left atrium, from where it may be ejected by the donor left ventricle as an embolus. All patients with HHT therefore need to be anticoagulated permanently. Coumadin administration is begun once the chest drains have been removed, and is continued for the lifetime of the patient. An antiplatelet agent, such as sulfinpyrazone or dipyridamole, may also be administered, but is not essential; aspirin or other salicylates have been avoided in view of the risk of gastric erosion, which is already increased in patients receiving corticosteriods. Relatively few patients, however, have been seriously troubled by emboli; noncompliance of the patient with regard to meticulous attention to anticoagulant therapy has been a major contributing factor in almost all such cases. As the risk of thrombus formation (and infection) is clearly increased when a valve prosthesis is present, the presence of a prosthetic valve in the native heart is considered an absolute contraindication to HHT.

HHT by the technique described above connects the donor heart in parallel with the recipient heart. Preferential flow to donor or recipient ventricle will be directly related to the respective ventricular compliance and contractility. Ejection of blood is asynchronous, depending on the different heart rates, but does not substantially interfere with the performance of either heart[34]. Pacing of the two hearts to coordinate ejection, either synchronously or asynchronously, has not been found to be beneficial, and is not advocated, although there is one experimental study which suggests otherwise[35]. Pacing of either heart may, of course, be necessary if there is a definite indication, such as atrioventricular dissociation or bradycardia.

Hemodynamic studies in patients with HHT, performed by Rigaud *et al.*[36], demonstrated a significant reduction in left and right ventricular filling pressures (by 41% and 36%, respectively) compared to pretransplant values. Cardiac index increased by 25%, and PVR was reduced by 61%. Patients could be divided into two groups according to the presence of one or two peaks on the aortic pressure curve. In the first group, because the native left ventricle could not generate enough pressure to open the aortic value, its entire stroke volume was ejected retrogradely into the common left atrium. In the severely dysfunctional native hearts, aortic valve regurgitation throughout the cardiac cycle was reported[37]. The donor heart assumed total left ventricular work and 80% of right ventricular work. In the second group, native left ventricular systolic pressure always exceeded aortic diastolic pressure. The donor left ventricle contributed 68% to systemic blood flow and the donor right ventricle 51% to pulmonary blood flow. Native left ventricular function deteriorated with time in all patients, but was more marked in those in the first subgroup.

The Cape Town group similarly documented a highly significant increase in cardiac output and a fall in transpulmonary gradient and PVR at a mean of 5 months after HHT[38]. The contribution of the donor heart to total cardiac output was estimated to be 72%. In both native and donor hearts, however, mitral and tricuspid valve regurgitation was observed, and was severe in some of the donor hearts, which was clearly a matter of some concern with regard to good long-term function of these hearts. Mitral regurgitation may be related to ejection of the recipient left ventricular blood retrogradely into the common left atrial cavity, leading to dilatation of the donor mitral ring. Interestingly, native aortic valve regurgitation was not detected in this group of patients.

Though the right middle lobe is often displaced superiorly by the donor heart, some collapse of the right lower lobe is nearly always present at the end of the operation, but with adequate respiratory therapy this lobe expands over the course of the next few days; in our experience this has not increased the incidence of postoperative pulmonary infection in this lobe. The presence of the heterotopic allograft in the right chest (Figures 14 and 15) leads to a slight reduction in right lung volume, but in no case has this been associated with symptoms of impaired ventilatory capacity. We have transplanted large adult hearts into two 14-year-old boys without problems in this respect.

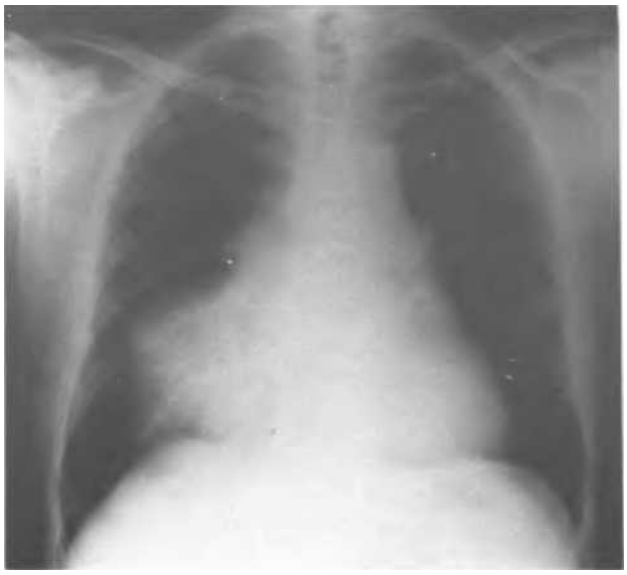

Figure 14 Postero-anterior chest radiograph of patient with heterotopic transplant: the donor heart lies in the right chest

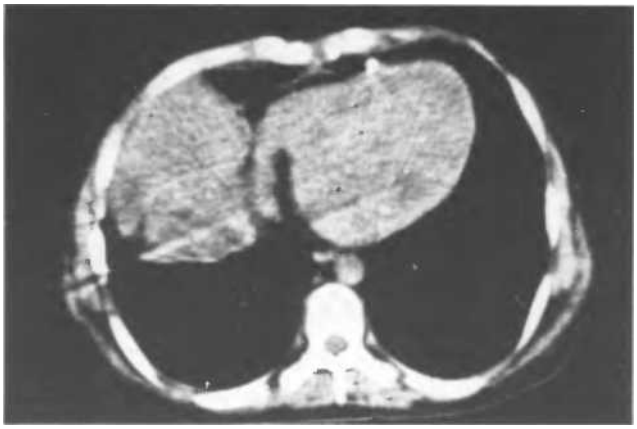

Figure 15 Computerized axial tomographic scan of the chest (viewed from below) showing the donor heart in the right chest anteriorly

In the early post-transplant period the effect of various inotropic and vasoactive agents on each heart should be monitored, and the dosage carefully balanced to prevent unwanted effects on either heart. If ventricular fibrillation of donor or recipient heart occurs after HHT, care must be taken in defibrillating, as this may lead to fibrillation of the other heart. If both hearts fibrillate, positioning the defibrillating paddles over the respective lateral walls of the chest (i.e. just below each axilla) is often successful in defibrillating both organs. Several centers, including Cape Town, have documented good donor heart function in the presence of malignant native heart ventricular dysrhythmias[39,40].

A few of our HHT patients with underlying ischemic heart disease have continued to show features of progressive atheroma of the coronary arteries of the native heart. One patient suffered a massive myocardial infarction of the recipient heart 3 months after HHT. He experienced diaphoresis and severe typical ischemic chest pain radiating to the arms; an electrocardiogram confirmed the sudden onset of ventricular fibrillation. Throughout this episode, however, the donor heart maintained a steady rhythm and a systemic blood pressure of 110/70 mmHg. He did not demonstrate any other clinical features of shock. Analgesia was all that was necessary in the form of therapy.

SEQUENTIAL HEART TRANSPLANTATION AND RETRANSPLANTATION IN PATIENTS WITH A HETEROTOPIC HEART TRANSPLANT

The indications for a second heart transplant in a patient with HHT, and the principles of selection of a patient suitable for such a procedure, are basically the same as those with regard to a patient with an OHT and are discussed in Chapter 36. There are, however, some differences that require consideration.

With regard to the timing of the second transplant in a patient with intractable acute rejection, HHT may allow patient survival even when the donor heart has ceased functioning entirely[18]. This is particularly likely in patients who undergo acute rejection of the graft within the first few weeks or months following transplantation, at which stage the patient's native heart is likely to remain sufficiently functional to allow patient survival until a second transplant procedure is performed.

In such patients, excision of the irreversibly acutely rejected heterotopic heart may be unnecessary or inadvisable (see below) or may be delayed (if possible) until the time of retransplantation. The patient is maintained on methylprednisolone 20 mg/kg per day orally, in an effort to prevent symptoms from the toxic effects of tissue necrosis. At Groote Schuur Hospital this policy was successful in all cases except one, in which the patient developed a high fever and was clearly unwell, necessitating immediate excision of the rejected donor heart[41].

Our experience and that of others[36] (though not all[21]) with patients with HHT has been that recipient heart function has steadily deteriorated during the months following transplantation, irrespective of the underlying cardiac pathology. The depression of native left ventricular function is mainly related to reduced preload (competitive filling) and increased afterload[36]. By the time graft vasculopathy (arteriosclerosis) develops, recipient heart function may have become inadequate to sustain life, or may have ceased altogether. Without retransplantation, therefore, complete graft failure is frequently followed by the death of the patient.

Operative considerations

It is our policy in all cases of sequential transplantation or retransplantation to prepare the femoral artery and vein. This allows immediate initiation of pump–oxygenator support through this route if cardiac function deteriorates before or while median sternotomy is performed. In all cases, inotropic agents should be available during induction of anesthesia.

After HHT the pericardium cannot be closed. Unless a sheet of prosthetic material, e.g. polytetrafluorethylene, has been inserted between heart and anterior chest wall, adhesions are likely to develop between the right ventricle of the recipient's own heart and the posterior aspect of the sternum; the donor heart, lying in the right chest, adheres to the right lung and anterior chest wall. Retransplantation may, therefore, be technically an extremely difficult procedure. Great care is required in opening the sternum and in dissecting out the structures of the chest.

In Cape Town the following operative procedures were employed in patients with HHT undergoing second transplants (Figure 16)[42–44]:

(1) Replacement of the heterotopic donor heart.
(2) Replacement of the native heart, leaving the first heterotopic donor heart *in situ*. Following excision of the recipient's heart, the standard operation of OHT was performed (Figures 17 and 18).
(3) Excision of both original donor and recipient hearts and insertion of an orthotopic heart transplant; the right lower lobe requires mobilization and re-expansion to fill the space vacated by the excised heterotopic heart.

Certain aspects of technique are worthy of note. Whenever it was decided to leave the heterotopic donor heart *in situ*, usually only its base and great vessels were mobilized. Similarly, whenever the recipient heart was to be left *in situ*, a full dissection was usually not carried out. If femoro-femoral bypass had already been initiated, a further single SVC cannula was all that was required. If cardiopulmonary bypass was not already in progress, an arterial cannula was inserted into the aortic arch, and two

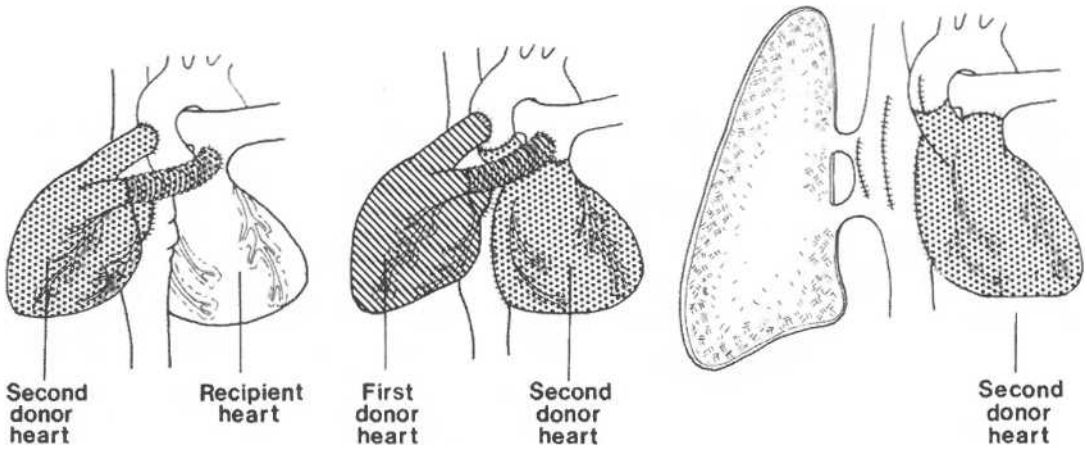

Figure 16 Different operative techniques used for retransplantation in patients with a heterotopic transplant. *Left*: Technique (1), replacement of the heterotopic donor heart. *Center*: Technique (2), replacement of the recipient heart leaving the first heterotopic donor heart *in situ*. *Right*: Technique (3), excision of both original donor and recipient hearts, and insertion of an orthotopic heart transplant; mobilization of the right lung is required to fill the space vacated by the heterotopic donor heart

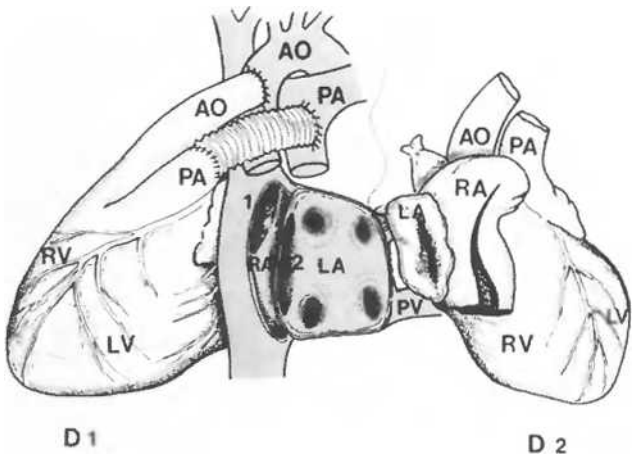

Figure 17 Replacement of the native heart in a recipient with a previous heterotopic transplant (D1) (Technique 2). The native heart has been excised, and the second donor heart (D2) is about to be sutured into the orthotopic position

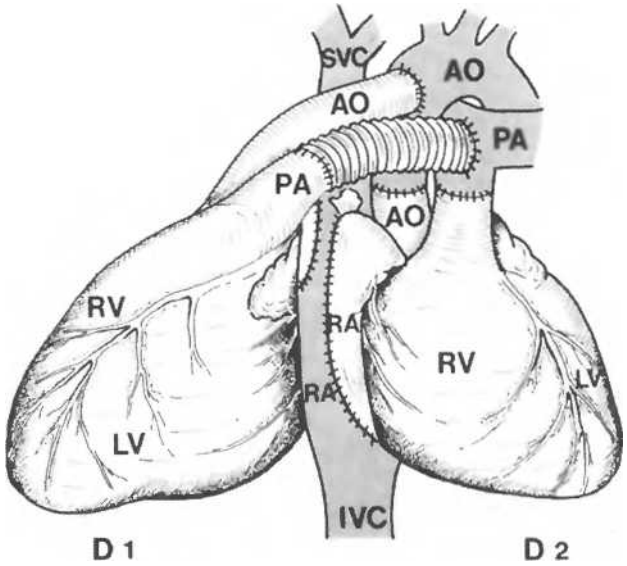

Figure 18 Replacement of the native heart in a recipient with a previous heterotopic transplant (D1) – the completed operation (Technique 2)

venous cannulae placed, one in the SVC, and one low in the right atrium or IVC. While handling the SVC, care was taken not to injure the azygos vein. Systemic cooling to 22°C was usually maintained.

Our approach to myocardial protection of the heart that was not to be replaced (i.e. either the heterotopic donor or the native heart) varied over the years. Usually the aortic crossclamp was applied cephalad to the anastomosis of the aortae, thus rendering both hearts ischemic. On occasion, arrest of the retained heart by cardioplegia was obtained, but on other occasions we relied on topical cooling only. As this heart (which was not to be removed) was badly diseased, and therefore of little value to the patient, we were not always meticulous in our efforts to protect the myocardium.

Decompression of the heart that was to be left *in situ* was readily achieved, as removal of the other heart rendered the left atrium open. At the end of the operative procedure, however, great care was taken to remove all air from both hearts. It has not been our policy to vent either heart, but a large air needle was placed in both aortic roots and in both left ventricles, either directly through the apex or, in the case of a heterotopic donor heart, through the anterior right ventricle and septum into the posteriorly placed left ventricle.

When the heterotopic donor heart required removal (techniques (1) and (3) above), this was performed once cardiopulmonary bypass had been initiated. On some occasions air leaks and oozing of blood from the right lower lobe proved unavoidable.

Excision of the first donor heart can be a difficult procedure in view of tight adhesions between this heart and the surrounding tissues, notably the lung. By leaving this heart *in situ*, the operating time is reduced and potential postoperative complications avoided.

Technique (3) is probably the least favorable, as removal of the original recipient heart, together with the donor organ (and insertion of an orthotopic graft), also requires expansion of the right lower lobe, necessitating an extrapleural mobilization of much of the right lung, thereby increasing blood loss significantly.

Excision of a heterotopic allograft in a patient with a functioning native heart

In (a) patients with an irreversibly acutely rejected heterotopic allograft (and a functioning recipient heart), when the donor heart requires excision as a semi-emergency following the development of toxic symptoms from tissue necrosis, and when retransplantation cannot yet be performed due to the absence of a suitable donor (or other contraindication), or (b) in the very rare event when the native heart has shown significant recovery and the donor assist is no longer required, excision can been achieved on occasions without the need for cardiopulmonary bypass. The operation is performed through a median sternotomy. Vascular clamps are applied across the four sites of anastomosis (i.e. aorta, pulmonary artery, right atrium and left atrium), the donor tissue divided and excised, and the residual cuffs of tissue oversewn. There have been no complications associated with leaving cuffs of donor aorta or atria in the chest.

If a second HHT is anticipated, the vacated cavity of the right chest has been maintained by filling it with a suitable foreign body, such as a silastic breast prosthesis. If this is not done, the space will fill with blood and other fluid and dense adhesions will form, making subsequent HHT exceedingly difficult, if not impossible.

Results of sequential transplantation or retransplantation

In the Cape Town series of nine sequential transplants[44], there were no operative deaths, though the procedures were difficult whenever the heterotopic donor heart required removal (techniques (1) and (3)). Two patients died, however, after 24 days and 3 months, from bacterial pneumonias, and one hyperimmunized patient lost graft function after 5 days from accelerated acute rejection, but survived a further 17 months before dying from his underlying cardiomyopathy. Of the two patients who underwent a third transplant, one died after 1 month from *Aspergillus* sepsis and the other died within the first post-transplant year from accelerated graft vasculopathy. The three surviving patients remained well several years after their second operation.

In this small series certain factors were noted to influence survival.

(1) *Operative technique*. Removal of the original donor heart, a technically difficult procedure with potential complications of bleeding and air leak, was followed by a higher morbidity and mortality than when this organ was left *in situ* and the second graft inserted in the orthotopic position.

(2) *Cause of failure of first transplant*. Patients who were retransplanted for acute rejection did less well than those retransplanted for chronic rejection. This was possibly related to the immune status of the patient at the time of retransplantation, but in this small series was more readily explained on the basis of the immunosuppressive therapy available to the patient at the time of transplantation (see below).

(3) *Immunosuppressive therapy*. Of the four patients who continued to receive immunosuppression with only azathioprine and methylprednisolone after retransplantation, only one was a long-term survivor. Of the four patients in whom therapy was changed to include CsA at the time of the second intervention, three proved to be long-term survivors.

From this experience we would recommend that the first donor organ is left *in situ*, and that the recipient's own heart should be replaced at the second operation (see Figures 17 and 18). This would also seem to be the operation of choice at third and subsequent procedures. The risk of major thromboembolism from a poorly or non-functioning heterotopic donor heart would seem small, as long as anticoagulation is maintained.

COMMENT

Though the technical problems faced in sequential transplantation or retransplantation in patients with an existing HHT are considerable, and therefore the risk of early postoperative complications probably increased, it would seem that a second transplant is certainly a worthwhile procedure, particularly since the introduction of CsA. However, the present indications for HHT are relatively few and, as reoperation in such patients is difficult, we believe that this procedure should be performed only when there is a definite contraindication to OHT.

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38
Heart Transplantation in Infants and Children – Indications, Surgical Techniques and Special Considerations

C.B. HUDDLESTON

INTRODUCTION

For some children with severe congenital malformations of the heart or end-stage myopathic processes, cardiac replacement offers the only hope of survival. The evolution of this treatment began in the late 1960s when Kantrowitz performed a heart transplant in a 16-day-old infant with tricuspid atresia[1]. Not long after that, Cooley performed a combination heart–lung transplant in a 2-month-old infant with an atrioventricular canal and pulmonary consolidation with pneumonia[2]. Neither of these infants survived their hospitalization, and in the 1970s fewer than 15 transplants in children were performed. The introduction of cyclosporin and the pioneering work of Bailey and associates at Loma Linda in neonatal transplantation were two significant developments of the 1980s which contributed to a steady increase in the volume of transplantation in children. According to the Registry for the International Society for Heart and Lung Transplantation[3] the number of children transplanted has plateaued since 1990 at 280–300 per year. Although heart transplantation (HTx) is a generally accepted therapy for children with medically refractory heart failure, controversies exist, such as the appropriateness of HTx for children with hypoplastic left heart syndrome. While it is true that HTx in children has been a direct extension from the experience gained in adults, there are substantial differences in practice from the standpoint of indications, surgical techniques, implications of immunosuppression on growth and response to infectious agents, and anticipated survival. This chapter will review the indications for HTx, the surgical techniques involved, the post-transplant management, and the results in children based on the published literature as well as the author's personal experience.

INDICATIONS

As with adults, children present for cardiac replacement as therapy for refractory heart failure or life-threatening arrhythmias. The specific indications for HTx in pediatrics are almost evenly divided between (a) congenital heart disease and (b) myopathic processes. In younger age groups, particularly infants, the congenital heart disease indication predominates and, as one approaches teenage years, the myopathic indication predominates[3].

CONGENITAL HEART DISEASE

It has been estimated that 10–20% of all children with congenital heart disease will ultimately require HTx over the course of their lives[4]. Long-term survival statistics (out to 15 years) in patients following surgical treatment of complex congenital heart disease are now available for entities such as tetralogy of Fallot[5], atrial repair of transposition of the great arteries[6,7], and Fontan procedure for single ventricle[8]. These all show a steady fall in survival that significantly exceeds that of the normal population. Patients with single ventricle anomalies status post-Fontan procedure represent a potentially large group of candidates for HTx. The downward slope of their survival curve is steeper than that of other surgically treated congenital heart diseases. Those with other complications of the Fontan procedure, such as protein-losing enteropathy and severe refractory atrial arrhythmias, may also be suitable candidates[9]. Further, children with single ventricle physiology who do not meet the strict criteria for the Fontan procedure, and for whom no other palliative option is available, can often be satisfactorily treated with HTx[10]. Heart failure occasionally occurs in teenagers and young adults when the anatomic right ventricle is serving as the systemic ventricle, such as following atrial correction of transposition of the great arteries or with 'congenitally corrected' transposition of the great arteries. HTx is a perfectly reasonable treatment option for these patients. Table 1 lists the specific congenital heart diagnoses treated with HTx at our institution.

Heart transplantation in neonates as primary therapy for hypoplastic left heart syndrome was introduced by Bailey and his colleagues at Loma Linda[11]. Their early and intermediate results have been good, with a 3-year actuarial survival >80% of all those surviving to HTx[12]. At the time, this compared quite favorably to previously reported results of reconstructive surgery for newborns with this diagnosis – <60% 1 year survival from Norwood's group[13] and 30% early survival from a multi-center group of surgeons[14]. This

Table 1 Diagnoses of children requiring transplantation for congenital heart disease at St Louis Children's Hospital

Hypoplastic left heart syndrome	
Primary	42
Following palliation	4
Failed Fontan procedure	3
Tetralogy of Fallot, S/P repair	2
Heterotaxy syndrome	2
c-TGA/VSD	
S/P repair; S/P MVR	3
Straddling mitral valve, CHB	1
d-TGA/VSD	
S/P Senning, VSD closure	1
Pulmonary atresia, IVS; s/p MI	1
Ebstein's anomaly, pulmonary atresia	1
S/P repair of atrioventricular canal	1
S/P repair of multiple VSD (left ventriculotomy)	1

c-TGA = congenitally corrected transposition of the great arteries; VSD = ventricular septal defect; MVR = mitral valve replacement; CHB = complete heart block; IVS = intact ventricular septum; MI = myocardial infarction

resulted in a progressive increase in the number of transplants in the infant population during the early 1990s. However, in the past 3–4 years two factors have made the results between HTx and reconstructive surgery for this entity increasingly similar: (a) the mortality while awaiting HTx and (b) the improved results with reconstructive surgery. In some centers the mortality on the waiting list for these critically ill infants is 15–20% as the time from listing to HTx has gradually increased to 4–6 weeks. If one is to add an 'acceptable' 5% operative mortality to this, the early mortality based on the planned intention becomes at least 25%. Some centers that have persisted with reconstructive surgery have improved their results considerably, with 1-year survival rates of 75–80%[15,16]. Thus, the most appropriate treatment for these infants remains controversial, with some centers continuing to use HTx as the treatment of choice, others employing reconstructive surgery exclusively, and others adopting a more selective approach. We have gone from offering HTx as the treatment of choice to a selective approach where risk factors for HTx or reconstruction are evaluated. Factors favoring HTx include: (a) blood type other than O, (b) severe tricuspid valve regurgitation, and (c) poor ventricular function. Factors favoring reconstruction include: (a) blood type O (because of the long waiting time for HTx), (b) good ventricular function, and (c) the presence of an intact atrial septum or a very small atrial septal defect not amenable to dilatation. The size of the ascending aorta, the anatomic subtype of hypoplastic left heart syndrome, and the size of the patient have not been used at our center to influence this decision.

CARDIOMYOPATHY

Cardiomyopathy is the dominant indication for HTx in children over 5 years of age. Although the etiology for the majority is unknown, viral (usually Coxsackie) infection accounts for 23%, hereditary cardiomyopathy 14%, hypertrophic cardiomyopathy 5%, and doxorubicin-induced cardiomyopathy 4%[17]. Ischemic cardiomyopathy, the dominant diagnosis for adult cardiac transplantation, is very rarely seen in children, and is limited to those with anomalous coronary artery syndrome with irreversible left ventricular dysfunction and hyperlipidemias. The indications to proceed with HTx in a patient with a cardiomyopathy are similar to those for adults. However, some factors have been noted which portend a poor prognosis for children with cardiomyopathies: (a) age >2 years at onset, (b) lack of improvement on medical therapy, and (c) associated arrhythmias[18].

CARDIAC TUMORS

Unresectable primary cardiac tumors, such as fibroma, are very rare. Cardiac transplantation is an option for therapy as long as there are no significant associated anomalies. These primary tumors rarely metastasize. Rhabdomyoma of the heart is the most common cardiac tumor in infants and is usually quite large at presentation. However, many of these patients have mental retardation related to tuberous sclerosis and most of the tumors spontaneously regress without treatment[19]. HTx is necessary medically when the tumor causes severe left ventricular outflow tract obstruction or life-threatening ventricular dysrhythmias.

RETRANSPLANTATION

Retransplantation accounts for slightly less than 5% of all heart transplants in children. Early graft failure, severe acute rejection, and coronary vasculopathy are the underlying reasons for this. The results with retransplantation are generally worse than for first-time transplants and one could argue that it is inappropriate to utilize this valuable resource in a situation where the anticipated result is less likely to be successful. The age range for which the donor : recipient ratio is >1.0 is 1–10 years. Thus, a child transplanted as an infant developing coronary vasculopathy as a 6-year-old is a perfectly reasonable retransplant candidate. All others are controversial and should be carefully examined on a case-by-case basis.

CONTRAINDICATIONS

Contraindications to HTx include those factors that preclude transplantation of any organ: (a) active malignancy, (b) uncontrolled infection, and (c) significant psychosocial issues. Severe renal and hepatic dysfunction that fails to improve with intensive medical treatment of heart failure is a relative contraindication, bearing in mind the balance between the nephrotoxic effects of cyclosporin and the potential reversal of these processes once the transplanted heart begins to provide satisfactory cardiac output. In neonates, however, renal insufficiency is a fairly strong contraindication because dialysis is difficult to manage in small children for a prolonged period of time.

The classic guidelines regarding pulmonary vascular disease and its impact on risk for HTx are that the indexed pulmonary vascular resistance should be <6–8 Wood units and the transpulmonary gradient should be <15 mmHg.[20,21] It should be emphasized that these are only guidelines, and are difficult values to obtain accurately in patients with congenital heart disease where intra- and/or extracardiac shunts significantly complicate the computations necessary to derive the pulmonary vascular resistance value. The patients identified as high-risk on the basis of pulmonary vascular disease should be evaluated extensively in the cardiac catheterization laboratory to examine their response to vasodilators such as nifedipine, oxygen, prostaglandin E_1, prostacyclin (where available) and inhaled nitric oxide. In addition,

inotropic agents should be evaluated as they frequently have both short- and long-term beneficial effects on elevated pulmonary vascular resistance, particularly when it is on the basis of poor ventricular function with a high end-diastolic pressure in the systemic ventricle or with atrioventricular valve regurgitation. Those patients with inadequate acute response to any of these measures should be treated for several days or weeks and re-evaluated prior to excluding them from isolated cardiac transplantation.

I personally do not consider any congenital cardiac lesion to be of such complexity that transplantation is impossible. That includes situs inversus and any of the heterotaxy syndromes. In general, anomalies of the systemic and pulmonary veins provide the major technical challenge in children with congenital heart disease. A number of novel solutions to these issues have been provided by various surgical groups and these will be discussed later in this chapter.

PRETRANSPLANT EVALUATION AND TREATMENT

This evaluation is much like that for adults. A series of tests screening for the presence of active infectious disease is performed. An evaluation for the presence of preformed cytotoxic antibodies is also undertaken. When present at a level of >15% either a prospective cross-match with the donor or treatment with plasmapheresis is necessary. The significance of these antibody levels in infants under 3 months of age is unknown as they most likely represent maternal antibodies. Except for the neonates with hypoplastic left heart syndrome, the potential candidate then undergoes a cardiac catheterization to confirm the diagnosis and also to evaluate the pulmonary vascular resistance. In an infant presenting with a presumptive diagnosis of cardiomyopathy, the diagnosis of anomalous left coronary artery originating from the pulmonary artery must be excluded. Children with complex congenital cardiac lesions, particularly those with heterotaxy syndromes, should have a thorough characterization of the systemic and pulmonary venous drainage to exclude anomalies.

Once listed and awaiting HTx, a number of potential complications can occur. Neonates with hypoplastic left heart syndrome are particularly complex patients to manage. They all require continuous treatment with prostaglandin E_1 to maintain patency of the ductus arteriosus. The pulmonary and systemic blood flows are balanced optimally by avoiding supplemental oxygen and hyperventilation, while constantly being on surveillance for additional hemodynamic problems, such as the closure of the patent foramen ovale[22]. In older children with myopathic processes clinical deterioration occurs due to either progressive pump failure or the development of refractory ventricular arrhythmias. Generally speaking, the intra-aortic balloon pump is not particularly effective in children, due in part to the greater compliance of the aorta and the small size of the vessels. When progressive heart failure is occurring and inotropic drugs are not providing the necessary support, mechanical support is indicated[23]. Ventricular assist devices may be inserted into older children; the lower limit of acceptable weight is not clearly defined. For very small infants, extracorporeal membrane oxygenator (ECMO) support is an option. Prior to embarking on the adventure involved with the use of one of these modalities (particularly ECMO) one must have some assurance of a relatively short anticipated waiting time. It is very difficult to maintain an infant on ECMO for more than 21 days without developing some sort of complication which would impact upon the candidacy of the patient for a transplant. An automatic cardioverter–defibrilliator or pacemaker–cardioverter– defibrillator may be used in children as small as 10 kg when ventricular arrhythmias occur that are poorly responsive to medical therapy[24].

SURGICAL TECHNIQUES

Anatomic considerations – general comments

The technique of HTx in children with myopathic processes is no different from that employed in adults, which is discussed elsewhere. I would comment, however, that there is some controversy regarding the nature of the venous anastomoses in HTx – atrial versus caval/pulmonary venous anastomoses. My preference, in general, is to perform caval as opposed to right atrial anastomoses for the systemic venous connections, except in two instances: (a) small infants in whom the superior vena cava (SVC) is very thin and at greater risk for kinking and narrowing due to purse-stringing the suture line, and (b) the presence of a left SVC. For the latter situation, my preference is to alter the recipient cardiectomy to maintain the patency of this vessel by leaving its course via the coronary sinus intact; the left atrial incision on the inferolateral free wall in the recipient is made near the atrioventricular groove[25]. Direct caval anastomoses require cannulation for cardiopulmonary bypass high in the SVC and quite low in the inferior vena cava (IVC). Cannulating low in the IVC often requires dissection of the IVC below the diaphragm so that it may be transected at its junction with the right atrium and still provide sufficient length for the anastomosis. Caval anastomoses provide a potentially better hemodynamic result, and also allow for the greater flexibility necessary for dealing with some of the complex anatomic entities presenting for HTx – particularly the heterotaxy syndromes and the post-Fontan patients.

The number of different combinations of congenital defects and their anatomic nuances preclude an encyclopedic description of each method of implanting the donor heart. However, with a discussion of some of the more unusual entities, modifications to meet the individual needs can be met. The major anatomic entities to be addressed are hypoplastic left heart syndrome, transplantation following the Fontan (total cavopulmonary connection) operation, situs inversus, and heterotaxy syndromes.

Donor assessment/management

Once a donor has passed the usual criteria for general acceptance for organ procurement on the basis of appropriate blood type and absence of transmissible disease, size match and organ function become the issues to be evaluated. The degree of size discrepancy allowable depends upon the size of the recipient. For neonates I will accept an organ from a donor as much as three times the weight of the recipient. For older children, the range by weight is usually of the order of 20% above or below. It is generally felt (without much objective data) that a heart from a larger donor will tolerate higher pulmonary vascular resistance in the recipient. I would agree that it is preferable to utilize a larger donor in this circumstance, but would not turn down an otherwise acceptable

donor for a patient with relatively high pulmonary vascular resistance due to somewhat small size in the donor alone. This would particularly apply to the situation where the donor is local and the anticipated ischemic time would be short.

Operative techniques

Hypoplastic left heart syndrome

Donor procurement (Figure 1). The major difference between donor harvest in this circumstance and any other harvest is to acquire sufficient donor aorta to allow for reconstruction of the recipient aorta as part of the transplant procedure. The ascending aorta, aortic arch including the head vessels, and proximal descending aorta are all dissected out. Following aortic clamping, cardioplegia administration, and cooling with topical cold saline and ice, the innominate, left common carotid, and left subclavian arteries are divided just beyond their origins. The proximal descending aorta is divided just beyond the origin of the ligamentum arteriosum. The remainder of the organ harvest proceeds as for any other cardiac procurement. At the time of transplant, the donor aorta is prepared by excising the superior portion of the aortic arch beginning just proximal to the origin of the innominate artery and going all the way to the ligamentum arteriosum.

Recipient operation (Figure 2). The ductus arteriosus, branch pulmonary arteries, and aortic arch with its branches are all dissected out extensively. When dissecting out the ductus arteriosus and during the distal arch reconstruction, care must be taken to avoid injury to the recurrent laryngeal nerve. Following systemic heparinization, the proximal pulmonary artery and right atrial appendage are cannulated. Once cardiopulmonary bypass has commenced, the left and right pulmonary arteries are occluded with snares and the patient is cooled to 18°C. Circulation is then shut down, the patient is exsanguinated into the cardiopulmonary bypass circuit, the snares are removed from the pulmonary arteries, and the innominate and left carotid arteries are occluded. The arterial and venous cannulae are removed and the recipient cardiectomy is performed. The pulmonary artery end of the ductus arteriosus is ligated and the remainder of it is excised from the aorta. The ascending aorta, which is generally very small, is resected with the heart. The underside of the aortic arch is opened down to the area of the ductus arteriosus. The aortotomy is extended approximately 1 cm distal to the region of the ductus because coarctation of the aorta is extremely common with this disease.

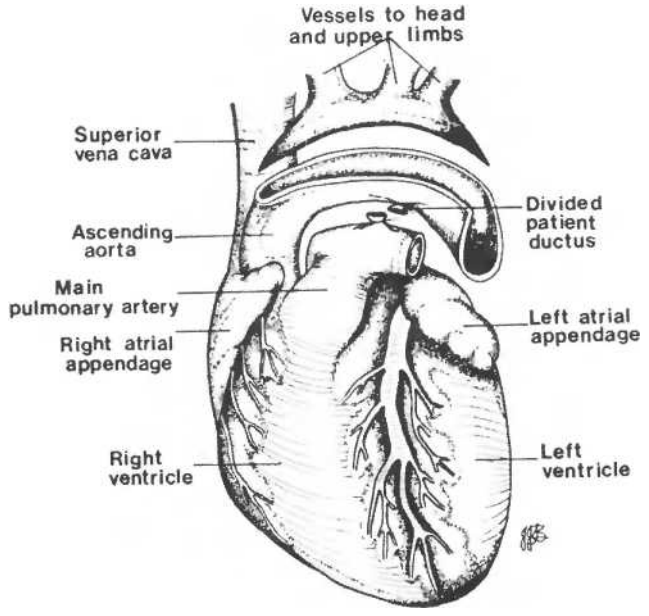

Figure 1 Donor heart prepared for transplantation in a patient with hypoplastic left heart syndrome. The aortic arch and descending aorta are prepared for the aortic reconstruction by removing the superior aspect, including the origins of the arch vessels

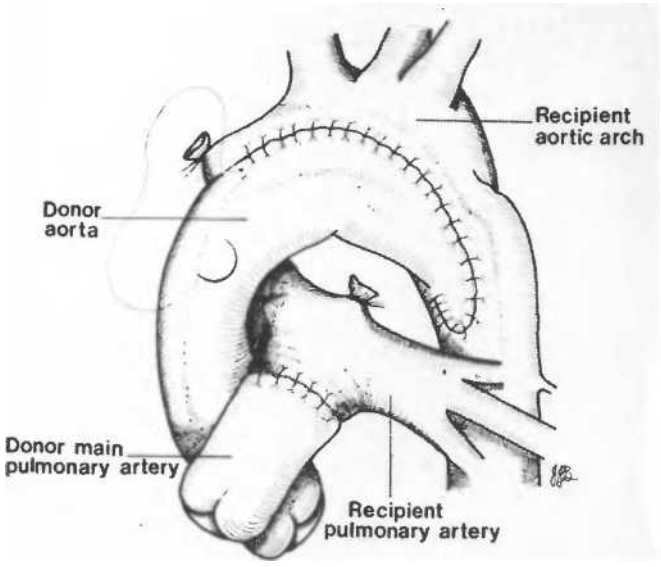

Figure 2 Completed arch reconstruction. The donor aortic flap is anastomosed to the recipient aorta in such a way to re-create aortic continuity and to repair the coarctation

The transplant is then commenced with the left atrial anastomosis performed in the usual fashion. The aortic anastomosis is performed next in such a fashion as to reconstruct the aortic arch and proximal descending aorta. Generally, this anastomosis is begun at the distal extent, with downward traction applied to the left pulmonary artery to provide exposure. The pulmonary artery anastomosis is then performed in the usual fashion, as is the right atrial anastomosis. Cannulation stitches are then placed into the new ascending aorta and right atrial appendage. De-airing the aorta is extremely important and can generally be done through the aortotomy site for cannulation. Rewarming is commenced, the patient weaned from cardiopulmonary bypass, and decannulation carried out[26].

Frequently, the donors used for these newborn infants are considerably larger than the recipients – in our series approximately twice as large by weight. Most of the time the donor heart fits satisfactorily but, when there is a particularly large donor–recipient mismatch, opening the pleural spaces and resecting a large portion of the pericardium provides more space for the heart. Avoidance of positive end-expiratory pressure and the use of small tidal volumes will also assist in getting the chest to close.

Failing this, it may be necessary to leave the chest open for a few days until edema has resolved.

Approximately 15% of all patients with hypoplastic left heart syndrome will have a left SVC. As mentioned above, a very simple method of handling this is to alter the reciplent cardiectomy such that the left SVC continues to drain via the recipient coronary sinus into the new right atrium[25] (Figures 3 and 4).

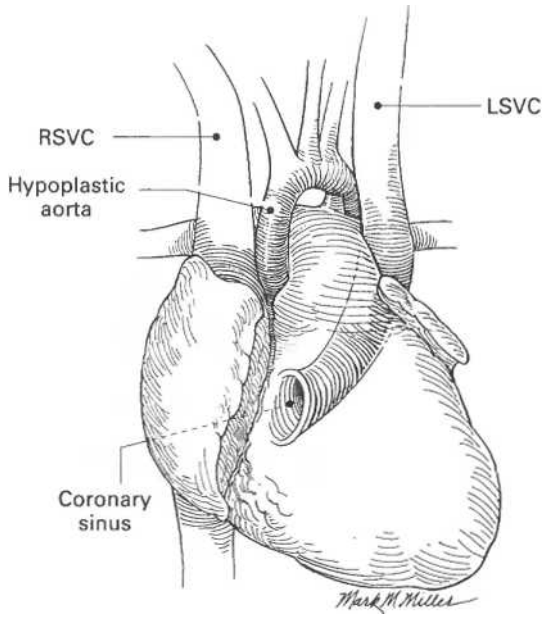

Figure 3 Anatomy of hypoplastic left heart syndrome with a left superior vena cava. This empties via the coronary sinus into the right atrium. LSVC = left superior vena cava, RSVC = right superior vena cava

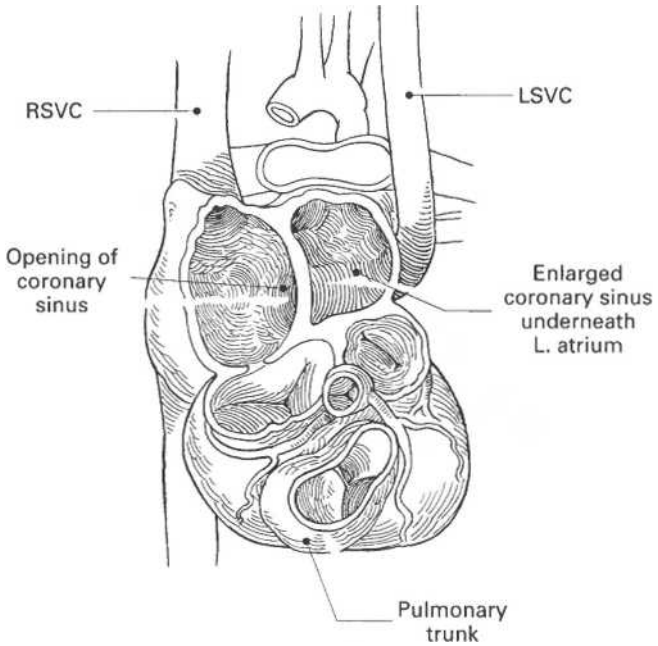

Figure 4 Hypoplastic left heart syndrome. The recipient cardiectomy is performed so that the coronary sinus is left intact along the postero-inferior left atrioventricular groove. This leaves the left superior vena cava draining into the right atrium via the recipient coronary sinus. The donor heart is then sewn into place in the usual fashion with bi-atrial anastomoses

The postoperative management of these infants is similar to that for any neonate undergoing major cardiac reconstructive surgery, with the possible exception that isoproterenol is used more frequently for chronotropic and inotropic effects. For patients who have waited for significant periods of time, the pulmonary vascular resistance will be somewhat elevated and will predispose to either right heart failure or pulmonary hypertensive crises. Therefore, we manage those infants at risk for this problem postoperatively with 24 hours of paralysis, sedation, and hyperventilation.

Status post-Fontan procedure

Donor procurement. Depending upon the precise anatomic nature of the congenital anomaly and the technical aspects of the Fontan procedure, pulmonary artery reconstruction and correction of systemic venous anomalies are the most common problems requiring modification of the standard technique in these patients. Thus, if there is not an associated lung harvest, one should include as much of the branch pulmonary arteries as possible. If this is not feasible, harvesting a section of the donor descending thoracic aorta also allows for excellent flexibility for pulmonary artery reconstruction when necessary, especially when the recipient has had a right 'classical' Glenn anastomosis in combination with a right atrial-to-left (or main) pulmonary artery connection as the Fontan technique; this results in a significant gap between the orifice of the right pulmonary artery and the main or left pulmonary artery. When the recipient has a left SVC, the innominate vein should be harvested in continuity with the SVC.

Recipient operation. Figures 5 and 6 demonstrate a patient with bilateral SVC, each anastomosed to the pulmonary artery directly.

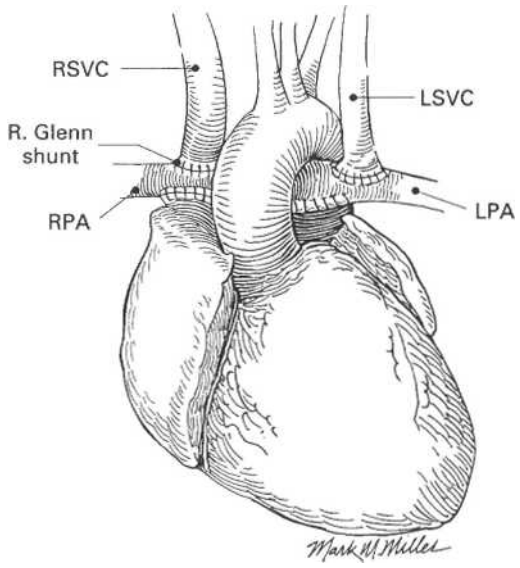

Figure 5 Anatomy in a patient with bilateral superior venae cavae, status post-Fontan correction by the total cavopulmonary connection technique. Both superior venae cavae are connected directly to the pulmonary arteries. The inferior vena cava is connected via an intra-atrial baffle to the orifice of the superior vena cava, the cardiac end of which is anastomosed to the underside of the pulmonary artery. LPA = left pulmonary artery, RPA = right pulmonary artery

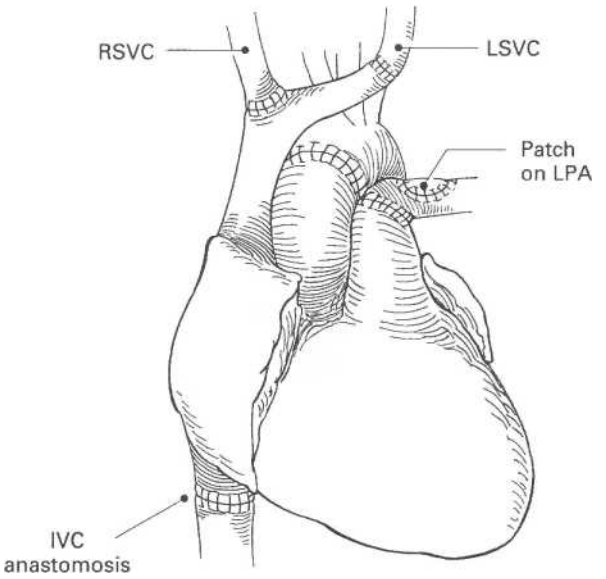

Figure 6 The completed transplant (in the anatomy illustrated in Figure 5). The left superior vena cava has been directly connected to the donor innominate vein, and the right superior vena cava is connected to the distal donor superior vena cava. The inferior vena cava is anastomosed directly to the donor inferior vena cava. The defects in the pulmonary arteries at the sites of the prior Fontan connections usually require patches. IVC = inferior vena cava

The blood from the IVC reaches the pulmonary artery via a lateral atrial baffle (cavopulmonary connection) and anastomosis of the cardiac end of the SVC and pulmonary artery. Venous cannulation will need to be high in both SVC and low in the IVC. The recipient cardiectomy is performed, removing most of the right atrium and all of the cavopulmonary baffle. A small cuff of right atrial tissue may be left on the orifice of the IVC to provide a little more length for this anastomosis. The SVC are removed from their anastomotic sites on the pulmonary artery; in most cases, the sites of the caval anastomoses to the pulmonary artery need to be patched, rather than directly oversewn. The remainder of the cardiectomy is performed in the usual fashion.

The transplant procedure is modified to allow for an anastomosis between the donor innominate vein and the left SVC. The right SVC is anastomosed directly to the donor SVC, and the IVC is connected directly to the donor IVC at its junction with the right atrium. The pulmonary artery anastomosis is placed at the appropriate position, most often utilizing a long segment of donor main pulmonary artery. It is frequently necessary to perform some sort of reconstruction of the right and left pulmonary arteries or the bifurcation. In the presence of a 'classical' Glenn shunt, the pulmonary artery reconstruction can be done with a long segment of harvested right pulmonary artery, if available. Alternatively, the donor descending thoracic aorta is an excellent conduct and can be easily harvested with the heart, regardless of the needs of the other procurement teams. It can then be interposed between the right and left pulmonary arteries to re-establish continuity. The donor main pulmonary artery may then be connected to an arteriotomy made in a convenient section of donor thoracic aorta. The pulmonary artery reconstruction and anastomosis will usually need to be performed prior to the aortic anastomosis since much of this reconstruction is located in what would be directly posterior to the aorta[10].

Situs inversus

Patients with situs inversus may present for HTx because of associated severe congenital cardiac defects, such as single ventricle physiology with failed Fontan or occasionally with an isolated cardiomyopathy and no other anomalies. In either instance this entity is a significant technical challenge primarily related to handling of the systemic venous return. Certainly, the expectation of finding a donor with situs inversus is extremely low, given that it is estimated to occur at a rate of only 2 per 10 000 population. Thus, techniques designed to modify the recipient so that a heart with normal situs may be implanted are necessary.

Donor procurement. Donor procurement will depend on how the surgeon specifically plans to handle the systemic and pulmonary venous connections as well as needs based on the associated anomalies present and previous palliative procedures. To allow for greatest flexibility, the harvest should include all of the SVC and a long segment of innominate vein. If the recipient has had a prior Fontan or Glenn procedure, modifications of the amount of pulmonary artery harvested should be made. A long segment of ascending aorta should be harvested. The left atrial portion of the procurement is usually standard.

Recipient operation (Figures 7–9). A number of techniques have been described that successfully handle the problems presented by situs inversus. The major adjustment is in the area of connection for the systemic venous return. Cannulation directly into the SVC and IVC at points far distant from the right atrium is advised. The aorta should also be cannulated in a distal location. The incision in the right atrium is near the atrioventricular groove, leaving sufficient tissue to allow for the modifications necessary for implantation of the donor heart. The incision in the left atrium is also near the atrioventricular groove. As much of the atrial septum as possible should be retained. The pulmonary artery is transected close to the bifurcation. The aorta is transected distally to move the anastomosis closer to the midline.

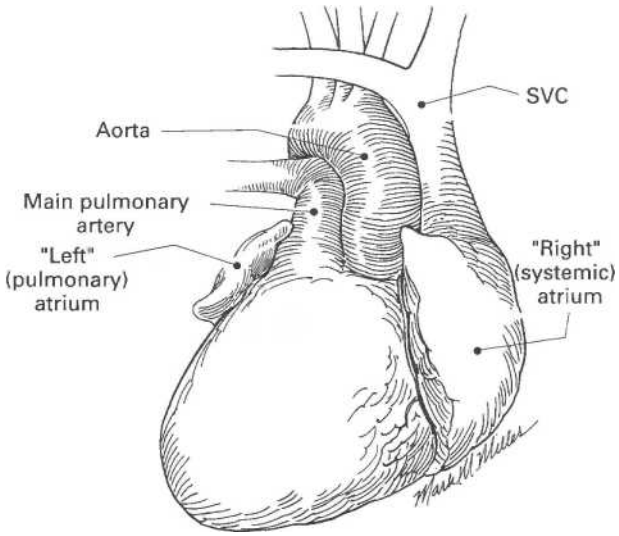

Figure 7 Anatomy of situs inversus totalis

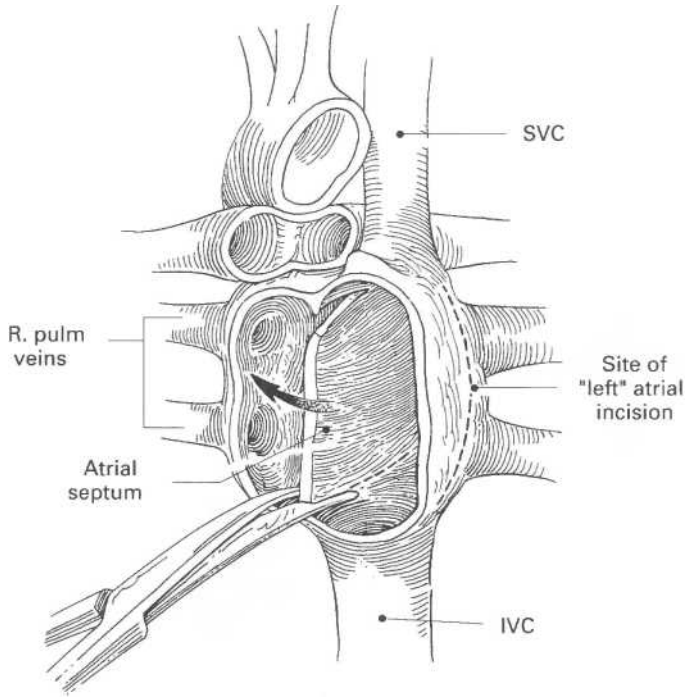

Figure 8 Situs inversus. Preparation of the recipient. The cardiectomy has been performed. The atrial septum is mobilized by incising the cephalad and caudad portions. The left atriotomy for the ultimate left atrial anastomosis is performed near the inter-atrial groove (indicated by dotted line)

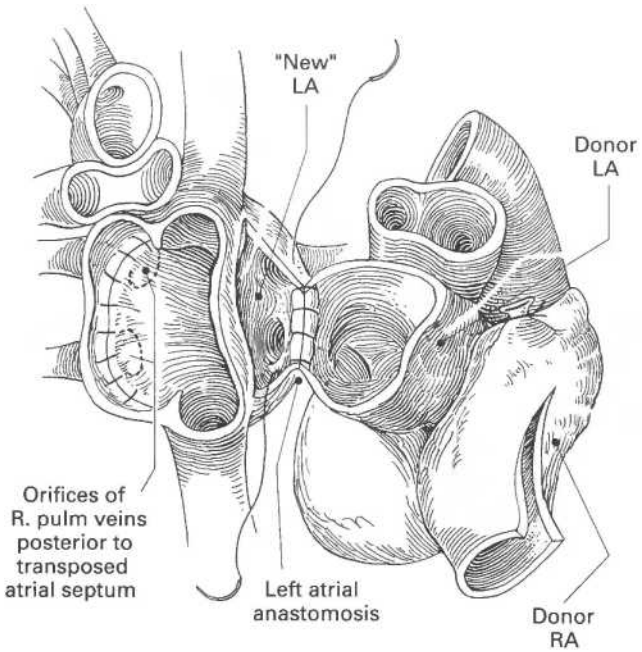

Figure 9 Situs inversus. The mobilized atrial septum is then sewn to the lateral free wall of the left atrium anterior to the right pulmonary veins so that the pulmonary venous blood will be directed to the new atriotomy. The donor left atrium is then sewn to the atriotomy near the inter-atrial groove. The right atrium is sewn to the atrial orifice on the right, to which the superior and inferior venae cavae have been directed. The pulmonary artery anastomosis should be performed prior to the aorta because it will lie posterior to the aorta. LA = left atrium, RA = right atrium

My preferred technique for implantation of the donor heart is the one illustrated and is reminiscent of the Senning procedure for transposition of the great arteries[21]. The atrial septum is mobilized by dividing it at its caudad and cephalad portions. It is then reattached to the free wall of the left atrium anterior to the right-sided pulmonary veins. When an atrial septal defect is present, pericardium or prosthetic patch material may be used in addition to, or in place of, the atrial septum. This directs the pulmonary venous return from the right lung to the left, across the midline. The interatrial groove is then mobilized extensively. An atriotomy is placed anterior to the left-sided pulmonary veins. The left atrial anastomosis is thus placed on the left side of the mediastinum. It is usually necessary to perform the right atrial anastomosis next. A portion of the left superior aspect of the new right atriotomy can be closed primarily to move more of this anastomosis to the right and to better match the size of the donor right atrial cuff. The recipient pulmonary artery is usually positioned to the patient's right. This can be effectively moved to the left by mobilizing the branches or by extending the arteriotomy out onto the left pulmonary artery, while partially closing the right side. The pulmonary artery anastomosis should be performed prior to the aortic anastomosis so that the pulmonary artery can be accurately seen. The aortic anastomosis is performed in the usual fashion.

There are other techniques (not illustrated) which may be applied, and may serve as better options depending upon the associated anomalies. Two of these will be described. The first is a modification of that described above, in which the atrial groove is dissected extensively and split so that two separate atria result. Frequently, the anterior portion of the atrial septum is too thin to split precisely; in that case, the septum should be devoted to the pulmonary venous atrium. These two atrial orifices are then transposed, moving the systemic (left-sided) atrium anterior and to the right, and moving the pulmonary (right-sided) atrium posterior and to the left. The SVC and IVC will need to be extensively mobilized by dividing the azygous vein above and by mobilizing subdiaphragmatic veins below. The atrial and arterial connections are then performed as described above.

The next modification is based on the principle of devoting the atrial mass to the pulmonary venous connection and re-establishing systemic venous flow with bicaval anastomoses[28]. This may be the preferred technique when the patient has palliated single-ventricle physiology or other major intracardiac anomalies, particularly one of the heterotaxy syndromes. Cannulation in the SVC must be at or above the entry of the innominate vein, and in the IVC below the diaphragm. The left-sided SVC is removed by transecting it at its junction with the heart and just below the entry of the innominate vein. This short segment is then anastomosed to the base of the innominate vein on the patient's right side. The donor SVC can then be anastomosed to the newly constructed right-sided SVC; alternatively, a long segment of donor SVC may be anastomosed directly to the innominate vein on the right side. The IVC is effectively moved across the midline to the right by utilizing a flap of right atrial tissue. The inferolateral portion of the right atrium is separated from the left atrium and the septum is resected. The incision in the right atrium is carried down into the IVC orifice medially to the level of the junction of the pericardium with the diaphragm. From there, the IVC is then sewn to the pericardium to create a tunnel which continues across the midline, utilizing the flap of right atrium to continue on to the

right where the donor IVC can be sewn to this tunnel; some of the IVC is sewn to the pericardial portion of the newly created 'tunnel'. The left atrial anastomosis is carried out by using the lateral portion of the recipient right atrium as the left side of the anastomosis, and the rest carried out in the usual fashion.

Heterotaxy syndromes

Patients with heterotaxy syndromes (or splenic syndromes) have ambiguous visceral and atrial situs associated with anomalies of systemic and pulmonary venous drainage. Virtually all these patients have single-ventricle physiology and most will have undergone prior palliative procedures. In addition, they have an endocardial cushion defect with its associated single common atrioventricular valve. This valve frequently becomes insufficient, resulting in either failed Fontan procedures or lack of candidacy thereof, thus leading to HTx as a treatment option.

The key issue once again pertains to the venous drainage, either systemic or pulmonary. Anomalies of the pulmonary venous drainage are associated with both polysplenia (sometimes referred to as left atrial isomerism) and asplenia (sometimes referred to as right atrial isomerism) syndromes, although the extracardiac forms occur almost exclusively with the asplenia syndrome. Anomalies of the systemic venous drainage occur with both asplenia and polysplenia, but are more common with polysplenia, where the vast majority of patients will have bilateral SVC as well as continuation of the IVC to the SVC via the azygous system. The hepatic veins may enter the atrium separately. It is crucial that both systemic and pulmonary venous drainage be carefully defined, either by cardiac catheterization or by other imaging studies such as a magnetic resonance.

Donor procurement. This is performed much like procurement for situs inversus. Sufficient length of SVC is necessary to allow for flexibility in the transplant operation. Other considerations for the harvest of the great arteries will be dependent on the associated anatomic defects and prior palliative procedures. The left atrial portion of the harvest does not need to be altered significantly. Although it may be more convenient to obtain the entire left atrium, including the orifices of the pulmonary veins, this would preclude lung harvest for transplantation of this organ into another recipient. It is entirely possible to perform isolated HTx in these patients with only a small left atrial cuff from the donor.

Recipient operation (Figures 10 and 11). The transplant procedure should in general be based on the principle of devoting the atrial mass to the pulmonary atrial anastomosis, and connecting the venae cavae by direct anastomoses. The illustrations provided are for a patient with total anomalous pulmonary venous connection to the right SVC and a midline IVC. The SVC is of necessity very large just as it enters a large common atrium, which these patients frequently have. Cannulation again is high in the SVC (above the entry for the pulmonary veins) and very low in the IVC. The heart is excised with an atrial incision that proceeds around the heart near the atrioventricular groove. The SVC is divided below the cannula and above the entry of the pulmonary veins, and the cardiac end is then oversewn. The IVC is separated from the atrium, leaving a short cuff of atrium attached for added

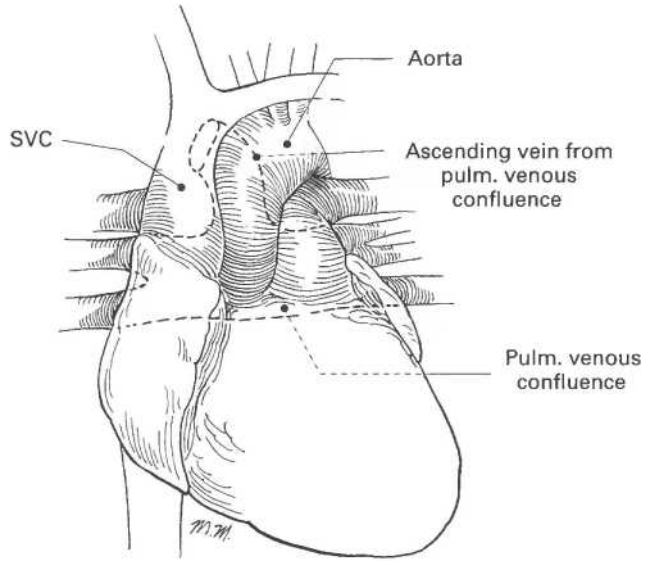

Figure 10 Example of anatomy of a patient with heterotaxy syndrome manifest as total anomalous pulmonary venous drainage to the superior vena cava via an ascending vein. The pulmonary venous confluence is in the posterior mediastinum behind the parietal pericardium and in a relatively superior position

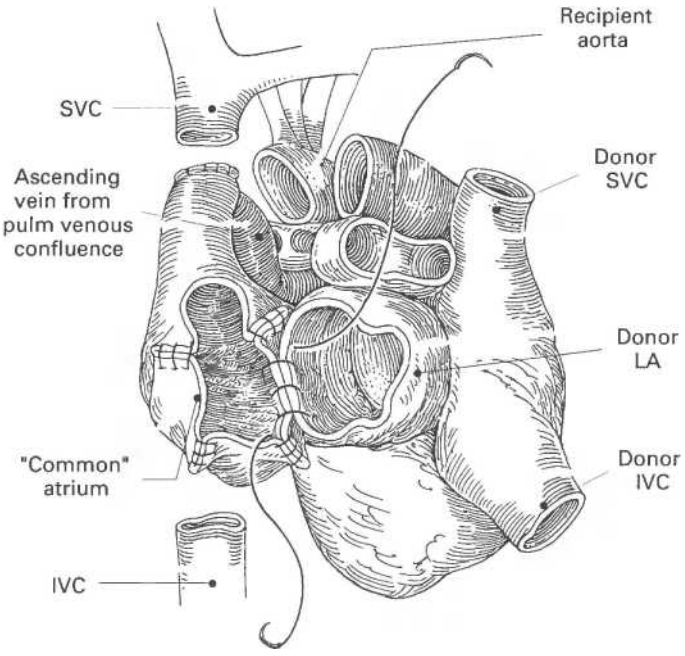

Figure 11 Heterotaxy syndrome. The recipient cardiectomy is performed such that the ascending vein from the pulmonary venous confluence is left intact into the proximal superior vena cava. The common atrium will become the left atrium. It is generally very large and, when the recipient cardiectomy is performed, a large orifice is present with the opening frequently more to the patient's right side. This opening is reduced in size by sewing up the corners in such a way as to shift the opening more to the left side. The systemic veins are connected by direct caval anastomoses

length. This leaves only the pulmonary venous drainage to empty into the atrial mass. This very large atrial orifice will frequently need to be reduced in size by sewing up the corners, especially those on the right upper and lower corners. This effectively moves the recipient left atrial orifice to the left. The transplant is then performed, beginning with the left atrial anastomosis. The connections for the pulmonary artery and aorta will depend upon the associated anomalies, but are generally not difficult to accomplish. Finally, the systemic venous connections are made with caval anastomoses[29].

IMMUNOSUPPRESSION/REJECTION

Although some have suggested that neonates may be at lower risk for rejection due to an immature immune system[30], the rate of rejection is the same in this age group as in any other[31]. It is our policy to maintain the same level of immunosuppression in infants as we do in teenagers. All are given an oral dose of cyclosporin pretransplant, and begun on a continuous infusion post-transplant to establish blood levels of 250–350 ng/ml. Azathioprine is given pretransplant in a dose of 2 mg/kg and then post-transplant at a dose of 1.5–2.0 mg/kg per day, modifying this as necessary to maintain a white blood cell count at >4500/mm^3. Methylprednisolone (20 mg/kg) is given while on cardiopulmonary bypass prior to removing the cross-clamp. The steroid dose post-transplant is weaned from 1 mg/kg per day to 0.1 mg/kg per day over 3–6 months, and then discontinued in selected patients. The cyclosporin dose is gradually reduced such that the trough blood level at 1 year post-transplant is 150–200 ng/ml. We reserve cytolytic therapy for recurrent or refractory rejection, but many centers use it early as 'induction therapy'. The first endomyocardial biopsy is taken at 7–10 days post-transplant. Because there are no absolutely reliable non-invasive means for detecting rejection, apart from histologic examination of the myocardium, endomyocardial biopsies are performed in all patients from the smallest infants to teenagers at regular intervals[31]. Acute rejection (>grade 2) is treated with bolus methylprednisolone (20 mg/kg) daily for 3 days.

POST-TRANSPLANT COMPLICATIONS – EARLY

Graft failure

This is the most common cause of death, particularly in the neonatal age group. Mechanical support for infants is generally limited to extracorporeal membrane oxygenation (ECMO). Intra-aortic balloon counterpulsation is generally not effective in small children because of relatively small size, high heart rates, and the distensibility of the aorta preventing effective counterpulsation. Small size is also the limiting factor for the use of ventricular assist devices. Although an oxygenator may not be necessary, ECMO provides an excellent means of mechanical support. As with other non-transplant cardiac procedures, it usually requires 5–7 days for recovery of the heart to be able to wean from ECMO. The patient should probably be relisted for HTx when mechanical support is necessary, bearing in mind that the heart might still recover, and that the results of retransplantation for acute graft failure are poor.

Right heart failure with tricuspid valve regurgitation may be treated in a number of ways. However, prior to initiating treatment one must ensure that there is no technical problem leading to obstruction of the main pulmonary artery or one of its major branches; this is particularly true in the setting of prior Fontan or other palliative procedures where the transplant has involved reconstruction of the pulmonary arteries. The combination of prostaglandin E_1, administered via a central venous catheter, and norepinephrine and/or epinephrine, administered via a left atrial line, is reasonably effective treatment. Prostacyclin and inhaled nitric oxide are particularly potent selective pulmonary vasodilators that are available in some centers and will likely become generally so in the near future. These agents are so effective that they may extend the limit of pulmonary vascular resistance acceptable for isolated HTx. It has been our experience that over the first 4–6 days following HTx the pulmonary vascular resistance falls somewhat, and the tolerance of the graft to this afterload requirement on the right side increases, in part due to recovery from the ischemic interval. Thus, one should make every effort to get through this interval, including the use of a right ventricular assist device when necessary.

Recurrent coarctation of the aorta is a potential surgical complication in infants transplanted for hypoplastic left heart syndrome. It should be suspected in the presence of upper extremity hypertension. This generally occurs late enough that it can be effectively treated with balloon dilatation[32].

Bleeding

This is particularly an issue in patients that have had multiple prior palliative procedures. The use of aprotinin is now routine in our center for these patients, and has had significant benefit at least on a subjective level. I do not believe that the number of prior cardiac procedures should impact on candidacy for HTx. We have successfully transplanted patients with as many as five prior sternotomies.

Neurologic

These complications include headaches, tremors, mental status changes, and seizures. Some of these may be related to the prolonged periods of circulatory arrest necessary for some of the transplant procedures. However, most of these complications are related to cyclosporin therapy and are not associated with a long-term neurologic problem.

Other

Gastrointestinal complications include gastric and duodenal ulcers, acalculous cholecystitis, and pancreatitis. We have encountered a number of patients with chylous pleural effusions that ultimately required thoracic duct ligation. Most of these were related to leaving the left-sided central venous catheter in place for a prolonged period of time, with subsequent occlusion of the subclavian or internal jugular veins. Phrenic nerve injury can occur from direct surgical trauma, particularly in patients requiring extensive dissection of the right SVC or of the branch pul-

monary arteries. This is generally well tolerated except in young infants who depend almost exclusively on the diaphragm for inspiration; they will likely require diaphragm plication for weaning from the ventilator.

POST-TRANSPLANT COMPLICATIONS – LATE

Infection

Infections in these children are relatively common, but are generally due to agents seen in otherwise normal children[12]. Respiratory syncytial virus occurs but has responded appropriately to either supportive therapy for mild clinical infections or ribavirin for more symptomatic cases. Symptomatic cytomegalovirus infections are unusual, but seroconversion is common. All children receive prophylaxis against *Pneumocystis carinii*, and infections due to this agent are rare. Fungal infections are also unusual. Immunizations are an important consideration. All children can receive inactivated vaccines – diphtheria–tetanus–pertussis and Salk polio. The measles–mumps–rubella vaccine is to be avoided, as is the oral (Sabin) polio vaccine. If the patient has time to complete his/her vaccination schedule prior to HTx, this is optimal. Children exposed to chickenpox should be treated with varicella–zoster immune globulin. The safety of the varicella vaccine in these patients is unknown.

Lymphoproliferative disease

In our experience this complication occurs rarely. It has usually been associated with Epstein–Barr viral infection. The management of this complication is the same as for adults, described elsewhere in this volume.

Graft vasculopathy

Some have suggested that the incidence of this complication in small children is less than that seen in adults[12]. However, in a multi-center study, some evidence of atherosclerosis was seen in 35% of children at a mean follow-up of 28 months post-transplant[33]. In our own series of 40 infants, two have been retransplanted, one has died, and another has angiographic evidence of coronary artery disease.

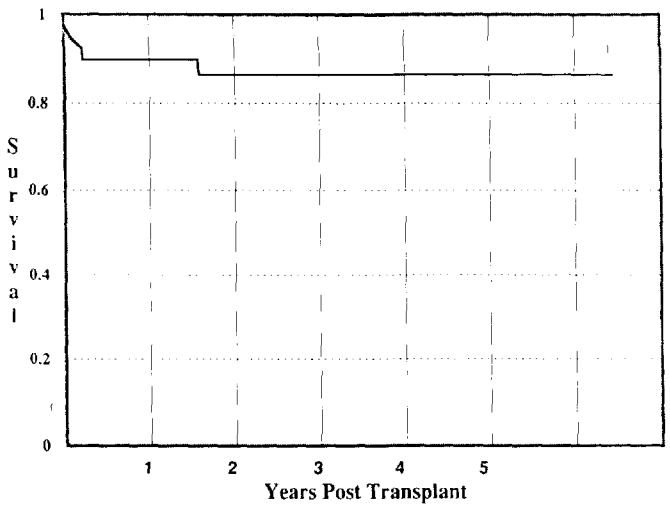

Figure 12 Kaplan–Meier actuarial survival curve of neonates undergoing heart transplantation at St Louis Children's Hospital. The vast majority had hypoplastic left heart syndrome

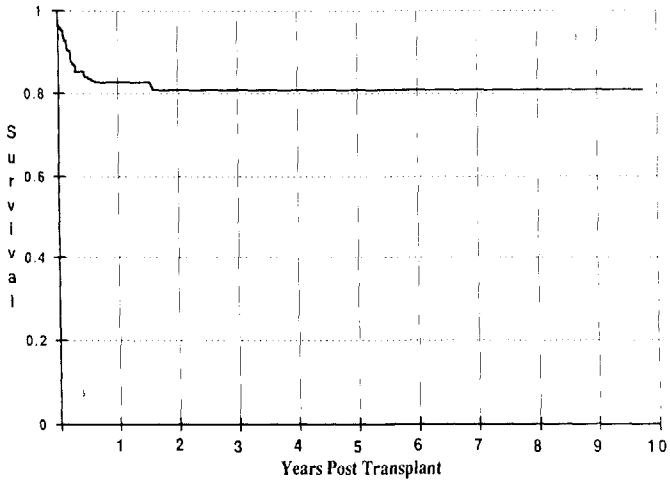

Figure 13 Kaplan–Meier actuarial survival curve for all children (age <18 years) undergoing heart transplantation at St Louis Children's Hospital

RESULTS

Survival

The actuarial survival for all pediatric patients undergoing HTx is around 78% at 1 year and 70% at 2 years post-transplant, figures similar to those obtained in adults. For infants transplanted at <1 year of age, the aotuarial survival is 65% at 1 year and 62% at 2 years. These data come from the Registry of the International Society for Heart and Lung Transplantation[3]. Most of the deaths in these infants occur in the first month following HTx and are due to graft failure. Our results at St Louis Children's Hospital are a little more favorable, with an overall survival of 90% at 1 year and 80% at 5 years. The survival in infants is 82% at 1 year and 80% at 5 years (Figures 12 and 13)

Growth

Apart from survival, quality of life is a concern. One determinant of this is the growth of the individual. A review of our series revealed that the infants grow along the 25th percentile for height and weight, with 4 years of follow-up available post-transplant[34]. Results from the Loma Linda group are similar[35]. We have also demonstrated that the left ventricle grows appropriately in these infants. When the donor heart is larger than the recipient, the left ventricular chamber adapts rapidly to the appropriate size for the stroke volume required. The wall thickness is initially significantly greater than normal, but gradually reduces to normal in the first year post-transplant[34].

COMMENT

It is now clear that, with intermediate follow-up available from several centers, cardiac transplantation in children has results similar to these seen in adults. There are no anatomic contraindications to HTx, although it may be necessary to alter the recipient anatomy and donor procurement to meet the individual needs. It is essential that a full anatomic evaluation of the recipient be performed so that appropriate plans can be made for donor procurement as well as the transplant procedure itself. The surgeon should have a thorough understanding of congenital heart disease and experience in the various palliative and corrective procedures employed therein.

Small size should not be an excuse for reliance on non-invasive means of diagnosis of rejection; if anything, the clinical diagnosis of rejection is more difficult to make in infants than in adults. Thus, one must be able to perform endomyocardial biopsies in children as small as 3 kg to provide appropriate care. An awareness of the impact of the usual childhood illnesses on the immunosuppressed patient is also necessary. The major impediment for this form of therapy remains the shortage of donors, a problem likely to be solved only with the evolution of xenotransplantation.

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39
Exercise Rehabilitation of Cardiac Transplant Recipients

E.W. DERMAN, K.L. DERMAN AND T.D. NOAKES

INTRODUCTION

Patients with chronic heart failure have impaired exercise tolerance. An expected outcome of cardiac transplantation in such patients is an improvement in functional capacity. However, despite normal left ventricular ejection fraction after cardiac transplantation, compared to control subjects the exercise capacity of these patients remains impaired both during symptom-limited maximal exercise and during static exercise[1–6]. Indeed, the exercise capacity of patients after cardiac transplantation is often comparable to that of patients with medically stabilized heart failure[7]. The reasons for this phenomenon remain unclear, but it could indicate that the exercise capacity of these patients is limited by peripheral alterations, perhaps in skeletal muscle, which develop during heart failure and which are not reversed immediately after cardiac transplantation.

There are a number of possible reasons why cardiac transplant recipients will have significantly impaired functional capacity and exercise tolerance.

Firstly, because of the incapacity caused by their progressive disease process, cardiac transplant recipients have frequently participated in little or no meaningful physical exercise for many months or years prior to transplantation. Ultimately they may be bedridden. The deleterious effects of bed rest on cardiovascular and skeletal muscular function and on bone mineral content are well described[8–12].

Secondly, the function[13] and structure of the skeletal muscle are abnormal following cardiac transplantation (Figures 1 and 2). This skeletal myopathy was originally ascribed to the use of corticosteroids following cardiac transplantation[14–16].

The main feature of the steroid myopathy is atrophy of both fiber types, particularly of type IIB fibers[17]. Electron microscopic analysis of skeletal muscle with steroid myopathy shows enlarged and degenerate mitochondria, dilatation of the sarcolemma, loss of myofibrils, and marked thickening of the basement membrane. Lipid-filled vacuoles and glycogen accumulation, especially in the type I fibers, are also prominent features[18]. The dose of corticosteroids necessary to induce myopathy is variable; prednisone 15–100 mg daily for periods ranging from 1 month to 5 years[19,20] has been shown to cause a skeletal myopathy.

Research from this laboratory shows that profound abnormalities of skeletal muscle structure, that are different from those described above, exist in patients with chronic heart failure and persist after cardiac transplantation[21]. These abnormalities include muscle fiber splitting, type II fiber predominance, myelin inclusion whorls within the mitochondria and various abnormalities of the cell nucleus. These findings suggest that the skeletal muscle structure and function in the transplant recipient are present before the chronic ingestion of corticosteroids, and are caused primarily by the condition of chronic cardiac failure. We have performed biopsies of skeletal muscle of cardiac recipients up to 2 years following cardiac transplantation, and have shown abnormalities similar to those identified prior to transplant. These findings suggest that the skeletal myopathy in cardiac transplant recipients is reversed slowly, and might indeed be inhibited or slowed by the ingestion of corticosteroids.

Thirdly, even after surgery, cardiac recipients can potentially have a residual central (cardiovascular) functional limitation as a result of the altered pattern of response of the denervated heart to exercise. As a result of denervation, the heart rate of cardiac recipients rises more gradually after the onset of exercise, reaches a lower peak, and decreases more slowly after cessation of exercise than does that of the subjects with normally innervated hearts[3–5,22,23]. As a result, maintenance of an adequate cardiac output in cardiac transplant recipients during submaximal exercise is achieved by augmenting preload and activating the Frank–Starling mechanism[4]. At high workloads, cardiac output increases secondary to chronotropic and inotropic effects induced by steeply rising circulating norepinephrine concentrations[4,24–27]. Whether these adaptations can ensure that the maximal cardiac output of transplant recipients equals that of normal subjects is not clear[5,23,28–30], but seems unlikely[1]. However, it is unlikely that these alterations in cardiac performance could be responsible for impaired performance during submaximal exercise when cardiac output is not limiting.

It should be noted that the prescription of β-receptor antagonist agents severely restricts the exercise tolerance of cardiac transplant recipients, in part because it prevents the essential chronotropic and inotropic actions of circulating catecholamines

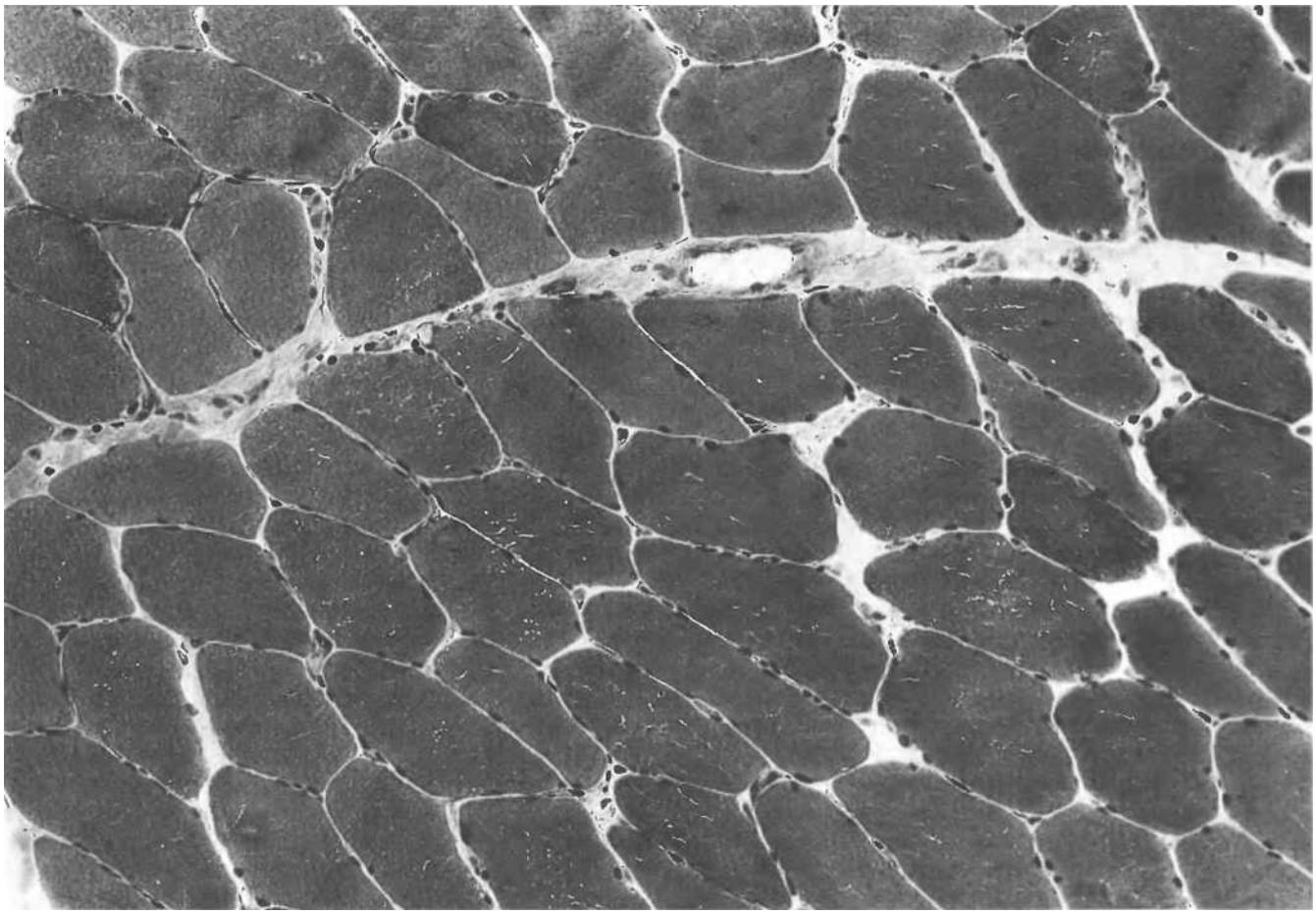

Figure 1 Light photomicrograph showing normal skeletal muscle from a control subject (H&E, × 300)

on the denervated myocardium[31,32]. However, not all studies have found β-blockers to have a deleterious effect on exercise tolerance in transplant recipients[3].

Fourthly, alterations in peripheral blood flow are present following cardiac transplantation[2,33]. Haywood *et al.*[33] measured forearm and renal blood flow during short-duration maximal upright bicycle exercise in transplant recipients. They reported increased renal and forearm vascular resistance with decreased renal and forearm blood flow, indicating that vasoconstriction is increased in renal vessels and non-exercising skeletal muscle during exercise. Morgan *et al.*[2] measured forearm blood flow in the non-exercising forearm of these patients during 30% maximal static handgrip exercise and also found that vasoconstriction, rather than the normal vasodilatory response, occurs in the non-exercising forearm during static exercise. These peripheral vascular changes might contribute to the exercise intolerance and skeletal muscle abnormalities in patients both before and after cardiac transplantation.

Fifthly, the ventilatory response to exercise is similar in transplant recipients to that observed in patients with chronic heart failure. The increased ventilation seems to be due to pulmonary ventilation/perfusion mismatch, or a restrictive pattern of breathing[34,35] or altered pulmonary diffusion capacity[13].

However, less than 80% of the maximal minute ventilation is utilized at peak exercise in transplant recipients. Thus patients terminate the exercise test before maximal ventilation is attained. Furthermore, arterial pO_2, pCO_2 and pH during graded exercise is not different in transplant recipients from controls[36]. These findings suggest that ventilatory abnormalities during exercise are generally not the factor limiting exercise performance in transplant recipients[3,37].

Finally, a major long-term limitation is the process of chronic rejection, manifested by accelerated atherosclerosis of the donor coronary arteries, and myocardial necrosis. Thus, work time during maximal exercise in cardiac transplant recipients was inversely related to the history of rejection, and was least in those with the most frequent and severe episodes of rejection[5]. This relationship could be explained either by more frequent episodes of myocardial necrosis, or by the administration of higher doses of immunosuppressive agents in those experiencing frequent episodes of rejection.

The result of any and all of these processes is that the exercise tolerance of cardiac transplant recipients is subnormal[1,5,28,38].

In this review we present an approach to the evaluation of the cardiovascular and skeletal muscle function of cardiac transplant recipients, and describe how this information can be used to pre-

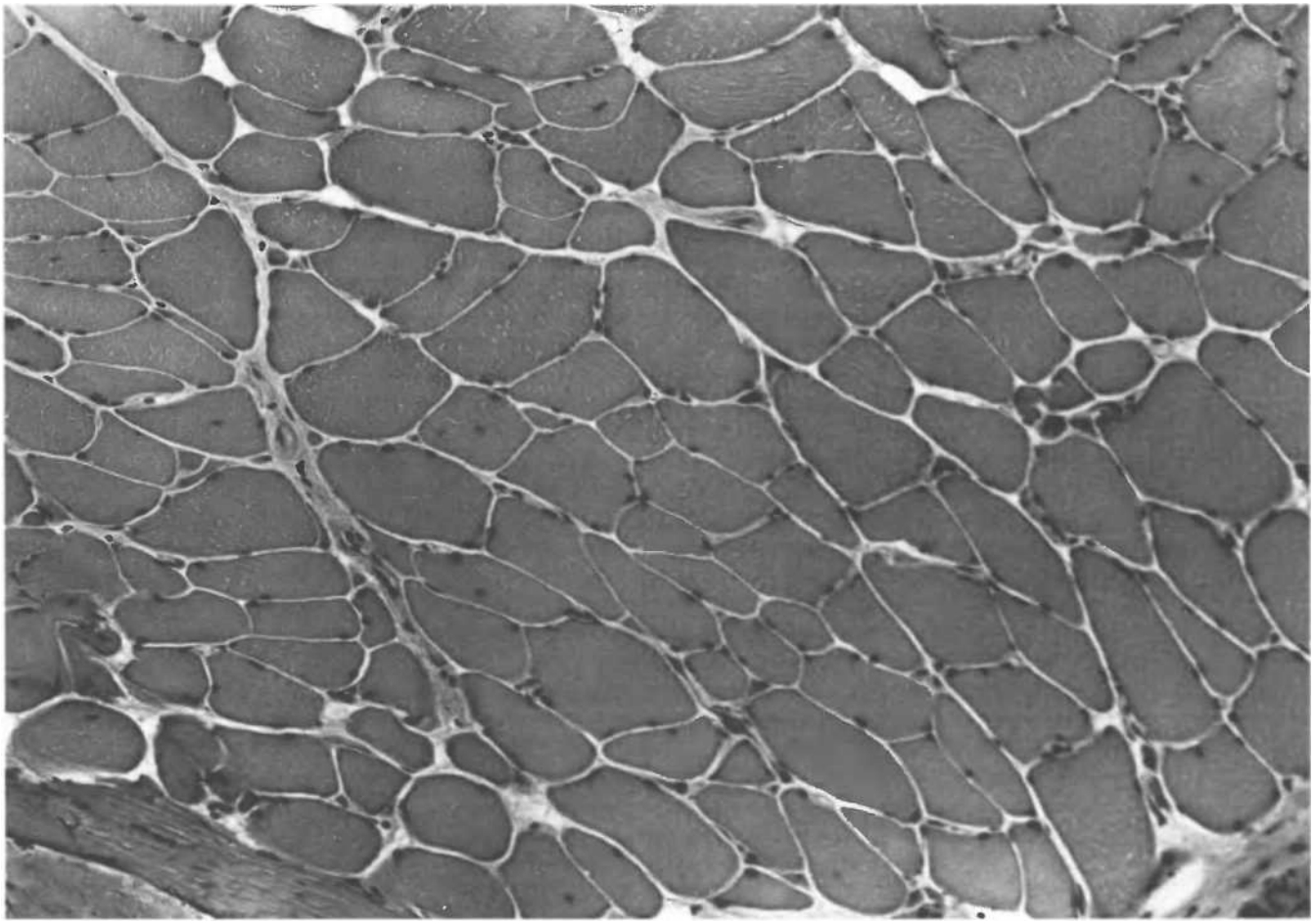

Figure 2 Light photomicrograph showing prominent atrophy, hypertrophy, and split skeletal muscle fibers from a heart transplant recipient. There are occasional central nuclei. Capillaries with very thick walls can be seen (H&E, × 200)

scribe appropriate individualized exercise programs for such patients. It is likely that the benefits of such an exercise program for cardiac recipients are similar to those enjoyed by patients with coronary artery disease[39–45].

EXERCISE TESTING AND FACTORS LIMITING EXERCISE PERFORMANCE IN CARDIAC TRANSPLANT RECIPIENTS

The prescription of the appropriate individualized exercise training program hinges on a correct understanding of the physiological and pathological factors that limit the exercise tolerance of cardiac transplant recipients. These factors are identified during short-duration exercise of progressively increasing intensity, maintained by the patient until volitional exhaustion or until the onset of identifiable medical endpoints, such as electrocardiographic changes compatible with myocardial ischemia, significant arrhythmias, hypotension, or angina pectoris.

The exercise test starts at a low work rate, with progressive linear increases in oxygen consumption and heart rate and with linear increases in work rate.

Figure 3 depicts the physiological response of cardiac transplant patients to exercise of progressively increasing intensity, and that of age-matched, sedentary control subjects. None of the transplant recipients terminated exercise prematurely because of the development of medical endpoints, and most complained of leg fatigue.

The features of note are that the cardiac transplant recipients stopped exercising at significantly lower work rates than did controls. However, the cardiac transplant patients stopped exercising when their rates of oxygen consumption, rates of ventilation and blood lactate concentrations were comparable to those of the control subjects at that same workload. Furthermore, blood lactate concentrations were also similar in both groups, with no evidence for a 'lactate turnpoint' during exercise in the transplant recipients. These findings suggest that the cardiac recipients stopped exercising before the delivery of oxygen to the active skeletal muscles became limiting.

Thus, somewhat paradoxically, one must assume that the exercise performance of the majority of cardiac recipients is limited either by peripheral, skeletal muscular factors, as also concluded by Kavanagh and his colleagues[1] or by a failure of skeletal muscle recruitment (central neural drive). It is clear, however,

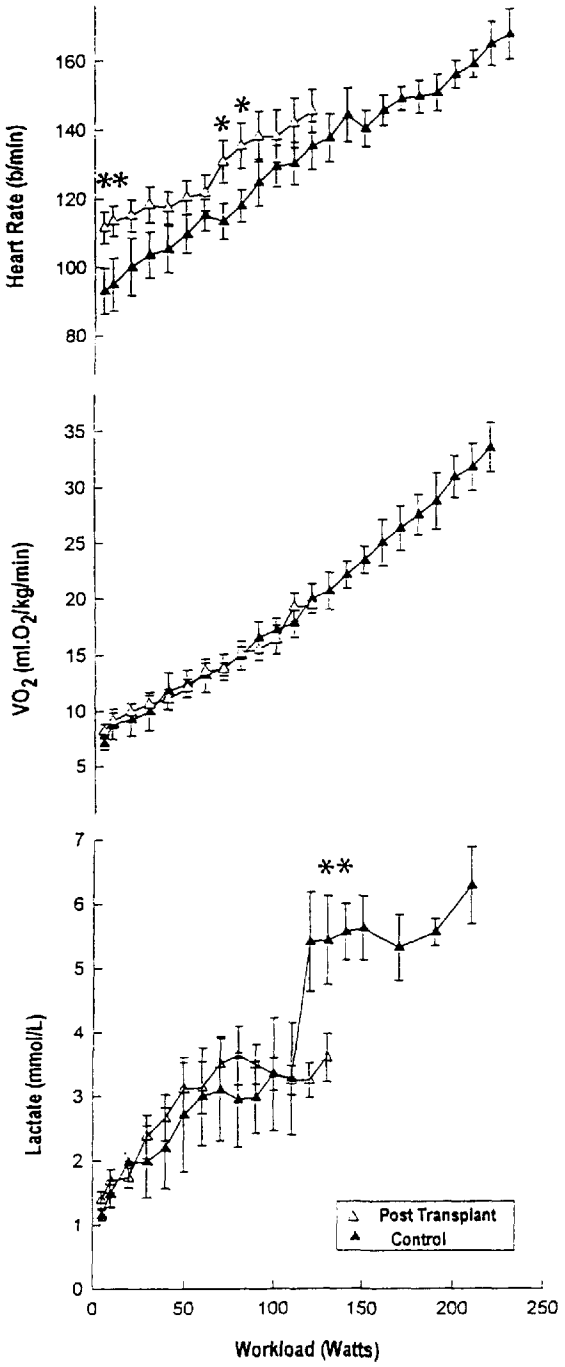

Figure 3 Heart rate, oxygen consumption and blood lactate concentrations depicted against increasing workload during graded exercise to exhaustion in patients after heart transplant (n = 15) and age-matched sedentary controls (n = 10). Data are displayed until >50% of the patients and controls terminated the exercise test. Abbreviations: b/min = beats per minute; V_{O_2} = volume of oxygen; ml O_2/kg/min = milliliters of oxygen consumed per kilogram of body weight per minute; * p <0.05 post-transplant vs control. All values are expressed as mean and standard error of the mean

that patients who terminate exercise at low rates of oxygen consumption and low venous blood lactate concentrations are unlikely to have a central cardiovascular limitation of their exercise tolerance.

These data indicate that an evaluation of skeletal muscle function would be appropriate in these patients.

ASSESSMENT OF SKELETAL MUSCLE FUNCTION IN CARDIAC TRANSPLANT RECIPIENTS

Currently *in vivo* skeletal muscle function can be evaluated by isokinetic or isometric testing.

Measurement of isokinetic skeletal muscle function

Isokinetic muscle strength and endurance can be measured using the Cybex isokinetic dynamometer (Lumex Inc., New York, USA). This system measures the dynamic muscular performance of different muscle groups during reciprocal contractions at different functional speeds.

A strength test measures maximum isokinetic torque of the quadriceps and the hamstring muscle groups of the dominant limb recorded during three maximal contractions through a full range of motion at a limb contraction speed of 60°/s. An endurance test of the dominant leg, involving repeated full knee extensions for 25s at a rate of 180°/s, is also usually performed. The total work and power generated by the leg muscles at this speed are measured.

Measurement of isometric skeletal muscle function

Isometric skeletal muscle strength of the quadriceps muscle can be measured by a strain gauge mounted on a custom-made leg-stabilizing chair.

In this chair the patient sits with his or her arms folded, back angled at 90°, knees at 90° flexion, and pelvis secured to the chair by an adjustable belt. Once the use of the hip flexors has been limited by securing the pelvis, a cuff is placed above the malleoli of the right ankle, and linked via a chain to a precalibrated strain gauge for the measurement of torque during contraction of the knee extensors.

Patients perform (a) maximum voluntary contractions (MVC) for the measurement of peak isometric torque and (b) repeated cycles of maximal contraction/relaxation until the patient is unable to generate a torque of 70% of the initial MVC. The time to reach this point is recorded as the time to fatigue, and is a measure of resistance of the skeletal muscle to the development of fatigue[46].

Results of tests of isometric and isokinetic skeletal muscle function in patients after cardiac transplantation and in age-matched, sedentary controls are depicted in Figure 4. Both isometric and isokinetic skeletal muscle function is impaired in patients after cardiac transplantation. Furthermore, the ability of the skeletal muscle to generate peak isokinetic torque and maximal isometric voluntary contraction, as well as the ability of the skeletal muscles to resist the development of fatigue, is reduced in patients following cardiac transplantation.

Whilst neither of these tests has yet been used for exercise prescription, they can be used (a) to quantify the peripheral component of the impaired exercise tolerance of transplant organ recipients; and (b) to follow the changes in muscle power that develop with training[15].

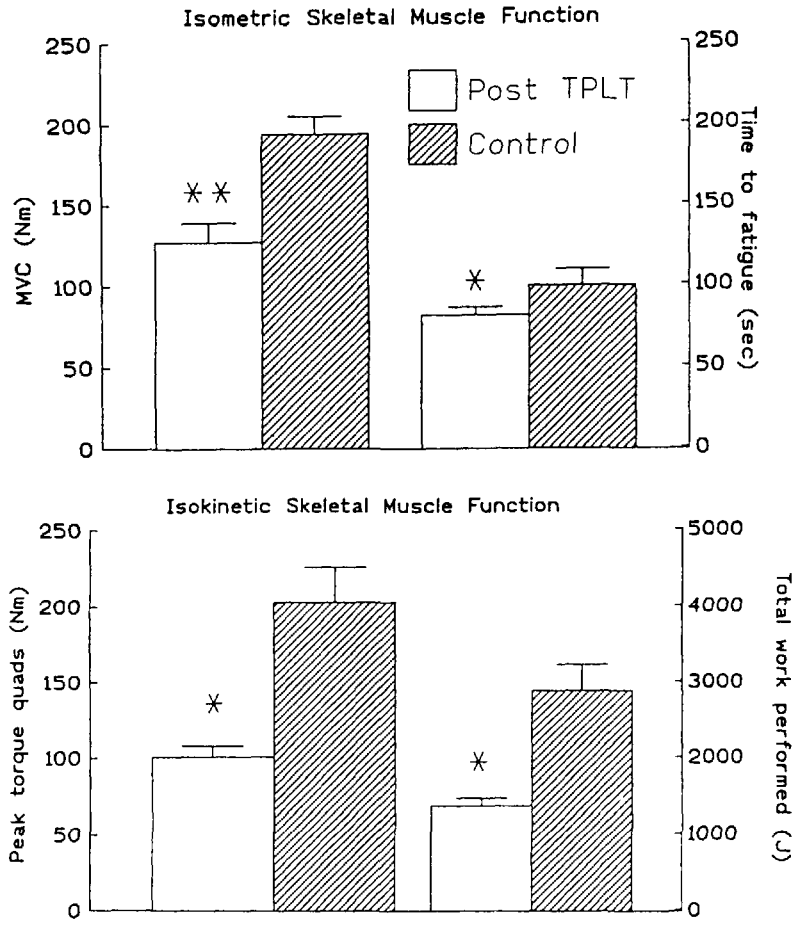

Figure 4 Isometric and isokinetic tests of skeletal muscle function in patients after heart transplant (n = 15) and age-matched sedentary controls (n = 10). Abbreviations: MVC = maximum voluntary isometric contraction; Nm = Newton metres; sec = seconds; quads=quadriceps; J = Joules; TPLT = transplant. * $p<0.05$ post-transplant vs control. All values are expressed as mean and standard error of the mean

EXERCISE REHABILITATION PROGRAM FOR THE CARDIAC TRANSPLANT RECIPIENT

The general principles for the exercise rehabilitation of the transplant recipient are similar to those for patients who have undergone coronary artery bypass surgery, coronary angioplasty or stent procedure, or valve replacement surgery, or who are recovering from myocardial infarction. Concise, formal guidelines for exercise rehabilitation of these patients are available[47]; however, special considerations for the rehabilitation of the transplant recipient exist and will be detailed.

The exercise rehabilitation program provides the transplant recipient with a graduated exercise training program and is divided into four phases: phase I, in hospital; phase II, post-hospital exercise intervention; and phases III and IV, extended outpatient and exercise maintenance[47].

Phase I usually begins as soon after surgery as possible and lasts about 10–15 days. Phase II, the convalescent stage, should begin within 4 weeks of discharge from hospital and usually lasts 8–12 weeks. Phase III, the supervised portion of the maintenance program, is usually of 4–6 months duration, and phase IV, the unsupervised portion of the ongoing maintenance program, is of indefinite duration.

Phase I – the intensive-care unit and hospital inpatient phase

The intensive-care unit phase of the rehabilitation program starts shortly after surgery, under the supervision of a physical therapist and the physician. When the patient's cardiac status is considered to be stable, he or she may start to perform a series of simple exercises. The aims of these exercises are to diminish the patient's risk of developing hypostatic pneumonia and thromboembolic phenomena, and to restrict the detrimental physical and psychological effects of deconditioning.

The formal exercise program will probably be conducted by a physiotherapist, and will usually be confined to exercises conducted in the bed or at the bedside[48].

Examples would be breathing exercises, upper limb exercises for the arms and shoulders, leg raises and ankle exercise, all performed once a day. The energy cost of these activities is very low, increasing the heart rate of the order of 8 beats/min and the rate of oxygen consumption by about 2 ml/kg per minute, or less than one metabolic equivalent (MET, which equals 3.5 ml O_2/kg per minute)[49,50].

Depending on the length of stay in hospital, and the patient's response to these exercises, additional exercises are added and the session increased to twice daily. 'Armchair mobilization' and

standing at the side of the bed are encouraged, and walking is introduced. The intensity of all activities is carefully controlled by monitoring the patient's level of perceived exertion[49] and watching for the development of cardiac arrhythmias or the onset of inappropriate dyspnea.

Unresisted cycling, treadmill walking, and resisted cycling may be introduced subsequently. Progression at all levels is carefully monitored in accordance with the criteria that are well established for the exercise rehabilitation of patients with ischemic heart disease[47]. Phase I rehabilitation usually lasts 10–15 days following cardiac transplantation.

Phases II and III rehabilitation: the post-hospital phase

Our own experience has been with out-of-hospital rehabilitation, particularly of patients with ischemic heart disease, but also of renal[15] and cardiac transplant recipients. The requirements of all these patients are quite similar. Experience with patients with ischemic heart disease has established the safety and value of early low-intensity exercise testing within 3–6 weeks after acute myocardial infarction, before hospital discharge[51]. It is more likely that, because of their lower risk of developing ventricular fibrillation during exercise, such criteria need not be applied with equal rigidity to all cardiac transplant recipients.

Early, low-intensity exercise testing may be performed prior to hospital discharge. In patients with ischemic heart disease such testing usually stops at a heart rate of 120–130 beats/min, or at 30 beats/min above the resting heart rate in persons receiving β-receptor antagonist agents, or at 60% of the age-predicted maximum heart rate. Criteria for terminating the test in cardiac transplant recipients who lack the normal chronotropic response to exercise will, of necessity, be different. The use of the Borg Scale of Perceived Exertion would be most appropriate; the test should probably be terminated when the subject reaches a perceived exertion of 12–13 units, equivalent to a perceived exertion described as 'light to somewhat hard', or a workload equivalent to about 9 MET[52]. Patients with ischemic heart disease who achieve a low maximum workload, or who show evidence of poor myocardial function during such low-intensity testing, have a 1-year mortality rate of between 15% and 25%, and require further cardiologic investigation. Cardiac transplant recipients are unlikely to show any of these abnormalities during low-level exercise testing. Those who do, would likely constitute poor candidates for an exercise rehabilitation program, and would require further, more invasive cardiac evaluation prior to referral to a formal exercise program.

Patients who show none of these abnormalities during low-intensity exercise testing may undergo symptom- and sign-limited maximal exercise testing some time later, possibly within 2–6 months after transplantation[1,52].

It is very useful, specifically in cardiac recipients, to sample expired respiratory gases during such maximal exercise testing, in order to measure minute ventilation and oxygen consumption on-line, for the following reasons:

(1) A failure of oxygen consumption to rise with further increases in workload would indicate that the patient had reached maximum aerobic workload, perhaps as a result of a limiting capacity to increase cardiac output, and that no extra information can be obtained by continuing the test further. As described, this is an unusual endpoint in most cardiac transplant recipients, but could be present in patients with advanced heart failure, in which case it is a dire prognostic sign.
(2) The workload corresponding to the ventilatory threshold can, at times, be identified. It serves little purpose to exercise the patient much beyond the ventilatory threshold, as it is inappropriate to prescribe exercise at intensities that exceed this threshold, at least initially.

The medical criteria for terminating the exercise test are the same as those used in patients with ischemic heart disease, and have been detailed by Kavanagh and his colleagues[1]. These are:

(1) Adverse symptoms. It is important to recognize that a patient with a denervated transplanted heart does not (usually) sense anginal pain. Significant other symptoms included severe dyspnea, light-headedness, faintness, confusion, and severe fatigue.
(2) Adverse signs, including facial pallor; either a fall in heart rate or blood pressure, or the failure of either or both to rise with increasing effort, systolic blood pressure exceeding 280 mmHg, or diastolic blood pressure exceeding 140 mmHg.
(3) Adverse electrocardiographic changes, including frequent complex ventricular extrasystoles, ventricular tachycardia, sustained supraventricular tachycardia, atrial fibrillation, second-or third-degree heart block, or severe ST segment depression (horizontal or downsloping of greater than 4 mm).

Determining the appropriate exercise intensity

The symptom-limited maximal exercise test is used as the basis for determining the appropriate exercise intensity for the cardiac transplant recipient. A popular approach for patients with ischemic heart disease is to limit the patient to a maximum of 90% of the maximum symptom- or sign-limited heart rate achieved during the maximal exercise test, and to allow a 6-week training period before the patient is allowed to exercise regularly at that heart rate.

However, this is clearly inappropriate for the cardiac transplant recipient who has a blunted chronotropic response to exercise. Accordingly, in these patients it is more appropriate to prescribe exercise on the basis of the measured oxygen consumption, the ventilatory threshold[53], the Borg Scale of Perceived Exertion[49], or a combination of percentage of maximally achieved heart rate and perceived exertion[54]. The approach of Kavanagh *et al.*[1] has been to allow patients to exercise either at 60–70% of their peak oxygen consumption or peak METS measured during the maximal exercise test, or at the exercise intensity corresponding to the ventilation threshold, or at an effort rating of 14 on the Borg Scale of Perceived Exertion, equivalent to a perceived exertion described as 'somewhat hard'. A detailed walking/jogging program and the method of progression has been fully detailed[1]. As the heart rate response of the transplant recipient is slower at the onset of exercise and remains elevated longer into the recovery than that of normal program participants, a longer warm-up and cool-down is prescribed. Progression of the intensity of exercise is individualized and is according to exercise tolerance and adaptation to the exercise program.

Table 1 The UCT/SSISA 24-week initial phase II–III training program for cardiac transplant recipients

Week	*Stretching (min/week)*	*Stationary cycling (min/week)*	*Low-impact aerobics (min/week)*	*Walking (min/week)*	*Jogging (min/week)*	*Low intensity muscle strengthening (min/week)*	*Circuit weight training (min/week)*	*High intensity interval training (min/week)*	*Perceived exertion rating (units)*
1	45	30	0	15	0	0	0	0	10
2	45	30	0	15	0	0	0	0	10
3	40	40	0	15	0	0	0	0	11
4	40	45	0	20	0	0	0	0	11
5	40	50	10	20	0	15	0	0	12
6	30	60	10	20	0	15	0	0	12
7	25	60	15	20	0	15	0	0	12
8	20	60	20	20	0	15	0	0	13
9	20	40	30	20	10	20	0	0	13
10	20	30	30	20	10	20	0	0	13
11	20	30	30	15	15	20	0	0	13
12	20	30	40	15	15	20	0	0	13
13	20	30	40	15	15	0	30	0	13
14	20	30	50	10	20	0	30	0	13
15	20	30	50	10	20	0	30	0	13
16	20	30	50	10	20	0	30	0	13
17	20	30	50	10	20	0	30	0	14
18	20	30	50	10	20	0	30	0	14
19	20	30	50	10	20	0	30	0	14
20	20	30	50	0	30	0	30	0	14
21	20	30	50	0	30	0	35	15	14
22	20	30	50	0	30	0	35	15	14
23	20	30	50	0	30	0	35	15	14
24	20	30	50	0	30	0	35	15	14

Exercise times are displayed in minutes/week. Patients participate in three structured exercise sessions per week. At each session a different activity may be performed so that the total weekly exercise duration for each activity would be represented by the above times.

The University of Cape Town/Sports Science Institute of South Africa cardiac rehabilitation program

Our own approach has been to devise a 24-week graded program that includes stretching, walking, jogging, low-impact aerobic dance, high-intensity interval training (in selected patients) and circuit weight training (Table 1). Dietary advice and psychological support form an essential part of this program. The program is overseen by a sports medicine physician, physiotherapists and exercise physiologists. We have found that these persons have special expertise for developing interesting and enjoyable exercise programs that are meaningful for all participants. They are also able to adapt the programs to new ideas that are fashionable amongst the popular exercise movement. For example, the inclusion of circuit weight training or high-intensity interval training in any exercise program would have been unthinkable 10 years ago; yet it is perfectly acceptable today.

At the start of our program, emphasis is placed on stretching and aerobic exercise. The aerobic component of our program is achieved through stationary cycling, walking, jogging and low-impact aerobic dance.

Prescription of aerobic exercise

The prescribed intensity is determined by the individual patient's response to graded exercise as described above, according to the Borg Scale of Perceived Exertion, and increases gradually as the program progresses. Table 2 lists equivalent values for the percentage maximum heart rate, the percentage Vo_2 max., and the rating of perceived exertion according to the date of Ekblom and Goldbarg[55], and others[56]. It shows that, to a first approximation, the Vo_2 max. is 10% lower than the percentage maximum heart rate at any exercise intensity, and that the rating of perceived exertion can be calculated as the percentage Vo_2 max. multiplied by 0.2. Thus the training heart rate zone of between 60% and 80% maximum heart rate, within which the patient should maintain his heart rate during exer-

Table 2 Comparative values for percentage maximum heart rate, percentage maximum oxygen consumption (Vo_2max), and rating of perceived exertion

Maximum heart rate (%)	*Vo_2max (%)*	*Rating of perceived exertion (units)*	*Subjective description*
50	36	7.2	Very, very light
60	46	9.2	Very light
70	58	11.6	Light
80	70	14.0	Somewhat hard
90	82	16.4	Very hard
100	100	19.2	Very, very hard

Data from ref. 55.

cise, corresponds to 50–79% V_{O_2} max. and ratings of perceived exertion of 10–14, equivalent to subjective feelings of 'light to somewhat hard'.

Whilst this approach is not as exact as that of Kavanagh *et al.*[1], it accommodates a great diversity of sporting interests amongst the patients, who may be less committed to a program that includes only walking and jogging.

Low-intensity skeletal muscle strengthening

This form of exercise intervention is employed from the fifth week of our program and consists of a light resistance placed on a limb which is exercised through the natural range of motion. Light resistance exercise is facilitated with the use of Cliniband, thin surgical tubing or light dumbbells.

Circuit weight training

The use of circuit weight training in the rehabilitation of cardiac patients has gained popularity in recent years. Not only does this form of training improve skeletal muscle strength and functional capacity, but it also provides a safe and novel alternative to aerobic exercise[57]. Guidelines for patient selection and resistance exercise prescription have been published previously[57–59]. Selection criteria include:

(1) Patients should have participated in a cardiac rehabilitation program involving aerobic exercise for a period of 12 weeks before participating in a circuit weight training program.
(2) A second graded exercise test should be performed before starting the circuit weight training program.
(3) The patient should have an exercise capacity of at least 6–7 METS as measured during the graded exercise test.
(4) Resting blood pressure should not exceed 150 mmHg systolic and 100 mmHg diastolic.

Exclusion criteria in the transplant recipient include:

(1) Persisting discomfort experienced in the area of the thoracotomy scar.
(2) Uncontrolled arrhythmias or hypertension.
(3) The usual exclusion criteria for participation in aerobic exercise.

Selected patients in our program begin circuit weight training after 12 weeks of conventional (aerobic) exercise. Resistance is set at a mass which can be lifted/pushed 15 times without undue strain or breath-holding. This mass usually equates to between 40% and 60% of a one-repetition maximal effort.

Patients typically make use of eight stations of selected resistance machines, including seated bench press, pectoral squeeze, triceps push down, biceps curl, seated rowing, latissimus pull-down, seated leg press, and leg extension apparatus.

High-intensity interval training

Our belief is that there is no evidence that patients who exercise more vigorously necessarily derive greater benefit than those who exercise more conservatively. On the other hand, it would seem that the risk of cardiac complications arising during exercise, at least in patients with ischemic heart disease, increases with intensity of exercise[60,61]. Thus, our approach has been to encourage most patients to exercise at a lower intensity for a longer time rather than at a higher intensity for a shorter time. However, the safety of high-intensity short-duration interval training has recently been established[62,63]. Patients who participate in this form of training typically cycle at higher workloads which increase the perception of effort to level 16 on the Borg Scale Perceived Exertion. This intensity is maintained for up to 2 minutes followed by a 2-minute recovery period at a lower workload. This form of exercise training is popular in selected patients who have participated in the program for at least 20 weeks, have a superior functional capacity, and express a desire to exercise at a higher intensity.

Monitoring during the exercise sessions

During each exercise session, careful attention should be paid to the following:

(1) Each patient's level of perceived exertion and heart rate is regularly checked. At first these are measured every few minutes, but later, as the patient's ability to monitor exercise intensity improves, only once every session. The heart rate checks are essential to identify abnormal heart rhythms. Patients are instructed to report any cardiac rhythm abnormalities to the attending exercise specialists. To reinforce these practices all patients are required to fill out an activity card at the end of each exercise session. The card includes information on resting heart rate and blood pressure, on exercising heart rates, on time spent in each activity, on the presence of symptoms, and any medications that might have been taken.
(2) Blood pressure at rest and during exercise should be measured regularly, as cyclosporine ingestion and chronic neuroendocrine hyperactivity induce hypertension in most transplant recipients[64–67]. Furthermore, an abnormal blood pressure response may help identify incipient or established heart failure, which may be an indication of rejection.
(3) All symptoms must be immediately reported. Our experience in patients with ischemic heart disease is that the majority of patients at risk of sudden death will develop warning symptoms[60] and, if these symptoms are ignored, problems will develop. Symptoms such as excessive dyspnea, unusual fatigue, general malaise and tiredness, and light-headedness must be taken seriously. They are an immediate indication to reduce the training load, and for further cardiac evaluation. As angina does not develop in those with denervated hearts, the presence of myocardial ischemia must be detected by careful attention to these other symptoms.
(4) During circuit weight training the patients are monitored to ensure that the selected weight stack is managed with relative ease and that the patient does not hold his or her breath during the bout. Tenderness over the thoracotomy scar is monitored during the first 2 weeks of circuit weight training.
(5) The patient is not allowed to exercise during or within 10 days of a pyrexial illness, due to the possible, albeit low, risk of fatal myocarditis.
(6) The results of the most recent endomyocardial biopsy score should be known, as rejection will decrease exercise tolerance.

Exercise training should be stopped during episodes of rejection, and the training schedule modified on return of the recipient to the program[68]. Alternatively, a reduced exercise capacity should alert to the possibility of an episode of rejection.

(7) Patients are also instructed that they must train regularly without peaks of activity, must avoid competition, and must reduce their exercise training load should mental tension and depression develop. It is our feeling that work tension and business stress, particularly when travel is involved, are important causes of transient exacerbation of symptoms. Smoking is prohibited. Particular attention is paid to patients with type A personalities because they are notoriously difficult to control in any exercise rehabilitation program. They will frequently exceed their exercise prescription and fail to report symptoms. Thus they may be more likely to be at risk of complications, and require particular attention[61].

BENEFITS OF TRAINING

There are relatively few reports of the effects of exercise training in organ transplant recipients.

Squires *et al.*[52] trained two patients, beginning 6 weeks after each had undergone orthotopic cardiac transplantation. After 8 weeks of low-intensity exercise training the maximal exercise capacity of both patients had increased, and their heart rates and rating of perceived exertion were decreased at all submaximal workloads.

In the most comprehensive study yet reported, Kavanagh *et al.*[1] followed 36 cardiac transplant recipients who participated in a more vigorous walking/jogging training program for up to 17 months. The average peak power output of the transplant recipient was less than one-half that of the untrained normal controls in the pretraining maximal exercise test. After training, lean body mass was increased, heart rate and blood pressure at rest were reduced, as were heart rate, minute ventilation and perceived exertion, but not cardiac output, during submaximal exercise. At exhaustion, peak heart rate, peak power output, and peak rate of oxygen consumption (V_{O_2} max.) were increased.

More recently, Keteyian *et al.*[54] reported greater benefits of a 10-week exercise training program in heart recipients than in a non-training control group. These investigators reported that V_{O_2} peak, peak respiratory rate, and maximum ventilation were increased after exercise training compared to the controls. Ratings of the perception of effort at submaximal workloads were also reduced through exercise training.

Cardiac output during submaximal exercise is, however, not altered after exercise training in transplant recipients[1].

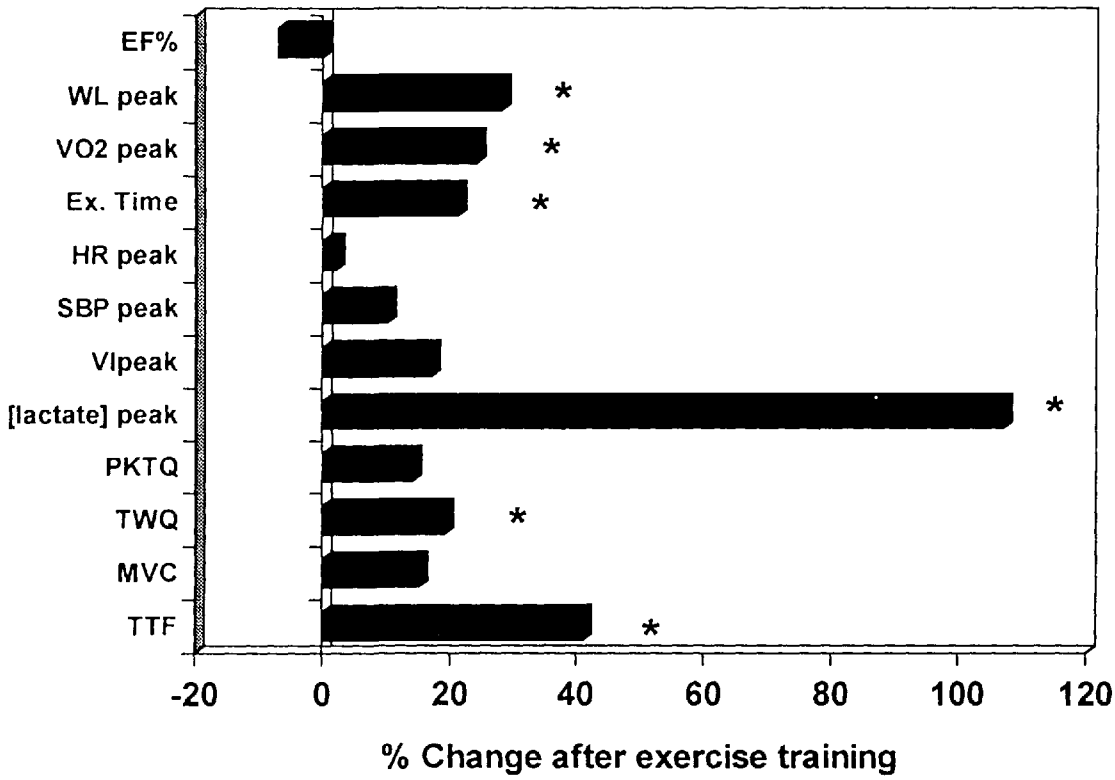

Figure 5 Effects of a 7-month aerobic exercise training program on cardiorespiratory, metabolic and skeletal muscle function in heart transplant recipients (n = 8). Abbreviations: EF% = ejection fraction; WL peak = peak workload during graded cycle exercise to exhaustion; V_{O_2} peak = peak oxygen consumption during graded cycle exercise to exhaustion; Ex time = exercise time during graded cycle exercise to exhaustion; HR peak = peak heart rate achieved during graded cycle exercise to exhaustion; SBP peak = peak systolic blood pressure recorded during graded cycle exercise to exhaustion; VI peak = peak minute ventilation during graded cycle exercise to exhaustion; [lactate] peak = peak blood lactate concentration during graded cycle exercise to exhaustion; PKTQ = peak torque generated by the quadriceps muscle during a maximal isokinetic test of skeletal muscle function; TWQ = total work performed by the quadriceps muscle during a 25-second test of isokinetic skeletal muscle function; MVC = maximum voluntary isometric contraction; TTF = time to fatigue during repeated isometric contraction/relaxation cycles of the quadriceps muscle. * $p<0.05$ post-training vs pre-training. All values are expressed as a percentage change from pre-training values

We have recently described changes in physiological parameters in a group of cardiac transplant recipients participating in a moderate-intensity aerobic training program for a period of 7 months[69]. Figure 5 depicts changes in cardiorespiratory and skeletal muscle function during exercise in transplant recipients following exercise training. Resting ejection fraction, peak heart rate, peak systolic blood pressure, and peak minute ventilation did not change significantly after exercise training. However, peak workload, peak V_{O_2}, peak exercise time, and peak blood lactate concentration during a graded exercise test to exhaustion increased significantly after training. Indeed, peak blood lactate concentration increased by over 100% of pretraining values. This finding is in accordance with the higher workload achieved after exercise training. Whilst the total isokinetic work produced by the quadriceps muscles during 25 maximal contractions increased by 20%, and time to reach fatigue during the test of isometric skeletal muscle function improved by more than 40%, peak isokinetic torque and the maximal voluntary contraction produced by the quadriceps muscles was not different after exercise training in these patients.

Histological analysis of skeletal muscle biopsies from the vastus lateralis performed before, and again after, the exercise training program revealed that the features of the myopathy, described above, had improved significantly, so that the histological appearance of the muscle was more normal after training.

We have concluded that a significant factor limiting the exercise performance of these patients was a peripheral myopathy, and that the major effect of training was to increase the resistance of the trained skeletal muscle to the onset of fatigue, rather than a direct training effect on the transplanted heart. We are currently investigating whether a program designed specifically to increase the patients' muscle strength, rather than their 'cardiorespiratory endurance', would not be an equally effective training method.

COMMENT

Certain physiological and pathological features of the cardiac transplant recipient demand that adaptations be made to the conventional principles underlying exercise prescription for patients with ischemic heart disease. In particular, the use of heart rate monitoring to control the exercise intensity is less applicable, and an alternate method using the Borg Scale of Perceived Exertion would appear to be more appropriate.

In addition, the cardiac transplant recipient is more likely to have a peripheral limitation to his/her exercise capacity, probably due to alteration of skeletal muscle structure and function during heart failure and the myopathic effects of the drugs used to control rejection.

It would seem that cardiac transplant recipients adapt in the normal way to exercise training, with the exception that the peripheral skeletal muscular adaptations would appear to dominate. The use of additional or alternate training programs specifically to increase skeletal muscle strength would seem justified.

Whether continued exercise training can prevent progressive drug-induced myopathic changes, and can reduce or delay the onset of hypertension or rejection-related accelerated coronary atherosclerosis, would seem worthy of further study.

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Non-cardiac Surgery in Patients with Heart Transplants – Anesthetic and Operative Considerations

E. BECERRA AND D.K.C. COOPER

INTRODUCTION

As cardiac transplantation becomes increasingly successful there is a growing possibility that patients with heart transplants may require surgery for conditions unrelated to the heart. Such patients clearly present special management problems, which include, in particular, atypical responses to both stress and certain pharmacological agents, and increased susceptibility to infection.

CONDITIONS FOR WHICH SURGERY MAY BE NECESSARY IN PATIENTS WITH HEART TRANSPLANTS

Between 12% and 30% of the patients who undergo heart transplantation may develop a pathological condition requiring one or more non-cardiac operations[1–8]. The need for such an operation has been documented from 2 hours to more than 10 years after transplantation[2]. The incidence of significant general surgical complications developing within 30 days after transplantation has been reported to be between 4.8%[7] and 7%[1].

Patients with cardiac transplants may, of course, develop any unrelated disease requiring surgical intervention (e.g. carcinoma of the stomach[2], head and neck surgery[9], aortic coarctation[10,11] or kidney transplantation[4]), as may any member of the population, but, in addition, they are at special risk of requiring surgery for several reasons (Table 1).

Such patients may develop a complication of the transplant operation (e.g. incisional hernia, wound infection, gastric outlet obstruction secondary to vagus nerve injury occurring during heart–lung transplantation[1]), or of a subsequent diagnostic procedure (e.g. right ventricular perforation, pneumothorax or hemothorax following endomyocardial biopsy).

Systemic thromboembolism may occur in patients with a heterotopic heart transplant from a poorly functioning recipient (native) left ventricle, but may also occur rarely in patients with an orthotopic transplant when donor heart function is decreased either during an acute rejection episode or when chronic rejection is advanced. Anticoagulant therapy and antiplatelet agents may increase the risk of gastrointestinal bleeding and hematoma formation following trauma. When cardiac transplantation has been performed for ischemic heart disease, the atheromatous disease process may progress in peripheral vessels and lead to ischemic complications, particularly in the lower limbs or brain. Aortic dissection may also occur. New or previous aortic aneurysms are seen almost exclusively in recipients with underlying ischemic heart disease[5,12,13] in whom there may be a rapid increase in size of the aneurysm[12]. A high incidence of cholelithiasis (30–42%) has been documented after heart transplantation[14–16], especially in older patients[14] and those with high cyclosporin levels[16]. All of these conditions may require surgery.

In addition, however, certain complications of long-term immunosuppressive therapy may require a surgical procedure, notably because immunosuppressed patients are more susceptible to infection (Chapter 32). The immunosuppressive agents themselves, particularly the corticosteroids, may lead to complications which require surgical treatment (Chapter 8). Corticosteroid

Table 1 Conditions for which patients with heart transplants may require surgery

1. Unrelated
2. Complications of the transplant operation
3. Complications of diagnostic procedures (e.g. endomyocardial biopsy)
4. Complications of immunosuppressive therapy, particularly corticosteroids
5. Complications of other drug therapy (e.g. anticoagulation)
6. Systemic thromboembolism (especially in patients with a heterotopic heart transplant)
7. Continuing atheromatous disease or disease related to hypercholesterolemia (particularly in patients with previous ischemic heart disease)

therapy may result in musculoskeletal disorders (e.g. osteoporosis, vertebral compression fractures, pathological bone fractures, aseptic necrosis), gastrointestinal disorders (e.g. peptic ulceration and pancreatitis) and ophthalmic disorders (e.g. cataract, glaucoma, exophthalmos), all of which may require operative procedures.

The differential diagnosis of an acute abdominal complication in a patient with a heart transplant may prove difficult, but requires urgent assessment in order to avoid delay of treatment. Despite steroid therapy the history and physical examination are generally reliable, though the white blood count may be misleading[1]. When perforation of the bowel is present, the condition is almost always manifest by pain, tenderness and muscular rigidity[1]. Peptic ulcer disease and *Cytomegalovirus* gastritis or duodenitis should be included in the differential diagnosis of abdominal pain[4,17]. Free intraperitoneal air on an abdominal radiograph suggests bowel or stomach perforation. In the early postoperative period, however, if no pain or muscular rigidity is present, free air may be associated with accidental opening of the abdominal cavity at the time of sternotomy. For the same reason, pneumothorax in the early post-transplant period (or even later[1]), or possibly as a complication of endomyocardial biopsy, may also progress and present as air in the abdomen. Assessment of the acute abdomen may include gastrointestinal endoscopy, computerized tomographic scanning, gastrografin contrast radiography, and ultrasound studies. Pancreatitis is a not-unusual complication of immunosuppressive therapy[18-24] and of *Cytomegalovirus* infection[7,25,26], but it should be noted that the serum amylase is often increased after cardiopulmonary bypass[27]. In doubtful cases, early exploratory laparotomy is advocated[1,7].

PREOPERATIVE ASSESSMENT

If time permits, before any major surgical procedure is undertaken, the status of the patient with regard to both acute and chronic rejection should be checked. This may involve clinical examination for features of cardiac failure or dysrhythmias, blood cell counts and plasma chemistry, electrocardiographic and/or echocardiographic studies (the latter to demonstrate adequate left ventricular function), endomyocardial biopsy to detect acute rejection, or even coronary angiography, thallium scanning or dobutamine stress echocardiography if significant chronic rejection is suspected. Elective surgical procedures should be postponed if the total white blood cell count is particularly low (less than 2000–3000 cells/mm^3).

Patients receiving long-term anticoagulation therapy should have this therapy reduced to a safe level for the period of operation, but it should be instigated again 48 hours after operation unless there is a contraindication. Antiplatelet therapy should be discontinued for the day of operation only. In an emergency, fresh-frozen plasma can be administered to normalize the coagulation state of the patient before surgery.

Unless the operative procedure is being undertaken for an infective complication, e.g. the drainage of an abscess, and a specific antibiotic is therefore indicated, our policy has been to prescribe an anti-staphylococcal antibiotic as prophylaxis over the period of the operation; this should be administered initially approximately 1 hour before the surgical procedure begins, so that high blood and tissue levels are present, and discontinued within 24–48 hours to minimize the risk of growth of resistant bacterial or fungal organisms.

SPECIAL PROBLEMS OF ANESTHESIA AND SURGICAL CONSIDERATIONS

The special problems faced in managing patients with cardiac transplants who require operative procedures include: (a) atypical responses to stress and to certain drugs, since the transplanted heart remains denervated; (b) increased susceptibility to infection; (c) increased tendency to arrhythmias, particularly during the first 3 months after transplantation or when acute or chronic rejection is occurring[28]; and (d) risk of complications related to drugs such as anticoagulants, corticosteroids, and cyclosporin. Furthermore, account has to be taken of the possibility that some of these patients are in a state of 'stress' from such conditions as acute rejection or infection, or from being in the early post-transplant period, or from surgical shock or other condition[5].

With regard to drug-related complications, the increased risks of managing a patient who has been on long-term anticoagulation therapy are obvious. The need for increased therapy in patients receiving corticosteroids over a long period of time, since their own adrenal cortical response to stress is suppressed, is also well known. Hydrocortisone 50–100 mg should be given intravenously immediately before the induction of general anesthesia, and may be required after operation every 8 hours for at least one or two doses[2,29]. Cyclosporine may result in impaired renal and/or hepatic function, which may complicate the perioperative period, and may also have resulted in systemic hypertension, for which the patient may be receiving additional antihypertensive therapy.

In an attempt to reduce septic complications and avoid wound healing problems, some centers, such as the Utah group, have at times advocated a decrease in corticosteroid therapy, substituting this with a short course of OKT3 or ALG[5]. Our own policy has been to maintain azathioprine, cyclosporin and corticosteroid therapy. In cases of pancreatitis, azathioprine is generally replaced with cyclophosphamide[5].

As cyclosporin does not appear to modify the effect of the commonly used anesthetic agents in humans[6,30], standard general anesthetic technique may be used, care being taken to maintain good oxygenation. (Orotracheal intubation has been suggested as being preferable to nasotracheal, to diminish the risk of lung infection[31,32].) Muscle relaxation, where necessary, may sometimes require larger doses than usual, since azathioprine antagonizes neuromuscular blocking agents by its phosphodiesterase-inhibiting properties[33]. Agents such as morphine may be used as necessary[2]. Halothane, a potent myocardial depressant, has been used in induction of anesthesia in the pediatric patient without untoward effect[6].

Particularly when a major surgical procedure is undertaken, adequate hemodynamic monitoring is essential. Arterial and central venous pressure lines are inserted, employing strict sterile technique. Continuous ECG monitoring is necessary. Percutaneous suprapublic rather than transurethral catheterization was advocated in the past to avoid urinary tract infections[34], though this is no longer considered necessary.

Since transplantation results in complete and usually permanent denervation of the heart, it can no longer respond to neurally-

mediated stimuli. During stress or exercise the heart initially increases cardiac output by an increase in stroke volume rather than by cardioacceleration (Chapter 27). To avoid hypotension and maintain cardiac output during stress, therefore, an adequate preload must be available, especially when spinal anesthesia is employed[6]. In addition, steps may be required to increase heart rate rapidly and also enhance contractile force, namely by the administration of inotropic agents; the response of myocardial adrenergic receptors has been shown to be normal or increased.

In the early post-transplant period any abdominal incision required should preferably be made in such a way that it does not communicate with the previous sternotomy. This may help avoid potential contamination of the mediastinum, especially when the abdominal procedure is for an infective condition. It has been recommended that the surgical management of emergency abdominal complications should, when possible, be technically conservative (e.g. by simple peptic ulcer plication instead of the performance of acid-reducing procedures, the fashioning of temporary intestinal stomas rather than the use of primary anastomosis, the use of retention sutures, etc.)[5]. Although this is probably wise counsel, modern developments in surgery, in particular the use of laparoscopic techniques, have reduced the risk of surgical procedures in the heart transplant recipient, particularly when performed electively.

POSTOPERATIVE MANAGEMENT

To reduce the risk of infection the patient should be extubated, and all drains and vascular and urinary catheters removed as soon as possible after operation. Since pulmonary infection is particularly common in immunosuppressed patients, they should receive respiratory therapy until fully mobilized; chest radiographs should be taken frequently during the early postoperative days, to monitor pulmonary status. Since these patients are frequently receiving long-term corticosteroid therapy, this should be supplemented to cover the operative procedure; it is not necessary to continue this extra therapy for longer than 48 hours after operation unless there is some specific indication. As corticosteroids may impair wound healing, sutures should be left *in situ* for rather longer than usual. For a similar reason, when a gastrointestinal or biliary leak is present, it is advisable to maintain the drain for a longer period of time than usual, to allow adequate healing[15].

After gastrointestinal surgery it may prove necessary to administer cyclosporin intravenously rather than orally[4], since absorption can be variable. To avoid nephrotoxicity the intravenous dosage should initially be small and adjusted when blood levels have been measured. It is rarely necessary to administer more than 1 mg/kg per day over the course of 24 hours. In patients beyond the first post-transplant year, even smaller doses (0.3–0.6 mg/kg per day) are generally sufficient. An easy guide to remember is to administer a dose of 1–3 mg per hour (NB, *not* 1–3 mg/*kg* per hour); this will generally provide continuous CSA whole blood levels of 125–300 ng/ml, which are usually sufficient to prevent acute rejection from developing.

Alternatively, most patients can be managed for several days, if necessary, without cyclosporin if ALG is administered on a daily basis to suppress the T-11 lymphocyte subset. AZA and corticosteroids can also be administered intravenously, AZA at the same dose as when given orally (although some groups advocate a reduction in dose)[4] with methylprednisolone being substituted for prednisone at the equivalent dose.

Early postoperative mobilization of the patient, to minimize the risk of venous thrombosis and pulmonary embolism, is as important as in other patients undergoing surgery; this complication has been documented in most of the published series[29,35]. Subcutaneous heparin therapy may be indicated in patients who are likely to be immobilized for a long period.

RESULTS OF NON-CARDIAC SURGICAL PROCEDURES IN PATIENTS WITH HEART TRANSPLANTS

The surgical technique, timing and indication for cholecystectomy all remain controversial[14,16,36,38]. At present, however, it is generally agreed that laparoscopic cholecystectomy is the best technique to employ. Symptomatic patients should ideally undergo cholecystectomy before heart transplantation, while asymptomatic patients can be left to undergo the procedure post-transplantation, usually only if they become symptomatic. When performed electively the operative mortality is 0%, whereas there is a significant risk (of possibly even 40%) when it is performed as an emergency operation in patients with heart transplants[39].

Surgical procedures to resect and replace abdominal aortic aneurysms, whether from a degenerative[12,13,34,41] or a mycotic[40] cause, have been associated with a surprisingly low risk. Furthermore, we have been unable to identify any cases of infection of the prosthetic graft used to replace the aneurysm. Although our own policy has been to replace the aneurysm pre-transplant whenever feasible (particularly in patients with previous cardiac surgery who may require initiation of cardiopulmonary bypass through the femoral route), this reported low mortality and morbidity make it reasonable not to eliminate heart transplant candidates with abdominal aneurysms, but to plan the surgery after transplantation, when the risks of acute myocardial infarction are clearly reduced[42–45]. However, with careful surgical technique and meticulous postoperative care, at our own center Chaffin and his colleagues have performed a number of abdominal aneurysmectomies and/or carotid endarterectomies in patients awaiting heart transplantation with no mortality and little morbidity (J. Chaffin, unpublished results).

A summary of published results of surgery in patients with heart transplants is shown in Table 2. As some patients at Stanford may have been included in more than one study, it is not possible to estimate accurately the combined mortality in these series. It is clear, however, that the mortality has been relatively low. Most of the deaths were related to the underlying pathology rather than to any anesthetic or surgical complication. The highest mortality was reported in a series which included complex surgery for serious abdominal pathologies; this mortality would probably have been lower if some of the patients had presented for medical consultation earlier in the course of their illness. The authors of this paper stress the importance of early diagnosis. Pulmonary infections and embolism were the most frequent postoperative complications.

COMMENT

General anesthesia and surgical intervention in patients with heart transplants would therefore appear to be relatively safe pro-

Table 2 Mortality of non-cardiac surgery in patients with heart transplants: published results

Center	*Year of publication (reference)*	*Number of patients*	*Number of operations*	*Early mortality (%)*
Stanford, USA	1977[33]	2	2	0
Stanford, USA	1977[46]	16(?)	24	4
New York, USA	1981[29]	1	2	1
Pittsburgh, USA	1985[1]	17	17	4
Cape Town, SA	1986[2]	15	39	1
Hershey, USA	1989[4]	14	16	1
Utah, USA	1991[5]	17	20	0
New York, USA	1991[6]	28	35	(?)
Cambridge, UK	1991[7]	20	21	4
Loyola, USA	1993[8]	23	33	4

cedures, if care is taken to monitor the patient and avoid the special complications that may be associated with immunosuppressive therapy. Neither a satisfactorily functioning heart transplant, even in the early postoperative period, nor immunosuppressive therapy need therefore be considered a contraindication to any other surgical procedure whenever it is clearly indicated.

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Recurrence of Myocardial Disease in the Transplanted Heart

A.M. KEOGH

INTRODUCTION

The question of the potential for recurrence of the underlying disease in the cardiac allograft arises most frequently, in Westernized countries at least, in relation to amyloid and sarcoid heart disease and giant cell myocarditis. It is about these that the literature contains the most information. Far less information is available on disease recurrence in hemochromatosis, Chagas' disease, and tumors involving the myocardium. One limitation of the literature is that follow-up is often relatively limited, particularly as the emphasis on results in cardiac transplantation is shifting away from 1-year survival towards long-term survival.

When assessing a recipient in whom there is the chance of disease recurrence, there are two considerations to weigh up. The first is whether transplantation is likely to improve the short-, medium- or long-term survival of that individual. The second is whether transplanting such a patient is the best use of a particular organ, bearing in mind the worldwide donor organ shortage (currently growing worse yearly) and the excellent results obtainable in those with more traditional diseases requiring transplantation. Not unimportantly, the cost of the procedure and likelihood of a long healthy survival, which may increase return to useful community participation, should be considered.

AMYLOID HEART DISEASE

Background

Amyloidosis is a generic term for the deposition of various different fibrous proteins in vital organs. These proteins display a characteristic spatial orientation conforming to a B-pleated sheet. The traditional classification includes: (a) primary, i.e. no pre-existent or coexistent disease, (b) secondary to a plasma cell dyscrasia, (c) secondary to chronic infection or inflammation, (d) hereditofamilial (familial Mediterranean fever), (e) local amyloidosis without systemic amyloid, and (f) age-related amyloid deposition (AS1 protein) in those over 65 years old[1]. It is important to differentiate clearly in the pretransplant assessment which of these applies, in order to best assess risk. The somewhat confusing terminology, and sometimes limited workup of amyloid patients, have tended to obscure the small amount of data reported to date. Many cases are classified as 'primary' amyloid, when in fact there was evidence of plasmacytosis or abnormal plasma cells in bone marrow. It has been suggested that a form of isolated cardiac amyloid exists, but in all likelihood this represents an early predominance of amyloid in one organ rather than true freedom from systemic multiorgan disease[2,3].

Chemical types of amyloid

The chemical class of amyloid protein is identifiable using monoclonal and polyclonal antibodies directed against purified and chemically characterized amyloid fibril proteins in up to 70% of amyloid disease[1].

AA amyloidosis

This is usually a reactive process in the wake of inflammation or infection. The AA protein is probably derived from serum amyloid-A protein (SAA) produced in response to B cell stimulation. The concentration of SAA can be elevated 1000-fold during an acute-phase reaction[1]. AA amyloidosis occurs predominantly in the kidneys. If progressive, the heart may become involved, but underlying renal disease would usually preclude consideration of cardiac transplantation.

AL amyloidosis

A lambda or, less commonly, A kappa amyloid protein deposition derives from a monoclonal light chain and very rarely from a defective heavy chain. The underlying disease may be benign (local plasmacytoma, benign monoclonal gammopathy) or a malignant plasma cell dyscrasia such as Waldenström's disease, multiple myeloma, lymphoma or Bence–Jones plasmacytoma[1].

Clinical and natural history

Amyloid heart disease generally takes the form of a restrictive cardiomyopathy with poor prognosis, leading to death within 2 years of diagnosis but sometimes as quickly as 4 months after diagnosis[4]. At least one report documents slower progression of disease before the development of congestive heart failure[5]. Roberts and Waller report mean duration of heart failure as being 18 months (range 1–108 months) and lasting <12 months in 64% of 54 necropsy cases[6]. Cardiac amyloidosis is the leading cause of death in patients with primary systemic amyloidosis[7]. The restrictive cardiomyopathy with resultant cardiac failure arises from intermyocyte amyloid deposition, compounded by narrowing of intramural coronary arteries. There may also be mural and valvular endocardial deposits and conduction pathway involvement[4,6].

Diagnosis

The most consistent echocardiographic features which should arouse suspicion of amyloidosis pretransplant are symmetrical increase in left ventricular wall thickness in the absence of a history of systemic hypertension or aortic valve disease, hypokinesis and decreased systolic thickening of the interventricular septum and posterior left ventricular wall, and small to normal ventricular cavity size[8]. Clinically significant cardiac amyloid is almost always associated with a low-voltage electrocardiogram. Amyloid is often an elusive diagnosis and should be considered where there is the question of a restrictive cardiomyopathy, constrictive pericardial disease or hypertrophic cardiomyopathy. It is important to make the diagnosis in order to avoid inadvertent transplantation of a patient with undetected amyloid.

Hematoxylin and eosin examination of cardiac biopsies may not be adequate. A patient with known monoclonal gammopathy had no detectable amyloid disease in biopsies of the heart, rectum or bone marrow, yet post-transplant rapidly developed widespread multi-organ amyloidosis[9]. As a result, multi-organ biopsy, along with electron microscopic examination of cardiac tissue, has been recommended[9].

Amyloid workup

A comprehensive diagnostic workup for amyloid should include a search for cardiac amyloid and for systemic involvement. The cardiac biopsy should be stained by the Congo Red method with polarized light viewing, looking for apple-green birefringence and dichroism. Tissue should also be stained with antisera against amyloid p, amyloid SAA and lambda and kappa light chains. Supplemental electron microscopy has been recommended[9]. Characteristic electron microscopic changes are amyloid banding around myocytes with scalloping of myocyte edges and amyloid fibril deposition.

Systemic disease should be sought by kidney, liver and rectal biopsies, and bone marrow aspirate and biopsy to rule out myeloma or other plasma cell dyscrasia, which are present in 45% of all cases of cardiac amyloidosis. Additional testing includes ESR, urine and serum immunoelectrophoretogram, full blood count, technetium bone scan for osteolytic lesions, and serum calcium.

Treatment

Deposits of amyloid fibrillar protein in myocytes and capillaries, and the mechanical interference with contractility, render amyloid heart disease relatively resistant to treatment with conventional heart failure medications[2,3]. In fact, conventional therapy, such as lanoxin[4,10] and calcium-channel-blocking agents[11], may have deleterious effects[10]. Intermittent-dose melphelan and prednisolone have been shown to prolong life only marginally in primary AL amyloid[12]. Because of its poor prognosis and the often relatively young age of those affected, the question of transplantation is still often raised, despite the fact that amyloid heart disease has been 'established' as a contraindication for many years.

Recurrence after transplantation

Amyloid heart disease has been considered a contraindication to transplantation because it tends to be a systemic disease, and it was expected that amyloid deposition would recur in the allograft. The available recent literature indeed confirms a generally malignant course post-transplant, either by recurrence in the allograft (as early as 2 months post-transplant) or by progression in other organs[13].

The most comprehensive experience is reported by Hosenpud *et al.*[14]. A survey of 24 transplant centers yielded 10 patients transplanted for the diagnosis of cardiac amyloid. This collective experience has the longest follow-up and involved many cases previously reported. Its results and conclusions largely supersede prior individual case reports in which less malignant outcomes were noted[15,16]. In this collective report the diagnosis of cardiac amyloid was made on diagnostic biopsy or on examination of the explanted heart[14] (Figure 1). All 10 patients had serum or urine protein electrophoretogram performed pretransplant, with an amyloid light chain demonstrable in eight patients (seven lambda and one kappa). Rectal biopsy was positive in three of seven, where performed, renal biopsy positive in two of three, liver

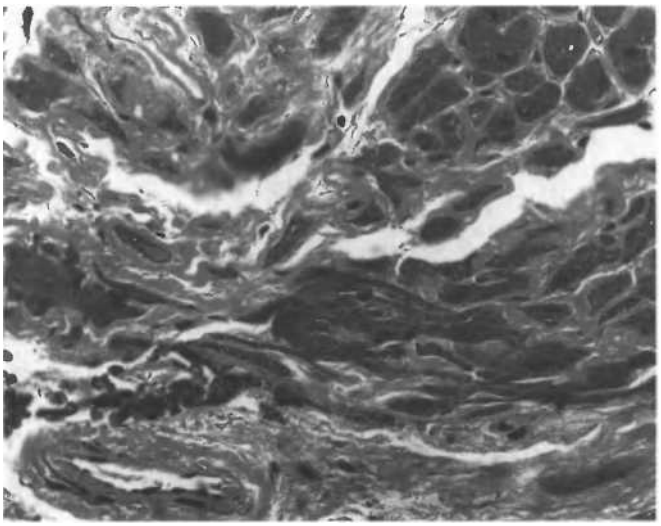

Figure 1 Cardiac amyloidosis (Masson's trichrome, original magnification × 400)

biopsy positive in two of three, and gingival biopsy positive in two of two, but marrow did not show plasma cell proliferation in any of nine where this was performed.

Of the 10 patients transplanted, follow-up was available in eight. One developed restrictive cardiomyopathy due to amyloid, one required dialysis for amyloid renal disease, one had extensive gastrointestinal amyloid, and one patient with 9-month follow-up was free of disease. Four had died – one of right ventricular failure in the perioperative period (unrelated to amyloid), one of amyloid liver failure, one of amyloid malabsorption and neuropathy, and one at home with malnutrition due to amyloid esophageal involvement and airway obstruction. By 4 years post-transplant, survival was only 39% in this group, and 66% had developed other major organ involvement[14].

Four of these nine survivors demonstrated recurrent amyloid deposition in the graft, in one patient as early as 14 weeks post-transplant[15]. However, in two of these four the diagnosis was solely an electron microscopic one, and there were no clinical sequelae, with right heart hemodynamics unaffected at a mean of 22 months post-transplant. One patient developed restrictive physiology on echocardiography[17]. The obligatory immunosuppression appeared to do little to ameliorate the progression of systemic amyloid disease.

The authors concluded that transplantation for amyloid heart disease was associated with a suboptimal survival, caused by either recurrence of local cardiac disease or progression of systemic organ involvement. Survival at 4 years was only half that anticipated in the general heart transplantation population.

Data pooling makes it difficult to distinguish whether systemic progression post-transplant was related to demonstrable disease in particular organs. It remains unclear, therefore, whether a patient with 'localized' cardiac amyloid, and who is biopsy-proven free of disease in the liver, kidney and bone marrow, may have a different long-term prognosis from that of the reported group. There are occasional reports indicating that patients with cardiac amyloid, but with negative rectal and renal biopsies and no evidence for myeloma, may do well over a 12-month period[15]. Long-term follow-up is needed. Additionally, there are no data indicating whether the specific protein, or the absolute serum or urine level, predicts recurrence or subsequent systemic manifestations.

Amyloid heart disease must be considered a virtual contraindication to transplantation, or at best only a palliative approach. The presence of myeloma must also be considered a total contraindication to transplantation.

Comment

Cardiac transplantation for amyloid heart disease is associated with less than half the usual survival post-transplant by 4 years. In 66%, other organ involvement occurs within 4 years. A comprehensive workup should be performed to exclude amyloid cardiac or systemic disease in potential transplant recipients in order to avoid inadvertent transplantation of such a patient, with its consequent suboptimal outcome. Amyloid heart disease should be considered a contraindication to cardiac transplantation; however, if this is undertaken, it should be done with the expectation of only short-term palliation.

SARCOID HEART DISEASE

Background

Sarcoid is a granulomatous systemic condition of unknown etiology. In an autopsy series of patients with systemic sarcoid, 27% had myocardial granulomas and, of these, 65% had clinical heart failure or arrhythmias during life[18]. On the other hand, most patients with cardiac sarcoid demonstrate systemic sarcoidosis[19]. Sarcoid is an uncommon cause of cardiac failure, making up only 0.03% of one center's large experience with patients in heart failure[20]. It more usually manifests itself as conduction disturbances[21] or arrhythmias[22]. Twenty-two percent of patients with cardiac sarcoid have ventricular tachycardia. Conduction disturbances are often transient and may be self-limiting, rendering response to treatment difficult to assess. Occasionally pulmonary fibrosis may result in right heart failure. Other clinical features include left ventricular aneurysm, papillary muscle dysfunction, and recurrent pericardial effusion[23]. In the small proportion of patients who develop cardiac failure, there is a poor correlation between myocardial biopsy changes and cardiac function.

Etiology

Several microorganisms have been implicated in the causation of sarcoidosis, including *Mycobacterium tuberculosis*[24], corynebacteria[25], and viruses[26]. Space–time clustering provides indirect epidemiologic evidence for an infectious agent[27]. This causation, however, remains somewhat speculative.

Natural history

Myocardial sarcoid may have a natural history of 15 years or more, but in most patients the interval from the onset of cardiac symptoms to death is less than 2 years, with 50% dying within 12 months[23]. In a large autopsy series of sarcoid heart disease, two-thirds had died suddenly, 23% of congestive cardiac failure, 3% of recurrent pericardial effusion, and 7% of unknown cause[23].

Histopathology

The finding of non-caseating granulomas on cardiac biopsy leads to the diagnosis of sarcoidosis in a patient if there are granulomas involving at least one other site (Figure 2). The most common cardiac sites for granulomas are the mitral valve and papillary muscle, upper interventricular septum, and left ventricular free wall. Granulomas are thought to coalesce and progress to fibrosis, resulting in transmural scarring in the absence of coronary artery narrowing[23]. The classical lesion is a central collection of monocytes and macrophages from which multinucleated and epithelioid cells are derived. T and B lymphocytes, immunoglobulins and complement may be present.

Systemically, most patients with sarcoid show negative responses to tuberculin, and abnormal cell-mediated and humoral immunity. Circulating atypical and activated T lymphocytes may be demonstrated in 50%. There is evidence of B cell hyperactivity with significant depression in T cell function.

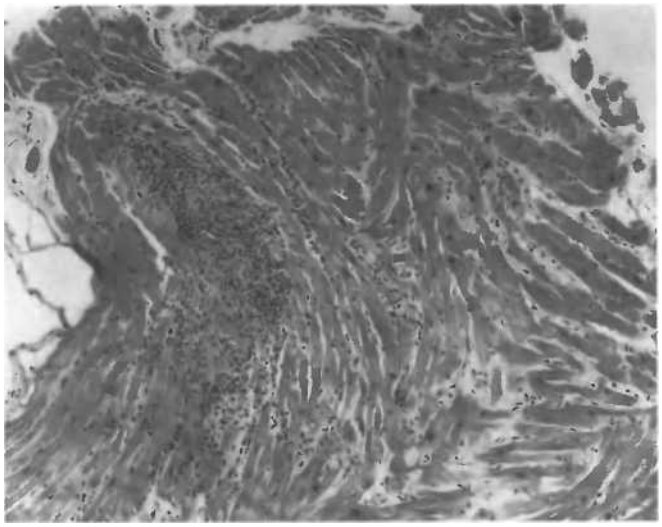

Figure 2 Cardiac sarcoidosis showing a non-caseating granuloma (H&E, original magnification × 200)

Pathophysiologically, left ventricular dysfunction is relatively easy to understand when the myocardium is massively infiltrated, but dilated cardiomyopathies with non-specific changes are just as common as restrictive myopathies. One report proposes that myocyte degeneration may be related to amines released from adjacent mast cells in an attempt to explain diffuse dysfunction in the presence of only localized foci of sarcoid[28]. Improvement in left ventricular function may be seen even in those with marked myocyte hypertrophy, myofibrillar loss and fibrosis, making prognostication difficult[20].

Diagnosis

Myocardial sarcoidosis is often difficult to diagnose because of the patchy nature of myocardial involvement. The definitive diagnosis is made on biopsy in only 25%, even when the affected areas should have been accessible[29]. Repeated cardiac biopsies are indicated in a patient with granulomas in at least one other organ or lymph node, in order to make the diagnosis. Repeated biopsies, and sampling from multiple sites (including both right and left ventricle), may increase the chance of sampling a granuloma. However, even multiple negative biopsies do not rule out sarcoidosis[20,30].

Angiotensin-converting enzyme level[31] and thallium scanning (showing patchy perfusion defects at rest which reduce during exercise[32]) are said to be useful, but are only indirect evidence for sarcoid involvement, and a histopathological diagnosis is still required.

Several specimens should be sent for culture and staining (Ziehl–Nielsen) for atypical mycobacterium. In a patient in our own unit, transplanted for cardiac 'sarcoidosis' with associated pulmonary infiltrate, non-caseating pulmonary granulomas recurred post-transplant and *Mycobacterium haemophilum* was grown postoperatively. Coronary vasculopathy led to death, and autopsy revealed mediastinal and hilar lymph nodes with extensive nodular hyaline fibrosis, raising the possibility the atypical mycobacteria may have been the causative process pretransplant.

Cardiac sarcoid may not be differentiable from idiopathic giant cell myocarditis on cardiac histology alone[24]. The presence of granulomas in at least one other organ, or in a lymph node, confirms the diagnosis of sarcoidosis. The presence of granulomas in the myocardium only raises the possibility of giant cell myocarditis. It is necessary, however, for differentiation, to biopsy other potentially, albeit covertly, affected areas.

Treatment

No treatment is of proven benefit in sarcoid heart disease. It remains unclear whether treatment with steroids is effective, as there are no randomized or controlled trials of steroids in sarcoidosis. In a retrospective report of 113 patients, only 15 had received steroids. However, four of these 15 patients (and these were the only four in the entire series) showed clearance of granulomas with steroid therapy leaving myocardial scarring[23]. It is of concern, however, that three of these four displayed ventricular aneurysm formation. Other reports also document clinical improvement in small numbers of cases, as a consequence of steroid therapy, in the form of reduction in arrhythmias, conduction disturbances, and electrocardiographic evidence of myocardial damage[20,33,34]. On the other hand, in a retrospective review of 145 patients with cardiac sarcoidosis, 80 were given steroids and 65 were not[35]. Electrocardiographic abnormalities improved in 14% of treated and 2% of untreated patients, were unchanged in 62% of treated and 81% of untreated, and worsened in 24% of treated and 17% of untreated. It was concluded that steroids were not indicated for isolated asymptomatic electrocardiographic abnormalities[35]. In the absence of proven therapies, however, there seems to be a consensus that a trial of steroids is indicated before committing a patient to transplantation.

Recurrence after transplantation

The literature contains only four case reports of patients transplanted for cardiac sarcoid. In three of these cases, sarcoid was said to be localized to the heart and the post-transplant course was benign. In the fourth case, definite systemic sarcoid was documented preoperatively and cardiac granulomas recurred post-transplant but without clinical sequelae.

Case 1 was transplanted for drug-resistant ventricular tachycardia without systolic dysfunction. Cardiac granulomas were discovered in the explanted heart but no recurrence was seen on post-transplant biopsy over a 3.5-year period. This patient died of mycoplasma pneumonia[20]. *Case 2* was transplanted for severe heart failure. Preoperative biopsy showed only mild myocyte hypertrophy and interstitial fibrosis. Histologic examination of the explanted heart, however, revealed numerous non-caseating granulomas. She remained well at 20 months with no evidence of sarcoid on biopsy[20]. *Case 3*, with a granuloma on pretransplant biopsy, was transplanted after progressive cardiac failure failed to respond to steroids. The explanted heart showed multiple granulomas. No granulomas were seen on surveillance biopsies subsequent to the transplant[20]. Systemic features of sarcoid were lacking in these three cases, leaving some doubt that the localized cardiac granulomatous process represented sarcoid.

Case 4 differs from the above in that the patient had definite bilateral hilar lymphadenopathy and interstitial pulmonary changes with granulomas on biopsy of heart, lymph nodes and lung. He was transplanted for cardiac failure, having failed a trial of steroids. Routine endomyocardial biopsy at 6 months (on triple therapy) showed non-caseating granulomas with negative staining for acid-fast bacilli. Steroid augmentation to 1 mg/kg per day for 2 months, then down to 20 mg per day, was associated with gradual resolution of granulomas on biopsy over a 6-month period. Two months after a reduction in steroid dosage, granulomas again recurred on biopsy. Cardiac function was unaffected each time[30].

There are no data as yet to support the suggestion that sarcoid patients should receive steroids as part of their maintenance immunosuppression post-transplant, just as there is no support for the suggestion to reduce immunosuppression in systemic sarcoid cases because of the intrinsic immune defects related to systemic sarcoidosis.

A unique report raises the possibility of transmissibility of sarcoid via transplanted organs[36]. A recipient with ischemic cardiomyopathy received a heart from a donor with probable sarcoid. The donor was noted at procurement to have hilar lymphadenopathy and, on examination of the unused lung, culture-negative pulmonary granulomas were found. At week 18 the cardiac recipient developed bilateral lower zone lung infiltrates and fevers with granulomatous pattern on lung biopsy. Repeated cultures failed to uncover an infectious agent. No specific treatment was given, and 8 months later the radiographic and biopsy appearances had resolved spontaneously. The recipients of the liver and kidney from the same donor did not demonstrate granulomatous disease at any stage. Although in no way conclusive, the possibility remains that sarcoid was implanted with the donor organ, reminiscent of Mitchell's and Rees's transmission of sarcoid from humans to animals by inoculation of sarcoid tissue[37].

Comment

Sarcoid heart disease, where there is evidence of systemic granulomatous involvement, may recur post-transplant, but not necessarily with clinical consequence, and possibly with steroid responsiveness[30]. Cases of granulomatous disease without documentation of a systemic search for granulomata may possibly represent idiopathic granulomatous or giant-cell myocarditis, and less can be concluded from these reports. Transplantation for sarcoid should probably still be considered experimental[30].

GIANT-CELL MYOCARDITIS

Alternative terminology

This condition is also known as idiopathic giant-cell myocarditis, giant-cell granulomatous myocarditis, and Fiedler's myocarditis.

Background

Idiopathic giant-cell myocarditis (GCM) is largely a diagnosis of exclusion. As with all granulomatous diseases it is necessary to exclude sarcoid, and tuberculosis, syphilis and fungi (where specific therapies are available). The workup on biopsy will include staining for fungi, acid-fast bacilli, spirochetes, polarized light examination for birefringent crystals, immunofluorescence, serum angiotensin-converting enzyme levels, ANA and DNA (to exclude systemic lupus), rheumatoid factor, ASOT (to exclude acute rheumatic fever), and electron microscopy for viral particles.

GCM can be associated with Wegener's granulomatosis[38], which is important to exclude because of its intense responsiveness to cyclophosphamide and steroids[39]. It is useful, therefore, to perform antineutrophil cytoplasmic (ANCA) testing in addition to ENT examination, chest radiograph, renal function testing and search for casts. Look also for thymoma and mediastinal lymphadenopathy. Once these have been excluded, idiopathic GCM represents a distinct clinicopathologic entity characterized by a sudden onset and rapid decline to death within 1–3 months of diagnosis, although prolonged survival has also occasionally been reported[38]. Ventricular dysfunction may be focal or diffuse[40]. Heart failure tends to be fulminant, often requiring inotropes or intra-aortic balloon pump support. Heart block (inflammatory lesions in the ventricular septum with biventricular bundle branch block) or arrhythmias also occur[40–45]. By definition, it is confined to the heart.

In the absence of myocardial biopsy the condition has previously often been diagnosed only at post-mortem[46]. Diagnostic biopsy is subject to the usual problem of sampling error. In patients with myocarditis proven by autopsy or on examination of the explanted heart, the diagnosis was made on diagnostic biopsy in less than 50%. GCM comprises up to 22% of all cases of biopsy-proven myocarditis in some series[38].

Etiology

It has been suggested that the disorder is autoimmune in origin because of its association with thymoma[47], giant-cell arteritis[48], thyrotoxicosis[49], systemic lupus erythematosus, dermatomyositis, Sjögren's syndrome, pernicious anemia, rheumatoid arthritis, infective endocarditis and Takayasu's arteritis[38,50]. Fungi, bacteria, parasites or protozoa have never been found. The clinical course is, however, thought to be most consistent with a viral causation[51].

Histopathology

The histologic changes on cardiac biopsy are of a granulomatous myocarditis with dense mononuclear infiltrate, distinctive *multinucleate giant cells* and central myocardial cell necrosis (Figure 3). Multinucleate giant cells probably either originate from myocytes[52] or differentiate from monocytes via macrophages[51]. Central eosinophils and plasma cells may be present. Fibrosis is said to be absent. GCM is a somewhat descriptive diagnosis, however, as giant cells are seen in other cardiac conditions. The histologic features of each are said to be distinctive and diagnostic as follows: infectious granulomatous myocarditis (palisading histiocytes around necrotic tissue centers), rheumatic myocarditis (interstitial Aschoff bodies, often adjacent to small intramyocardial arteries, but no myocyte necrosis) and rheumatoid heart disease (where the granulomas are said to have a palisading appearance). Lymphocytic myocarditis may have rare giant cells.

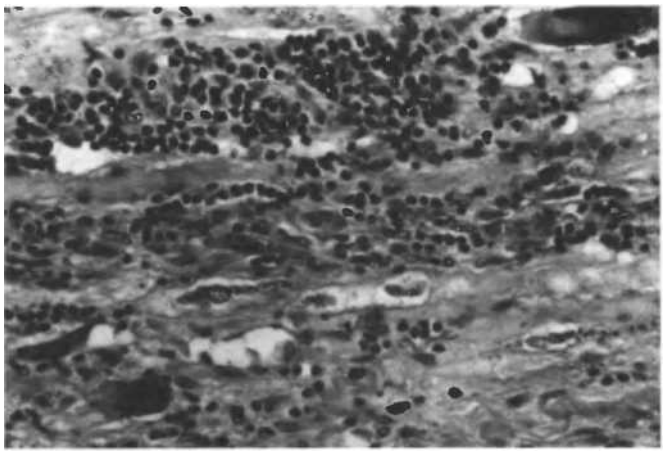

Figure 3 Giant-cell myocarditis (H&E, original magnification × 400)

The most common disease, however, from which GCM must be differentiated in the pre-transplant setting is sarcoidosis as sarcoid granulomas may have a similar appearance. In practice the diagnosis rests on the presence of systemic features in sarcoid and their absence in GCM.

Treatment

Treatment with steroids and azathioprine is seldom successful[40,43,53], although there are occasional anecdotal reports of response[54].

Recurrence after transplantation

The literature provides eight case reports of patients with GCM undergoing cardiac transplant. The outcome was favourable in six of the eight cases.

Case 1: a 35-year-old man was transplanted for rhythm disturbances and fulminating heart failure requiring intra-aortic balloon pump support. Granulomas recurred on allograft biopsies at 5 months post-transplant. Syncope, bifascicular block, and sino-atrial arrest with ventricular escape rhythm necessitated pacemaker insertion. Left ventricular function was impaired. A complete workup including culture for mycobacteria, autoantibodies and viral serology, Kveim test and serum ACE levels, liver and bone marrow biopsies was negative. There were no eye or nasal granulomas. Complete resolution of the inflammatory process occurred with high-dose steroids within 1 month. The recurrence was thus not prevented by maintenance immunosuppression with cyclosporin and azathioprine, but did prove dramatically steroid-responsive. The conclusion was that transplantation was a reasonable option in GCM, although a steroid-inclusive immunosuppression schedule was advised[55].

Case 2: a 58-year-old man with fulminant heart failure was transplanted and GCM found subsequently in the explanted heart. Triple-therapy immunosuppression was used with antithymocyte globulin induction. Prednisone was withdrawn at 3.5 years. Recurrence of GCM occurred histologically just over 4 years post-transplant when a sole giant-cell granuloma was noted on cardiac biopsy[56].

Cases 3 and 4: five patients with GCM are reported from Stanford. All received pretransplant immunosuppression with various combinations of methylprednisolone and azathioprine, but failed to respond, and all displayed progressive failure and ventricular arrhythmias. Of five listed for transplant, two were transplanted. One showed disease recurrence at 9 years post-transplant, but was asymptomatic and the histological changes resolved with steroids. In the other there was no recurrence[40].

Case 5: a 51-year-old female with biopsy-proven GCM preoperatively had rapid progression of cardiac failure despite high-dose intravenous and oral steroid therapy. There was no evidence of extracardiac disease. Twenty-one months post-transplant she developed ventricular arrhythmias and systolic impairment, failing again to respond to high-dose intravenous and oral steroids. Other causes of granulomatous disease were rigidly excluded. Myocyte necrosis and giant cells did resolve histologically, although she required an implantable defibrillator. In this case the same response to immunosuppression was seen before and after transplant, in that inflammation resolved with steroids but arrhythmias were not abolished[43].

Case 6: a 34-year-old patient with rapidly progressive heart failure over 4 months and monomorphic ventricular tachycardia had biopsy-proven GCM, unresponsive to immunosuppression with cyclosporin and prednisolone. Following cardiac transplant and maintenance immunosuppression with triple therapy (with OKT3 induction) there had been no recurrence of GCM by 3 years. A localized non-Hodgkin's lymphoma (kappa variety) was in evidence, however, at 6 months post-transplant[42].

Case 7: a 47-year-old male was transplanted for rapidly progressive heart failure and life-threatening arrhythmias with no response to steroids. Biopsy-proven GCM was present on pretransplant biopsy, with other potential sites and systemic disease reasonably excluded. There was no recurrence on cardiac biopsy over 23 months[45].

Case 8: a 15-year-old boy with fulminant cardiac failure and ventricular arrhythmias was transplanted after a period of Hemopump support. On postoperative triple therapy there was no GCM recurrence up to 5 months of follow-up[44].

Post-transplant, there may be an overlap in the appearance of cellular rejection and possible recurrence of GCM. An episode of hemodynamic compromise associated with GCM-like biopsy appearance occurred at 6 and 9 weeks post-transplant in a patient with rheumatoid arthritis. The episode responded clinically and histologically to steroids, and is put forward as a case of GCM-like rejection[57].

Comment

GCM has a benign post-transplant course in 75% of cases reported to date, and appears to be an intrinsic cardiac disease process suited to transplantation. Long-term data are awaited.

HEMOCHROMATOSIS

Alternative terminology

This condition is also referred to as genetic, hereditary, primary or idiopathic hemochromastosis.

Background

Genetic hemochromatosis (GH) is a common autosomal recessive inherited disorder in which excessive iron absorption leads to iron deposition in parenchymal liver cells, heart, pancreas and other organs. Homozygous hemochromatosis occurs in 0.04% of the population – only homozygotes develop iron overload sufficient to cause clinical disease. The male : female ratio for clinical disease is 5 : 1, with women probably protected by menstrual blood loss. Presentation is between 30 and 60 years of age with lethargy, arthralgias, loss of libido and upper abdominal discomfort. Clinical signs include grey skin pigmentation and testicular atrophy. In advanced disease, diabetes mellitus, liver disease (fibrosis or cirrhosis) and cardiac failure or dysrhythmias may occur. Hepatocellular carcinoma occurs in about 30% with cirrhosis.

The cardiomyopathy in hemochromatosis is due to a direct free iron effect on myocytes, rather than interstitial infiltration. Accordingly, cardiac function worsens or improves in proportion to the degree of iron accumulation in cardiac myocytes[58]. Iron overloading induces lipid peroxidation, known to produce membrane distortion, disruption and altered membrane permeability of lysosomes. Leakage of hydrolytic enzymes leads to cell death.

It seems that a hepatic defect in iron metabolism is present in GH, but there is also a suggestion of an extrahepatic defect in iron metabolism. Liver transplantation may need to be considered where cardiac failure and cirrhosis coexist[59]. The overall liver transplant experience, however, is of reduced survival compared with that of other liver transplant recipients[60], with cardiac, malignant and infectious complications accounting for the excess morbidity and mortality post-transplant[60].

Histology

The only single constant histopathologic finding is iron deposition in the form of pigmented granules within myocytes or in the interstitium (highlighted by Gomori's iron reaction).

Diagnosis

The differential diagnosis is secondary iron overload due to recurrent transfusion or to iron-loading anemias, e.g. thalassemia major, hereditary spherocytosis. Screening for GH is with transferrin saturation (ratio of serum iron and iron-binding capacity) which is elevated early in the disease, and serum ferritin concentration (each taken after an overnight fast). Transferrin saturation is abnormal if above 55%, and serum ferritin abnormal if above 200 μg/l in women or above 350 μg/l in men.

If transferrin saturation and serum ferritin are found to be abnormal, liver biopsy is strongly advised, irrespective of liver function tests. It is the hepatic iron index on liver biopsy (measure of hepatic iron relative to age) which discriminates primary from secondary hemochromatosis. The hepatic iron index is the hepatic iron concentration (μmol/g dry weight) divided by age. Homozygous patients have a value >2.0, whereas alcoholic siderotic and heterozygous hemochromatosis patients have values <1.5.

Treatment

Treatment with life-long venesection is effective, titrated until excess iron stores are removed (i.e. serum ferritin falling to the low normal range). This may take 1–2 years and maintenance venesection is subsequently required. If cardiac patients do not tolerate this, then desferrioxamine may be used, although this is costly and in practice rarely needed. Alcohol intake should be minimized and vitamin C (which increases iron absorption) avoided. New oral chelators are available. Phlebotomy depletes myocardial iron stores as documented by serial myocardial biopsy specimens[61], and is also associated with improved cardiac function[62]. Ultimately, however, this relation may be lost and cardiac dysfunction will continue to deteriorate despite iron unloading[63].

Those patients with cirrhosis have a risk of primary liver cancer even when complete iron depletion is achieved, and should be followed with α-fetoprotein levels and hepatic ultrasound. Primary carcinoma of the liver (not cardiac failure) is the leading cause of death in idiopathic hemochromatosis, and this should be borne in mind when considering such a patient[64].

Recurrence after transplantation

Despite GH being a common disease, there are only two case reports in the literature of cardiac transplantation. A patient who underwent a liver transplant for cirrhosis developed cardiac failure at 7 months post-liver transplant. At 3 years he underwent heart transplantation. No follow-up is available as he died of infection within 3 weeks of heart transplant[63]. The second cardiac transplant was in a juvenile with heart failure despite venesections and desferrioxamine. At 18 months post-transplant, myocardial iron content was still within reference range on biopsy and he remained well[65].

Comment

There are inadequate data at present to support hemochromatosis as a reasonable indication for cardiac transplantation. The likely detrimental effect of cirrhosis and the potential to develop hepatocellular carcinoma must be remembered.

CHAGAS' DISEASE (TRYPANOSOMIASIS)

Background

Chagas' disease is caused by the protozoan *Trypanosoma cruzi* and is prevalent in Brazil, Argentina and Chile. It manifests as an extensive myocarditis becoming evident years after initial infection. Three stages are described: acute, latent and chronic.

The acute disease is transmitted to humans by the bite of the reduviid bug (colloq. 'vinchuca') which acquires the organism by feeding on infected armadillos, racoons, opossums, skunks and domestic dogs and cats. The bite allows transmission of trypanosomes from the bug's feces to the person through the disrupted skin. The bite is often around the eye, causing unilateral periorbital edema (Romana's sign) or a skin lesion (*chagoma*). Congenital and transfusion-related transmission can also occur. In the acute phase, parasite multiplication and widespread

migration occur throughout the body. T cell function is depressed in those with inapparent acute infections, while those with clinically apparent acute infection (fever, myalgia, hepatosplenomegaly and myocarditis) have intact T cell function. The parasites are seen within cardiac myocytes, accompanied by a marked cellular infiltrate, with or without fiber degeneration. Cell lysis is due to cell- and antibody-mediated immunity directed against antigens released from *T. cruzi*-infected cells. Clinically, heart failure, left ventricular apical and right atrial thromboses, and pericardial effusions are seen. Heart failure may resolve over months or be fulminant if there is panmyocarditis.

After a latent period of 10–30 years, 30% of patients develop chronic Chagas' disease with predominantly right (but also left) ventricular failure with tricuspid regurgitation and ventricular or atrial arrhythmias. Syncope and sudden death can occur. Conduction system involvement is common, resulting in right bundle branch block or left hemiblock. The echocardiogram shows a right- and left-sided dilated cardiomyopathy but with relatively preserved interventricular septal motion, often with apical left ventricular aneurysm and thrombosis. Other features may include autonomic megaesophagus, megacolon, and dilatation of the stomach, duodenum, ureter or bronchi. Parasites may not be detectable at this stage of the disease in 75% of cases. The process appears to be due to development of self-directed cytotoxic T lymphocytes capable of lysing normal host cells in the absence of parasite antigens.

Histologically, in the chronic stage, myocyte necrosis is seen with extensive fibrosis together with a chronic cellular infiltrate of lymphocytes, plasma cells and macrophages, and occasional granulomas or even arteritis. Immunohistochemical markers identify the infiltrate as being 96% T lymphocytes (predominantly $CD8^+$, with only a smattering of $CD4^+$ antigen expression[66].

Diagnosis

The Machado–Guerreiro complement fixation test has a sensitivity of 90% and specificity of 99% for chronic Chagas' disease. Indirect immunofluorescent antibody, ELISA, and hemagglutination tests are also useful. To detect parasites in the blood, 'clean' reduviid bugs raised in a laboratory are allowed to bite the patient, and identification of the parasite in the bug's intestine is proof of parasitemia in the infected human (xenodiagnosis).

Treatment

Antiparasitic treatment with nifurtimox and benzimidazole reduce parasitemia but do not affect chronic disease once it has developed.

Recurrence after transplantation

There are at least 12 cases in the English literature of cardiac transplantation for Chagas' disease. Bocchi *et al.* reported 12 patients with Chagas' disease who underwent cardiac transplant with follow-up from 1 to 81 months. Patients with megacolon or megaesophagus were not transplanted. All but one patient received triple immunosuppressive therapy. Only four received antilymphocyte globulin induction therapy. All patients were monitored for *T. cruzi* by serological tests, examination of blood or leukocyte concentrate for parasite, xenodiagnosis, culture and mouse inoculation. Five of 12 patients received benzimidazole for 2–3 months as prophylaxis. In nine patients, *T. cruzi* reactivated, with the parasite detected in blood or other tissues, and in five of these nine there was clinical disease recurrence with myocarditis, fever and panniculitis accompanied by a rise in hemagglutination titers. All reactivations responded to treatment with benzimidazole. Six of the 12 patients died, but in no case was death apparently related to *T. cruzi* infection. Curiously, three of the 12 developed a lymphoproliferative disorder and a fourth developed pulmonary Kaposi's sarcoma[67].

In one other report, a 54-year-old woman with Chagas' cardiomyopathy presented at 5 months post-cardiac transplant with a progressive rise in liver enzymes with biopsy-confirmed chronic active hepatitis. This was improved by a temporary cessation of azathioprine, later reintroduced in small doses, and reduction in cyclosporin. The cause of the hepatitis was not determined[68].

Comment

Most patients transplanted for Chagas' disease show reactivation of *T. cruzi* infection with myocarditis. Follow-up is too short to determine whether chronic Chagas' disease can also recur. Antiparasitic prophylaxis was unsuccessful when given for up to 3 months. The occurrence of lymphoproliferative disorders in 25% of transplanted patients raises the possibility that the underlying immune changes related to Chagas' disease may predispose this group to malignancies when further immunosuppressed.

CARDIAC TUMORS

Cardiac tumors deserve mention as an indication for transplantation, there being a small literature available on this topic[69]. Primary cardiac tumors occur only rarely (0.3%). Atrial myxomas are the most common benign form. Benign tumors may be amenable to surgical excision while malignant tumors are seldom resectable.

Recurrence after transplantation

In a collective report from Goldstein *et al.*[69], nine patients were reviewed who underwent cardiac transplantation for primary cardiac tumors. In each of these the tumor was unresectable without cardiac explantation. Of the nine, four had malignant tumors (comprising three sarcomas and one lymphoma) and five had locally invasive benign tumors (four extensive but benign fibromas and one phaeochromocytoma). In seven of these nine, cardiectomy allowed total resection of the tumor, and all of these patients were alive at follow-up 12 months to 6 years post-transplant without recurrence, despite immunosuppression. One of these nine patients died beyond 6 years post-transplant from rejection due to non-compliance, without tumor recurrence. The two patients in whom tumor was present at the surgical margins (one large-cell lymphoma and one angiosarcoma) died at 14 and 15 months, respectively, of tumor recurrence despite adjuvant chemotherapy.

Comment

There is a potential for at least short-to-medium-term cure for benign tumors with cardiac transplantation, providing the tumor can be totally resected by cardiac explantation.

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42
Quality of Life After Heart Transplantation

C.E. SKOTZKO

INTRODUCTION

The number of patients undergoing heart transplantation (HTx) has increased over the years as transplantation has become a therapeutic option for individuals with end-stage cardiac disease. One-year survival rates now approach 80–90% (Chapters 43 and 44), providing a real 'gift of life' to those who are fortunate enough to receive a donor organ.

The lack of donor organs is severe, but the advances in pharmacologic management of patients with severe heart failure provide hope that the HTx candidate will maintain relative stability during the indefinite waiting period for a donor organ. With such scarce resources (of both organs and health-care financing) increasing scrutiny has been focused on the return of the HTx recipient to a reasonable quality of life. Attempting to determine if an individual who was near death is better off after a life-saving procedure may sound simple but, in actuality, the results are mixed.

Although HTx imparts almost immediate improvement in ejection fraction and cardiac output, Kuhn *et al.*[1] found that it was the first anniversary that served as a significant marker. Full social and emotional adjustment did not begin to occur until 12 months after implantation. This can be better understood when acknowledging the many tasks that are negotiated after HTx, including: (a) readjusting to family and vocational roles, (b) adapting to changes in body image, (c) controlling anxiety or fear of rejection and infection, (d) tolerating immunosuppressant drug side-effects, and (e) coping with disabilities and/or costs[2].

While medical staff look at objective hemodynamic data to denote improvement, the recipient's perception is far more subjective, driven by personal experience and cultural expectations. In an effort to better examine the complex nature of quality of life after HTx, examination of particular aspects of a recipient's functioning helps to unravel what is known with respect to outcome after HTx. The functions to be examined are: (a) - physical, (b) vocational, (c) psychological, (d) social, and (e) sexual functions.

PHYSICAL FUNCTION

The National Transplantation Study in the USA[3] found that 80–85% of surviving HTx recipients are physically active when global measures of activity are considered. Harvison *et al.*[4] found that 77% of recipients felt that their exercise ability had improved, while 15% reported no change. This certainly sounds promising, but it is worthwhile comparing individuals post-HTx with a comparable group with severe heart failure who are being vigorously managed medically, many of whom are awaiting HTx themselves. Walden *et al.*[5] provided such a comparison. At 6 months post-HTx no difference was seen between the two groups by the results of the 6-minute walk test, despite a statistically significant improvement in ejection fraction in the HTx group. Stevenson *et al.*[6] also compared such groups and, despite dramatic improvement in ejection fraction in the HTx group, there were no significant differences between the two groups in: (a) maximum work load, (b) oxygen uptake, (c) anaerobic threshold, or (d) maximum oxygen pulse. There appears, therefore, to be little difference in pre- and post-HTx physical function.

Kobashigawa *et al.*[7], however, found that it is possible to measurably improve the physical status of HTx recipients. Cardiac rehabilitation was documented to be beneficial in improving physical function and significantly improving Vo_{2max} (in comparison to a group of HTx recipients who did not receive such rehabilitation.

This brief overview indicates that, while HTx does improve cardiac function and the recipient's perception of physical function, overall fitness does not follow unless focused training occurs.

VOCATIONAL FUNCTION

The data on physical function may partially help explain the relatively low rate of return to employment found after HTx (Chapter

15). Recipients state almost universally that a stable financial situation is required for an acceptable quality of life. Examining the findings of Paris *et al.*[8,9], who reviewed the employment status of HTx recipients at seven HTx centers in the USA, we find that 45% were employed, 13% medically disabled, 6% retired, and 36% unemployed. Of the unemployed group, 63% had no plan to look for work. The policy of the individual transplant center with regard to providing continuing disability claims had an impact on the number of recipients who sought and secured new employment.

In Australia, Harvison *et al.*[4] documented that 53% of HTx recipients were employed, 28% received a pension, 9% were voluntarily retired, 6% were unemployed receiving benefits, and 4% were on paid leave. (The figures do not indicate the number of medically disabled individuals.) While it would appear that only 6% of HTx recipients are unemployed in Australia, the large number receiving a pension probably correlates with the unemployed in the USA.

Walden *et al.*[5] reported that there was no difference in the rate of return-to-work between HTx patients and those on tailored medical therapy for heart failure. It may be that the 6-month assessment mark is too early after HTx to allow maximum return to employment, but the possibility exists that there is actually no increased employment rate after HTx.

An unfortunate reality in the present health-care environment in the USA is the link of insurability with disability. Meister *et al.*[10] found recipients to be in one of four groups with respect to employment: (a) 32% employed, (b) 25% retired, (c) 7% medically disabled, and (d) 36% insurance-disabled. The insurance-disabled population, although capable of working, is dependent on disability and government health-care benefits. Paris *et al.*[8,9] found that 71% of the unemployed population would lose disability income, while 29% would lose health insurance, by returning to the workforce. The potential loss of income and health insurance associated with recovery after HTx is a major obstacle for many recipients. Indeed, many who present with anxiety and depression have extreme financial stresses that at times induce psychological disability.

PSYCHOLOGICAL FUNCTION

Psychological function is an area with potential for heavy overlay of subjective experience. Post-HTx perceptions often rely on pre-transplant personality. Shapiro *et al.*[11] supported this when they reported that pre-HTx life satisfaction correlated closely with post-HTx satisfaction. In addition to personality factors, there is clear evidence that HTx directly impacts the recipient's psyche.

Depressive disorders were noted in HTx recipients as early as 1969. At that time Lunde[12] noted that one in nine HTx recipients had problems with depression. As experience with HTx was in its infancy at that time, and survival rates were low, many might dismiss these numbers as a logical fear of an uncertain outcome. However, in 1989 Shapiro and Kornfeld[13] found 51% of HTx recipients had an affective illness characterized by irritability and mood lability. Additionally, Jones *et al.* in 1988[14] reported that individuals were more anxious 4 and 12 months after HTx than at discharge from hospital immediately post-HTx. As survival rates improved dramatically between 1969 and the late 1980s, it is difficult to dismiss these findings.

Such high rates of psychological distress are not surprising to medical personnel involved in after-care clinics who provide counseling to HTx recipients. Immunosuppressive agents are known to act centrally, with depression and anxiety as prominent side-effects[15–17]. Added to this are accumulating data showing that chronic administration of cyclosporin may contribute to dementia in some patients.

Corticosteroids are commonly linked with anxiety and depression in non-HTx populations. This link is supported by Jones *et al.*[18], who found that HTx patients not receiving steroids had significantly less anxiety than a matched group receiving steroids as part of their immunosuppressive regimen.

The link of pretransplant personality with post-transplant course was addressed by Brennan *et al.*[19], who reported that individuals with personality disorders had greater documented non-compliance after HTx, although this did not affect survival. This finding was supported by Shapiro and Fingeroth (personal communication, 1993), although an early study from Cape Town suggested that survival was affected[20].

It should be acknowledged that many programs utilize data regarding pre-existing psychopathology in decision-making regarding candidacy for HTx[21]. These findings do not generalize to a non-personality-impaired group. Skotzko *et al.*[22] found that there was no impact of pre-existing psychiatric disorders on survival or on medical outcome after HTx.

The above studies provide evidence for the impact of immunosuppressive agents on a recipient's emotional state, as well as acknowledge that personality factors may have a lasting impact on post-HTx course. Caution needs to be exercised to ensure that candidacy decisions are made with attention to factors affecting compliance, and not from prejudice toward a psychiatrically impaired population.

SOCIAL FUNCTION

The ability of recipients to interact with family and friends is an important domain to consider in outcome after HTx. Harvison *et al.*[4] noted a link between social satisfaction and productivity. Individuals who had successfully gained employment after HTx reported increased social satisfaction. Lough *et al.*[23] determined that recipients acknowledge a significant ($p < 0.01$) positive sense of accomplishment in the following areas: (a) future outlook, (b) feelings about self, (c) social support, (d) sense of achievement, and (e) decision-making. Recipients additionally acknowledged a significant ($p < 0.01$) positive sense of accomplishment in their relationships with family, friends, work associates, and health professionals. Emotional behavior, social interaction, alertness, and communication have all been documented to be diminished after HTx[24].

Walden *et al.*[5] found psychosocial functioning to be comparable between HTx recipients and those with heart failure undergoing tailored medical therapy, although the latter group did acknowledge greater dysfunction in social activities.

The importance of social ties toward the outcome after HTx was demonstrated by Skotzko *et al.*[22], who documented a statisti-

cally significant association between marriage and (a) fewer hospitalizations and (b) less severe rejection episodes. This underscores the importance of relationships in maintaining health and diminishing the impact of illness on perceptions of quality of life.

What is often not addressed in social functioning is the vital link between doctor and patient. Patients who are unable to form a strong working relationship with their doctors are often lost from follow-up in heart failure clinics and, at times, in HTx clinics.

SEXUAL FUNCTION

Concerns regarding ability to perform sexually after HTx are often raised before the procedure is undertaken. Some of the vasodilators used to manage heart failure render many men impotent while awaiting HTx.

Mulligan *et al.*[25] surveyed a population of men (predominantly white and married) with a mean age of 47.9 years, and found that pretransplant libido was strong and remained unchanged after HTx. Unfortunately, erectile function and the ability to achieve orgasm were impaired before, and declined further, after transplantation. Harvison *et al.*[4] found that only 50% of transplanted patients were nearly or completely satisfied with their sexual life, and Lough *et al.*[23] supported this finding of a negative trend in sexual function after HTx.

In an assessment of immunosuppressive therapy (double versus triple therapy) Jones *et al.*[14] found that recipients who were not receiving steroids had higher levels of sexual satisfaction. The United Kingdom Study[26], however, indicated that, while 84% of patients awaiting HTx had problems with sexual function, only 29% had difficulty after HTx.

The data available regarding sexual function in men after HTx are therefore mixed. The impact of immunosuppressive medications on sexual function is becoming more apparent, as are the effects of pre-existing conditions which may impede sexual function even in the absence of transplantation.

The impact of HTx on women is deserving of further investigation. Awareness of immunosuppressive medications and their impact on female fertility and hormonal cycles is in its infancy.

COMMENT

Many patients make remarkable recoveries after HTx, going on to compete in athletic events, manage businesses, nurture relationships, and raise families. Dramatic recoveries are most apparent in the sickest individuals with severe physical limitations. The data reviewed support the conclusion that HTx helps people to sustain life and hope for the future, but also indicate that HTx recipients need assistance in returning to full functional lives.

In this time of dwindling fiscal resources, a wish-list of sorts can be gleaned from the information reviewed. The data available suggest that return to optimal physical function requires focused attention. Physical rehabilitation would appear to be an area worthy of further investigation, and patients should be given every encouragement and assistance to maximize their fitness.

Physical, fiscal, and psychological barriers impede the return of HTx recipients to the workforce. Legislative reform to foster improved access of transplant recipients to employment without endangering their access to health care and sustenance income deserves further attention.

Increasing awareness of the psychological impact of immunosuppressive agents and appropriate referral to mental health providers would dramatically improve the quality of life of many HTx recipients. Attention to the need to reaffirm social ties after HTx, and renegotiate roles with family members and health-care providers, deserves further promotion.

Finally, impediments to sexual function in men and women deserve further examination. While many HTx recipients are reluctant to bring their sexuality into the examination room, many have concerns that deserve attention.

It has been shown possible to save the lives of patients by this miraculous procedure. It is now time to devote resources to help this population contribute their full potential to future generations.

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Results of Cardiac Transplantation and Factors Influencing Survival based on the Registry of the International Society for Heart and Lung Transplantation and the Cardiac Transplant Research Database

M.P. CINQUEGRANI AND J.D. HOSENPUD

INTRODUCTION

Despite significant progress in lengthening survival following heart transplantation (HTx) since the introduction of cyclosporin, patients who undergo this procedure do not face a normal life expectancy for their age. Information derived from a number of databases has been useful in identifying risk factors for mortality following transplantation. This chapter will review those clinical variables associated with decreased survival for heart transplant patients.

PATIENT SURVIVAL

Survival data from the latest report of the Registry of the International Society for Heart and Lung Transplantation (ISHLT) provide an overview of post-transplant mortality rates[1]. Of the 27 977 patients followed for a 12-year period through February 1995, 76% survived for at least 1 year after HTx (Figure 1). These data reveal a 4% per year subsequent mortality rate, suggesting a maximum graft survival of $18\frac{1}{2}$ years. When broken down by era of transplantation (Figure 2), there has been a clear improvement in 1-year survival rate over time. The 1- and 3-year survival rates for patients transplanted in the pre-cyclosporin era of 1975 through 1981 are far below those of more contemporary time periods. That progress has been made in enhancing survival is evident in the 1- and 3-year survival rates of 81% and 73%, respectively, for the 1988–1994 interval, versus roughly 55% and 40% 1- and 3-year survival rates for patients transplanted between

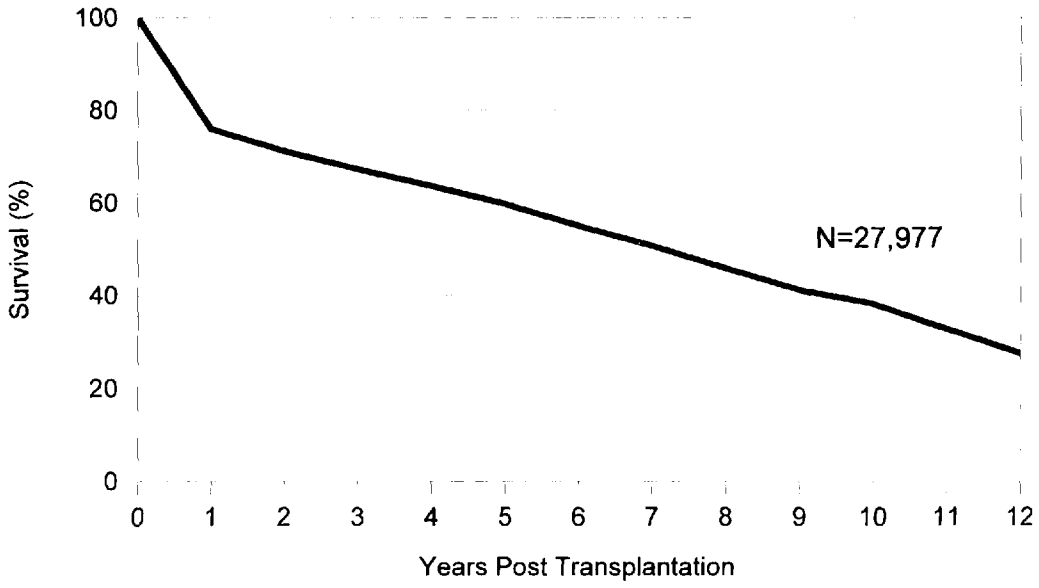

Figure 1 Survival following heart transplantation (International Society for Heart and Lung Transplantation (ISHLT) data, 1995)

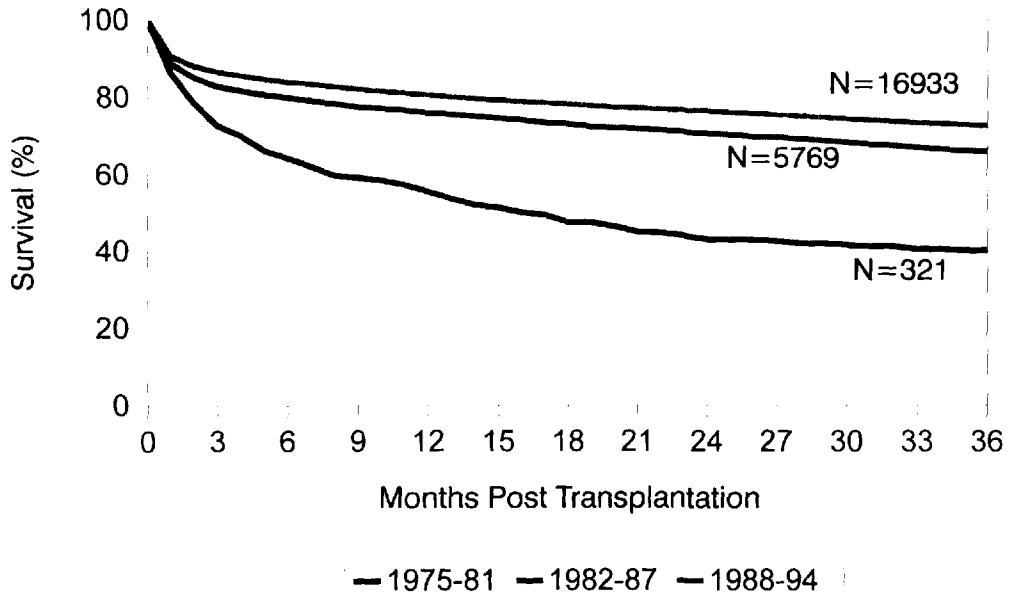

Figure 2 Cardiac transplant survival by era of transplant (ISHLT, 1995)

1975 and 1981. The introduction of cyclosporin has been credited with the survival advantage of HTx subsequent to 1982. Other factors, such as increased clinical experience at active transplant centers, may also play a role in improved survival[2].

CAUSES OF DEATH

In the first year following HTx, infection and rejection are the predominant causes of mortality. Bourge's analysis of the Cardiac Transplant Research Database Group's (CTRD) data, which included data on 911 transplanted patients, revealed a 1-year mortality rate of 16%, with infection and rejection being nearly equal in frequency as the cause of death in 45% of the non-survivors[3]. Most of the mortality observed in this database occurred in the first 6 months following transplantation. Data from both the CTRD and the ISHLT have identified a number of clinical characteristics in the donor and recipient which appear to predispose the recipient to mortality post-transplant.

FACTORS INFLUENCING SURVIVAL

Table 1 lists risk factor data from the ISHLT registry for 10 782 patients who underwent HTx in the USA between 1987 and 1994. In addition to the ISHLT risk factors, the CTRD data also revealed modest negative effects on survival for a blood group O donor matched to a non-O recipient, as well as negative effects for lower pretransplantation cardiac output in the recipient, and impaired renal function in the recipient and, in children, high pulmonary vascular resistance[3]. Clearly, it is easy to appreciate the impact of some of these factors on a recipient's risk for infection

Table 1 Risk factors for 1-year cardiac transplant mortality; n = 10 782 (US data, 1987–94)

Variable	*Odds ratio*	*p-Value*	*95% confidence interval*
Previous Tx	3.55	< 0.001	2.78–4.53
Ventricular assist device	1.88	< 0.001	1.45–2.42
Ventilator	1.83	< 0.001	1.50–2.24
Hospitalized	1.16	0.006	1.04–1.29
Recipient < 5 years	1.62	< 0.001	1.25–2.09
Recipient 50–59 years	1.23	< 0.001	1.09–1.38
Recipient ≥ 60 years	1.73	< 0.001	1.49–1.99
Recipient female	1.14	0.03	1.01–1.29
Congenital	1.47	0.002	1.15–1.87
CTR Vol < 9Tx/year	1.34	< 0.001	1.18–1.53
Recipient ABo type A	0.87	0.008	0.79–0.96
Donor female	1.2	0.001	1.08–1.34
Donor 35–44 years	1.26	< 0.001	1.10–1.44
Donor 45–59 years	1.73	< 0.001	1.47–2.04
Donor ≥ 60 years	3.49	< 0.001	1.91–6.39
Ischemic time/hour	1.1	< 0.001	1.05–1.16
CMV: DNR-positive/Recipient-negative	1.2	0.029	1.02–1.42

or rejection, or mechanical graft failure in the case of long ischemic times. For some risk factors, such as gender, the interaction between risk factor and recipient mortality is more obscure.

Recipient pretransplant clinical status

Recipient health status plays a significant role in survival post-transplantation. Of all recipient-related factors, prior HTx is the most potent in terms of predicting mortality (Table 1), even when subselecting for patients transplanted more remote (>6 months) from their primary transplant (Figure 3). Patients undergoing any second cardiac transplant operation face a higher mortality rate[4]. The negative survival risk of repeat cardiac surgery, together with a multitude of other factors, including the degree of illness associated with the need for retransplantation, immunologic factors, and the morbidity associated with prolonged immunosuppression, probably account for the overwhelmingly poor outcomes that have been reported with second-time HTx. The subgroup of patients who undergo repeat HTx within 6 months of the initial procedure are at particularly high risk for mortality compared to patients receiving a second heart transplant at a later time (Figure 4). This observation identifies a group of patients who are likely

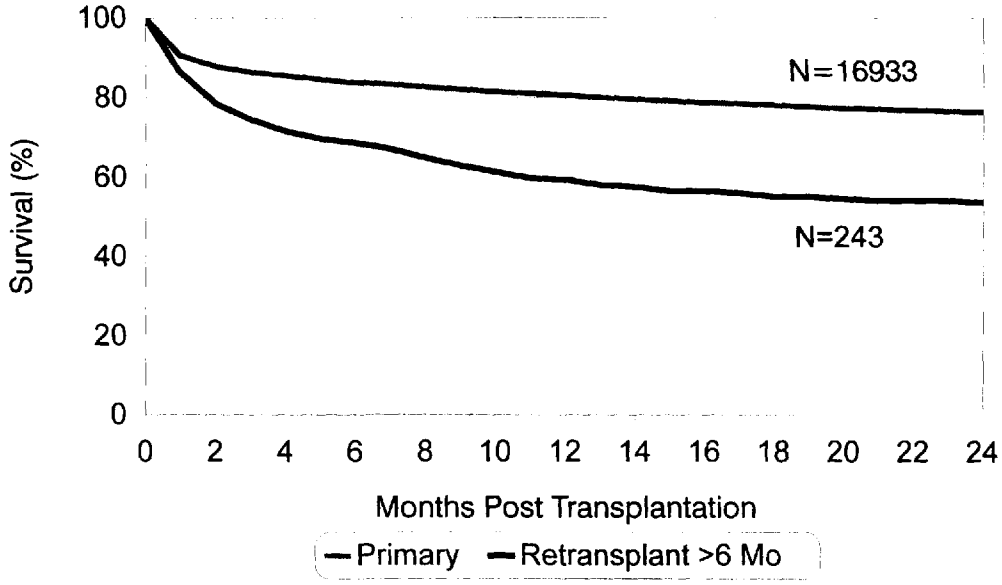

Figure 3 Cardiac transplant survival for retransplantation more than 6 months after initial transplant compared to survival for primary transplant (ISHLT, 1995)

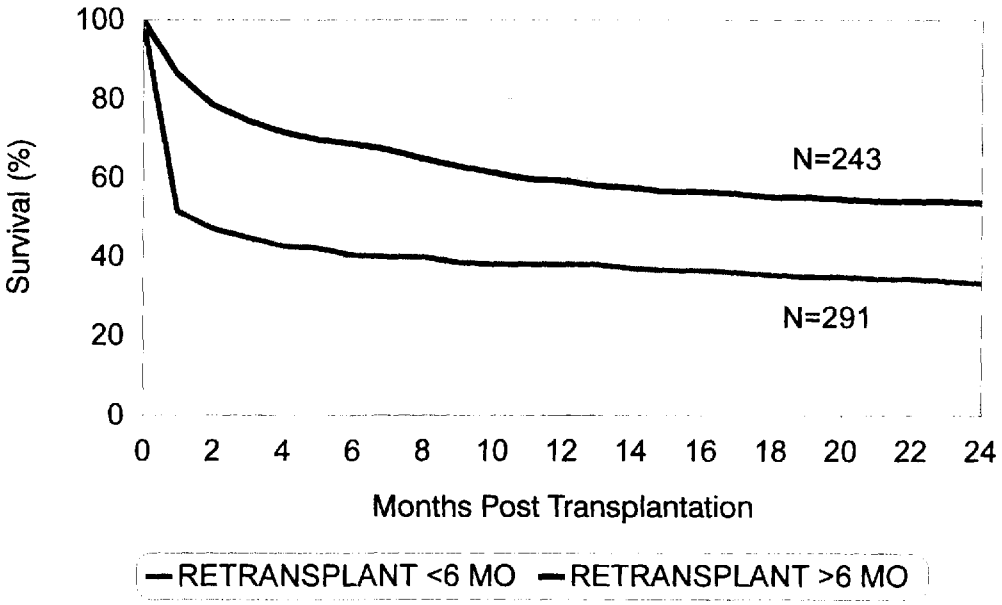

Figure 4 Cardiac transplant survival for retransplantation more than 6 months after initial transplant compared to patients with early retransplantation (ISHLT, 1995)

more susceptible to graft failure due to rejection or primary graft failure, which in turn may play a role in their poor outcome with a subsequent transplantation. The very poor survival of recipients of second heart transplants within 6 months of the first procedure should be taken into consideration before committing to repeat HTx in light of the limited availability of donor hearts.

The need for intensive-care support prior to HTx identifies patients at higher risk for post-transplant mortality. Individuals requiring hospitalization for intravenous inotropic support represent a group of patients with advanced congestive heart failure who are likely to be physically debilitated and subject to postoperative infections. Those patients who eventually require mechanical ventilation or ventricular assist devices prior to transplantation are at the far end of the spectrum of illness and, not surprisingly, are at increased risk for post-transplant mortality. Both mechanical ventilatory support and ventricular assist devices are potential conduits for infection. Miller *et al.* report that the lung is the most common site for post-transplant infection, with a mortality rate of 23% in infected patients[5]. It is not surprising, in this light, that patients on long-term ventilatory support are at increased risk for death post-transplant. Three-month survival post-transplant has been reported to be 65% for patients on pretransplant ventilatory support[3]. In addition to the infectious risk of mechanical ventricular assist devices, these patients may be subject to compromised end-organ function such as renal insufficiency. A combination of factors probably suggests an additive risk for post-transplant mortality in patients who were preoperatively on mechanical ventricular assist devices.

Recipient age

Recipient age, at both the young and the old ends of the spectrum, negatively influences survival following HTx. As with adult HTx, there has been an improvement in survival for pediatric patients transplanted in the more recent era (Figure 5). Post-transplant survival in the pediatric age group is very age-dependent, as shown in Figure 6, with patients in the cohort of less than 1 year of age fairing worst and the 6–18-year age group having 24-month survival rates similar to the general adult population. Bourge *et al.* also found the recipient age factor to be the greatest negative predictor of survival in recipients under age 5[3]. This group experienced a 1-year survival of only 68%, compared to 85% for their entire patient population. High pulmonary vascular resistance appeared to be a significant risk factor for post-transplant mortality in children. In part, the very high mortality rates for the recipients less than 1 year of age may be reflected in the presence of congenital heart disease as the predominant indication for HTx in that age group (Figure 7). The stepwise decrease in mortality risk noted in older age cohorts of children may represent a shift away from congenital heart disease, being replaced by cardiomyopathy as the principal indication for HTx. In addition to hemodynamic factors such as high pulmonary vascular resistance, other factors, such as the presence of an immature immune system and enhanced susceptibility to infection, are probably active in increasing the mortality risk of the youngest transplant recipients.

Recipients over the age of 50 begin to experience the negative effects of age on post-transplant mortality. The mortality risk of age is particularly marked in patients over the age of 65. In this group the increased mortality risk was evident within 3 months of transplantation and remained significant throughout the 36-month time period reported by the ISHLT (Figure 8). The presence of concomitant complicating problems such as vascular disease or a predisposition to diabetes mellitus, in addition to greater susceptibility to infection, probably contribute to the enhanced mortality risk associated with older age.

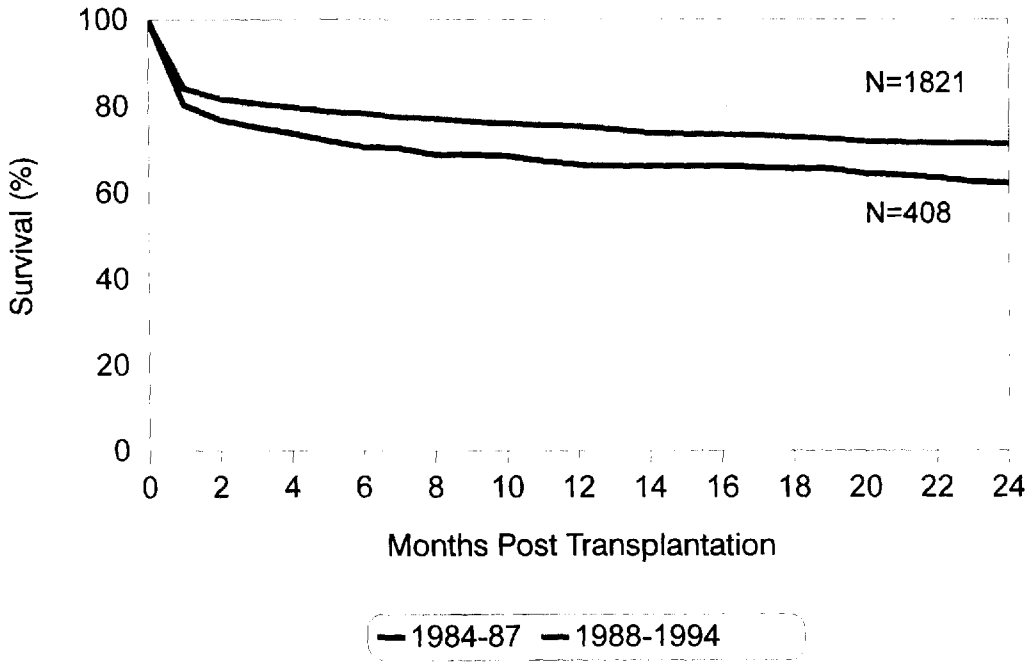

Figure 5 Pediatric heart transplant survival by era of transplant (ISHLT, 1995)

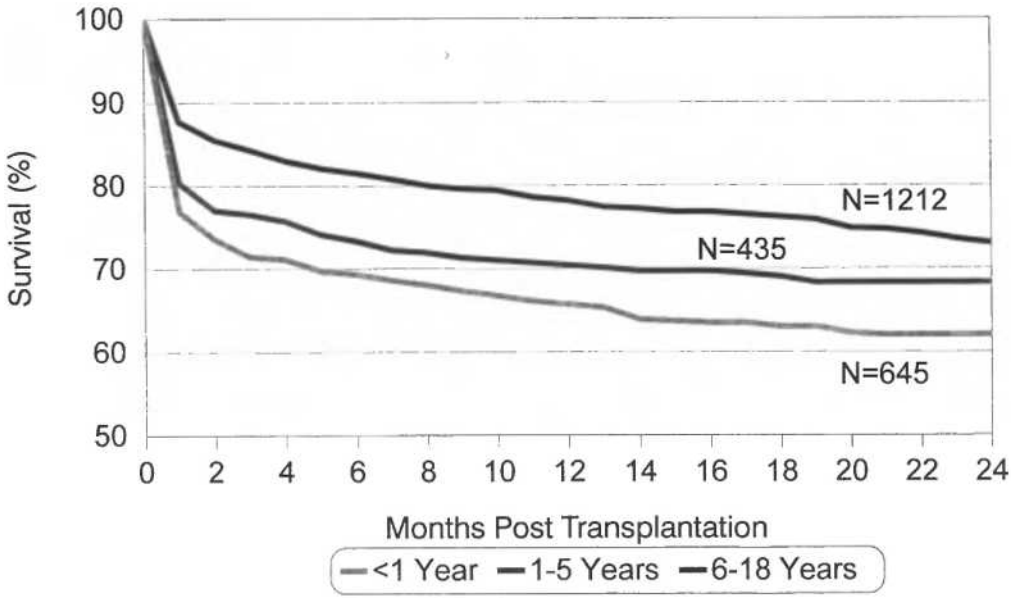

Figure 6 Pediatric heart transplant survival by age at time of transplant (ISHLT, 1995)

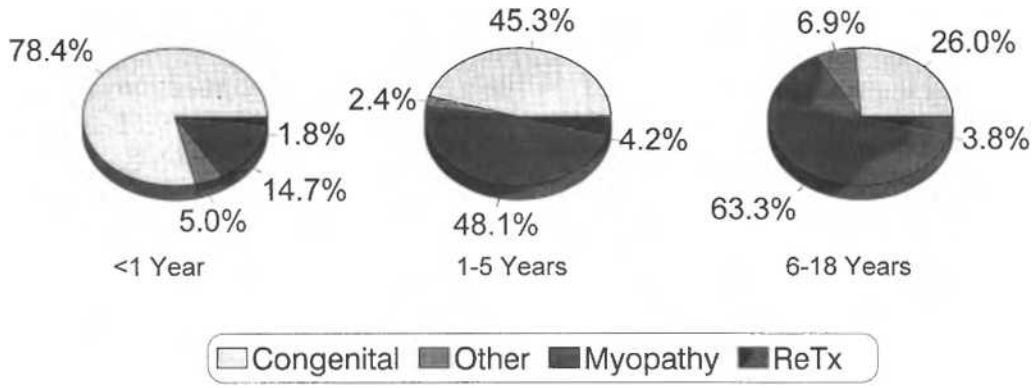

Figure 7 Indications for pediatric heart transplant by age cohorts (ISHLT, 1995)

Donor factors

Age of the donor heart also greatly influences post-transplant mortality. Both the CTRD and ISHLT databases recognize donor heart age as a significant risk factor for mortality post-transplant. The increased mortality odds ratio is present for donors over age 35, but it is most noticeable in the donor group over age 60. Indeed, the odds ratio for mortality in the group over age 60 is nearly equivalent to the mortality risk found in retransplantation (Table 1). Another potent donor risk factor is associated with donor hearts from women. Some of this effect may be explained by higher cumulative rejection rates for recipients of female hearts and mismatches between donor and recipient body surface areas[6,7]. The immunological reasons for an increased susceptibility to rejection in female donor hearts are not understood. Donor–recipient body surface area mismatch can potentially lead to complications due to restrictive cardiac physiology in the recipient. Young *et al.* studied their patient population for donor–recipient interactions that may add to the risk of post-transplant mortality. In addition to the well-known risk factors, they observed that high donor inotropic support, donor diabetes mellitus, diffuse donor heart wall motion abnormalities and, in pediatric donors, death from causes other than closed-head trauma, all contributed to post-transplant mortality risk. These investigators found their risk factor profile was additive in nature, allowing them to predict recipient mortality rates based on donor and recipient characteristics.

Factors influencing late mortality

Certain factors confer decreased survival potential on recipients well after the first year of transplantation. Data presented in Figure 9 assess survival over a 48-month period for patients who have already survived 1 year. Three factors were identified which increased mortality risk in 1-year survivors of HTx. In the recipient group a history of prior HTx remains a potent ongoing risk

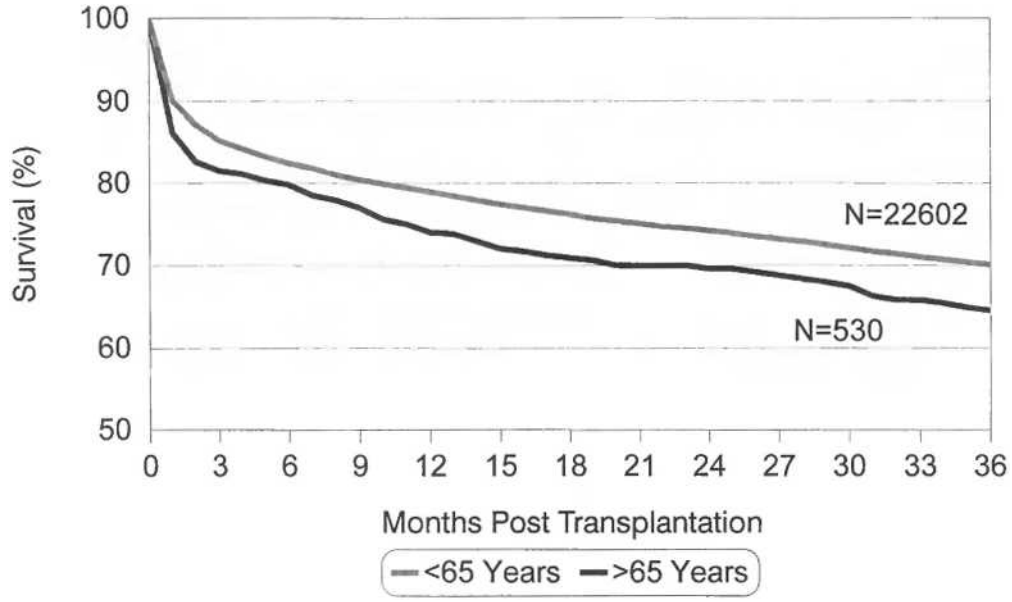

Figure 8 Cardiac transplant survival by age at time of transplant (ISHLT, 1995)

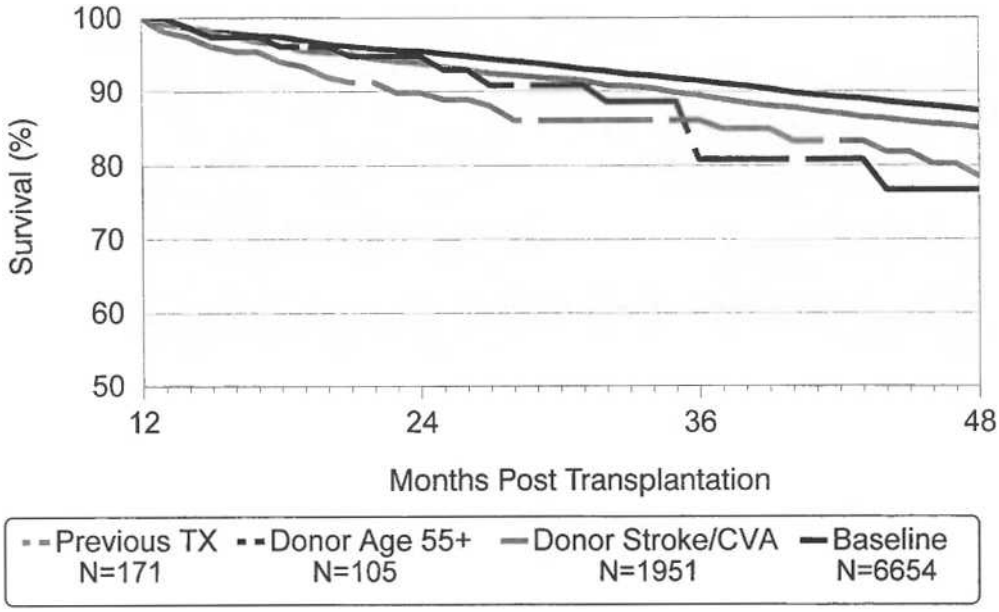

Figure 9 Risk factors for mortality in patients who survive more than 1 year after cardiac transplantation. Stroke/CVA refers to these as etiology of death in the donor. Baseline refers to the entire transplant database (ISHLT, 1995)

factor for mortality. From the donor perspective, donor age greater than 55 and the presence of cerebral vascular accident as a cause of death in the donor significantly increases the risk of mortality well after HTx. These findings indicate the long-term and persistent nature of certain risk factors, and place added importance on taking them into consideration when making decisions regarding HTx. This is particularly true in the case of donor age, since long-term survival in recipients of donors over age 55 is as poor as that found in recipients of a prior heart transplant.

Infection

On the whole, bacterial and viral infections are the most common agents of infection post-transplant, accounting for 47% and 41%, respectively, of all events[5]. Fungi and protozoa make up the remainder of infectious agents, but these agents carry a much higher risk of mortality at 36% versus approximately 13% for the aggregate group of infections.

Cytomegalovirus is the most common infecting organism in the post-transplant patient, accounting for up to 26% of all infections.

Miller *et al.* found that CMV infection was perhaps more frequent during the first year post-transplant in CMV-negative recipients of a CMV-positive donor heart. Approximately 27% of CMV-negative recipients who received a CMV-positive heart developed active CMV infections versus 15% of all other post-heart transplant patients. They also found that the rate of infection with any organism was higher in patients who received OKT3 or antithymocyte globulin induction therapy (41% with induction therapy versus 35% without induction therapy), and that induction therapy enhanced the risk of CMV infection during the first year post-transplant (19% with induction versus 12% without induction). Kirklin *et al.* also reported the risk for early post-transplant CMV infection to be increased for CMV-negative recipients of a CMV-positive heart, with enhancement of that risk by cytolytic therapy[8]. The risk of mortality from CMV was noted to be greatest among those patients who had frequent infections with any other organism, suggesting that these patients were particularly susceptible to infection. In the group of all patients with CMV infections, those who were CMV-negative recipients of CMV-positive hearts appeared to be at no greater risk for mortality (approximately 6%) regardless of the use of cytolytic therapy, compared to others with CMV infections.

While this database suggests that the risk of mortality in post-transplant patients with CMV may be equivalent between patient groups (CMV-negative recipient/positive donor, CMV-positive recipient/positive donor, or CMV-positive recipient/negative donor), the ISHLT database identifies the CMV-negative recipient/CMV-positive donor to be a risk factor for post-transplant mortality (Table 1). This is not surprising given that CMV colonization carries with it an increased rate of active infection and mortality, and that the CMV-negative recipient may be receiving the organism at a particularly vulnerable time relative to his or her degree of immunosuppression.

Gender

Women compose a much smaller proportion of patients who undergo Htx, but their mortality risk is much higher than that of men[9]. Wechsler's report on actuarial survival of the 75 adult women in their total group of 379 patients receiving HTx revealed significantly lower survival for women as compared to men at 6 months (75% vs 84%) and 36 months (64% vs 76%). In addition, the ISHLT database shows a significant mortality risk for women recipients of heart transplants at 1 year compared to the entire group of recipients (Table 1). The CTRD data identified female recipient gender as a risk factor for death or retransplantation due to rejection[10].

In the Wechsler study, infection and rejection were the principal causes of early mortality in women, similar to findings in men. In this study, several factors were found to be associated with reduced survival in women receiving heart transplants. Transplantation of a CMV-positive donor into a CMV-negative recipient increased mortality risk, as did the use of OKT3 induction therapy. These investigators also found that the use of a CMV-positive donor and OKT3 together had an additive effect on mortality risk in female recipients. The authors propose that, since women are more susceptible to autoimmune disease than men, they may be immunologically more reactive and thus more likely to experience life-threatening rejection episodes. Multiparity has been implicated as a mechanism for increasing immune reactivity in female heart transplant recipients[11]. Wechsler's female patient population was largely multiparous, but this did not appear as a risk factor for mortality.

The increased mortality risk for HTx in women also extends to recipients of female donor hearts (Table 1). Female donor hearts have been associated with the early development of rejection as well as a greater cumulative incidence of rejection[6,10]. The susceptibility of female donor hearts to rejection is unknown, especially when placed in male recipients. It is conceivable that there is something unique immunologically with allografts obtained from female donors. More likely, however, mechanical problems due to mismatch between a heart from a small-body-surface-area woman transplanted into a larger male are at work in increasing mortality associated with the female donor[7].

HLA matching

Cross-matching kidney donors and recipients relative to their HLA tissue type has resulted in significantly prolonged graft survival with overall less rejection[12]. Unfortunately, HLA typing for donors and recipients in HTx has not been considered feasible given the length of time the testing takes, and the inadequacy of organ preservation techniques, which allow for a limited ischemic window of 4 hours following explantation. Heart graft failure rates due to ischemic injury become unacceptably high if the duration of ischemia is greater than 4 hours[3]. Nonetheless, there remains great interest in more closely matching the immunological profile of donor and recipient than the current practice of ABO typing allows, since rejection is a major cause of morbidity and mortality following heart transplantation.

Opelz's and Wujciak's prospective study of the role of HLA compatibility between donor and recipient, and its effect on heart graft survival, demonstrated significant improvement in patient survival in closely matched individuals[13]. As would be expected, of the 8331 patients entered into follow-up, only 128 had a perfect match or one mismatch of the HLA-A, B, or DR loci with their donor heart. This group experienced an 83% survival 3 years following transplant, in contrast to the significantly lower survival rates of 76% for patients with two mismatches and 71% for three to six mismatches. The degree of HLA match was found to be a predictor of survival that was independent of other factors known to influence graft survival, such as age, sex, or the duration of organ ischemia.

A retrospective analysis of the ISHLT database covering 10 752 heart transplants also revealed a significant reduction in mortality risk for patients with more than three matches at the HLA-A or DR loci[14]. Similar findings were reported by the Cardiac Transplant Research Database Group[10]. Interestingly, the ISHLT data did not demonstrate an independent role for matching at the HLA-B locus, suggesting that the HLA-A and DR loci are more involved in mediating rejection-related mortality. While these data support the value of closely matching donor and recipient HLA loci, the balancing of practical issues, such as the time required to perform tests and graft ischemic time, remain limiting factors in developing strategies to take advantage of HLA typing. It is possible, however, that more rapid testing and im-

proved heart graft preservation techniques will make feasible the use of HLA typing, and better matching of the immunological profiles of donor and recipient.

Recipient race

In addition to gender, race also appears to play a role in survival following heart transplantation. Jarcho *et al.*, reporting for the Cardiac Transplant Research Database Group, found that among the independent risk factors for time to first rejection and rejection-related mortality was black recipient race[10]. Hosenpud *et al.* found an increased mortality risk not only for black recipients but also for Asian recipients (risk ratios of 1.4 and 1.35 respectively, from Proportional Hazards Model)[14]. The mortality risk associated with race was found to be independent of HLA matching status. The reasons for race affecting survival post-heart transplant are not clear. Butkus' study of graft survival in cadaveric renal transplantation suggested that black recipients were negatively affected by socioeconomic factors and problems with drug regimen compliance[15].

In Wechsler *et al.*'s study cohort, there was a disproportionate number of non-white women (31%) compared to men (11%), but the authors were not able to conclude that race was the factor that decreased survival in women as opposed to men[9]. The relatively small number of patients included in this study (379, with 75 women and 304 men) may have limited the authors' ability to observe the effects of race on survival in either women or men. More studies from the large transplant registries will be required to gain insight into the mechanisms driving reduced survival in non-white recipients.

COMMENT

This chapter has reviewed many of the recognized clinical factors associated with decreased recipient survival following HTx. An understanding of the factors which affect clinical outcomes in HTx is of great value, since donor hearts are a limited resource and should be allocated very carefully with an eye on the potential for successful results of transplantation. Certainly, it is unlikely that post-transplant mortality risk will ever be reduced close to zero, but by matching donor and recipient risk profiles as much as it is feasible to do so, the best possible outcome for an individual recipient can be expected.

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Results of Cardiac Transplantation and Factors Influencing Survival based on the Collaborative Heart Transplant Study

G. OPELZ

INTRODUCTION

This report provides an overview of transplant results obtained during the past 10 years at centers participating in the Collaborative Transplant Study. The Collaborative Transplant Study was initiated for renal transplants in 1982 and extended to cardiac transplants in 1984. Rather than aiming at being a comprehensive registry for worldwide transplantation activities, the Collaborative Transplant Study was intended from the outset to be a scientific registry. Its aim was to identify and characterize factors that influence graft outcome, associated with the hope that knowledge concerning such factors would be useful for improving the results of future transplantations. Anticipation that meaningful results would be obtained more rapidly if many transplant centers cooperated formed the basis for a collaborative multicenter approach. Remarkably, 104 transplant centers in 23 countries are currently participating in the study, expending considerable resources on the collection of data and reporting them to the study center at the University of Heidelberg.

METHODS

The transplants on which this analysis was based were performed from January 1985 to September 1994 at the following transplant centers: Austria: Graz, Innsbruck; Australia: Melbourne (Alfred H., Childrens H.), Sydney; Belgium: Aalst, Leuven, Liege; Brazil: Belo Horizonte; Canada: Edmonton, Halifax, London, Montreal (Inst. Cardiol., Royal Victoria H.), Ottawa, Quebec, Toronto, Vancouver, Winnipeg; Colombia: Medellin; Croatia: Zagreb; Czech Republic: Prague; Finland: Helsinki (adult and pediatric); France: Nancy, Nantes, Paris, Rennes, Strasbourg; Germany: Essen, Hamburg, Hannover, Heidelberg, Kaiserslautern; Great Britain: Manchester, Newcastle upon Tyne, Papworth–Cambridge, Sheffield; Italy: North Italy Transplant (Bergamo, Bologna, Milan, Padova, Pavia, Udine, Verona), Turin; Lithuania: Vilnius; New Zealand: Auckland; Norway: Oslo; Russia: Moscow; Saudi Arabia: Riyadh; Slovenia: Ljubljana; South Africa: Cape Town; Spain: Pamplona; Sweden: Goeteborg; Switzerland: Geneva, Lausanne, Zurich; USA: Ann Arbor, Baltimore, Birmingham, Charleston, Charlotte, Chicago, Columbus, Dallas (Baylor, Childrens H., Methodist H., St Paul's H.), Detroit, Falls Church, Fort Wayne, Hershey, Indianapolis, Iowa City, Kansas City, Loma Linda, Louisville (Humana H., Kosair H.), Madison, Maywood, Miami, Milwaukee, Nashville (Tenn. Heart I., Vanderbilt), New Haven, New Orleans, New York, Oklahoma City (Baptist H., University H.), Philadelphia, Pittsburgh (Allegheny H., University H.), Portland, Rochester, Salt Lake City (LDS H., University H., VA H.), San Francisco, Seattle, Toledo, Tucson, Washington, Wichita.

The transplants were reported to the study center shortly after transplantation. Clinical follow-up information was obtained 3, 6 and 12 months after transplantation and yearly thereafter. The centers were assured of data confidentiality and of the anonymity of results obtained at individual participating centers.

Graft survival rates were computed according to the Kaplan–Meier method. No exclusions were made for any reason. Statistical significance was estimated using the log rank test or weighted regression.

RESULTS AND DISCUSSION

The overall 5-year results of first and second orthotopic heart transplants are shown in Figure 1. The survival rate of first grafts was 31% higher at 5 years than that of second grafts (64 ± 1% vs. 33 ± 3%, $p<0.0001$). In addition, there were 136 heterotopic first heart transplants which had a 5-year survival rate of 42 ± 5% ($p<0.0001$ as compared to orthotopic first grafts). All subsequent data given in this report refer to orthotopic transplants.

Based on the 5-year results depicted in Figure 1 it is possible to estimate survival half-life times for the period from the first to the fifth post-transplant year, and to extrapolate from these to project 10-year survival rates. As illustrated in Figure 2, the 10-year projected graft survival rates are approximately 48% for first heart transplants and 19% for second heart transplants. It should be pointed out that these 10-year rates are based on the hypothetical assumption that graft failures occur at the same rate from the 5th to the 10th post-transplant year as from the 1st to the 5th post-transplant year.

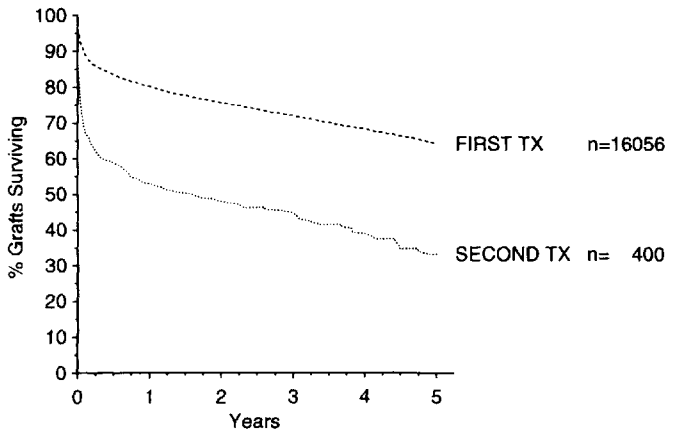

Figure 1 Graft survival of first and second orthotopic heart transplants. Numbers of patients studied are indicated. First grafts survived at a significantly higher rate than second grafts (log rank $p < 0.0001$)

Figure 2 shows that, as indicated by the straight lines on logarithmic scale, the risk of failure of a kidney or heart transplant remains fairly constant over time. In other words, there does not appear to be a process of 'graft adaptation' whereby a transplant would become less and less likely to fail the longer it functioned. Another way of illustrating that is shown in Figure 3. Heart transplants that were reported with excellent function at 3 months, 1 year, 2, 3 or 4 years, were analyzed. During subsequent follow-up it is evident that grafts with excellent function at 3 years had about the same risk of failing during the following year as grafts with excellent function at 3 months.

It is interesting that the graft survival rate of first heart transplants is slightly better than that of first cadaver kidney transplants. Second kidney grafts, however, have a much higher success rate than second heart transplants (Figure 2). It was noted many years ago that the success rate of second kidney transplants depended on whether the first graft was rejected rapidly or slowly[1]. As shown in Figure 4, heart transplants appear to follow the same rule. Retransplants that were carried out after a period of more than 12 months of initial graft function did exceedingly

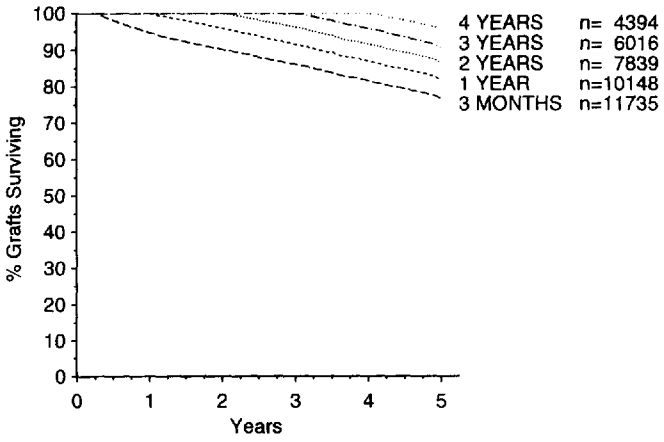

Figure 3 Survival curves for transplants with excellent clinical outcome at various post-transplant intervals. For each interval, subsequent graft survival was plotted starting at 100%. Intervals and numbers of patients studied are indicated for each curve. Note that the survival rates show a similar decline regardless of the length of follow-up

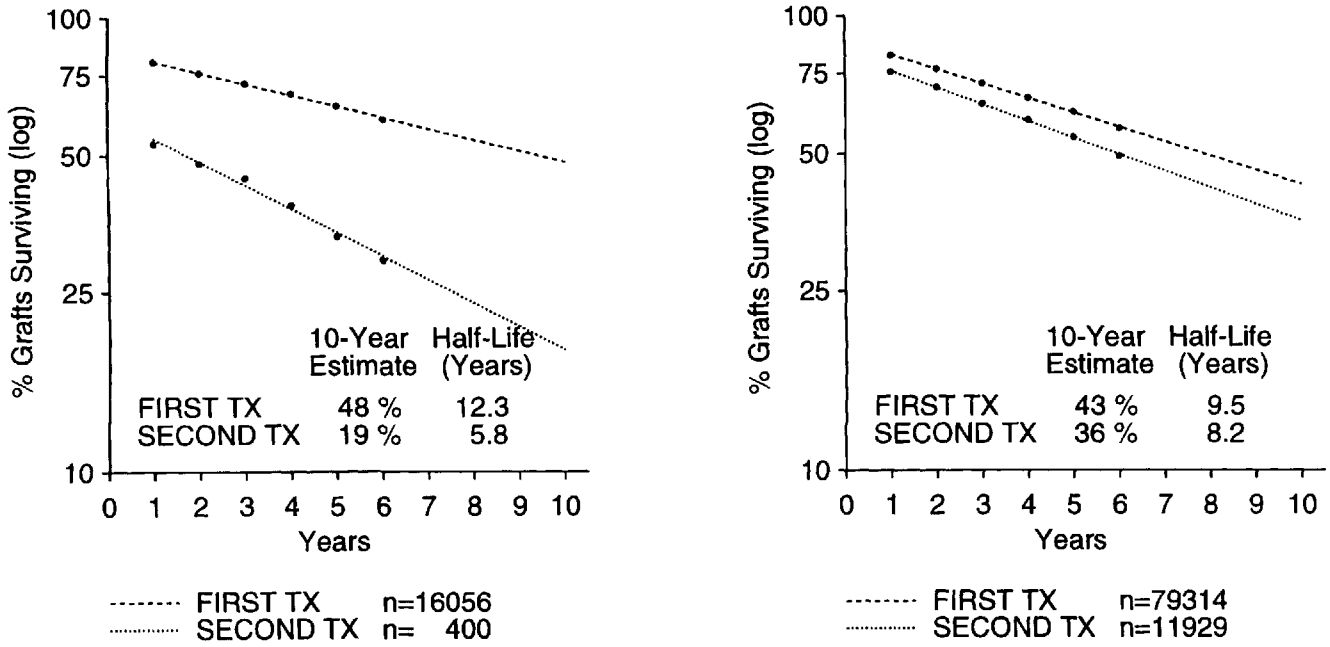

Figure 2 Long-term estimation of graft survival for heart transplants (left) and cadaver kidney transplants (right). Observed survival rates are indicated by solid dots. Note logarithmic scale of y axis. The fact that the dots fall into straight lines indicates a constant risk of failure after the first year. The risk is expressed in half-life times. Estimated 10-year survival rates for first and second transplants are indicated. Numbers of patients studied are given at the foot

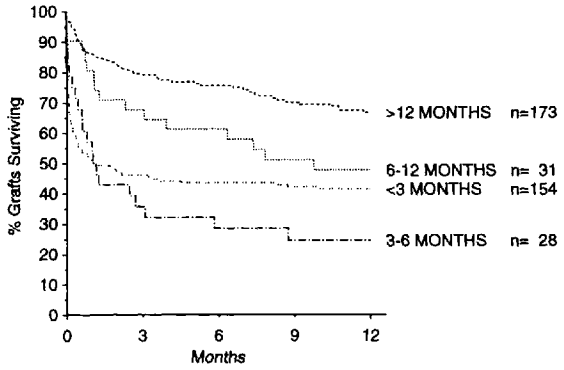

Figure 4 Survival of second heart transplants separated according to the duration of function of first transplants. First graft survival in months and numbers of patients studied are indicated

well. If the first graft had functioned for only a few weeks or months, second transplants showed a high early failure rate, indicating that many of these retransplants were rejected acutely (Figure 4).

The results of first heart transplants were remarkably stable throughout the study period. Figure 5 shows the very similar outcome of transplants performed during different time intervals. This consistency of results justifies the combined use of all data in the present analysis of risk factors. Moreover, the results were also consistent with respect to the geographical region in which the transplants were performed. The majority of transplants were reported from North America and Europe, and their 5-year outcome was identical (Figure 6).

Figure 7 shows the results of first heart transplants in relation to the patients' underlying disease. The curve for patients with congenital heart disease shows a relatively rapid decline early after transplantation, possibly due to technical problems associated with transplantation in children. At 5 years, however, the result of patients with congenital heart disease was only marginally lower than that of other disease categories. The survival curve of patients with valvular heart disease also showed a relatively steep initial decline.

A comparison of survival after heart transplantation with that after heart–lung or lung transplantation is shown in Figure 8.

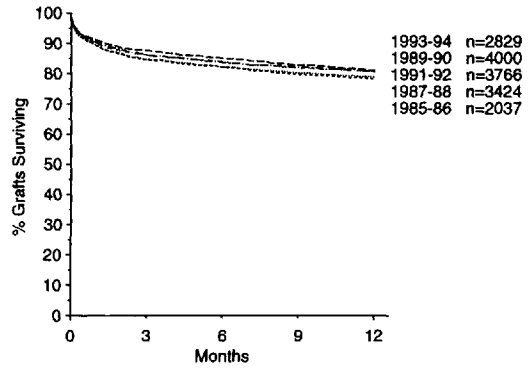

Figure 5 One-year graft survival rates for transplants performed during 2-year intervals since 1985. The latest interval includes transplants done from January 1993 to September 1994

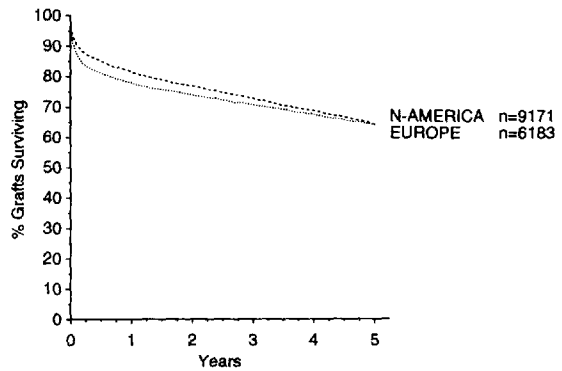

Figure 6 Comparison of first heart transplant survival for patients transplanted in North America or Europe. The 5-year outcome was identical

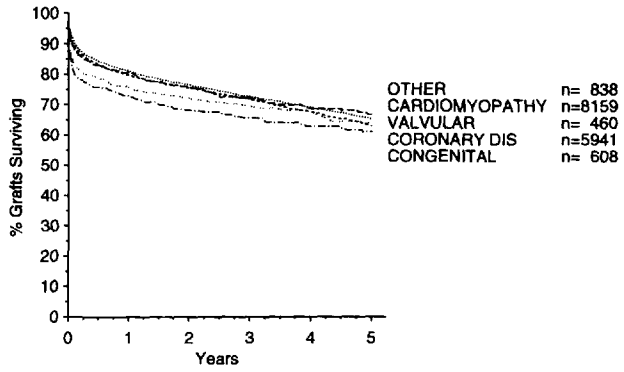

Figure 7 Survival of first heart transplants according to the patients' underlying disease. Note the steep initial decline for patients with congenital and valvular heart disease

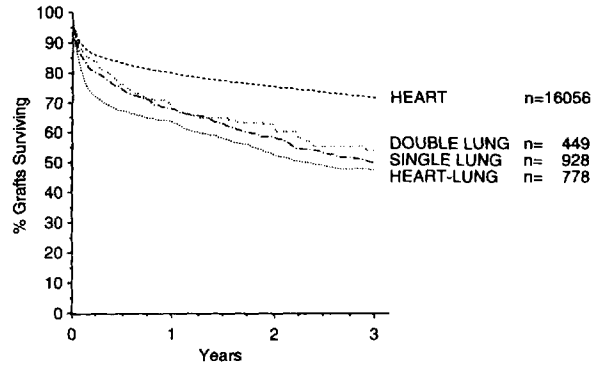

Figure 8 Three-year graft survival rates of heart, heart–lung, single lung or double lung transplants. Numbers of patients studied are indicated

Heart transplants appear to have both a lower initial failure rate and a lower long-term attrition rate. Similar to the heart transplant results (see Figure 5), the success rate of heart–lung transplants appeared to remain constant during the years from 1990 to 1993 (Figure 9). Patients with primary pulmonary hypertension had a somewhat lower survival rate than patients with other underlying diseases (Figure 10).

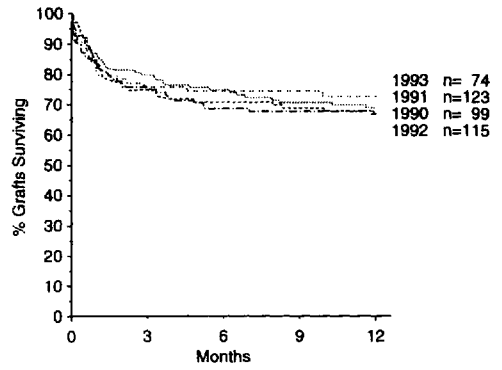

Figure 9 One-year survival rates of heart–lung transplants performed during each of the calendar years from 1990 to 1993

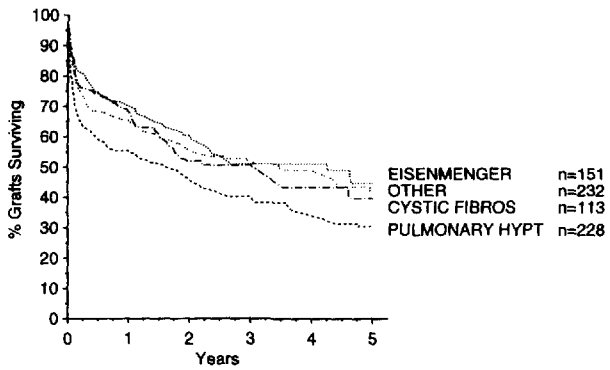

Figure 10 Survival rates of heart–lung transplants for the most common indications. CYSTIC FIBROS = cystic fibrosis; PULMONARY HYPT = primary pulmonary hypertension

Single or double lung transplants have been performed at an increasing frequency and with increasing success since 1990 (Figure 11).

Analysis of factors influencing heart transplant survival

Recipient and donor gender

The survival rate of male-to-male transplants was 3% higher at 5 years than that of female-to-male grafts, or that of male or female donor grafts into female recipients (Figure 12). While the lower success rate of female donor hearts in male recipients might be explainable by a comparatively lower physiological muscle capacity of female donor hearts, which on average are smaller than male hearts, this does not explain the lower survival of male-to-female or female-to-female grafts.

Recipient and donor race

There is a strong racial influence on heart transplant survival which appears to be related to both the recipient's and the donor's

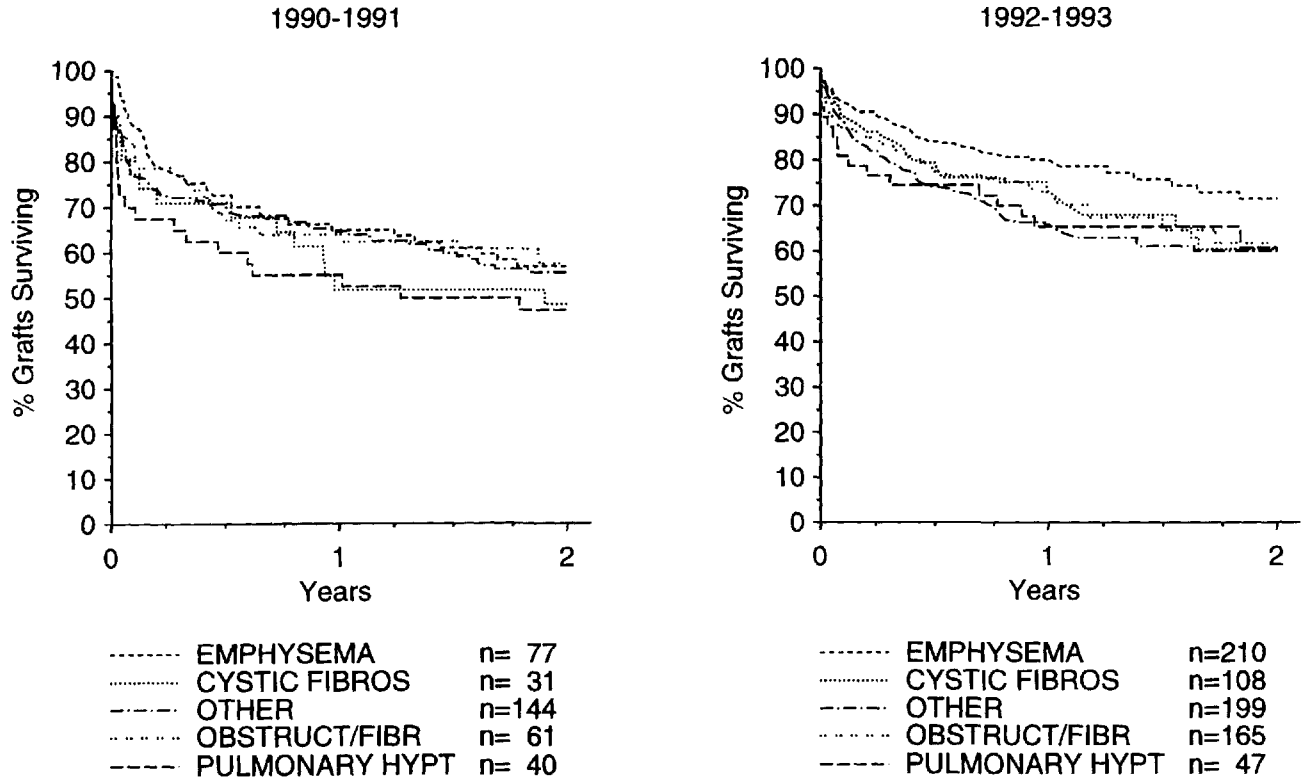

Figure 11 Survival of first lung transplants performed during the years 1990–91 and 1992–93. Curves are plotted separately for the most common indications. CYSTIC FIBROS = cystic fibrosis; OBSTRUCT/FIBR = obstructive lung disease, pulmonary fibrosis; PULMONARY HYPT = primary pulmonary hypertension

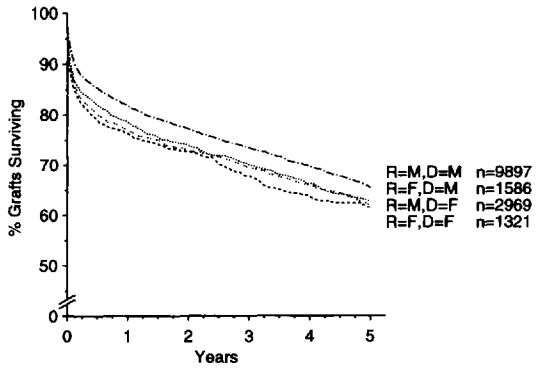

Figure 12 Survival rates of first heart transplants according to the gender of recipient and donor. Male-to-male transplants had a 3% better 5-year success rate than the other combinations. R = recipient; D = donor; M = male; F = female

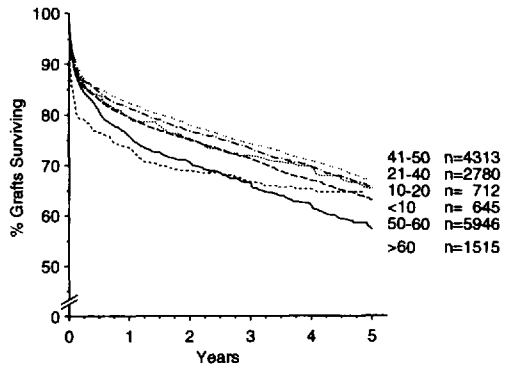

Figure 14 Influence of recipient age on survival of first heart transplants. Recipient age group (in years) is indicated for each curve, together with the number of patients studied. Note the peculiar curve for patients younger than 10, which shows a steep early but slow subsequent decline

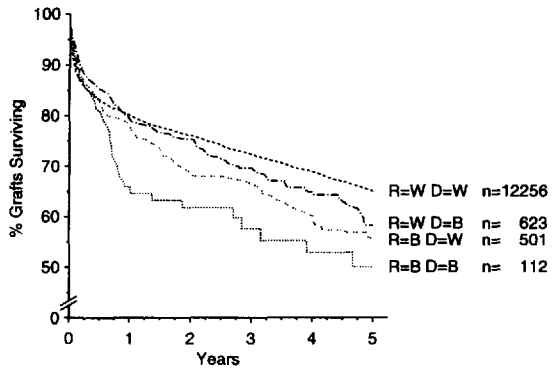

Figure 13 Influence of recipient and donor race on outcome of first heart transplants. R = recipient; D = donor; W = white; B = black

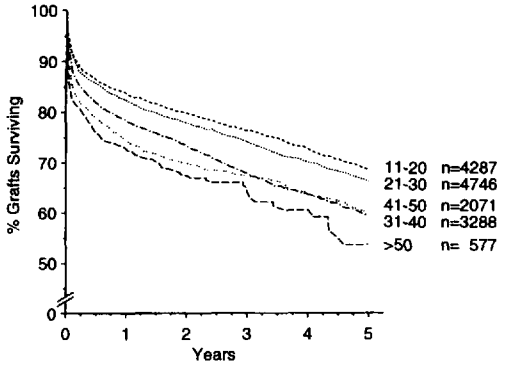

Figure 15 Influence of donor age on survival of first heart transplants. Donor age in years and numbers of patients studied are indicated

race. Transplants from white donors into white recipients had a 15% higher survival rate at 5 years than transplants from black donors into black recipients (65 ± 1% vs 50 ± 6%, $p<0.001$). Transplants from black donors into white recipients or white donors into black recipients had intermediate survival rates (Figure 13). These results are very similar to results obtained with cadaver kidney transplants[2]. The reasons for these differences in results are not clear. Socioeconomic factors, a differential expression of histocompatibility antigens, or genetic differences in immunoresponsiveness have been discussed.

Recipient and donor age

The age of the recipient appears to have relatively little influence up to 60 years. Patients older than 60 have an inferior graft success rate (Figure 14). The survival curve of pediatric patients (<10 years) shows a steep early decline, probably related to technical problems, followed by a particularly good subsequent outcome course (compare also with Figure 7).

The influence of donor age on graft survival is much more pronounced than that of recipient age. An analysis by decades of donor age shows that, among adult donors, graft survival declines significantly with donor age (regression $p<0.0001$) (Figure 15). Moreover, this can be shown to apply to transplants performed both in relatively young adult recipients (age 20–50) and in older recipients (age >50) (Figure 16). As the indications for transplantation have widened, the demand for donor organs has increased. Understandably, in life-threatening situations one cannot wait for an organ obtained from a young donor under ideal circumstances. Nevertheless, the striking association of donor age with graft outcome must be reason for concern. It would seem prudent to direct research efforts towards identifying risk factors in potential donors. Age *per se* cannot be a suitable endpoint measure. However, the strong correlation of donor age with the graft failure rate provides a clue which should stimulate research with the aim of eliminating those potential donors whose organs can be predicted to have a very high likelihood of failure.

ABO compatibility

Although heart transplants are generally done from ABO-compatible donors, 21 ABO-incompatible heart transplants have been reported to the Collaborative Transplant Study. For each of these transplants, written confirmation of the ABO typing results was obtained in order to exclude clerical reporting errors. In addition, information was obtained on the ABO subtype of blood group A donors, and whether plasmapheresis or splenectomy were performed. Based on the small number of cases available for analysis

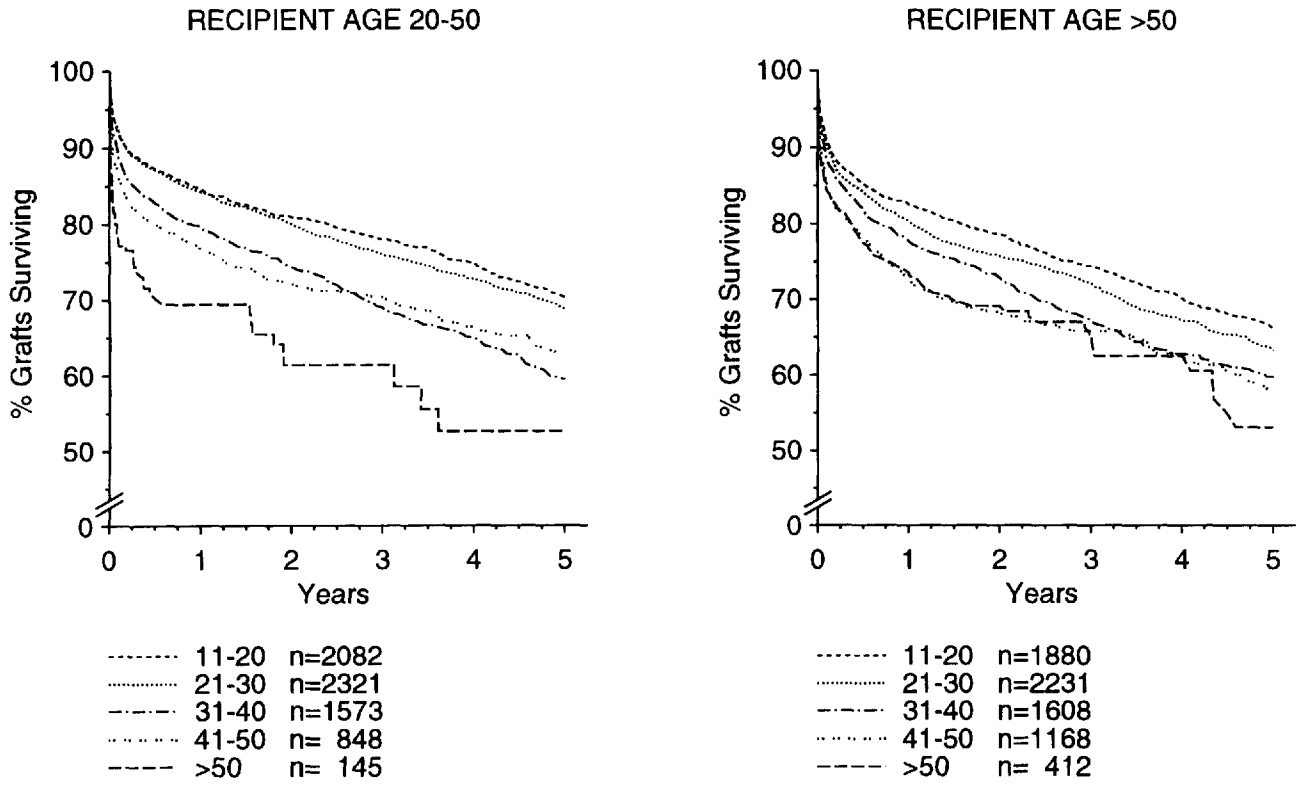

Figure 16 Analysis of donor age in two age subgroups of adult heart transplant recipients. Donor age groups and numbers of patients studied are indicated at the foot

it was not possible to identify risk factors. However, it is remarkable that 10 of the 21 transplants continued to function at 1 year (Figure 17). The steep initial decline in the graft survival rate shows that ABO incompatibility is a strong histocompatibility barrier in heart transplantation. However, if the early post-transplant period, during which the danger of antibody-mediated graft rejection is very high, is overcome, ABO-incompatible hearts can apparently survive for long periods.

There have been reports that ABO-identical heart transplants have a better survival rate than ABO-compatible transplants. These claims are not supported by the Collaborative Transplant Study data. ABO-identical and ABO-compatible transplants had virtually the same success rate (Figure 18).

HLA compatibility

Heart transplants are carried out without consideration of the HLA match. Many transplant centers are not even typing their patients. From the outset it was a declared goal of the Collaborative Transplant Study to investigate whether HLA matching had an influence on heart transplant survival. As shown in Figure 19, there is a significant correlation of graft outcome with matching

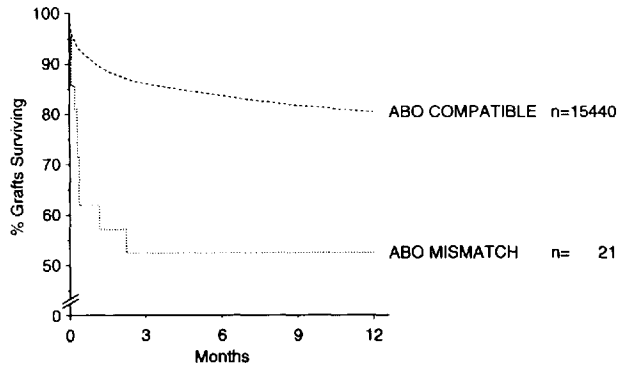

Figure 17 Influence of ABO incompatibility on heart transplant survival. All types of ABO mismatches were included

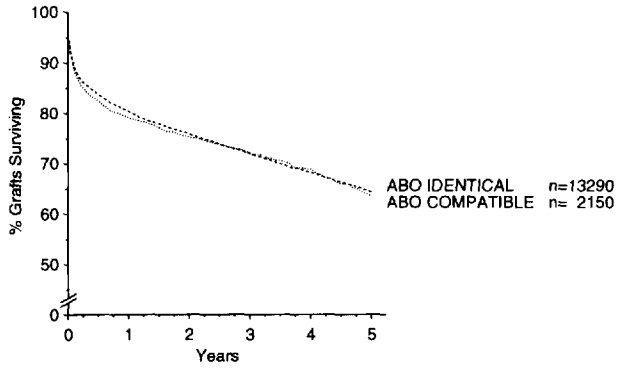

Figure 18 Comparison of graft survival for ABO-identical and ABO-compatible first heart transplants

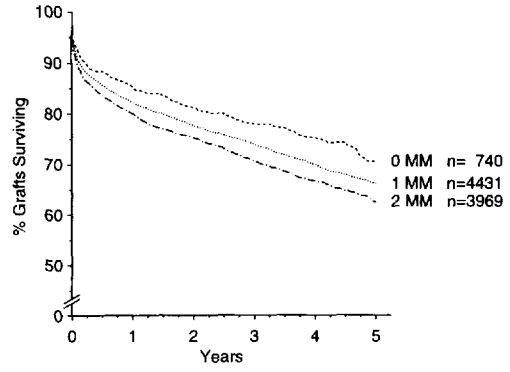

Figure 19 Influence of HLA-DR antigen mismatches on heart transplant survival. MM = mismatches. Numbers of mismatches and numbers of patients studied are indicated. The association of survival with the number of mismatched antigens is statistically highly significant (weighted regression $p < 0.0001$)

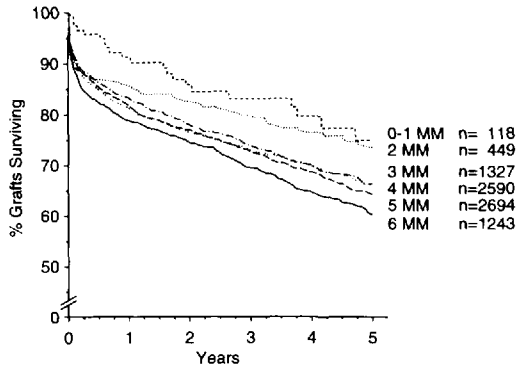

Figure 20 Influence of mismatches at the HLA-A, -B and -DR loci on heart transplant survival. Grafts with no or one mismatch were combined. Note the initial steep but subsequent slow decline of the group with two mismatches. The association of survival with the number of mismatched HLA antigens was highly significant (weighted regression $p < 0.0001$)

for the HLA-DR antigens. The influence of matching for the HLA-A and HLA-B antigens was smaller. However, considering the three loci together (A+B+DR) further improves the HLA matching effect, as shown in Figure 20.

With respect to the previous Collaborative Transplant Study publication on HLA matching[3], the results of the current analysis are interesting for two reasons: (a) the superior outcome of transplants with none, one, or two mismatches is sustained; (b) whereas transplants with three, four, five, or six mismatches had identical survival rates in the previous study, the current analysis performed on a larger number of transplants shows a separation of graft survival as the number of HLA mismatches increases (weighted regression $p<0.0001$). The difference between the survival rates of none or one mismatch and six mismatch grafts is 15% at 5 years (75 ± 5% vs 60 ± 2%, $p<0.003$).

An analysis of patients who, at the time of transplantation, were rated as 'good or medium risk' recipients (based on recipient center criteria) shows an even more impressive correlation of matching with graft outcome. The none or one mismatch grafts now separate clearly from the two mismatch grafts (Figure 21).

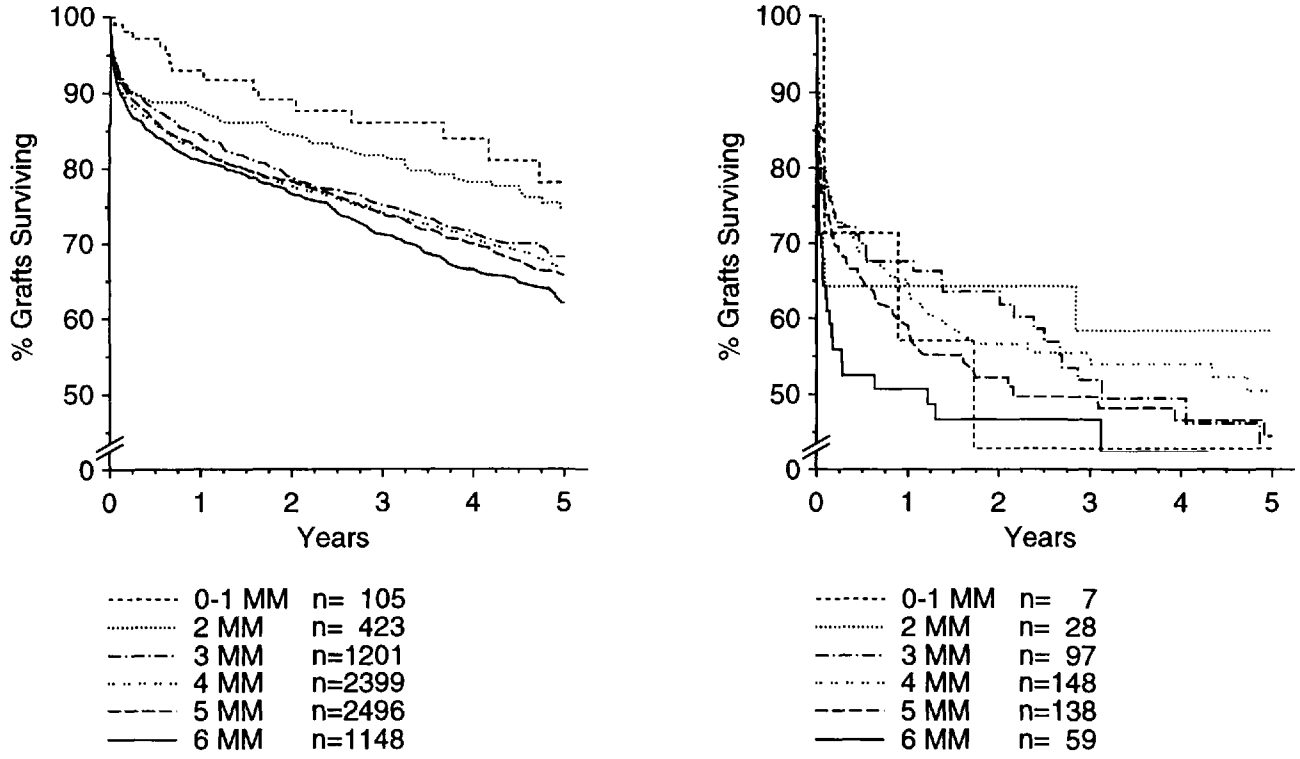

Figure 21 Analysis of HLA matching in patients who were categorized at the time of transplantation as 'good or medium risk' recipients (left) and in patients categorized as 'poor-risk' recipients (right)

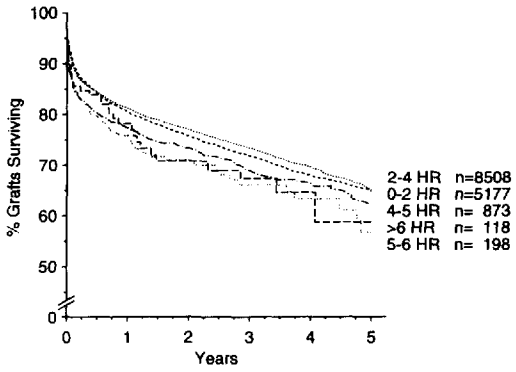

Figure 22 Effect of cold ischemic heart preservation time on transplant outcome. Ischemia times in hours are indicated, together with the numbers of patients studied

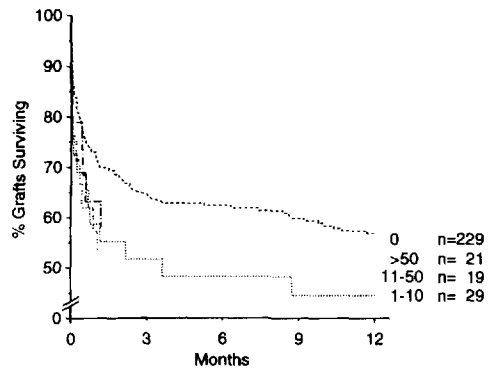

Figure 24 Influence of preformed lymphocytotoxic antibodies on survival of second heart transplants. Even low-level reactivity was associated with poor graft outcome

The survival rate difference at 5 years between no or one mismatch and six mismatch transplants is 17% (79 ± 6% vs 62 ± 2%, p=0.001). In contrast, and not surprisingly, HLA matching does not show an influence on the outcome of 'poor-risk recipients' in whom the influence of other risk factors was obviously dominant (Figure 21).

Preservation time

Because prospective HLA matching would have to involve the transportation of hearts between transplant centers, the acceptable time limit of organ preservation is important. Figure 22 shows that hearts are optimally preserved with current preservation methods for up to 4 hours. The rate of function declines noticeably, but not dramatically, with longer preservation times. It will be interesting to follow the progress of research in the preservation field. Extended preservation limits would make organ sharing and HLA matching more practical.

Preformed lymphocytotoxic antibodies

The Collaborative Transplant Study data provide evidence that presensitization in the form of serum lymphocytotoxic antibodies is associated with decreased heart transplant survival. This is shown for first heart transplants in Figure 23. Unfortunately, the available data do not allow a separation of antibodies into those of the IgG or IgM class. From the kidney transplant experience it is known that IgG antibodies are deleterious, whereas IgM antibodies are not[4]. Because antibodies in recipients who have rejected a previous graft are more likely to be of the IgG class (second set response), the analysis of second transplants is particularly interesting in this respect. As shown in Figure 24, even low-level antibody reactivity appeared to be associated with poor graft outcome in retransplants.

Figure 25 shows that, among first heart transplants, patients with a positive lymphocytotoxic crossmatch had a 5% lower graft survival rate at 1 year than patients with a negative crossmatch (82 ± 1% vs 77 ± 2%, p=0.04). The same argument as discussed above with respect to preformed IgG and IgM antibodies applies to the crossmatch results. Unfortunately, it was not possible in this analysis to differentiate whether the positive crossmatches were due to IgG or IgM reactivity. The results of second transplants in which a positive crossmatch clearly was as-

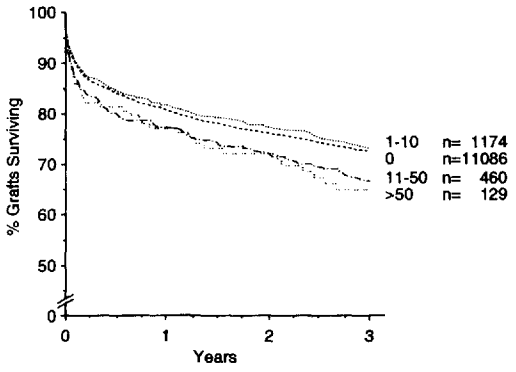

Figure 23 Influence of preformed lymphocytotoxic antibodies in the patient's pretransplant serum. Percentage reactivity against the test panel and numbers of patients studied are indicated. The difference in graft survival between patients with 0–10% and >10% reactivity was statistically significant (log rank p <0.01)

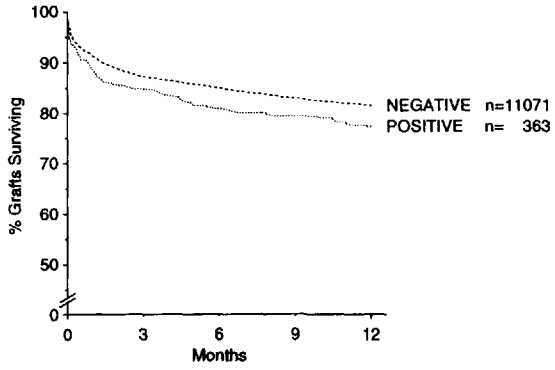

Figure 25 Influence of a positive crossmatch test at the time of transplantation on graft survival. The lower success rate in patients with a positive crossmatch was statistically significant (log rank p = 0.04)

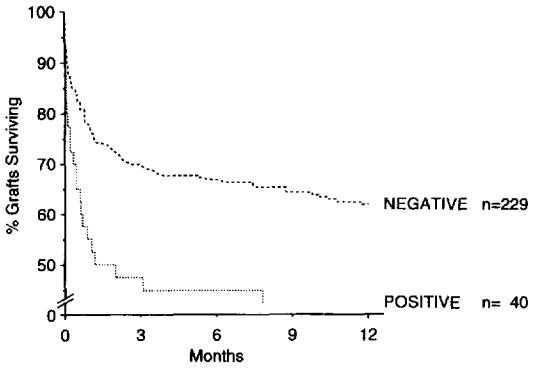

Figure 26 Influence of a positive crossmatch result on outcome of second heart transplants. The result was statistically significant (log rank p <0.01)

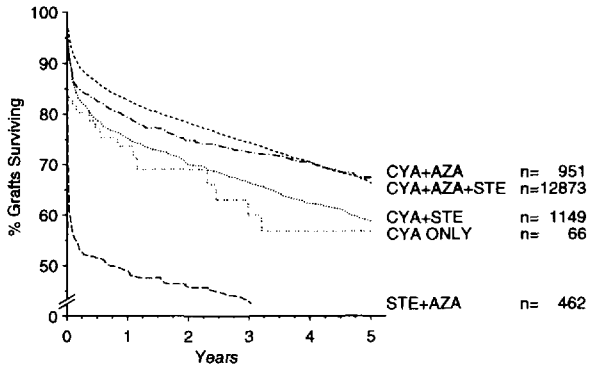

Figure 27 Analysis of heart transplant survival according to the initial immunosuppressive protocol. CYA = cyclosporine; AZA = azathioprine; STE = steroids

sociated with poor graft survival (p<0.01) (Figure 26) are also very interesting.

Immunosuppression

An analysis of immunosuppressive induction protocols shows that most heart transplant recipients received triple drug immunosuppression (cyclosporin, azathioprine, steroids) and that this type of protocol, together with the cyclosporin and azathioprine protocol, resulted in the highest 5-year success rate. Patients who received cyclosporin monotherapy or a combination of cyclosporin and steroids without azathioprine had an approximately 10% lower 5-year success rate. Patients who did not receive cyclosporin, and were treated only with steroids and azathioprine, had an extremely high early failure rate, most likely due to rapid immunological rejection (Figure 27).

Figure 28 demonstrates that induction therapy with prophylactic OKT3 monoclonal antibodies did not improve the results in cyclosporin-treated recipients. In patients not receiving

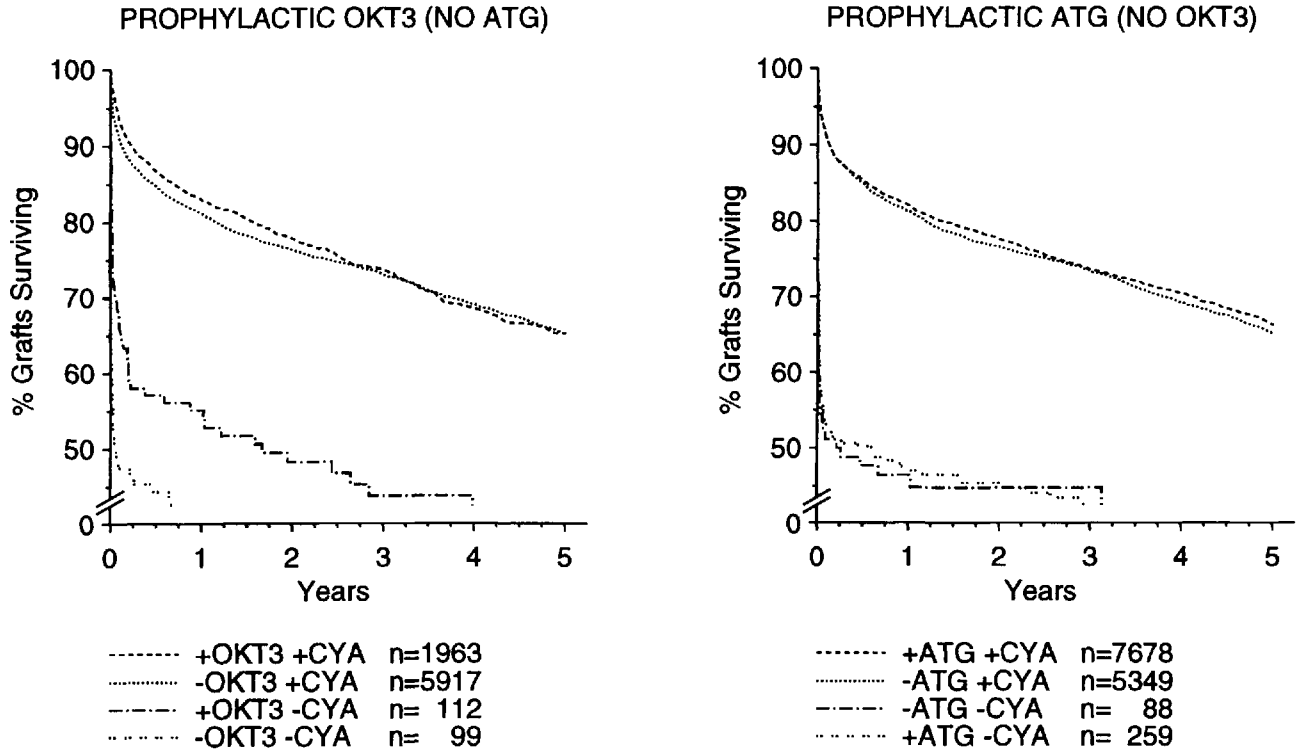

Figure 28 Effect of rejection prophylaxis with monoclonal OKT3 or polyclonal ATG on heart transplant survival. Patients receiving both types of antibodies were excluded from the analysis. +OKT3 = with OKT3 prophylaxis; –OKT3 = without OKT3 prophylaxis; +CYA = with cyclosporine treatment; –CYA = without cyclosporine treatment. Note that the main influence on the success rate was exerted by cyclosporine and not by antibody prophylaxis

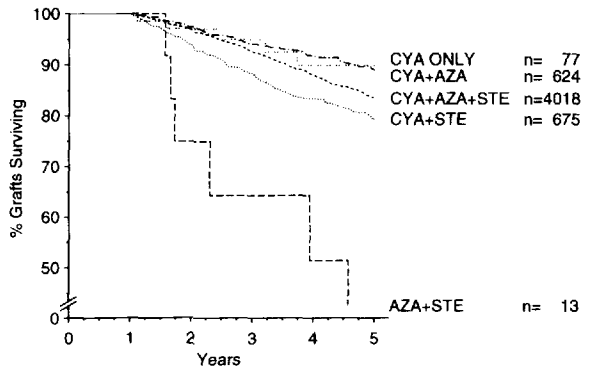

Figure 29 Influence of the immunosuppressive maintenance protocol on long-term graft outcome. All patients in this analysis had excellent graft function at 1 year. Subsequent survival was best in patients who were maintained on a steroid-free cyclosporine protocol. CYA = cyclosporine; AZA = azathioprine; STE = steroids

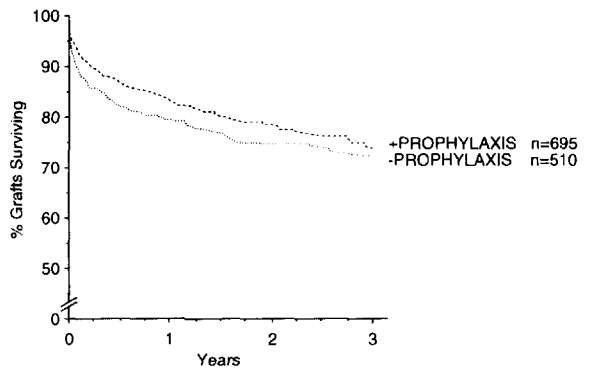

Figure 31 Effect of CMV prophylaxis on graft outcome in heart transplants from CMV-positive donors into CMV-negative recipients. The difference between the two curves was not statistically significant

cyclosporin, OKT3 prophylaxis appeared to confer a small but unsatisfactory benefit.

The results of ATG induction therapy were very similar to those obtained with OKT3 induction. Neither among cyclosporin-treated patients nor in patients treated without cyclosporin was there a noticeable benefit (Figure 28).

Figure 29 shows graft survival rates from 1 to 5 years depending on the patients maintenance protocol of immunosuppression. Patients who were reported to have excellent function at 1 year were separated according to the immunosuppressive protocol they received at 1 year, and followed for another 4 years. It is evident that patients on steroid-free maintenance on cyclosporin (with or without azathioprine) had the best 5-year outcome. This result parallels the results recently obtained in a Collaborative Transplant Study analysis of cadaver kidney transplants[5].

CMV status of recipient and donor

Cytomegalovirus infection is of considerable concern in transplant recipients, because of both the clinical significance of CMV disease and the CMV-induced enhancement of immunological rejection mechanisms[6]. As shown in Figure 30, there was no striking effect of pretransplant CMV status on graft survival. Of course, these results must be considered in the context of CMV prophylaxis. An analysis comparing transplants of CMV-positive donor organs into CMV-negative recipients with or without CMV prophylaxis showed a small but statistically not significant advantage of CMV prophylaxis (Figure 31). This analysis did not take into account the type of agent used for CMV prophylaxis. Detailed information on prophylactic agents is currently being collected, and the results will be reported at some later time.

Incidence of non-Hodgkin lymphomas

Transplant recipients are at an increased risk of developing non-Hodgkin lymphomas. Whereas the yearly incidence of NHL is approximately 10 per 100 000 in the general background population, the Collaborative Transplant Study data show a strikingly increased rate of lymphomas during the first post-transplant year in recipients of thoracic organ transplants. Whereas cadaver kidney recipients demonstrate a lymphoma rate which is approximately 20 times higher than the background rate, the rate is approximately 100 times background in heart transplant recipients, 300 times background in lung recipients, and 500 times background in heart–lung recipients (Figure 32). As reported

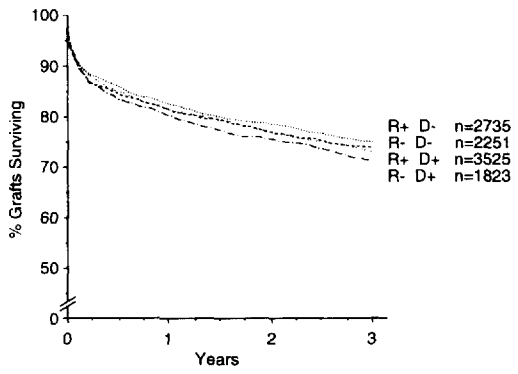

Figure 30 Influence of the cytomegalovirus status of recipient and donor on heart transplant survival. R+ = CMV-positive recipient; R– = CMV-negative recipient; D+ = CMV-positive donor; D– = CMV-negative donor

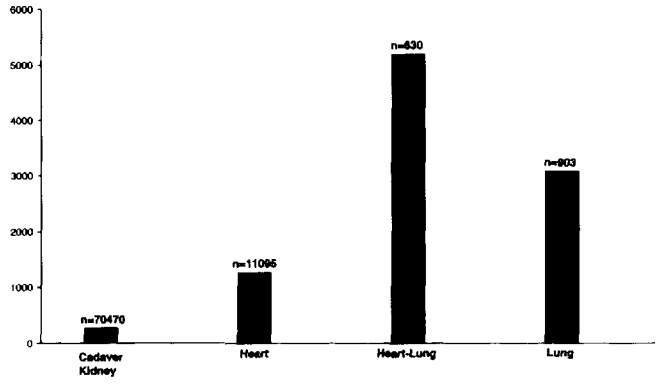

Figure 32 Incidence of non-Hodgkin lymphomas during the first post-transplant year. Incidence per 100 000 patients is shown. Numbers of patients studied are indicated for each type of organ transplant

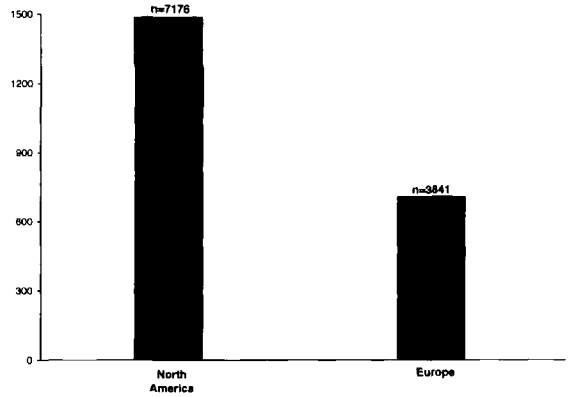

Figure 33 Incidence of non-Hodgkin lymphomas during the first post-transplant year in heart transplant recipients transplanted in North America or Europe

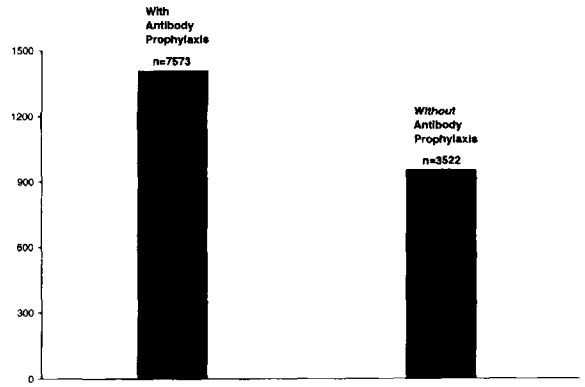

Figure 34 Influence of treatment with prophylactic antilymphocyte antibodies on incidence of non-Hodgkin lymphomas during the first post-transplant year. Patients treated with monoclonal or polyclonal antibodies were combined for this analysis. First heart transplants were analyzed. Numbers of patients studied are indicated

previously[7], the incidence of lymphomas was twice as high in patients transplanted in North America than in patients transplanted in Europe (Figure 33). There is circumstancial evidence for a more aggressive use of immunosuppressive drugs in North America, and this may be related to the higher incidence of lymphomas[7].

That more potent immunosuppression leads to a higher rate of lymphomas is demonstrated by the results shown in Figure 34. Patients who received prophylactic treatment with monoclonal or polyclonal antilymphocyte antibodies had a significantly higher incidence of lymphomas than patients without antibody prophylaxis. However, the increased incidence observed in North America could not be attributed to a more frequent administration of antilymphocyte antibodies. Even among patients who received antibody prophylaxis, North American patients had a higher lymphoma incidence during the first year (1.895 per 100 000) than European patients (1.068 per 100 000). The data were not sufficient to allow a judgement as to whether treatment with monoclonal antibodies was associated with a higher lymphoma rate than treatment with polyclonal antibodies.

Acknowledgments

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45
Lung Transplantation – Experimental Background and Early Clinical Experience

J.D. HARDY

INTRODUCTION

Early developmental studies of lung transplantation will be reviewed briefly, as well as the first lung allotransplant in a human. In addition, the first 22 clinical lung allotransplants are tabulated (Table 1).

Genesis

Experimental lung transplantation was begun at least in the 1940s[1] and perhaps even before then. Many investigators entered the field, but space limits acknowledgment to only a few[2–11]. In our laboratories at the University of Mississippi, Webb and associates[12] began canine heart–lung transplantations in the later 1950s, which led ultimately to the first heart transplantation in a human in 1964[13]. In parallel studies my group transplanted the single lung[14] and, later, both lungs at the same operation, primarily in dogs and monkeys.

Meanwhile, two clinical cases brought special focus and immediacy to our laboratory lung transplantation program. These patients had each represented a circumstance in which, if a single lung could be transplanted into a patient who was in potentially temporary, but surely otherwise fatal, hypoxia, then the patient might later survive on his contralateral lung even if the allograft had to be removed. The first patient (1960) was a man with small-bowel obstruction who vomited as anesthesia was being induced, with resulting massive bilateral pulmonary aspiration; he died of hypoxia several days later. If it had been possible to transplant a single functioning lung, to provide time for the patient's own remaining lung to clear, the transplant could have been removed

Table 1 Early experience with clinical lung transplantation

Investigator (year)	*Indications for transplantation*	*Length of survival (days)*	*Cause of death*
(1) Hardy *et al.* (1963)	Carcinoma/emphysema	18	Renal failure
(2) Magovern and Yates (1963)	Emphysema/cor pulmonale	7	Pneumonia
(3) Shinoi *et al.* (1965)	Bronchiectasis*	18 (lobe removed)	Patient survived
(4) Neville (1965)	Carcinoma	< 1	Pulmonary edema
(5) White *et al.* (1965)	Silicosis	7	Pneumonia
(6) Tsuji (1966)	Unknown*	Unknown	Unknown
(7) Morris and Gago (1967)	Pulmonary hypertension*	< 1	Hemorrhagic congestion
(8) Burcherl (1967)	Acute exposure hydrochloric acid	< 1	Cardiac arrest
(9) Burcherl (1967)	Trauma	2	Unknown
(10) Hayata (1967)	Bronchiectasis*	Status of lobe unknown	Patient survived
(11) Matthews *et al.* (1968)	Paraquat poisoning	13	Paraquat poisoning
(12) Haglin (1968)	Carcinoma	< 1	Bleeding diathesis
(13) Beall (1968)	Emphysema	26	Viral pneumonia
(14) Hallman (1968)	Emphysema	4	Atelectasis
(15) Derom *et al.* (1968)	Silicosis	10 months	Patient survived
(16) Hardy (1969)	Emphysema	28	Pneumonia
(17) Veith (1969)	Carcinoma/emphysema	8	Pneumonia
(18) Ross (1969)	Emphysema	10	Pneumonia
(19) Beall *et al.* (1969)	Emphysema	10	Pneumonia with abscess
(20) Vanderhoeft (1969)	COPD	11	Uncertain
(21) Kahn (1969)	COPD	4	Unknown
(22) Haglin (1970)	COPD†	11	Infection? rejection

*One lobe only transplanted; † both lungs transplanted; COPD = chronic obstructive airways disease

later if rejected. Azathioprine and steroids for immunosuppression were already in use in our kidney transplant patients.

The second patient (1962) was a 73-year-old man with extensive bilateral alveolar carcinoma of the lungs. Already severely dyspneic, he sustained a spontaneous left pneumothorax that soon proved fatal. Here again, a situation presented in which a lung transplant might have prolonged life.

The younger first patient – with a benign condition – might have been considered as having been 'morally assaulted' by a lung transplant (since conceivably he might have survived without the risk of a lung transplant). In the instance of the second patient, with imminently fatal hypoxia and no other effective treatment available for the extensive bilateral malignant pulmonary infiltration, the only major ethical objections to lung transplantation might have been that (a) there was no precedent, (b) there was no guarantee that a lung allograft would function in the new human host and prolong comfortable life, and (c) in any event such an 'experiment' was not justified because of his dismal cancer prognosis.

However, by this time we had transplanted lungs in several hundred animals, and good early function of a transplanted lung(s) had been well established; this finding had also been demonstrated by research groups elsewhere. I was now satisfied in my own mind that either of these two clinical circumstances – massive bilateral pulmonary aspiration or extensive pulmonary cancer with hypoxia – would likely present again, as would terminal respiratory insufficiency due to emphysema or pulmonary fibrosis. Therefore, we believed we should press on with our laboratory work so as to be prepared if and when such a clinical need arose again. Webb and I cleared the prospect of a clinical lung transplant with the officials of the University of Mississippi Medical Center.

EXPERIMENTAL STUDIES

Lung reimplantation

Operative technique

A wide variety of questions confronted lung transplant groups in the late 1950s and early 1960s. To begin with, a consistently dependable autotransplant (reimplant) operative technique had to be developed and mastered by the lung transplant team in each laboratory. This achievement of consistently successful reimplantation was important, because it was desirable to establish first the functional characteristics of the *autotransplanted* lung, before moving on to the *allotransplanted* lung, the function of which was certain to be influenced adversely by the allograft immunological rejection process. Appropriate transoperative and postoperative management of the animal was also important, in order to achieve statistically valid results.

Our own experimental investigations[14–21] will be briefly reviewed.

Initially the pulmonary veins were anastomosed individually, but soon a left atrial cuff containing all four ostia of the pulmonary veins was anastomosed to the atrium of the recipient, thus reducing the incidence of stenosis and thrombosis of the pulmonary veins. The pulmonary artery caused few problems, but it was anticipated that stricture of the bronchial anastomosis would commonly occur. This did occur occasionally, and some animals did exhibit bronchial necrosis and fistula. On the whole, however, bronchial problems were less frequent than expected, even though no effort was made to anastomose the bronchial arteries. In subsequent experimental and human lung allotransplants, healing of bronchial (or tracheal) anastomoses was found to be problematic when corticosteroids were included in the early post-transplant immunosuppressive regimen.

Function of the reimplanted lung

The successfully reimplanted lung was studied variously by auscultation, chest radiography, arterial blood gases, bronchoscopy and bronchography, bronchospirometry, isotope scans, pulmonary arteriography (angiocardiography), and immediate or subsequent ligation of the pulmonary artery to the opposite (unoperated) lung[14–16]. Extensive gross anatomical and microscopic studies were also performed. Ligation of the pulmonary artery to the contralateral lung (instead of performing contralateral pneumonectomy) preserved more nearly the normal pulmonary respiratory reflexes, which afforded much more effective pulmonary ventilatory mechanics than those observed later when both lungs were reimplanted at the same operation.

When, in later experiments, neural connections were severed completely by bilateral lung reimplantation, the animal usually exhibited a slow and deep pattern of respiration and, although commonly able to survive the operation, overall respiratory efficiency was much impaired. Microscopic studies demonstrated vagal nerve degeneration, and the Hering–Breuer reflex was abolished in the reimplanted lung. However, within weeks, early nerve regeneration was demonstrated microscopically, and in chronic dogs studied months or years later, the Hering–Breuer reflex was commonly found to have returned.

The respiratory efficiency of the single reimplanted lung, where respiratory reflexes were essentially normal because of the 'normal' contralateral lung, was good initially, but then declined over the following 7–10 days to about one-half the normal level; thereafter, it gradually improved to regain a low normal level of respiratory efficiency at approximately 2 weeks. Large numbers of animals were studied in the investigation of this decline–recovery pattern. Structures of the pulmonary hilum – pulmonary artery, bronchial arteries, nerves, bronchus, pulmonary veins, lymphatics – were divided individually in various series of animals, to determine the effect on pulmonary function. Suffice it to say that definitive conclusions were hard to come by, but a positive temporal correlation was demonstrated between impaired function and the time required for regeneration of the lymphatics[19]. Regeneration of pulmonary lymphatics following lung reimplantation (and allotransplantation, if the animal were adequately immunosuppressed) could be demonstrated across the bronchial anastomosis by 7–12 days. The ischemia and hypoxia to which the lung was subjected during reimplantation was another potentially significant factor.

In puppies it was found that the reimplanted lung grew in both size and function[20].

Many studies were directed toward short-term cold storage and also longer cold and hyperbaric oxygen storage of the lung, with either delayed reimplantation (occasionally) or, later, allotransplantation of the preserved lung. However, these efforts resulted in only limited extension of the safe storage time.

Lung allotransplantation

The large number of lung reimplantation experiments paved the way for similar anatomical and functional studies of lung allotransplants[17,18]. In brief, it was found in a substantial series that in the untreated dog the allotransplant was rejected in an average of approximately 7 days. In contrast, immunosuppressive therapy, consisting of various regimens involving azathioprine, prednisone, and mediastinal radiation, produced an average allograft survival of approximately 35 days. In some instances, animals that had undergone unilateral or single-operation bilateral lung allotransplantation lived many months. In the process of rejection, the normal lung anatomical structure was replaced by disordered architecture and necrosis. In the occasional animal the rejected and necrotic lung became encased in protective fibrin and fibrous tissue, permitting long survival of the host.

THE FIRST LUNG TRANSPLANT IN A HUMAN

Following approximately 7 years of lung transplant research involving hundreds of animals, Webb and I obtained permission from the University of Mississippi Medical Center administration to perform a lung transplant in a human patient should the need and the appropriate set of ethical circumstances arise, as noted above. The principal criteria set for selecting a recipient were as follows:

(1) The patient must have a probably fatal disease, so that in the event that untoward results were encountered, his or her life would not have been materially shortened.
(2) There must be a reasonable possibility that the patient would benefit from the lung transplant.
(3) The removal of the patient's own lung must not result in the sacrifice of any of his or her own functioning lung tissue.
(4) Transplantation of the left lung had been found to be somewhat simpler technically than transplantation of the right, and thus it was elected to initiate the clinical phase of the work by transplanting a left lung.

The recipient

On 15 April 1963 a 58-year-old man with carcinoma of the left lung and dyspnea at rest from emphysema was admitted to the University of Mississippi Hospital. He had borderline renal failure, secondary to long-standing chronic glomerulonephritis. Details of diagnosis, general evaluation, ethical considerations, and other matters were published at the time[21]. Suffice it to say here that the four pre-set criteria for a potential lung transplant recipient were essentially fulfilled.

There followed a period during which antibiotics were administered in an attempt to clear the pneumonitis distal to the obstructing carcinoma of the left main bronchus. This was only partially successful. The indications for *left pneumonectomy* were the apparently localized carcinoma of the left main stem bronchus and the persistent sepsis distal to this obstructing lesion. The indication for *lung transplantation* was that, already dyspneic on even mild exertion, it was considered vital to replace even the very limited function being provided by the left upper lobe.

The donor

At approximately 7.30 p.m. on 11 June 1963, a patient entered the emergency room of University Hospital in shock and pulmonary edema secondary to massive myocardial infarction. All resuscitative efforts failed, and the family members permitted autopsy and donation of the left lung for transplantation.

The operation

The organ was transplanted into the left hemithorax of the recipient with only moderate difficulty, caused by the prior and persisting infection and the fact that the hilar carcinoma had invaded surrounding tissues more extensively than had been detected preoperatively[21]. Blood samples taken from the transplant pulmonary artery and vein demonstrated immediate excellent respiratory function of the transplant, as reflected in the pulmonary venous versus pulmonary arterial blood gases. This effective function continued during the 18 days he lived.

Postoperative course

Immunosuppression was with azathioprine, prednisone, and mediastinal radiation. Unfortunately his renal function declined steadily postoperatively, and this major problem, plus infection and the preoperative state of general debility due to the extensive cancer, caused his death.

Postmortem studies

Gross examination of the transplanted lung revealed a well-ventilated organ, with patent anastomoses. A small defect in the membranous portion of the transplant bronchus had been noted at bronchoscopy postoperatively, but this defect was found to have been sealed off by the inflammatory reaction in surrounding tissues. Arteriograms demonstrated excellent patency of the pulmonary vasculature. Microscopy disclosed virtually no evidence of allograft rejection[21].

COMMENT

This first case had demonstrated the technical feasibility of clinical lung transplantation. The transplant had functioned immediately and for the duration of the patient's life. There had been little or no rejection of the allograft under the immunosuppressive regimen administered – plus perhaps some degree of immunosuppression attributable to the gradual renal decompensation. It was concluded that clinical lung transplantation would eventually offer an effective form of management for otherwise terminal respiratory insufficiency.

SUBSEQUENT EARLY CLINICAL EXPERIENCE

By 1970, 22 known human lung allotransplants had been performed (Table 1)[22], and two more as units of heart–lung transplants (Chapter 66)[22]. The early results were disappointing but, with the advent of cyclosporin for improved immunosuppression,

Figure 1 Joel Cooper, who led the Toronto group which played a leading role in the establishment of single lung transplantation.

heart–lung transplant units later exhibited not only better heart transplant survival but better survival of the lungs as well. Finally, truly long-term survival after single lung transplantation was achieved by Joel Cooper (Figure 1) and his colleagues in Toronto, the first such operation being performed on 7 November 1983. With this source of encouragement the clinical transplantation of one or both lungs is now poised for widespread and successful application[23].

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Indications, Selection and Pretransplant Management of the Potential Recipient

J.R. MAURER

INTRODUCTION

Since the first edition of this textbook, technical advances in surgical approach and intraoperative management, combined with increasing experience in medical care of transplant recipients, have led to much-improved outcomes for lung transplant recipients. This increased experience in the care of the recipients has led both to changes in indications for specific surgeries and to improved criteria for choosing appropriate transplant candidates.

INDICATIONS

Single lung transplantation

Single lung transplantation quickly became the most common type of lung transplant performed in the world. In the April 1995 report of the St Louis International Lung Transplant Registry this procedure accounted for more than 2300 of the 3836 total reported lung transplants[1]. Bilateral sequential single and *en-bloc* double lung transplants accounted for approximately 1500 of the procedures. In the Registry report, single lung transplant recipients enjoyed nearly the same survival as bilateral lung recipients (71% vs 73% at 1 year and 62% vs 57% at 3 years).

In the late 1980s and early 1990s, indications for single lung transplant were rapidly extended to include a number of end-stage pulmonary and pulmonary vascular processes as transplant physicians learned that acceptable outcomes could be achieved with unilateral transplant. By far the largest number of single lung transplants – over 1200 – have been done in patients with end-stage emphysema/chronic obstructive pulmonary disease (COPD). Within this group, approximately 25% were performed in α_1-antitrypsin-deficient patients. The next largest number – more than 550 – have been performed in patients with pulmonary fibrosis, and the third-ranking indication has been primary or secondary pulmonary hypertension. There is a rather large group – more than 340 – of single-lung recipients with 'other' diagnoses, which include such entities as lymphangiomyomatosis, sarcoidosis, eosinophilic granulomatosis, retransplantation, bronchiolitis obliterans, etc. Occasionally, single lung procedures have been done in circumstances that would normally be contraindications, e.g. in patients with bilateral bronchiectasis[2,3]. In these reported cases a contralateral pneumonectomy of the remaining native lung has been performed to prevent spread of infection. These unusual indications for single lung transplant have been undertaken only in exceptional situations, and this type of patient should not generally be considered appropriate for unilateral lung replacement. There has also been reported unilateral lung transplant in combination with heart transplant[4]. This approach has rarely been used when both heart and lung replacement are necessary, to avoid excessive bleeding in the event of scarring of one pleural space (which would be left intact) and to make available more organs (the contralateral donor lung could be used in another donor) for transplant.

While virtually every potential pulmonary or pulmonary vascular diagnosis has been considered for unilateral lung transplant, the morbidity and survival data are better in some types of diseases. The best early survival results appear to be in obstructive lung disease patients. In this group the International Registry records a 1-year survival of 78%, almost the same as that of bilateral lung transplants; however, in pulmonary hypertension unilateral lung recipients the 1-year survival is 59% and in pulmonary fibrosis approximately 65%. It should be noted, however, that bilateral transplant survival in these groups is also slightly less than that achieved in emphysema patients. Longer-term survivals in each of these groups of patients are difficult to compare because of the small numbers of patients available for evaluation.

Issues in selection of patients for single lung transplant

While the overall success of single lung transplantation has been encouraging, care must be taken in selecting appropriate patients for a unilateral procedure, as some characteristics of the remaining native lung have been identified as having a potential negative impact on survival. In most patients undergoing unilateral transplant the native lung is structurally abnormal. In one report, pathology in the native lung caused morbidity in more than one-quarter of single lung transplants and was a factor in half of the deaths[5]. The presence of bullous changes (as in emphysema) or

cystic changes (as in sarcoid or pulmonary fibrosis) can predispose to colonization of the native lung with organisms which can result in fatal infections[5–7]. In pulmonary hypertensive patients, low blood flow to the remaining native lung may predispose to infection[7]. End-stage pulmonary fibrosis is often complicated by traction bronchiectasis, and areas of unsuspected bronchiectasis are not uncommon in patients with emphysema. While little can be done to predict which pulmonary hypertensive patients will have relatively ischemic remaining native lung and be at risk, areas of bronchiectasis are usually easily visualized on high resolution CT scan. Patients presenting for transplant with chronic sputum production may have colonized susceptible areas of native lung, and should be carefully evaluated before being approved for unilateral transplant.

Early in the era of successful lung transplantation there was concern about the possibility of overventilation of the highly compliant remaining native lung in emphysema patients in the instance of single lung transplantation. While overinflation of the remaining native lung with some mediastinal shift is not uncommon[8], it rarely compresses the transplanted lung to the extent that function is compromised. In some cases in which functional compromise has occurred, resection of part or all of the native lung has been tried[9]. It has been suggested that selecting patients without obvious large bullae in what will be the remaining lung may help prevent overinflation problems, but this has not been formally evaluated[10].

Another problem that has been identified involving the remaining native emphysematous lung – and could be a problem in pulmonary fibrosis, lymphangiomyomatosis, or any other structurally abnormal lung – is the development of persistent pneumothoraces. In one case this complication eventually required pneumonectomy of the native lung[11].

Bilateral lung transplantation

Of the approximately 1500 reported bilateral lung transplants, more than 1200 have been done via the bilateral sequential single surgical technique with the bulk of the remainder done as *en-bloc* double lung procedures[1]. Of the bilateral sequential transplants, 40% have been done in patients with cystic fibrosis, 26% in patients with COPD/emphysema, 11% in patients with pulmonary hypertension and the remainder in patients with a variety of diagnoses.

Patients with cystic fibrosis and other forms of end-stage disease with chronic colonization or infection (usually bronchiectasis) are the ideal candidates for this type of lung replacement surgery. It is generally felt that removing both lungs in this type of patient will greatly reduce the chances of disseminated infection post-transplant. The overall success rate with bilateral lung transplant has been slightly better than that with single lung transplant; 1-year survivals are 73% compared with 71%, and 2-year survivals are 68% compared with 62%. *En-bloc* double lung transplant survival rates are lower than both the bilateral sequential and single lung transplant with 1- and 2-year survivals reported at 65% and 58%, respectively.

Other indications for bilateral lung transplant are more controversial. In a general sense it seems reasonable that a person receiving two lungs rather than one lung might have better exercise capacity, but clearly the function gained with one lung is adequate for normal activities of daily life[8,12,13]. It has also been argued that the long-term outcome is likely to be better if a person receives two lungs rather than one, but there are no long-term studies to validate this[14]. The argument, however, has been used to support use of bilateral lung grafts in younger patients with emphysema, even though reported functional results, morbidity and mortality are comparable with single lung grafts.

The question of the 'best' type of transplant has been most discussed in the literature for patients with pulmonary hypertension. A number of reports have documented good results with single lung transplantation, or single lung transplantation with simultaneous cardiac repair, in patients with primary and secondary pulmonary hypertension[15–18]. At least two series, however, suggest that long-term outcomes might be less favorable in single-lung recipients. Bando *et al.* reported that in 57 consecutive pulmonary hypertensive and Eisenmenger's patients transplanted in the Pittsburgh program, bilateral lung recipients had a larger fall in pulmonary pressures, a greater functional recovery and lower graft-related mortality when compared to single-lung recipients[19]. Lupinetti *et al.* from Michigan reported a small series of Eisenmenger's patients who underwent cardiac repair and single lung replacement. In this group of five patients, only one lived for more than 18 months after transplant[20]. King *et al.* reported significantly longer ICU stays in pulmonary hypertensive patients, but 1- and 3-year survivals comparable to those of patients with obstructive lung diseases[21]. Others also[22,23] report results comparable to those in patients with other diagnoses. The International Registry lists a 1-year survival for pulmonary hypertensives with bilateral transplants at 66% (n=54) and a 2-year survival at 61% (n=20); the survivals for single-transplant recipients are only slightly less at 59% (n=105) and 53% (n=80)[1]. Among the arguments that have been used to support the choice of bilateral lung transplant over single lung transplant are that ventilation of a bilateral transplant may be easier during the notoriously difficult postoperative course with the development of pulmonary edema in many of these patients thus enhancing early outcomes, and that long-term outcome and exercise tolerance might be enhanced, since this is usually a very young group of patients[14,19]. These claims, however, have not been formally evaluated.

At present there are inadequate data to suggest that either unilateral or bilateral lung transplant is the preferred graft choice in pulmonary hypertensive and Eisenmenger's patients. Longer-term follow-up of larger numbers of patients is necessary to resolve this question. Another issue which has been considered in selecting transplant type for pulmonary hypertensives is the degree of impairment of right heart function. Many institutions preoperatively evaluate right ventricular function, presumably to detect a 'maximum' degree of dysfunction that would mandate heart–lung transplant. However, the maximal degree of right heart dysfunction beyond which recovery in impossible is not known, since significant improvement from relatively low ejection fractions has been recorded in both transplant and pulmonary thromboendarterectomy patients[19,24,25]. Generally, lower limits of function are based on arbitrary values of right ventricular ejection fraction, e.g., 15% or 20%, but some institutions do not set a lower limit[19]. At present it is not known if severely reduced right ventricular function implies non-recoverability which would mandate heart–lung transplant.

Cadaver and living-related lobar transplantation

The ongoing worldwide donor shortage has led to creative attempts to increase the donor pool. Recently success has been reported in the use of single lobe transplant from adults or older children to pediatric recipients[26] and bilateral lobe transplant to older children or adult recipients, most often with one lobe each donated from two living-related donors[27]. Outcomes reported from this group are mostly short term, but are comparable to those achieved in the overall lung transplant population. Most of the recipients of lobar transplants to date have been cystic fibrosis patients, but if this approach is successful in other centers, other end-stage patients will undoubtedly soon be included in the recipient pool, as will the inclusion of older patients who have willing related donors. Concerns have been raised about ethical and moral implications of this surgery and risk to donors, but this is probably no higher than that to living-related donors of other solid organs, as long as care is taken to choose healthy, low-risk donors[27,28]. Stringent criteria for donor physical and mental health should be outlined, and adhered to, in order to ensure minimal donor morbidity[29]. The ethical considerations of living-related lung donation were explored by Shaw *et al.*, who stress the importance of detailed informed consent and that the essential elements of the informed consent include disclosure, mental competence and voluntariness[30]. To ensure this they recommend informed consent be obtained at least twice, a consent-advocate be appointed to protect the potential donor's interests, and a 2-week waiting period be left between final consent and the actual surgery, to allow the donor time to reflect on his/her decision.

PATIENT SELECTION

Selection of appropriate candidates for lung transplantation is based on general criteria for the potential transplant group as a whole – as well as specific criteria for each disease entity – which seeks to identify patients who have exhausted available medical care, yet have progressive disease and a very limited projected lifespan and/or dismal quality of life.

We will first discuss general criteria.

Age

Initially several programs included strict age criteria for potential transplant candidates. Though these criteria were totally arbitrary, they did limit the number of candidates for a very scarce resource. In the past few years the age limits have gradually crept upward, so that in its most recent report the International Registry lists 208 transplant recipients between the ages of 61 and 70[1]. Snell *et al.* reported similar survivals in patients between the ages of 50 and 60 when compared to patients younger than 50[31].

The Registry data compare 1- and 2-year survival of patients over the age of 60 with those under 60. At 1 year the survivals are 71% and 61%, and at 2 years they are 64% and 50%, respectively. These early data suggest there may be a real difference in outcomes of patients over the age of 60; thus, if survival is lower in the setting of a very limited organ supply, imposing age restrictions on transplant recipients may well be reasonable.

Complicating medical illness/end-organ damage

Patients with multiple medical problems continue to be poor candidates for lung transplantation. However, motivated patients with chronic controlled medical problems such as hypertension or diabetes can generally undergo transplant successfully. These patients should undergo a thorough search for occult end-organ damage, e.g. coronary artery disease or renal and hepatic insufficiency, which may adversely affect outcomes before their being offered transplant (see evaluation section below).

Osteoporosis is a particularly common problem in many patients with end-stage disease, both as a component of the disease and as a complication of steroid therapy and immobility[32]. The incidence of fractures post-transplant and attendant morbidity is very high[33]. Low bone mineral density, especially with accompanying vertebral compression fractures, should be considered at least a relative contraindication to transplantation.

Systemic illness such as collagen vascular disease had initially been deemed a contraindication to lung transplant because of the presumed involvement of multiple organ systems and the potential for the underlying disease to progress. However, in many cases, patients with systemic diseases either have manifestations primarily or entirely in the lung or have 'burned-out' disease with end-stage lung pathology. Thus successful transplantation in patients in these disease categories has been reported[34]. It must be emphasized that a careful search for occult pathology in other vital organs is necessary in the evaluation of these potential candidates.

Extrapulmonary infection is considered by most programs to contraindicate transplant, because of the potential for dissemination in the face of intense immunosuppression[35]. The more troublesome question is how to assess patients who may be colonized with resistant mycobacterial or other organisms. There is increasing evidence of the negative impact on outcomes with single lung transplant in this circumstance, but it is not yet clear whether these organisms should contraindicate bilateral lung or heart–lung transplant. The issue has been more difficult in cystic fibrosis patients who frequently colonize multi-resistant organisms. In the Toronto group's experience, resistant *B. cepacia* has had significant impact on outcomes, though they still transplant these patients[36]. Another group transplanting large numbers of cystic fibrosis patients, however, does not accept patients with panresistant organisms[37]. This issue requires further clarification, but available data suggest that preoperative resistant organisms are likely to reappear postoperatively, and often produce serious or fatal infections.

Potential candidates with a past history of malignancy should be very carefully assessed before acceptance into a transplant program. Increasing data suggest that post-transplant patients are particularly prone to developing a variety of malignancies, and patients who have already demonstrated this tendency may be especially susceptible. Nevertheless, occasional patients with a presumed 'cure', e.g. adult survivor of childhood leukemia with chemotherapy-induced lung disease, may be acceptable candidates. Lung cancer is a contraindication to lung transplant.

Steroid use

The initial concerns about steroid use as an unacceptable impediment to adequate bronchial anastomotic healing have been shown to be largely unfounded[38], and patients are now routinely ac-

cepted for transplant who are taking small to moderate doses (generally up to 15 mg daily) of prednisone or the equivalent. Probably of more importance than healing concerns in these patients is bone mineral loss, and appropriate investigation for osteoporosis and its consequences should be undertaken (see above).

Previous thoracic surgery/significant pleural disease

Postoperative bleeding was a major cause of morbidity and mortality in the early reports of lung and heart–lung transplant outcomes. Major technical improvements, including the 'clamshell incision' and improved intraoperative management of bleeding, have greatly reduced this cause of early mortality. However, it has been difficult to decide when previous thoracic surgery or obvious pleural scarring should be considered an impediment to transplant. Dusmet *et al.* compared the outcomes of 18 patients with previous intrapleural interventions with 18 matched controls and found no significant difference in blood loss, chest tube drainage or overall hospital stay[39]. While pleural scarring may weigh in the decision about type of transplant, this should not be considered a contraindication when the newer surgical approaches are used.

Ambulatory status

Many programs require that candidates maintain a minimal ambulatory status[35,37], on the presumption that this predicts earlier and better postoperative mobility and may also be an indicator of patient motivation. While a comparison of ambulatory vs non-ambulatory candidate outcomes has not been formally looked at prospectively, data have been published on patients who were transplanted while on mechanical ventilation. Some scattered case reports[40,41] have suggested good outcomes for ventilator-dependent patients, but a close appraisal of two other series[42,43], which together included 16 patients, is less optimistic. Nine of the 16 patients died and four of those were early deaths. Of the seven survivors, two developed bronchiolitis and five were reported well. Significant neurologic complications and renal failure were common. These results are significantly worse than the overall group of patients undergoing transplant; in the face of donor shortages it is difficult to justify transplant in this population.

Nutritional status

Normal nutritional status is optimum in patients presenting for transplant, but typically patients with certain kinds of lung disease have low body mass indices at end-stage. Emphysema and cystic fibrosis candidates have been reported as a group to have reduced body mass indices, which improve without supplemental feeding post-transplant, whereas pulmonary hypertensive and pulmonary fibrosis patients as a group come to transplant with normal body mass indices[44]. It is not known whether outcomes are worse for more cachectic emphysema patients, but there is some evidence that this is a risk factor in cystic fibrosis patient outcomes[45]. For this reason it is advisable to hyperaliment those cystic patients that are significantly below normal body mass index. Similarly, obese candidates have all the risks of any obese patients coming to surgery, compounded with the addition of post-operative immunosuppression risks. Thus, obese patients are likely to mobilize more slowly, and are at significant risk of post-operative complications.

Psychosocial issues

Craven *et al.* studied in detail the rate of psychopathology in patients presenting for transplant, and found that approximately half of the patients had a past or present psychiatric disorder. Organic brain syndromes, depressive disorders, alcohol abuse and anxiety were the most common diagnoses, and most often had their onset after the onset of the pulmonary disease[46]. Anxiety disorders and panic anxiety were particularly common, and are important to recognize in these patients because they are often amenable to counselling, stress management techniques or even pharmacologic interventions. Occasionally, patients with frank psychoses, active or recent drug abuse, dysfunctional and potentially destructive family/support relationships, or active smoking are found. These are contraindications to transplant unless assurance of compliance with postoperative medication regimens and close medical follow-up can be assured. Patients must be able to refrain from smoking for a minimum of 6 months before transplant in most programs. In Craven *et al.*'s experience approximately 10% of patients presenting for transplant were ultimately refused for one of these reasons.

TRANSPLANT WINDOW FOR SPECIFIC DIAGNOSES

Early in the experience with lung transplantation it was often difficult to decide when a patient's disease was sufficiently advanced to consider transplant, and yet not so advanced that he or she was likely to die on the waiting list or in the perioperative period. In the past 10 years, however, experience in transplant centers, as well as increasing information from practitioners who regularly follow patients with diseases likely to progress to end-stage, have allowed the formulation of some guidelines in selecting potential transplant recipients.

Cystic fibrosis

Cystic fibrosis is one of the more difficult diagnoses to predict length of survival, probably because of the ever-present threat of lethal infectious exacerbations even in patients who have been functioning relatively well. One of the most helpful pieces of information in helping to decide when to choose cystic fibrosis patients for transplant was an epidemiological article by Kerem *et al.* published in 1992. In this longitudinal study of 673 cystic fibrosis patients followed between 1977 and 1989, the authors found that $FEV_1 \leq 30\%$ predicted; $Pa_{O_2} \leq 55$ mmHg or $Pa_{CO_2} \geq 50$ mmHg implied 2-year mortality rates in excess of 50%. Additional risk factors were developing these parameters at a younger age, or if the patient were female[47]. The usefulness of these criteria has been supported by data from transplant centers comparing parameters of patients dying while waiting, with those who survived to transplant[48]. The 'wild card' of infectious exacerbations, and multiple reports of high death rates in cystic fibrosis

patients awaiting transplant[49–51], support the policy of earlier rather than later referral. Another parameter which should trigger early referral is sudden worsening of previous stable disease and/or increasing numbers of hospitalizations[52].

Emphysema/COPD

This category accounts for the largest group of patients referred for lung transplantation[53] because obstructive lung is the most common lung disease which progresses to end-stage. In the United States it is the fourth leading cause of death[54]. It is difficult to predict survival in this group of patients, but from a different perspective than in cystic fibrosis patients. Patients with this group of diseases become very disabled from their disease, but tend to survive for relatively long periods of time, albeit often with an extremely poor quality of life. Data from various supplemental oxygen trials document that in patients with FEV_1 values $\leq 30\%$ under the age of 65 the 3-year survival can range from about 50% to more than 70%[55]. Enhanced medical attention, including appropriate oxygen supplementation and rehabilitation, as is carried out in many transplant programs, may further improve these percentages[56]. Thus, it is often difficult to predict survival in emphysema/COPD patients, and consideration of the degree of impairment of quality of life may be included in the decision-making process. Significant impairment of quality of life might include inability to independently complete normal toilet or basic activities of daily living, or the sensation of impending involuntary micturition or bowel evacuation whenever suddenly stressed or short of breath. This impairment usually occurs at FEV_1 values of $\leq 20\%$. Patients usually, but not always, require supplemental oxygen, and may or may not be hypercapnic. Six-minute walk values in this group are usually in the range of 200–350 meters.

Pulmonary fibrosis/restrictive lung disease

Unlike obstructive lung disease, the progression of this category of end-stage disease is relentlessly progressive, particularly in the case of idiopathic pulmonary fibrosis. Because of this characteristic many, if not most, of these patients die on the transplant waiting list[51,57]. Natural history studies document a median survival of under 5 years in these patients[58], which is only slightly higher if medical treatment is instituted[59,60]. Patients with total lung capacities of $\leq 60\%$ are nearly all dead within 2 years[59]. Probably of equal importance in predicting outcome is the diffusing capacity. In the Toronto experience of selecting patients for lung transplant, a subgroup of patients (approximately 10%) referred with pulmonary fibrosis have normal or near-normal lung volumes, but diffusing capacities of under 50%. The prognosis in this group has been no better than that of patients with total lung capacities of $\leq$ 60%. These patients invariably require supplemental oxygen, with particularly high requirements on exercise because of marked desaturation. Patients tend to remain relatively asymptomatic during the first half of the course of the disease (measured in time from diagnosis), but when they become symptomatic they deteriorate rapidly and relentlessly. They should be referred for evaluation for transplant as soon as the symptomatic deterioration begins, since the wait for donor organs may be many months. Intercurrent respiratory tract infections in this group of patients often appear to accelerate the course of the disease.

A major problem in patients with pulmonary fibrosis has been difficulty in tapering the large doses of prednisone used to treat the disease. Patients are often unable to be tapered off steroids because of an exacerbation of symptoms, but are able to tolerate doses of 15–20 mg of prednisone and can usually be transplanted at this level. Recently, Venuta *et al.* suggested concurrent use of cyclosporin in an attempt to reduce steroid dosage, and others have suggested concurrent use of azathioprine or cyclophosphamide[61].

Primary pulmonary hypertension/Eisenmenger's syndrome

Patients with primary and secondary pulmonary hypertension with similar degrees of elevated pressures have very different projected survivals.

Data from the National Prospective Registry of Primary Pulmonary Hypertension have been very helpful in establishing prognostic criteria for this group of patients. D'Alonzo *et al.* reported on survivals of 194 patients from 32 centers. All patients had right heart catheterization to establish hemodynamic parameters, and survivals were calculated from the time of this diagnostic evaluation[62]. Median survival of all patients was 2.8 years, and 1-year survival was approximately 68%. Specific hemodynamic parameters were useful in predicting outcome. A mean pulmonary pressure of 55 mmHg or less implied an approximate 4-year survival; a mean of 85 mmHg or more correlated with under 1-year survival. Other predictors of poor survival were mean right atrial pressure of more than 10 mmHg and mean cardiac index of less than 4 l min^{-1} m^{-2}. Hyperbilirubinemia may also be a sign of end-stage disease, and bilirubin levels of more than 1 mg/dl, and particularly higher than 2 mg/dl, have been associated with high post-transplant death rates[63]. A recent paper, noting that there is some evidence of improved survival in patients responding to vasodilator agents (calcium-channel blockers or prostacyclin), suggests that all patients with primary pulmonary hypertension undergo vasodilator trials with hemodynamic monitoring[64]. Soon inhaled nitric oxide might also be a possible treatment option[65]. In the event of a good response, referral for transplant might be delayed until patients meet the criteria noted above. However, only about 20% of patients have been reported to have this good response to vasodilators, and the death rate of pulmonary hypertensives while awaiting transplant has been high; in one paper the 6-month actuarial survival of primary pulmonary hypertensives on a waiting list was 60%[51]. Thus, we feel that it is prudent to refer for assessment primary pulmonary hypertensives who fit any of the criteria consistent with survival of under 4 years. Patients felt to be too early in the disease to transplant can be followed closely by the transplant center, as deterioration can occur rapidly and unexpectedly.

Survivals of Eisenmenger's patients with secondary pulmonary hypertension are much more difficult to predict. The 6-month actuarial survival of this group at 89% was the best of any diagnoses in Hayden *et al.*'s paper[51]. Even when pulmonary pressures are suprasystemic, and patients desaturate markedly on exercise, prolonged survivals are not uncommon. However, sudden death is

unpredictable and may occur in an otherwise stable patient. Thus, adequate criteria to predict survival are not yet available to make accurate predictions in this group of patients. Probably the best indicator at present for selection of candidates is progressive decline in exercise capacity to the point of difficulty in completing activities of daily living.

In any pulmonary hypertensive patient it is very important to assess for a secondary cause of the disease, to ensure that no medical or alternative surgical treatments are overlooked, to give the surgical team full information and to make as accurate as possible predictions about survival. Thus a thorough search for thromboembolic disease, as well as undetected congenital cardiac or vascular anomalies, should be undertaken. Gorcsan *et al.* reported that transesophageal echocardiography in 48 consecutive patients – all of whom had undergone cardiac catheterization and transthoracic echocardiography – revealed important data in 25% of patients. This included detection of proximal pulmonary artery thrombi, atrial septal defects and ventricular septal defects, among other things[66].

EVALUATION OF POTENTIAL TRANSPLANT CANDIDATES

Each patient presenting for transplant consideration should undergo complete pulmonary function testing to assess his/her status with respect to the 'transplant window' of his/her particular illness. It is also helpful to assess exercise tolerance in some way, to gain baseline information and to assist in designing a preoperative rehabilitation program. The most commonly used exercise tests are the 6-minute and 12-minute walk tests, which are adequate in testing patients who are very end-stage. However, for cystic fibrosis patients and patients who are less exercise-limited at end-stage, a graded exercise study such as the modified Bruce Protocol is more discriminating.

Other studies that are useful in the pulmonary evaluation are perfusion scans, especially when single lung transplant is anticipated, as such scans may dictate which side is transplanted. Chest CT scan is also a very valuable tool to assess degree of bullous disease in COPD patients, areas of occult bronchiectasis in a variety of diseases, unappreciated lung cancers in pulmonary fibrosis and COPD patients, mediastinal and hilar abnormalities and the extent of pleural disease.

The extent of cardiac work-up necessary pretransplant depends upon the candidate's diagnosis and the risk factors for cardiac disease. Pulmonary hypertensive patients, as noted above, require an extensive work-up to assess left- and right-sided cardiac function and pulmonary pressures and a search for secondary causes of the disease. The work-up for patients with other diagnoses is less clear-cut. All patients require minimal assessment of cardiac function – either echocardiogram or radionuclide study (or equivalent) or both. A mild degree of left ventricular dysfunction is usually tolerated by transplant recipients, but more serious left ventricular impairment is problematic. As noted previously, much greater degrees of right ventricular dysfunction are generally acceptable. More debate has centered around how aggressively the possibility of coronary artery disease should be pursued. A number of institutions have routinely performed coronary angiography on patients presenting for transplant who have specific risk factors such as significant smoking history, male sex and appropriate age for coronary disease. Leibowitz *et al.* recently reviewed data from 77 patients who underwent angiography during consideration for transplant[67]. This group found that eight of the nine patients who were found to have coronary artery disease had at least one risk factor other than smoking. This study suggests that a careful history can eliminate many expensive and superfluous angiography procedures.

In all patients a careful assessment of organ systems at risk of toxicity from immunosuppressive agents should be undertaken. Liver function studies are adequate hepatic assessment in most patients, but in those with a history of alcohol use or other hepatic disease an ultrasound, and possibly liver biopsy, may be necessary. Kidney function is the most critical of the vital organs to assess carefully pretransplant, because virtually every patient placed on cyslosporine will experience some nephrotoxicity; a small percentage will develop renal failure. Minimal preoperative assessment is a measurement of creatinine, but a creatinine clearance determination is far more useful and highly recommended. Patients having preoperative creatinine clearances less than 75% of normal in this population may have intrinsic renal disease and a high risk of postoperative renal insufficiency.

A psychosocial evaluation is indispensable in the evaluation of the pretransplant patient. The reliability of identified support systems can be verified, coping mechanisms evaluated, and anxiety, panic or other illness-related dysfunction can be assessed so that appropriate intervention is made. Occasionally psychiatric diagnoses, motivational problems or substance abuse is uncovered, factors that would make the patient a poor transplant candidate. Useful scales to use in evaluation of these patients are found in Kelly *et al*[46].

Nutritional assessment – including calculation of body mass index, calorie counts and review of serum iron indices, protein and albumin – can assist the dietitian in making recommendations to correct nutritional problems as much as possible pretransplant.

As with all transplant candidates, serology to determine previous exposure to cytomegalovirus, Epstein – Barr virus, HIV, toxoplasmosis, hepatitis B and hepatitis C is routinely obtained.

Specific pretransplant diagnoses may require more extensive evaluation; for example, patients with scleroderma should have an evaluation of esophageal motility, as significant loss of motility may predispose to aspiration and put the transplant at risk. In this group of patients a preoperative kidney biopsy may be indicated if there is a decrement in creatinine clearance. If the patient has significant Raynaud's phenomenon a preoperative trial of cyclosporin (which can cause vasospasm) might be tried, to ensure that it will not worsen the problem postoperatively. In each case the selection of specific assessments should be guided by the potential end-organ damage of the candidate's underlying diagnosis.

PRETRANSPLANT MANAGEMENT

The period of time from acceptance for transplant until the surgery can vary from a few days to many months. This is a very stressful time, not only for the patient, but also for his or her entire support network. Life is essentially on hold, for at any time the patient might receive a call to report to the transplant center. At the same time the disease is progressing, debility is increasing and the patient

is acutely aware that survival is a race against time. How best can the family and the patient cope and, at the same time, how can the patient remain a good transplant candidate?

It is very helpful for the transplant candidate if the transplant center has some type of organized or structured program in which he or she can participate. This type of program reminds the patient that he or she has not been forgotten, gives the patient and the family activities in which to focus their energies, and allows the center to keep abreast of changes in the patient's physical and emotional status. A good program can also help the patient become a better transplant candidate, or maintain his or her status.

The first and most common component of a transplant center program is a rehabilitation program for the patient. Outpatient programs in which the candidate participates two or three times a week have been shown to improve exercise tolerance and improve the patient's sense of well-being[68,69]. Endurance and strength exercises should be included in such a program, which is easily set up in a facility near the patient's home. Types of exercises might be treadmill walking, bicycling, lifting small weights and light aerobics.

A second useful component of the preoperative phase is a support group. This is useful for both the patient and the family. Such groups can meet monthly or weekly, or at other intervals, but should have set meeting times so that interested candidates can participate. It is sometimes useful to have support meetings for the families alone as the stress of waiting for transplant and living with a dying person can be overwhelming. Psychosocial personnel are ideal leaders for this type of group meeting. These meetings can also serve as a forum at which the transplant physicians or other team members can address issues which have been identified and are of concern to the transplant candidates.

A third essential component of the pretransplant program is an outpatient clinic. Pretransplant patients should be seen regularly in the clinic to assess progression of their disease, adjust medications and supplemental oxygen, and help identify any new problems which arise. Some patients who wait long periods of time may require interval evaluation of cardiac or other organ function and may progress beyond the transplant window. Each patient should discuss with the primary physician his or her wishes for end-of-life care and appropriate documentation, e.g. living will, should be made. Particular policies of the transplant program regarding management of acute problems, mechanical ventilation, or other end-of-life issues should be discussed with the patient early in his or her entry into the program.

RETRANSPLANTATION

Unfortunately a significant proportion of patients undergoing lung transplant will experience complications that greatly impair quality of life, and may request retransplantation. Because of the donor organ shortage a number of centers do not offer retransplantation. The results of retransplantation worldwide have been carefully looked at by Novick *et al.*[70], who found that the 12-month survival in patients undergoing retransplant for obliterative bronchiolitis was 41%, significantly lower than that of initial transplants. Patients who underwent unilateral retransplant with retention of an old contralateral graft had poorer survivals. Earlier data from Novick *et al.*[71] documented that results of retransplant after early graft failure are dismal. Patients presenting for retransplant should meet the same criteria as those presenting for initial transplant, since they are competing for the same small donor organ pool. This will essentially limit retransplant to relatively well-functioning intermediate- to long-term survivors of the initial transplant.

PEDIATRIC LUNG TRANSPLANT CANDIDATES

Increasing numbers of pediatric transplants are being reported in the literature. The most common illnesses for which transplantation has been indicated are cystic fibrosis, primary pulmonary hypertension and congenital heart disease. One-year survival in these patients has been reported as around 70%, approaching that of the adult population[72,73]. Death rates seem to be slightly higher in the cystic fibrosis group[74]. Most of the transplants in the pediatric population have been either heart–lung or bilateral lung and, more recently, bilateral lobar (see above). In general, criteria for selection of pediatric lung transplant patients should be similar to those of the adult population, e.g. absence of multisystem organ failure, good family support, etc. Support systems and ability/willingness to comply with post-transplant regimens are particularly important in this group of patients as the rate of bronchiolitis obliterans, infection and post-transplant lymphoproliferative disorders has been reported to be high[72]. Determining when a child is ill enough for transplantation may be more difficult than in adults. Steinberger *et al.* reviewed referrals for pediatric lung and heart transplant, and found that of 31 patients referred, nine improved with improvements in medical therapy that either obviated or delayed transplant surgery[75]. While minimal criteria have been published[74] denoting when a child should be considered for transplant, definitive guidelines have not yet been published.

RECURRENCE OF UNDERLYING DISEASE

A number of the diseases for which lung transplantation is done are either congenital or part of a systemic illness, and have the potential to recur. α_1-Antitrypsin emphysema, for example, would probably recur in recipients who live long enough as the enzyme deficiency is not primarily corrected by transplant. Recurrence in the survivals reported to date has not been reported as a problem. Interestingly, however, recurrence of underlying disease has been reported, most notably in patients with lymphangiomyomatosis[76] and sarcoidosis[77–79]. Sarcoidosis appears to recur regularly post-transplant, but has rarely been reported as clinically impairing the outcome of the recipient. To date, recurrence of these underlying diseases has not been considered a reason to refuse transplant.

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47
A Comment on Pretransplant Management of the Potential Lung Recipient

N.K. IMES

INTRODUCTION

The general principles of management of a patient with severe pulmonary disease apply equally to those patients who are being evaluated, or who are currently on the waiting list for lung transplantation (LTx). These principles are well known to the pulmonologist and will not be repeated here. In addition, a major objective of medical management of LTx candidates is to keep the patient in an optimal nutritional and physiologic condition prior to transplantation. Medications should be avoided which might have adverse effects on the patient at the time of surgery, or cause problems with toxicity and end-organ damage. Examples include corticosteroids and drugs which result in nephrotoxicity or fluid retention. The principles of good nutrition and maintenance of near-normal ideal body weight apply to these patients, since either excessive or inadequate body weight may adversely affect their ability to undergo the transplant with an acceptable risk. Physical rehabilitation in an exercise program several times a week is mandatory for all LTx candidates to optimize their muscular strength and endurance.

SPECIFIC THERAPY FOR THE UNDERLYING PULMONARY DISEASE

Specific therapy depends upon the underlying disorder for which the patient is to undergo LTx. In the case of patients with end-stage emphysematous lung disease, bronchodilator administration with albuterol and ipratropium is indicated and helpful. Inhaled corticosteroids may be beneficial in decreasing airway inflammation, and at the same time may reduce the need for oral corticosteroids.

Antibiotic use is frequently indicated in patients with emphysema and chronic bronchitis. When the patient develops respiratory infection, causing exacerbation of the lung disease, the use of broad-spectrum antibiotics is likely to be necessary and even lifesaving. This antibiotic coverage, however, should not be continued for long periods of time, in view of the danger of encouraging the growth of resistant microbial organisms. Avoidance of frequent antibiotics may not be possible in the patient with end-stage bronchiectasis where infection control may be the only means to control respiratory failure and maintain nutrition.

Oral corticosteroid therapy is not a contraindication to LTx *per se*, but heavy use of oral or parenteral corticosteroids will result in adverse physiologic effects. These complications include diabetes mellitus, osteoporosis, skin changes, truncal obesity, and muscle wasting, all of which may have detrimental effects post-transplant. Therefore, it is recommended that the dosage of oral corticosteroids be maintained within the range of 5–15 mg prednisone/day (or equivalent dose). Patients with pulmonary fibrosis may have been subjected to particularly high-dose long-term corticosteroids prior to the time they are considered for LTx. The obvious problems caused by high-dose corticosteroids may therefore be encountered. These drug side-effects need to be addressed and the corticosteroid dosage lowered to the smallest that can be tolerated by the patient.

Other drug therapies may also have been instituted before these patients were evaluated, such as the adjunct use of azathioprine or cyclophosphamide. Because of the immunosuppressive effects of these drug combinations, these patients are at high risk of developing infections similar to those seen in the post-transplant patient. Therefore, they must be carefully monitored and aggressively treated whenever a suspected infection is present. The potential toxicity of these drugs to the bone marrow must also be monitored closely, with periodic complete blood counts.

The patient who has primary pulmonary hypertension is likely to be chronically maintained on anticoagulants and possibly on agents to decrease the pulmonary artery pressure, such as calcium-channel blockers. Diuretic therapy and sodium restriction will be essential to control the patient's right ventricular failure secondary to pulmonary hypertension and prevent excessive ascites, liver congestion and edema.

REHABILITATION AND NUTRITION

All LTx candidates must participate in rehabilitation to maximize endurance and respiratory muscle strength. This is important not only to optimize the patient's ability to utilize limited lung

function pretransplantation, but also to enhance the chance of surviving the rigors of the surgical procedure and the postoperative period.

Nutrition must be carefully assessed (Chapter 16) and monitored since either deficient or excessive body weight may be detrimental to the success of the transplant. Patients who are malnourished or underweight must be placed on a regimen of vitamins and food supplements in order to improve their body weight to within 10–15% of their ideal. Patients who are overweight, on the other hand, must be placed on caloric restriction (but not to the point of starvation) to allow their body weight to decrease gradually. These patients should ideally decrease their body weight to within approximately 10–15% of ideal by the time of transplantation.

Appropriate use of sodium restriction is needed in most LTx candidates because of the presence of cor pulmonale. Diuretics may also be helpful in decreasing the amount of fluid retention caused by cor pulmonale, or by medications, such as calcium-channel blockers, that may be needed for treatment of systemic or pulmonary hypertension. Hepatic injury may result from poor control of cor pulmonale. Consequences of diuretic and fluid management require periodic monitoring of the patient's sodium, potassium and magnesium.

COMMENT

In summary, the specific management of LTx candidates preoperatively differs little from their usual care and treatment. However, since the exact date of their transplant remains unknown, every effort must be made to keep these patients in optimal physiologic condition at all times. Patients must continually participate in physical rehabilitation, maintain proper fluid balance, and make an effort to achieve ideal body weight and nutritional state. It is also important for the physician to encourage the patient to maintain morale and a positive outlook, during what is frequently a long delay until a suitable donor becomes available.

The physician must use medications judiciously to avoid functional impairment of the patient's organ systems and/or drug side-effects which may complicate the transplant or have long-term deleterious effects. Our policy has been to examine the patient and perform selective evaluations on potential recipients at least every 6 weeks before transplantation, and more frequently if indicated by the severity of the disease. This allows potential problems to be identified early and resolved quickly, and ensures that the LTx candidate is always in optimal condition when the time for transplantation occurs.

48
Excision and Storage of the Donor Lungs

S. KESHAVJEE AND T.R. TODD

INTRODUCTION

The expansion of organ transplantation over the past 10 years has led to further stresses on an already limited donor pool and to an increasing shortage of suitable organs[1]. In an effort to meet this need, centralized registries of potential recipients were developed to prioritize recipients and to ensure access to donors at distant sites. These programs were able to expand as improved means of donor organ preservation were developed. However, the demand for organ donors has exceeded the supply despite an intense educational campaign aimed at both the public and the profession. As a result the maximum number of transplantable organs must be retrieved from every available donor. In order to achieve this maximum number, the following points deserve consideration: (a) assessment and selection of the donor lungs, (b) maintenance of selected donors, and (c) excision and preservation of the donor lung(s).

ASSESSMENT AND SELECTION OF THE DONOR LUNGS

There are several reasons why the lungs of brain-dead donors might not be suitable for transplantation.

The precipitating cause of brain death may have led to significant direct pulmonary parenchymal or bronchial damage. Trauma is the commonest cause of brain death in an otherwise healthy young person; thus the presence of pulmonary contusion or bronchial trauma must be considered. In addition, the aspiration of gastric contents is a frequent accompaniment of a depressed level of consciousness. As intracranial pressure rises, neurogenic pulmonary edema may also be seen. These changes traditionally have resulted in the exclusion of pulmonary donation. However, the donor shortage dictates that assessment should be thorough, and interventions should be undertaken in an attempt to ensure maximum usage of both lungs (or at least a single lung) from every potential donor.

Moreover, these patients have all undergone tracheal intubation for the purpose of mechanical ventilatory support. They have been cared for in intensive-care units where the presence of highly resistant bacteria leads to colonization of the respiratory tract as early as 3 days[2]. As a result the early onset of pneumonia is a well-recognized feature in brain-dead subjects. In our experience this pulmonary infection can on occasion be rapidly progressive.

Radiographic appearances

These factors underscore the importance of careful assessment of potential lung donors. The portable chest radiograph supplies useful information provided that the technique employed yields a film of good quality. If a portable film taken in the intensive-care unit is inadequately exposed, subtle pulmonary infiltrates may not be visualized clearly. Radiographs of questionable quality should be repeated. A localized infiltrate should be of particular concern as this most likely represents pneumonitis rather than pulmonary edema (unless it is directly related to an area of chest trauma, in which case it may represent a pulmonary contusion). In our experience, if it is a pneumonia, the area of involvement will often progressively increase in size, and this will usually preclude donation of the lungs. On occasion, infiltrates will disappear or stabilize, emphasizing the importance of repeated radiographic assessment. When the infiltrate involves only one lung, the contralateral lung may be used for transplantation, provided that gas exchange is satisfactory[3]. (In such a case, if oxygenation is suboptimal, a test clamping of the pulmonary artery to the poor lung can be performed in the operating room at the time of harvest to confirm that the contralateral lung is indeed functioning well.) The presence of pulmonary edema demands a trial of diuresis. If the clinical diagnosis of edema is correct, the radiographic appearance and gas exchange may improve sufficiently to allow for lung donation.

Arterial blood gases

Arterial blood gases are measured with the donor receiving an F_iO_2 of 1.0 and a positive end-expiratory pressure (PEEP) of 5 cmH_2O. Under these circumstances the po_2 has traditionally

been maintained above 300 mmHg. Blood gases are repeated every 2 hours while awaiting donor organ extraction, to ensure continued suitability. If the arterial blood gases are not satisfactory, before excluding the donor for lung donation it should be ensured that the donor is being adequately ventilated. The endotracheal tube should be seen to be properly positioned, and it should be ensured that the tidal volume is appropriate for the donor size and that the PEEP and F_iO_2 are indeed 5 cmH_2O and 1.0, respectively. Suctioning bronchoscopically should also be performed prior to this assessment to rule out major airway obstruction by secretions. Only after the ventilation and fluid status of the donor have been optimized should the donor be turned down for donation due to inadequate gas exchange.

Bronchoscopy

Bronchoscopy is undertaken routinely. Aspirates are processed bacteriologically, and both donor and recipient receive antimicrobial agents based on the initial Gram stain. Few bronchoscopic findings will preclude the use of donor lungs. On occasion one may see severe tracheo-bronchitis, indicating aspiration of acid gastric content, which will preclude the use of the organs. Of course, the presence of food or vegetative matter will also preclude the use of the lungs. Frank pus is also an indication to exclude the use of the donor, especially if pus continues to well up out of segmental orifices after being suctioned out, indicating a pneumonia. Small amounts of mucopurulent material are commonly seen and do not preclude use of the lungs. Confirmatory evidence is received from the Gram stain assessment.

Assessment of lung size

The lungs are the only organs that are transplanted into a relatively rigid cavity. 'Relatively rigid' is indeed the correct description, as the primary disease process will have altered the volume and shape of the thoracic cage. In pulmonary fibrosis, lung volumes will be smaller than those predicted for the patient's age, height and weight. The diaphragms are high and the rib spaces compressed. In emphysematous conditions (idiopathic emphysema, α_1-antitrypsin deficiency) and in bronchiectatic conditions (bronchiectasis, cystic fibrosis) lung volumes are increased, resulting in low diaphragms and widened intercostal spaces. These factors must be considered in the selection of the appropriate donor. It is thus imperative to assess the thoracic volume accurately in both recipient and donor.

We currently use the following formulae to match the recipient's *predicted* (not actual) lung volume to the donor's *predicted* lung volume based on height, weight, sex and age. We have found the use of these formulae to be simpler and more accurate then our previous practice of measuring lung dimensions on the donor chest radiograph.

PREDICTED TOTAL LUNG CAPACITY (*L*)
Male = (0.094 × height in cm) – (0.015 × age in years) – 9.167
Female = (0.079 × height in cm) – (0.008 × age in years) – 7.49

The predicted lung volumes using the above formulae provide a guideline to be used in matching the donor lung to the recipient and, in general, have been found to be quite reliable. Some modification may be necessary at times. For example, in cases of pulmonary fibrosis, with a contracted lung volume, we may use a lung that is slightly smaller than the predicted volume, although larger than the recipient actual volume. Similarly, when the recipient has expanded lung volumes, we may use a lung that is slightly larger than the calculated size using the formula, knowing that we will not have a space problem in the chest. When a larger lung is used, if a significant space problem is encountered, the donor lung can be 'down-sized' or 'volume-reduced' using a linear stapler to excise lung tissue.

The diaphragm and chest wall readily conform to the size of the new donor lung(s). This change usually occurs over the first 2 postoperative weeks. A decrease in lung volume in an emphysematous lung recipient is accompanied by a more normal diaphragmatic contour and a reduction in the size of the intercostal spaces. On the other hand, a significant increase in thoracic volume will be noted on the chest radiograph after lung transplantation for pulmonary fibrosis. In fact, it was this experience following lung transplantation, where changes in the chest wall contour and mechanics were noted to improve postoperatively, that lead Joel Cooper[4] to re-explore the concept of chest wall and diaphragmatic dysfunction as a contribution to overall respiratory dysfunction in end-stage emphysema, leading to the development and ongoing study of lung volume reduction surgery[4].

MAINTENANCE OF THE DONOR

There are a few features of donor maintenance that are important when lung donation is considered. As noted above, frequent chest radiographic and blood gas assessments are important. In general, a euvolemic status is the aim of donor management in an effort to obtain hemodynamic stability and avoid pulmonary edema. Fluid restriction is maintained as long as urine output is adequate (greater than 30 ml/h). Diabetes insipidus is usually controlled with desmopressin (DDAVP)[5]. If fluid status monitoring is difficult, a central venous pressure line should also be inserted.

A dopamine infusion may help support blood pressure and renal perfusion, thus reducing the need for fluid administration. Following brain death, hemodynamic instability is common, particularly when the interval between declaration of brain death and organ extraction is prolonged. As a result, peripheral vascular resistance may be low and these patients may receive large quantities of intravenous fluid as preload. When a predisposition to capillary leak occurs, the accumulation of extravascular lung water is directly proportional to preload. In addition, an impairment in cardiac contractility has been described in experimental models of brain death and, in our own experience, has been seen in about 20% of declared organ donors. The administration of an inotrope is therefore of potential importance. The donor should be maintained at 37°C and a warming blanket should be used if required.

EXCISION AND PRESERVATION OF THE DONOR LUNGS

The preservation and excision of the donor organs are critical components on which the final outcome of the entire transplant operation rests. To quote John Wallwork, 'You can't make a

chicken out of a fried egg.' As much attention must be paid to the donor harvesting procedure as to the implantation procedure.

The technique of excision of the lungs is influenced by the need to preserve and excise the heart for transplantation into another recipient. A median sternotomy is performed and the pericardium is opened widely. Superiorly, the pericardium is incised to the origin of the innominate artery. The superior vena cava (SVC) is mobilized immediately caudal to the azygos vein and encircled with two 0-silk ties. The inferior vena cava (IVC) is dissected. The ascending aorta is freed circumferentially and dissected free of the pulmonary artery. This permits adequate identification of the pulmonary artery bifurcation and ensures that the right pulmonary artery is not injured during cardiac excision.

With the aorta retracted to the left and the SVC to the right, the posterior pericardium overlying the distal trachea is incised. If time permits, the trachea is mobilized well above the carina, usually at the level of the innominate artery, and a tape is placed around it. If the donor is unstable, or time is short, the isolation of the trachea can be performed after the pulmonary flush is completed. Both pleural spaces are widely opened so that the surgeon can examine the pleural spaces for adhesions and also inspect the lungs carefully. The lungs are inspected and palpated, primarily to rule out an area of consolidation and confirm complete expansion. If any atelectasis is noted, the anesthetist should reinflate the lungs by hand inflation.

A 4/0 prolene purse-string suture is placed in the ascending aorta for the insertion of a cardioplegia catheter (if the heart is to be extracted). A second 4/0 prolene purse-string suture, approximately 1 cm in diameter, is placed on the anterior surface of the pulmonary artery approximately half-way between the pulmonary valve and the bifurcation of the main pulmonary artery. At this point the procedure is frequently interrupted to allow the liver and/or kidney surgical teams to complete the preparation of the abdominal organs.

After the abdominal teams have completed their dissection and have their flush cannulae in place, the thoracic team returns to the field. The patient is heparinized (300 U/kg) and a cardioplegic needle or cannula is placed in the ascending aorta. The cardioplegia tubing is primed, de-aired and hung. A large-bore catheter (5 mm diameter) is then inserted into the main pulmonary artery within the previously placed purse-string suture, and secured in place with a tourniquet. The pulmonary flush lines are primed and de-aired. The flush solution is hung at a maximum height of 30 cm above the patient. Note that with the use of large-bore tubing (5 mm diameter) and a large-bore flush cannula (5 mm diameter) one can achieve high flow with a maximum pressure of 30 cmH_2O and the use of a roller pump or pressure bag to infuse the flush solution is not indicated. We use approximately 50 ml/kg of flush solution (3–3.5 liters for the average adult).

During insertion of these cannulae it may be helpful for the anesthetist to gently hand-ventilate the donor lungs. Once the flush lines are ready, however, the anesthetist re-inflates the lungs to remove any atelectasis and then places the donor back on the ventilator. Ventilation is to continue until the anesthetist is asked to discontinue it. When all teams are ready, 1 mg of prostaglandin E_1 (PGE_1) is injected directly into the main pulmonary artery. After a drop in systemic blood pressure is noted, implying a PGE_1 effect, the SVC is ligated, the IVC divided just above the diaphragm (to vent the right side of the heart) and the left atrial appendage truncated (to vent the left side). The hole in the left atrial appendage should be at least 2 cm wide to ensure that there is no obstruction to pulmonary venous outflow. The aorta is then cross-clamped and cardioplegia is initiated.

The pulmonary flush is then started. The surgeon controls the flow into the pulmonary artery with a clamp on the line and aims to have the entire 3 liters flushed within 3–5 minutes. During the flush period one must observe: (a) the heart – to ensure that there is no left ventricular distension; (b) the left atrial appendage – to ensure good outflow of the pulmonary flush; (c) the lungs – to ensure that uniform blanching is occurring in both lungs (occasionally if the pulmonary artery catheter is in too far, the flush may be directed to one lung only and the catheter will need to be pulled back to correct the situation); and (d) that the left atrial effluent is allowed to spill into both chest cavities to provide further topical cooling of the lungs. If the lung flush is completed before the cardioplegia infusion is completed, ventilation should be continued (F_iO_2 0.5 to 1.0) and the lungs left *in situ* in the cold flush solution in the chest.

After the pulmonary flush and cardioplegia infusions are completed, the cannulae are removed, and excision of the thoracic organs is performed. The heart is extracted first. The SVC is divided between the previously placed silk ties (taking care to avoid the sinoatrial node and to leave a generous of length of SVC for caval anastomosis if desired). The IVC division is completed, if not already done, once again taking care to leave some length of IVC for a caval anastomosis if desired (without compromising the suprahepatic cava for the liver team). The aorta is transected just below the aortic cross-clamp. Since we have switched to bilateral sequential lung implantation, the remaining length of the main pulmonary artery is no longer an issue. The main pulmonary artery is usually transected close to its bifurcation.

To initiate the left atrial division, an incision is made in the left atrium between the confluence of the left pulmonary veins and the coronary sinus (Figure 1). With traction on the heart, the incision

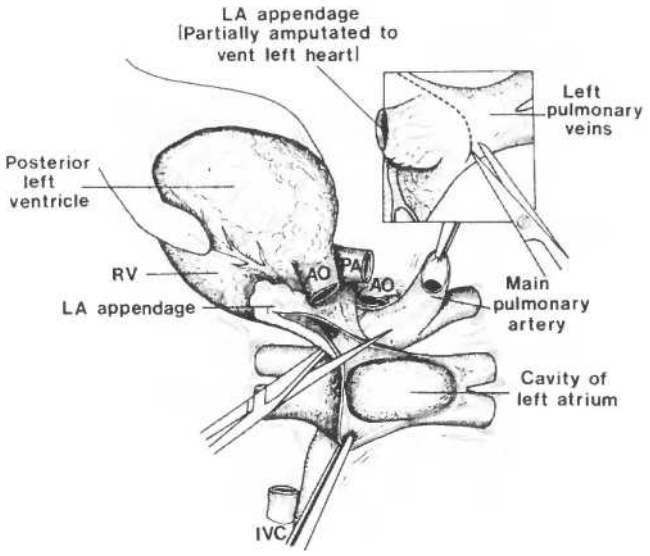

Figure 1 The left atrial incision is started half-way between the coronary sinus and the origin of the left inferior pulmonary vein. The dissection is continued circumferentially, viewing the atrium and veins from the inside and the outside to guide the direction of the incision

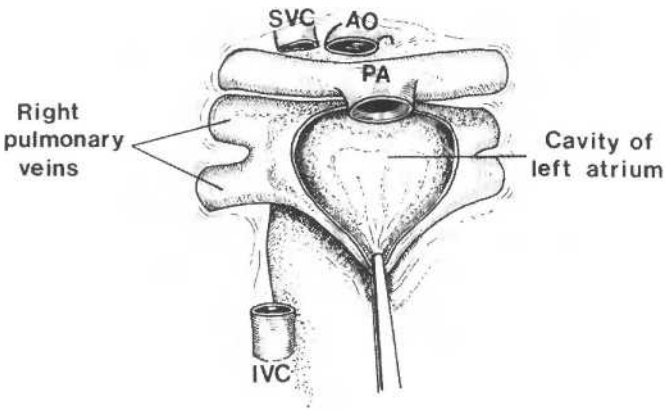

Figure 2 After excision of the heart, the left atrial cuff and pulmonary arterial bifurcation are clearly visible

is continued superiorly and inferiorly and then to the right, preserving a cuff of left atrium around the orifices of both the right and left pulmonary veins (Figure 1). The last cut in the atrium is guided by first incising the endocardium anterior to the right pulmonary veins. A single cut extending from the SVC to the IVC then completes the extraction. In this manner the right atrium remains intact, and yet a satisfactory cuff of left atrium is retained around the orifices of the right pulmonary veins. At this point, with the heart excised, the surgeon should be able to visualize the open main pulmonary artery at its bifurcation and a generous cuff of left atrium joining and surrounding both sets of pulmonary veins (Figure 2). Removal of the lung block then proceeds.

The inferior pericardial attachments to the diaphragm are divided down to the esophagus. Dissection in the pre-esophageal plane is then initiated, extending superiorly as far as possible (usually one can go well above the carina with this dissection).

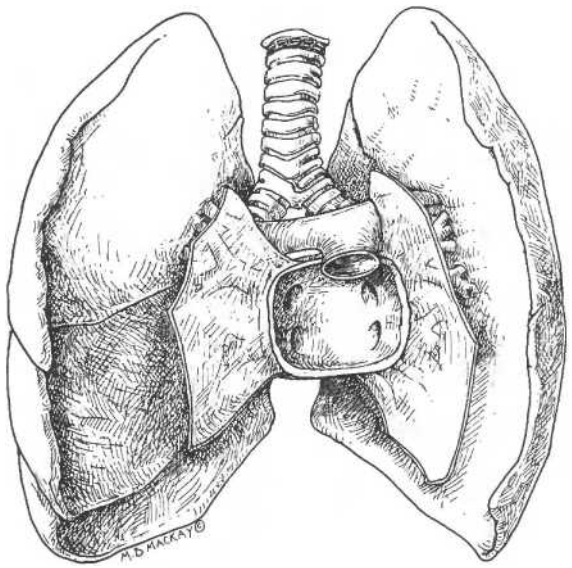

Figure 3 The lung block after removal from the chest. The trachea remains stapled and the lungs remain inflated

The mediastinal pleura superiorly is then divided. On the right side, this is initiated inferior to the azygos vein and on the left it is initiated over the aortic arch. The trachea is then clamped and stapled with a TA-30 bronchial stapling device. The clamping of the trachea is timed to occur after end-inspiration to ensure that the lungs are maintained in the inflated state. The anesthetist may discontinue ventilation only after the clamp is applied. The stapling device may be left on the distal trachea and used as a 'handle' for traction on the trachea during the extraction. After the trachea is divided, it is retracted forward and the tracheo-esophageal plane is developed and extended inferiorly as far as possible, using a combination of blunt and sharp dissection.

To complete the dissection in the mid-mediastinum, the right lung is rolled medially out of its pleural cavity to expose the posterior mediastinum and the dissection is carried out, once again in the pre-esophageal plain. If the ligamentum arteriosum was not divided initially, a 'button' of aorta can be taken with the lung block at this point to avoid damaging the now-flaccid pulmonary artery (Figure 3).

Once the lung block is completely excised, it is placed in a plastic bag with 2–3 liters of cold flush solution. This bag is, in turn, double-bagged for security and to maintain sterility. The bagged organs are then placed on ice in an insulated container (cooler) for transportation. Care is taken to remove as much of the air as possible when tying each of the plastic bags, to make sure that the entire graft is covered with the cold solution during storage and that the air does not insulate the organ from the ice in the cooler.

COMMENT

With respect to preservation of the lung for transplantation, the following factors are currently considered to be important: (a) flush cooling; (b) cold storage; and (c) storage in the inflated state with oxygen. There are many other drug interventions and metabolic substrate additions currently being evaluated that will likely prove to be important over time.

In the initial experience in lung transplantation in Toronto, lungs were preserved simply by cold atelectatic immersion. Over the years we have changed our technique considerably. The current technique of preservation involves a cold pulmonary vascular flush prior to extraction. The most commonly used technique involves the use of PGE1 (1 mg) injected into the pulmonary artery, followed by a flush of Euro-Collins solution (approximately 50 ml/kg) which also contains 1 mg of PGE1. The flush is carried out under low pressure. This is ensured by using a gravity system where the bag of flush solution is hung 30 cm above the donor; the maximum pressure generated, even in the event of an outflow obstruction problem, is 30 cmH_2O.

With respect to the flush solution, many varieties have been used with success. Evidence in the recent literature, however, suggests that low potassium extracellular solutions are superior in animal studies[6–9]. This remains to be confirmed in clinical studies. It is important to ensure that the lungs are fully inflated, with no atelectasis, prior to initiating the flush, to ensure uniform distribution.

It is clear that a cold temperature is beneficial and the ideal temperature has been described[10] to be in a range of 10°C.

Clinically, we feel that this leaves too narrow a margin for safety, so we aim for 4°C, knowing that the average temperature of the organ during the implantation process is likely to be higher.

The lung is a unique organ in that energy-efficient aerobic metabolism can continue, even in the absence of blood flow, if the lungs are inflated with oxygen[6,7]. This is because O_2 can diffuse directly from the alveoli to the endothelial and parenchymal cells. This concept is taken advantage of by ensuring that donor ventilation is continued to the last minute, and the trachea is clamped with the lungs in the inflated state.

Lung transplantation has clearly been established as effective therapy for patients with end-stage lung disease. The shortage of donor organs continues to be the single most important factor limiting the number of transplants that can be performed. Clearly, novel sources of donor organs such as xenografts, living-related donors and non-heart-beating donors will have to be explored further. Further research into strategies to improve the function of marginal donor lungs will give us the confidence to use marginal lungs that are currently turned down using existing criteria. In the meantime, however, we must strive to optimize every donor lung that is available to us, in an effort to provide as many viable grafts as possible for successful transplantation.

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Anesthesia for Lung Transplantation

W.A.P. DEMAJO

INTRODUCTION

Lung transplantation is now a viable treatment option for selected patients with end-stage lung disease. Up to 1994 over 2500 lung transplants had been performed world-wide.

There has occurred a change in the types of transplants performed, with a decrease in heart–lung procedures and a marked increase in bilateral lung transplants[1]. The preferred technique for the latter procedure is now, almost universally, that of sequential single lung transplantation (SSLT), utilizing a bilateral anterothoracosternotomy (Chevron incision) and bronchial anastomoses[2]. This procedure decreases, but does not obviate, the use of cardiopulmonary bypass (CPB), which was a requisite with the double lung transplant utilizing a tracheal anastomosis. A significant change has also occurred in the types of cases transplanted, with cystic fibrosis showing the greatest increase. The debate regarding the best choice of operation for patients with obstructive lung disease is still ongoing, the majority of these patients receiving a single lung[3]. As experience in management of lung transplant patients has increased, the frequency of use of CPB has decreased, although it remains an essential requisite in all centers performing these procedures.

The indications for transplantation have been broadened, as have those relating to donor acceptability[4]. A number of centers have operated on ventilator-dependent patients[5] and the number of retransplants has increased[6]. The criteria for acceptability have also been modified so that the previous policy of steroid prohibition preoperatively, and no routine administration in the first 3 weeks postoperatively, has been removed[7]. Indeed, improvement in postoperative lung function and better healing of the anastomoses has been shown to occur in patients treated with steroids routinely from the immediate postoperative period. No deleterious effect has been reported in patients who continue to be treated with steroids up to the time of transplantation.

HISTORY

The development of lung transplantation began in 1947 when Demikhov homografted canine pulmonary lobes[8]. In 1950 Metras[9], Juvenelle *et al.*, and Hardin and Kittle[10] published their techniques of canine lung allotransplantation. The first human lung transplant was performed by Hardy *et al.* in 1963; the recipient survived for 18 days[11] (Chapter 45). In the following 20 years approximately 50 lung transplants and two heart–lung transplants were performed.

Useful clinical data are available in 11 cases; of these, five were for obstructive lung disease and six for restrictive lung disease[12,13]. Pulmonary function tests revealed severe abnormalities in both groups. The diffusion capacity was 25% of predicted in the obstructive group, and 31% of predicted in the restrictive disease group. The data on arterial oxygenation (Pa_{O_2}) were not interpretable, as the inhaled oxygen concentration was not identified; however the ranges of Pa_{O_2} were 40–63 mmHg in the obstructive group and 37–60 mmHg in the restrictive group. The arterial carbon dioxide (Pa_{CO_2}) levels were elevated in both groups, with the obstructive group having a mean of 66 mmHg (range 49–100 mmHg), and the restrictive group showing a mean of 72 mmHg (range 34–100 mmHg); five patients were ventilated preoperatively.

Hemodynamic data are available for seven patients (four obstructive, three restrictive); all had pulmonary hypertension. Extracorporeal bypass was utilized in 15 patients; in four patients it was used because either heart–lung or double-lung transplantation was performed. In the other 11 patients, eight were placed on CPB electively, as it was considered to be the optimal way to perform single-lung transplantation; one required CPB because of intraoperative hypoxemia, while the remaining two came to operation with established veno-venous femoral partial bypass to ameliorate hypercarbia and/or hypoxemia. Of those in whom CPB was not used, three cardiac arrests occurred, one due to right ventricular failure; the cause of cardiac arrest in the other two patients was not reported.

The anesthetic technique used, and the intraoperative course, were reported in detail in only two cases. In the case reported by White *et al.* the patient had arterial and central venous pressure catheters inserted[14]. Induction was accomplished with thiopental; the patient was paralyzed with succinylcholine and intubated with a Carlen's double-lumen tube. Maintenance was with nitrous

oxide, halothane and oxygen. During one-lung ventilation and on an F_iO_2 of 1.0, arterial blood gases were PaO_2 130 mmHg, $PaCO_2$ 82 mmHg, and pH 7.15. Severe hypotension occurred at the time of one-lung ventilation and progressed to cardiac arrest with a central venous pressure (CVP) of 40 cmH_2O. The patient responded to isoproterenol, and the systolic blood pressure increased to 60 mmHg. Following reperfusion through the established anastomoses, CVP fell to 3 cmH_2O and blood pressure returned to normal. At this juncture, on an F_iO_2 of 1.0, PaO_2 was 211 mmHg, $PaCO_2$ was 60 mmHg, and pH was 7.25.

The patient reported by Rolly *et al.* was anesthetized in a similar manner; a CVP catheter was not used[15]. During one-lung ventilation (F_iO_2 = 1.0) PaO_2 was 160 mmHg, $PaCO_2$ was 110 mmHg, and pH 7.21. Episodes of hypotension or cardiac decompensation did not occur, and the operation was completed uneventfully.

These two cases indicated that the major potential intraoperative problems were hypoxemia, hypercarbia, and cardiac dysfunction. In addition, it was speculated that the use of CPB and anticoagulation increased the risk of bleeding in these cases (which are likely to be complicated by pleural adhesions). These are still the major problems encountered in modern transplantation, which was inaugurated in 1983 by a successful right lung transplant in a patient with idiopathic pulmonary fibrosis performed by the Transplant Group at the University of Toronto[16]. The improved success in lung transplantation has been attributed to a number of factors, including better immunosuppressive therapy (especially cyclosporin), more effective donor organ preservation techniques, improved patient selection, and improved surgical techniques, especially those related to bronchial anastomosis.

INDICATIONS FOR TRANSPLANTATION

Lung transplantation is indicated in patients with irreversible, progressively disabling lung disease with a life expectancy <12–18 months with appropriate therapy. The number of conditions that have been treated with lung transplantation has increased over the years (Table 1); they fall into four broad disease categories: restrictive, obstructive, infective, and pulmonary vascular. In addition, some centers have transplanted patients with 'irreversible' adult respiratory distress syndrome[17], and retransplanted patients with failed primary lung transplants; the results of retransplantation are discouraging[6] (Chapter 60) and inadequate numbers are available to define the former indication. Parameters to predict life expectancy, without transplantation, have been developed for some of the pulmonary diseases. However, predicting length of survival for the individual patient is inaccurate, and clinical judgement as to need for transplantation is necessary[18,19]. Mortality in most groups awaiting transplantation remains unacceptably high at 20–30%[20].

Table 1 Pulmonary diseases treated by lung transplantation

Emphysema including α_1-antitrypsin deficiency
Cystic fibrosis
Pulmonary hypertension – primary and secondary
Idiopathic pulmonary fibrosis/interstitial lung diseases
Obliterative bronchiolitis
Eosinophilic granuloma
Lymphangioleiomyomatosis
Sarcoidosis
Bronchiectasis
ARDS

Table 2 Selection criteria for lung transplant recipients

End-stage lung disease with evidence of progression of disease and life expectancy < 12 → 18 months
No other systemic disease
Demonstrated compliance with medical regimens
Psychologically stable; no recent history of alcohol or drug abuse
Must be ambulatory with O_2 as required
Abstinence from tobacco > 6 months
No extrapulmonary site of infection
Absence of marked obesity
Prednisone tapered to ≤ 20 mg per day
Single lung transplant
Age ≤ 65 years
No septic lung disease, such as bronchiectasis or cystic fibrosis
Bilateral lung transplant
Age < 60 years

The current guidelines for selection of recipients are listed in Table 2. Patients must have a realistic understanding of expectations and demands resulting from the waiting, the operation, and postoperative care. Patients who have demonstrated a pattern of non-compliance, including the inability to desist from smoking for a period of at least 6 months, unreliability, and dependence on drugs or alcohol, are not considered acceptable candidates. All non-pulmonary sites of infection must be cured before transplantation. Patients with persistent, resistant pulmonary pseudomonal or mycobacterial species have increased mortality post-transplantation; some centers consider them unacceptable risks.

Single lung transplantation

Single lung transplantation is recommended for patients less than 65 years old who are free from chronic infectious lung disease. Single lung transplantation has been performed mainly for patients suffering from restrictive lung disease. Some centers have done single lung retransplants in patients afflicted with bronchiolitis obliterans after double lung transplantation. Experience up to 1989 suggested that patients suffering from obstructive lung disease were not suitable candidates for single lung transplantation since postoperative ventilation–perfusion mismatch and hyperinflation of the more compliant native lung were considered highly likely. Stevens *et al.* reported that, in two emphysematous patients, perfusion to the implanted lung increased to 70% of total, while ventilation and lung volume decreased to approximately 30% of total[21]. Mal *et al.*, in 1989, reported a successful single lung transplant in an emphysematous patient. Since then, all centers have successfully transplanted single lungs in this patient group[22].

Patients suffering from pulmonary hypertension and cor pulmonale have traditionally undergone heart–lung transplantation. However, intervention with single lung transplantation, before the development of severe right heart failure, is now considered acceptable. The primary consideration in these patients is the degree

of reversibility of the right ventricular dysfunction. Experimental and clinical data suggest that a significant degree of right ventricular remodeling and improvement of function occurs, once there is an amelioration of the physiological environment[23,24]. Some centers preferentially use bilateral transplants in pulmonary hypertensive patients, to minimize the early postoperative hemodynamic instability and to increase tolerance of subsequent bronchiolitis obliterans.

In single lung transplantation the side transplanted is determined by the function of the native lung, as assessed by quantitative ventilation–perfusion scintigraphy, and the potential for finding pleural adhesions from previous surgery. In the absence of the suspicion of significant pleural adhesions the less functional lung is replaced.

Bilateral lung transplantation

Bilateral lung transplantation is primarily indicated in patients with pulmonary disease associated with persistent infection (e.g. cystic fibrosis or bronchiectasis), because of the risks related to the use of immunosuppression in the presence of infection. Patients with significant bullous lung disease are preferentially give a double lung transplantation, as they are more likely to have problems with native lung herniation and mediastinal shift after single lung transplant. The decision to perform single versus bilateral lung transplantation is determined by careful consideration of: (a) the advantage, to the individual patient, from the extra pulmonary reserve provided by bilateral lung replacement and (b) the reduction in availability of donor lungs for other patients. Due to the greater pulmonary reserve offered by bilateral lung transplantation, some centers preferentially offer double lungs to younger patients.

PREOPERATIVE ASSESSMENT

In addition to the routine assessment of the patients, with evaluation of those aspects of the history and examination that are relevant to lung transplantation, special emphasis is placed on the assessment of the pulmonary and cardiac status. Pulmonary function tests are routinely done, although they often have to be curtailed because the patients are incapable of performing at the level required for completion of the tests. They are done to define the type of pulmonary disease and to serve as a baseline for comparison of future measurements. Blood gases on room air and at various flow rates of oxygen are done to determine the supplemental oxygen requirements of the patient. The ventilation–perfusion scan is performed to decide on the relative function of the two lungs, and thus to help in the decision as to which single lung should be transplanted.

Cardiac assessment is through the use of echocardiography, radionuclide cardiac ejection scans, coronary angiography, and pulmonary angiography and catheterization, in appropriate cases. Radionuclide cardiac ejection scans, in conjunction with echocardiography, are performed to define the level of right and left ventricular function. The level of pulmonary pressure is determined, when feasible, by Doppler echocardiography. Additional investigations in the assessment of pulmonary hypertension are not performed routinely, as effective therapy is not available and a high complication rate occurs during instrumentation and pharmacological manipulation of these patients[25].

Finally, testing of exercise capability is carried out to assess overall functional competence and cardiac and pulmonary reserve. The 6-minute walk test and treadmill exercising (1 mile per hour and a 4% gradient) are utilized by most centers. This test serves as a baseline for future tests and may also help to pick out those patients who might require CPB intraoperatively. As it is not unusual for patients to double their exercise tolerance during a period of rehabilitation prior to transplantation, testing should be repeated after a suitable period of conditioning.

DONOR SELECTION

The shortage of donors has necessitated a relaxation of the previous criteria for potential organ donors (Table 3). Unfortunately, despite better donor management, <25% of donors of other solid organs are suitable for lung donation. This is due to the susceptibility of the lung to injury associated with: (a) brain death (particularly aspiration), (b) prolonged ventilation, (c) direct lung injury at trauma, and (d) hemodynamic instability and fluid accumulation. Conscientious attention to details of management is important in sustaining the integrity of potential donor organs. Management of the catecholamine burst, reported to occur at the time of brainstem ischemia, with control of the associated systemic and pulmonary hypertension, may attenuate pulmonary (neurogenic edema) and cardiac (myocardial dysfunction) complications. This is turn may minimize the exudation of fluid into the lungs. Nonetheless, it is advisable to monitor the patient, at least with a central venous catheter (CVP), and not to exceed a venous pressure of 7 cmH_2O[26].

The procurement of donor lungs involves the direct administration of 500 μg of prostaglandin E1 into the pulmonary artery. Modified Euro-Collins or University of Wisconsin flush solution (100–150 ml/kg) is delivered at 4°C through a large-bore cannula inserted into the main pulmonary artery. Decompression of the heart and lungs is achieved by division of the inferior vena cava and left atrial appendage. The lungs are ventilated with 100% oxygen before clamping of the trachea. It has become standard practice to harvest both lungs, whenever feasible, without affecting the use of the heart for transplantation. 'Twinning', the use of a donor lung block to provide two single lungs for separate patients, has increased the donor pool.

Table 3 Criteria of donor lung suitability

Age < 55 years
ABO compatibility
Chest roentgenogram
Clear
Adequate size match
History
Smoking ≤ 20 pack-years
No significant thoracic trauma (blunt, penetrating)
No aspiration/sepsis
Gram stain and culture data (if prolonged intubation)
No prior cardiac/pulmonary operation
Oxygenation
Arterial oxygen tension ≥ 300 mmHg, on inspired oxygen fraction of 1.0, 5 cmH_2O PEEP

OPERATIVE PROCEDURE

Single lung transplantation

Single lung transplantation is performed using a posterolateral thoracotomy. The patient is positioned to allow access to the ipsilateral femoral vessels in the event that CPB needs to be instituted because of hypoxemia, hypercarbia, and/or right ventricular decompensation on clamping of the pulmonary artery. (In the earlier lung transplant operations the first intervention, prior to the thoracotomy, involved the mobilization of the omentum, which was passed into the chest cavity and subsequently used to wrap the bronchial anastomosis of the implanted lung. Due to a high incidence of difficulties with gastrointestinal motility and feeding, and because of lack of proven benefit, this aspect of the technique has been abandoned[27].)

After thoracotomy the lung is collapsed, the ipsilateral pulmonary artery is isolated, and a trial clamping is performed. Significant right ventricular decompensation may ensue due to an increase in pulmonary artery pressure. If function does not improve with pulmonary vasodilators and/or inotropes, the clamp is removed and CPB is instituted. When a right thoracotomy has been performed, this can be done by cannulating the right atrium and the ascending aorta. In a left thoracotomy, cannulation of the descending aorta and main pulmonary artery can be easily carried out. Femoral cannulation is an alternative for both situations.

The pneumonectomy is performed by dividing the pulmonary artery beyond the level of the first upper lobe branch. The pulmonary veins are divided as distally as possible, and the bronchus is bisected. Particularly in patients with cystic fibrosis and Eisenmenger's syndrome, the bronchial arteries may be greatly enlarged and be a cause of significant bleeding.

After the removal of the native lung the recipient's pericardium surrounding the pulmonary veins is opened circumferentially to permit placement of a left atrial clamp as centrally as possible. The bronchus is next anastomosed, usually by 'telescoping' the smaller bronchus into the larger bronchus to a distance of one or two cartilaginous rings. This maneuver has obviated the need for the omental wrap, although most surgeons still surround the anastomosis with peribronchial tissue, pericardium, or parietal pleura. The pulmonary artery anastomosis is completed, followed by the atrial anastomosis. Before completion of this anastomosis the pulmonary artery clamp and, subsequently, the left atrial clamp, are removed to evacuate any residual air in the vasculature. Lung inflation may assist the process of evacuation of air.

Bilateral lung transplantation

The initial bilateral lung replacements, designated double lung transplantation, were performed via a median sternotomy en-bloc, with a tracheal anastomosis, necessitating CPB. Morbidity and mortality were significant due to hemorrhage from the poorly exposed posterior mediastinum, and poor healing of the tracheal anastomosis because of poor collateral blood flow from the pulmonary artery circulation. After en-bloc double lung transplantation, the latter is the only source of blood supply to the trachea and bronchi, as the bronchial arteries have been sacrificed.

The first modification introduced was bilateral bronchial anastomoses, through a median sternotomy, with CPB. Because collateral blood flow to the main bronchi is greater than to the trachea, it has been argued that healing of these anastomoses is better. Nowadays, sequential single lung transplantation with bilateral bronchial anastomoses through a bilateral anterothoracosternotomy is the preferred technique. The functionally worse lung is transplanted first, in the manner outlined above for a single lung transplant, followed by the second lung. During the transplantation of the first lung the patient is dependent on his/her other native lung, and during the second transplant is dependent on the newly implanted lung. CPB is often not necessary but, if required, cannulation of the right atrium and ascending thoracic aorta can be easily and quickly carried out.

ANESTHETIC MANAGEMENT

Induction and maintenance

All lung transplants are performed with CPB capabilities readily available. The anesthetic requirements are listed in Tables 4 and 5. Peripheral venous and arterial catheters are inserted prior to induction. A modified rapid sequence induction is performed as the recipient is not always appropriately fasted. The patient is preoxygenated and induction can be accomplished with fentanyl (10–20 μg/kg) and incremental sodium thiopental (50–70 mg) while maintaining cricoid pressure. Intubation is done after the administration of succinylcholine (1.5 mg/kg), and muscular paralysis is sustained with a long-acting muscle relaxant. Intubation is with a left double-lumen endotracheal tube, which is checked for proper placement. Frequent endobronchial suction is essential to prevent tube blockage and atelectasis, particularly in cystic fibrosis patients[28].

The patient is connected to a ventilator that has air/oxygen mixture capability and a variable positive-end-expiratory-pressure (PEEP) valve. Initially, the F_iO_2 is maintained at 1.0 and is subsequently adjusted to maintain an Sa_{O_2} >90%. Ventilation is adjusted based on the results of arterial blood gases or the continuous intra-arterial blood gas monitor. In patients with obstructive lung disease it is of the greatest importance to avoid hyperinflation by ventilating with a low inspiratory/expiratory

Table 4 Anesthetic and monitoring equipment

ECG monitor
Arterial and pulmonary artery pressure monitors
Extracorporeal cardiopulmonary bypass machine with oxygenator
Pulse oximeter and end-tidal CO_2 monitor
Ventilators (anesthetic and constant-flow generators)
Left double- and single-lumen endotracheal tubes
14F Fogarty venous catheter (bronchial blocker)
Peripheral intravenous, arterial and pulmonary artery catheters

Table 5 Anesthetic medications

Fentanyl
Sodium thiopental
Succinylcholine/pancuronium or vecuronium
Isoflurane
Benzodiazepines
Dopamine, nitroglycerine, and phenylephrine in separate infusion systems

ratio and avoiding large tidal volumes. A pulmonary artery catheter is inserted via the internal jugular vein with an 80 cm length protection sheath so that the catheter can be maintained sterile during the necessary adjustments required: (a) during placement, (b) during the surgical procedure, and (c) by the surgeon. A nasogastric tube and oropharyngeal temperature probe are inserted. A Foley catheter is placed into the urinary bladder. Baseline values are obtained of hemodynamics, arterial blood gases, activated clotting time, and potassium and glucose levels.

Anesthesia is maintained with supplemental fentanyl, valium and isoflurane. Nitrous oxide is avoided because of blunting of the hypoxic pulmonary vasoconstrictive reflex[29]. There are theoretical considerations in the use of volatile anesthetic agents because of their effects on the pulmonary vasoconstrictive response to alveolar hypoxia. However, their pulmonary vasodilator properties may be useful in right ventricular afterload reduction. In practice, low concentrations of halothane or isoflurane have been recommended for one lung anesthesia[30].

INTRAOPERATIVE PROBLEMS

Hypercarbia

Hypercarbia to some degree or other occurs in all patients, either because of inability to administer an adequate tidal volume due to high peak airway pressure, as is the case in patients with restrictive disease, or due to dead space ventilation and hyperinflation in patients with obstructive lung disease. The latter type of patient should be closely monitored, especially on attachment to the ventilator, for hemodynamic instability due to an increase in mean intrathoracic pressure from air trapping[31]. This phenomenon can be exacerbated by relative dehydration; during induction it is advisable to administer fluid volume liberally to patients with obstructive lung disease.

If hypercarbia is tolerated by the patient, attempts at achieving normal levels of CO_2 should be avoided. Blood gases should be monitored closely because, due to the large dead space in some of these patients, correlation between $PaCO_2$ and end-tidal PCO_2 is poor. Other possible causes of hypercarbia are atelectasis, pneumothorax, and a displaced double-lumen endotracheal tube. It is very infrequent that a patient requires CPB because of hypercarbia alone.

Hypoxemia

Hypoxemia may occur at various times during the procedure. Early, during one lung ventilation, hypoxemia may develop due to shunting; it often improves with clamping of the ipsilateral pulmonary artery. In some cases, which will be detailed later, hypoxemia may persist, despite various manipulations, necessitating the institution of CPB.

Hypoxemia may be noted in sequential single lung transplantation during the second lung transplant because of inadequate function of the first transplanted lung, usually due to pulmonary edema. During the second transplant, high pulmonary pressures and/or high pulmonary capillary wedge pressures are to be avoided because of the propensity of the first transplanted lung to edema formation[32]. Pulmonary vasodilators, inotropic agents and diuretics may be beneficial. CPB needs to be initiated if improvement does not result.

At the completion of the operation the double-lumen endotracheal tube is replaced with a single-lumen tube and the patient is transferred to the intensive-care unit. The post-transplant management of these patients is described elsewhere (Chapter 53).

Cardiopulmonary bypass

CPB is not used routinely in pulmonary transplantation, but should always be available. It increases technical complexity and prolongs operative time and the length of allograft ischemia. Furthermore, the necessary volume-loading and systemic anticoagulation may compromise hemodynamic stability and increase bleeding, with subsequent allograft deterioration. In addition, neurologic damage is an infrequent but distressing sequela of CPB.

Over a 10-year period from 1983 the Toronto Lung Transplant Group performed 153 lung transplants. In the single lung transplant group ($n = 53$), 13 patients required elective CPB because of primary or secondary pulmonary hypertension. Of the remaining 40 single-lung recipients, 12 (30%) required emergency CPB secondary to hemodynamic instability and/or hypoxemia. It has been suggested that a decrease in cardiac index >1.5 l/min per square meter after pulmonary artery clamping, rather than the degree of pulmonary hypertension, should be the criterion for institution of CPB[33]. All 12 patients requiring CPB had restrictive lung disease (12/29 patients). None of the 11 patients with obstructive lung disease required CPB. In the restrictive disease group of patients the pretransplant 6-minute walk test, arterial oxygen saturation, oxygen requirement on exercise, and right ventricular ejection fraction (<27%) were all significantly different between those requiring CPB and those not[34].

In the double lung transplant group ($n = 100$), 18 patients underwent en bloc double lung transplantation and 82 patients underwent SSLT. In the latter group, 13 patients underwent elective CPB. Of the remaining 69 SSLT recipients, 26 (38%) required emergency CPB secondary to hemodynamic instability and/or inadequate arterial gases. For SSLT no good clinical preoperative predictors for CPB were found; the need of CPB is dependent on intraoperative factors[34,35].

The decision to institute CPB is determined by the presence of hypoxemia, pulmonary hypertension, respiratory acidosis, or systemic hypertension. The former two factors are the most important, and attempts should be made to optimize cardiac function through the use of inotropes, maintaining adequate mean systemic blood pressure, and fluid administration. Pulmonary vasodilators are generally ineffective because of adverse systemic effects, although beneficial results from the use of inhaled nitric oxide have been reported. The use of transesophageal echocardiography has been recommended to assess right ventricular function during pulmonary artery clamping, and to evaluate the response to therapy.

COMMENT

It is, indeed, a credit to the development of anesthesia that the intraoperative mortality in these highly complex cases is low. The

major challenge in anesthetic management is still that of right ventricular function. Whether inhaled nitric oxide will facilitate the intraoperative management of these cases is still an unanswered hope.

A marked reduction in the previously common anastomotic complications has occurred. The greatest remaining challenges in lung transplantation are donor availability, cytomegalovirus infection, and bronchiolitis obliterans. Unfortunately, the incidence of bronchiolitis obliterans has increased considerably, and the evidence suggests that all transplants develop this complication to various degrees. This problem may be due to inadequate immune suppression.

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Surgical Techniques of Single and Bilateral Lung Transplantation

H.A. GAISSERT AND G.A. PATTERSON

INTRODUCTION

Over the past decade, single (SLTx) and bilateral (BLTx) lung transplantation have become accepted therapies for patients with end-stage lung disease. The choice between SLTx and BLTx has been primarily determined by the underlying disease process. Suppurative disorders, such as cystic fibrosis or bronchiectasis, require obligatory replacement of both lungs. Conversely, adequate correction of the physiologic defect in pulmonary fibrosis and pulmonary hypertension has been achieved with SLTx. Emphysema was originally treated with double lung or combined heart–lung transplantation so as to avoid compression of a single lung allograft by the overly compliant contralateral native lung following SLTx. Subsequent experience showed that replacement of a single lung was well tolerated. The persistent shortage of donor organs necessitates the use of single lung allografts in suitable patients to allow organ sharing. However, current data suggest that there is a survival advantage for recipients of bilateral grafts[1] (Chapters 65 and 69). Yet providing an individual patient with two grafts may conflict with the needs of the total recipient population. This issue is particularly acute for patients with chronic obstructive lung disease.

With increasing worldwide experience in lung transplantation, important technical modifications have been made. *En-bloc* double lung transplantation has been replaced by a bilateral sequential single lung technique because of technical difficulties and a high incidence of postoperative complications associated with the former approach. Coverage of the bronchial anastomosis with omentum, once part of every lung transplant, has been abandoned as a routine. Protection of the bronchial anastomosis with local peribronchial tissue and early use of steroids have since led to a marked decline in anastomotic complications. Routine bronchial artery revascularization has been advocated for single[2] and bilateral[3] procedures. However, long-term follow-up is lacking in these reports, and to date there is no compelling evidence that bronchial revascularization provides overall superior results.

In the following sections we will describe our approach to SLTx and BLTx. In recent years we have preferred bilateral transplant whenever possible, except for patients with pulmonary fibrosis. Therefore, the following discussion will emphasize that preference.

ANESTHESIA (see also Chapter 49)

Adequate hemodynamic monitoring during the entire procedure is critical. A systemic arterial catheter, Swan-Ganz pulmonary artery catheter and urinary catheter are routine. Inevitable systemic hypothermia reduces reliability of a radial artery catheter. Consequently we utilize a femoral artery catheter whenever we anticipate a lengthy or technically challenging procedure. Intraoperative transesophageal echocardiography is employed routinely. This has been particularly helpful in assessing the effect of elevated pulmonary artery pressures on right ventricular function so as to determine the need for cardiopulmonary bypass[4].

After induction of general anesthesia, mechanical ventilation is established. For patients with emphysema it is critical to monitor hemodynamics carefully at this point. Air trapping can occur quickly, with resultant decreased venous return and severe hypotension. Deliberate hypoventilation with small tidal volumes and a long expiratory phase avoids this problem. In experienced hands, manual ventilation best accomplishes this aim. We prefer selective lung ventilation in all patients. A left-sided Robertshaw endobronchial double lumen tube is placed, when necessary with bronchoscopic guidance. In children with smaller airways an inflatable bronchial blocker may prove helpful. However, routine use of cardiopulmonary bypass in this age group has been employed successfully and appears more practical[5].

Profuse bronchial secretions may totally obstruct the narrow airway of a double lumen tube in a relatively short time, particularly in suppurative lung disease such as cystic fibrosis. In such cases, bronchoscopic aspiration and irrigation of secretions is therefore performed immediately after intubation through a single lumen endotracheal tube, which is then exchanged for a double lumen tube. Suctioning with a catheter is continued repeatedly by the anesthesiologist until both recipient lungs are extracted.

CARDIOPULMONARY BYPASS

Cardiopulmonary bypass (CPB) is mandatory for the conduct of LTx in patients with primary or severe secondary pulmonary hypertension. For the rest of the adult population, CPB is not routinely employed during SLTx or BLTx. A number of intraoperative situations require the institution of CPB: (a) severe pulmonary hypertension, (b) significant hypercarbia, (c) persistent hypoxemia, or (d) unacceptable hypotension produced by the necessary retraction for dissection and implantation[4]. Test-clamping of the pulmonary artery is performed prior to recipient pneumonectomy, to assess pulmonary hypertension. Although patients with moderately elevated pulmonary artery pressures often respond to anticipatory use of prostaglandin E1 and systemic vasoconstrictors, the diseased contralateral vascular bed does not reliably respond to pharmacologic intervention, and right ventricular failure may develop. Transesophageal echocardiography has been very helpful in assessing right ventricular performance at this juncture.

Another reason for CPB is the failure to adequately ventilate the contralateral native lung. Most commonly this occurs as the result of sputum retention in suppurative lung disease. The purulent sputum is tenacious and cannot easily be aspirated through the small suction catheters passed through the bronchial or tracheal lumen of the double lumen tube.

In our experience the necessity for CPB arises most commonly in the setting of severe pulmonary hypertension during contralateral pneumonectomy following implantation of the first graft. The reason for intraoperatively impaired graft function is unclear, but could be related to incomplete graft preservation or to bacteremia during extraction of the native lung, particularly in patients with cystic fibrosis. Prolonged pulmonary hypertension will lead to edema of the freshly implanted graft and hypoxemia. Prior to committing the patient to the risk of CPB it is imperative to be sure that pressures are being recorded distal to the first pulmonary artery anastomosis. Pressures obtained proximal to the pulmonary arterial anastomosis may not reflect pulmonary artery pressures to which the graft is exposed. When the entire cardiac output flows to the transplanted lung, a large gradient may be present across the anastomosis despite the absence of obvious anastomotic narrowing[6]. Advancing the tip of the Swan-Ganz catheter across the anastomosis will provide a more accurate measurement. After implantation of the first lung, constant attention is devoted to hemodynamic parameters and gas exchange, to observe early signs of functional deterioration. When pulmonary hypertension and right ventricular dysfunction persist, early elective institution of CPB is preferable to emergency measures. It is also important to be certain that there is no metabolic cause of pulmonary hypertension, such as hypoxemia or acidosis.

The retraction necessary to accomplish safe pneumonectomy and implantation may produce severe hypotension and elevated central venous pressures – a dangerous combination especially for cerebral perfusion. Careful retraction can usually resolve this problem. However, prolonged hypotension is unacceptable. Unless the problem can be easily managed, CPB should be instituted.

Both lateral thoracotomy and bilateral thoracosternotomy incisions provide sufficient access for standard techniques of CPB. The ascending aorta and right atrial appendage are selected for cannulation during right lateral thoracotomy and anterior thoracosternotomy, whereas the descending thoracic aorta and main pulmonary artery are used when cannulation is required for left SLTx. We and other authors[7] have observed a dramatic reduction of intra- and postoperative bleeding with aprotinin (Trasylol), a protease inhibitor. When CPB is anticipated for any indication, we administer aprotinin routinely.

BILATERAL LUNG TRANSPLANTATION

Positioning

The patient is positioned supine. The arms are placed at the sides, which in almost every patient affords excellent exposure to the anterior and lateral chest. In patients with a normally shaped thorax, elevation of the chest on folded sheets improves access to the lateral chest wall. Alternatively, the arms may be raised and supported over the face. Another option is to abduct both arms. However, both of these positions risk traction injury to the brachial plexus or compression nerve injury elsewhere in the upper extremity. Furthermore, the operative field for the surgeon and an ipsilateral assistant is greatly compromised unless the arms are placed at the sides. The supine position exposes the peroneal nerves at risk, especially if the knees are extended. We have had several patients with transient postoperative 'foot drop'. Therefore, in all patients a knee-roll is used. In addition, ankles and elbows are carefully padded.

A generous operative field is created to include the entire chest, as far lateral as possible, the abdomen, and both groins. The patient is adequately secured to the table, since maximal rotation of the table away from the surgeon is employed to facilitate access to the hilum.

Incision

A bilateral anterior thoracosternotomy is performed connecting both mid-axillary lines through the fourth or fifth intercostal space (Figure 1). The level of incision is determined by the expected level of the hilum and the location of any anticipated pleural adhesions. In patients with chronic obstructive pulmonary disease the fifth intercostal space is usually adequate. However, during transplantation for cystic fibrosis, we prefer access through the fourth intercostal space to gain access to often extensive apical adhesions and to an upwardly displaced pulmonary hilum. Transverse division of the sternum is accomplished with the saw once the internal mammary vessels have been ligated and divided.

Recipient pneumonectomy

The lung with the least amount of function as determined by preoperative quantitative ventilation–perfusion scintigraphy is always transplanted first. If lung function is equal on both sides, the right side is transplanted first. Not only is the right lung larger, therefore perhaps decreasing the chance of having to use CPB, but access to the left hilum requires extensive retraction of the heart, and consequent hypotension. Such handling may be better tolerated with a well-functioning transplanted lung on the right side.

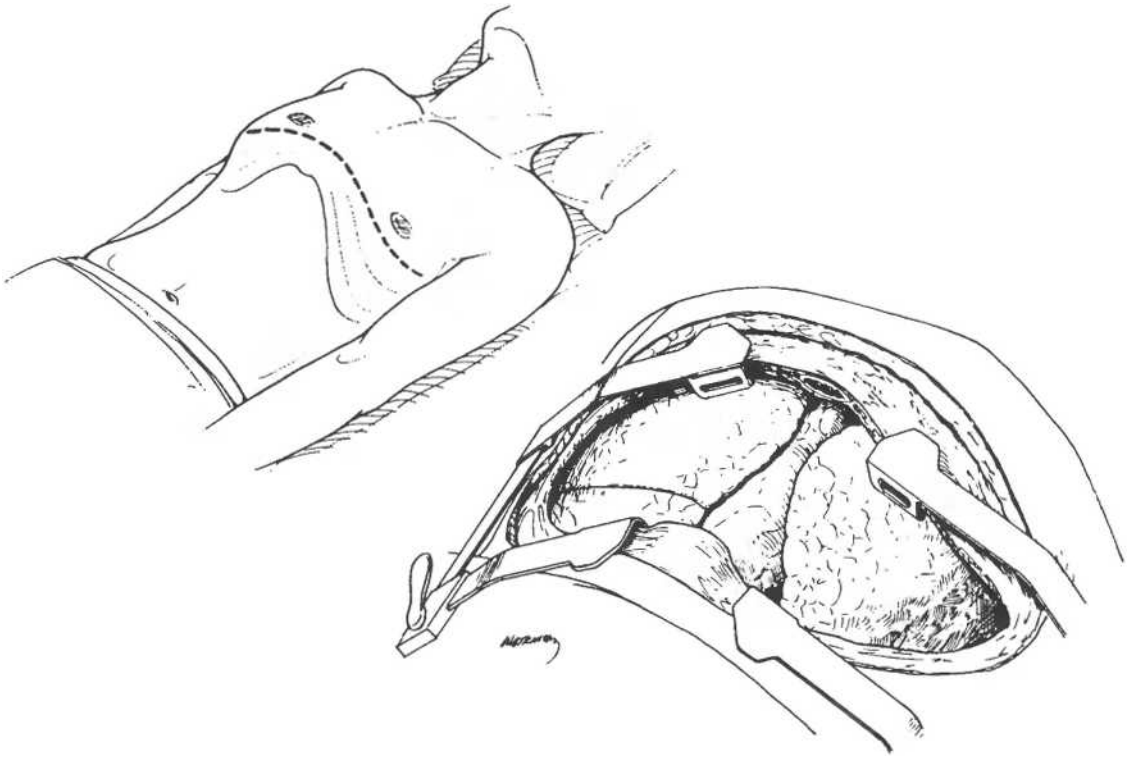

Figure 1 For bilateral lung transplantation, bilateral anterolateral thoracotomies are performed through the fourth or fifth intercostal spaces with transverse division of the sternum. This provides good exposure to both hilar regions. (All figures in this chapter are from Shields TW: General Thoracic Surgery. Malverne, PA: Williams & Wilkins; 1994, with permission)

If the patient will tolerate the necessary retraction, we prefer early mobilization of both hila and preliminary lysis of adhesions to decrease the ischemic time of the second lung to be implanted. It is imperative to avoid injury to either of the phrenic nerves or the left recurrent laryngeal nerve.

Pneumonectomy of the recipient lung is performed (Figure 2). The pulmonary artery is circumferentially mobilized and temporarily clamped, to assess the extent of pulmonary hypertension and right ventricular dysfunction. The superior and inferior pulmonary veins are mobilized and segmental branches divided between ligatures to create maximal length on the atrial stump. The upper lobe branch of the pulmonary artery is ligated and divided. The main arterial trunk is divided with a vascular stapler beyond the ligated upper lobe branch. Peribronchial and subcarinal nodal tissues are ligated and divided. In patients with cystic fibrosis, inflamed and vascular lymph nodes render this portion of the dissection tedious. Appropriate hemostasis at this point is nevertheless important. The main stem bronchus is divided proximal to its upper lobe branch, while avoiding lateral dissection to prevent devascularization. Individual bronchial arterial bleeders are cauterized or ligated. A complete pericardial release is performed, incising the pericardium close to its reflection on the pulmonary veins. The anterior pericardium on the left side is not incised until the atrial anastomosis is performed, to prevent the left atrial appendage from protruding over hilar structures. The release dramatically improves access to the left atrium. Meticulous hemostasis is achieved since visualization of this area is limited during the later part of the procedure.

Preparation of the donor lungs

The donor double lung block is divided immediately before implantation (Figure 3). Left atrium, pulmonary artery bifurcation, and proximal left main stem bronchus are divided. The pulmonary artery is circumferentially freed to the take-off of the first upper lobe branch. Hilar tissue superior to the pulmonary artery is divided to avoid kinking of the artery subsequent to anastomosis. Excess pericardium is trimmed. The donor bronchus is shortened to leave two cartilaginous rings from the upper lobe origin and a generous length of membranous portion. The peribronchial tissues are left undisturbed to preserve blood supply. Donor lungs are stored in iced saline while awaiting implantation.

Donor lung implantation

For implantation, the donor lung is placed on the posterior surface of the pleural cavity and packed in iced laparotomy sponges. In patients with a normal or small pleural space (i.e. pulmonary vascular disease or pulmonary fibrosis), access to the hilum may be somewhat difficult when too many sponges are used. Due to the thawing of ice, a constant flow of saline will gravitate toward the

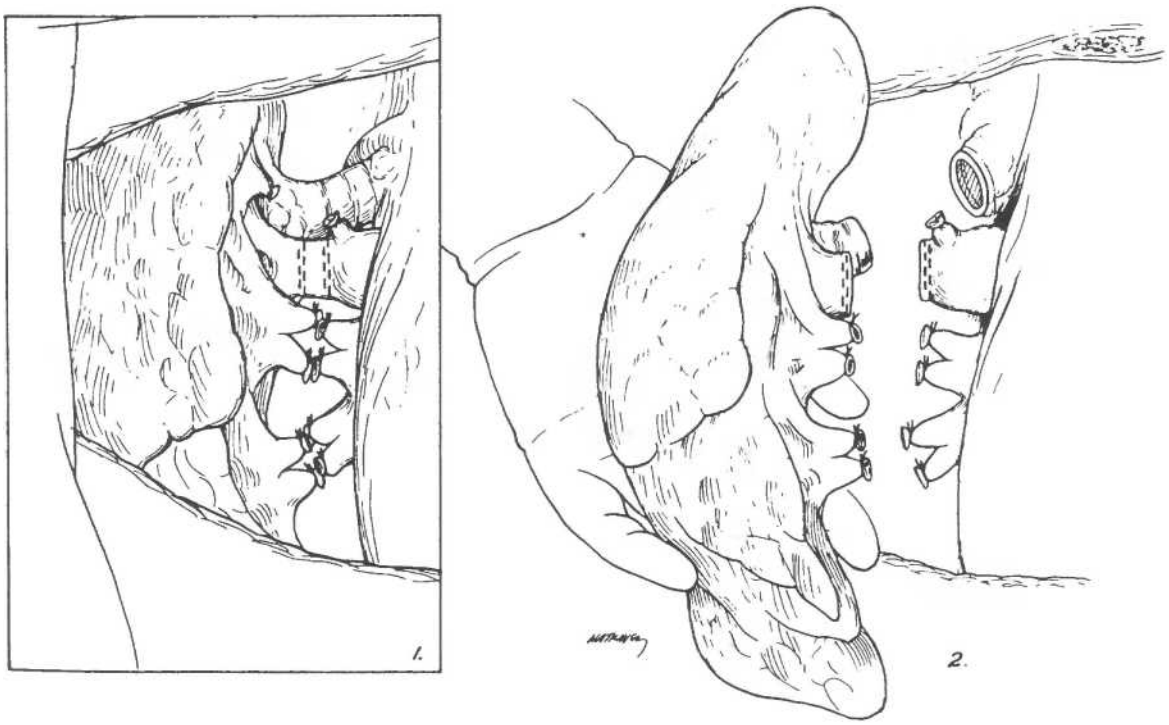

Figure 2 Excision of native right lung. The pulmonary artery (middle) is doubly stapled and divided beyond its first upper lobe branch. Segmental branches of the superior and inferior pulmonary veins (bottom) are divided between ligatures. The bronchus (top) is transected just proximal to the upper lobe orifice

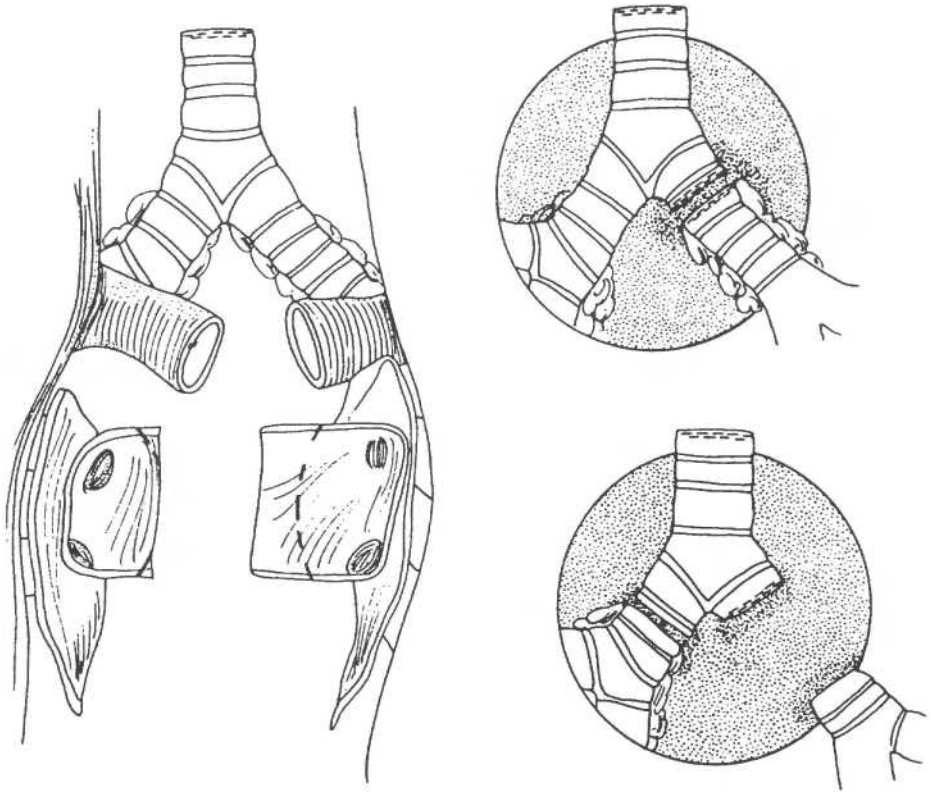

Figure 3 Preparation of the donor lungs. The pulmonary artery is divided at its bifurcation. The pericardium and left atrium are divided, with the left atrium being trimmed further (dotted lines). The airway is transected but kept sealed by using a GIA stapling device across the proximal left main stem bronchus. The donor right and left bronchi are further revised for implantation (as shown at the bottom right) leaving two cartilaginous rings from the upper lobe origin

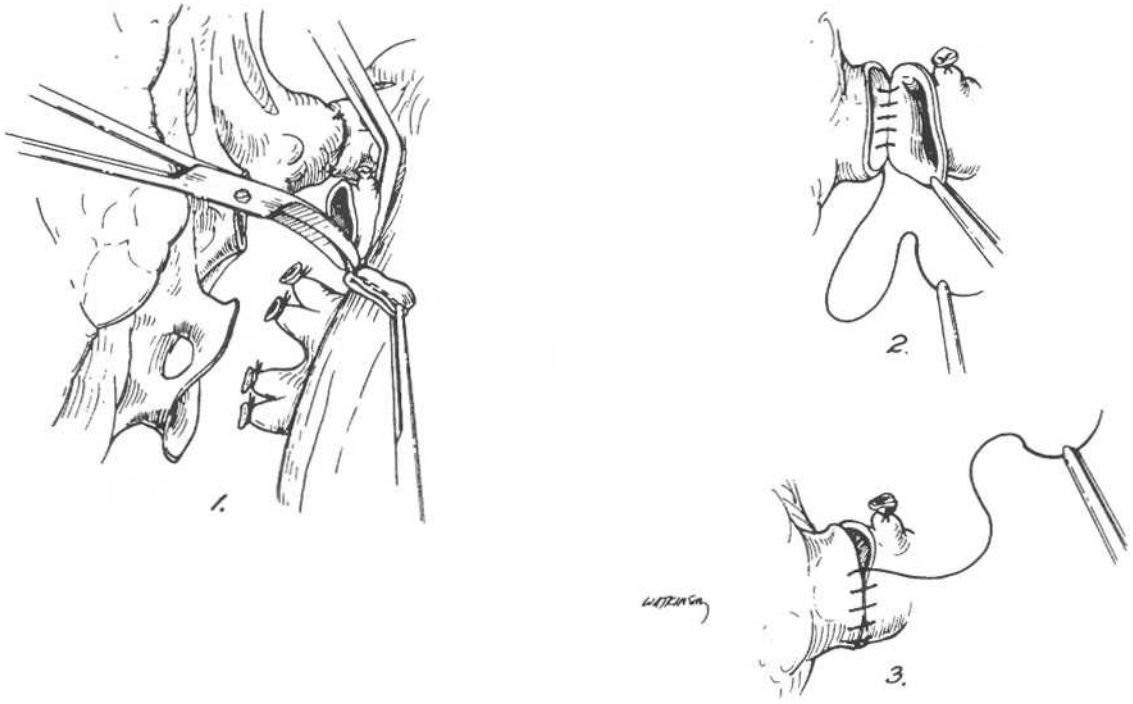

Figure 4 Implantation of donor right lung. The bronchial anastomosis has been completed. A vascular clamp is placed across the recipient pulmonary artery. The staple line is excised, and an end-to-end anastomosis is constructed with the donor pulmonary artery using 5/0 polypropylene

open recipient bronchus. The anesthetist therefore places a catheter on suction into the ipsilateral lumen of the endotracheal tube until the bronchial anastomosis is complete. The implantation proceeds with sequential construction of bronchial, arterial and left atrial anastomoses.

Exposure of the bronchus as the most posterior structure is aided by a traction suture through the bronchial wall and by retraction of the pulmonary arterial stump, which is gently grasped and displaced with a small Duvall clamp. A posterior layer of peribronchial tissue is gathered with a running 4/0 PDS suture. Donor and recipient bronchus are oriented by accurate placement of 4/0 PDS traction sutures through the corresponding junctions of membranous and cartilaginous wall. One traction suture is tied, then used to approximate the membranous wall in a running fashion, and tied to the other traction suture.

The anastomosis of the cartilaginous portion is constructed with five or six evenly spaced, figure-of-eight 4/0 PDS sutures. When both bronchi are small, a telescoping technique may obstruct the lumen, but if donor and recipient bronchi are sufficiently large, and of different diameter, they may be intussuscepted by telescoping one cartilaginous ring into the lumen of the larger bronchus. A recent review of bronchial anastomotic complications at our institution has discouraged us from using modified mattress sutures for this purpose, as this technique was associated with a higher incidence of stricture[8].

The integrity of the anastomosis is tested by inflating a small amount of air into the submerged bronchus. The anterior aspect of the bronchus is then covered with peribronchial tissue and pericardium using a running layer of 4/0 PDS sutures. We continue to believe that it is important to separate the bronchial and arterial suture lines by healthy tissue.

The recipient pulmonary artery is clamped centrally (Figure 4). The handle of the Satinsky clamp is temporarily secured to the chest wall to steady the artery. Both recipient and donor pulmonary arteries are maximally shortened to avoid inadvertent kinking of the anastomosis with graft inflation[9]. Correct orientation of recipient and donor pulmonary arteries is important, to avoid anastomotic torsion. The upper lobe branches are aligned and an anastomosis is performed with running 5/0 prolene. Deep bites of arterial wall are avoided, to minimize constriction at the anastomosis. While the artery remains clamped, the lumen is de-aired with cold saline before completing the anastomosis. At this point an initial bolus of methylprednisolone 500–1000 mg is administered in anticipation of allograft reperfusion.

The left atrial anastomosis is prepared by placing a large Satinsky clamp far onto the left atrial wall (Figure 5). This maneuver may be associated with a brief episode of hypotension. When systemic blood pressure has recovered, the recipient left atrial cuff is created by connecting all segmental vein branches.

Both atrial cuffs are trimmed again to prevent kinking. The anastomosis is created using running 4/0 prolene. During this phase a continuous infusion of PGE1 is commenced. We believe that PGE1 reduces reperfusion injury in preserved lung allografts[10].

The left atrial anastomosis is de-aired. The lung is gently inflated, the pulmonary arterial clamp is opened, and the left atrial clamp is briefly released. The anastomosis is then secured and the left atrial clamp removed.

Hemodynamic performance and gas exchange of the implanted lung are observed before proceeding with the contralateral pneumonectomy. The technique of pneumonectomy and implantation of the second lung is otherwise identical.

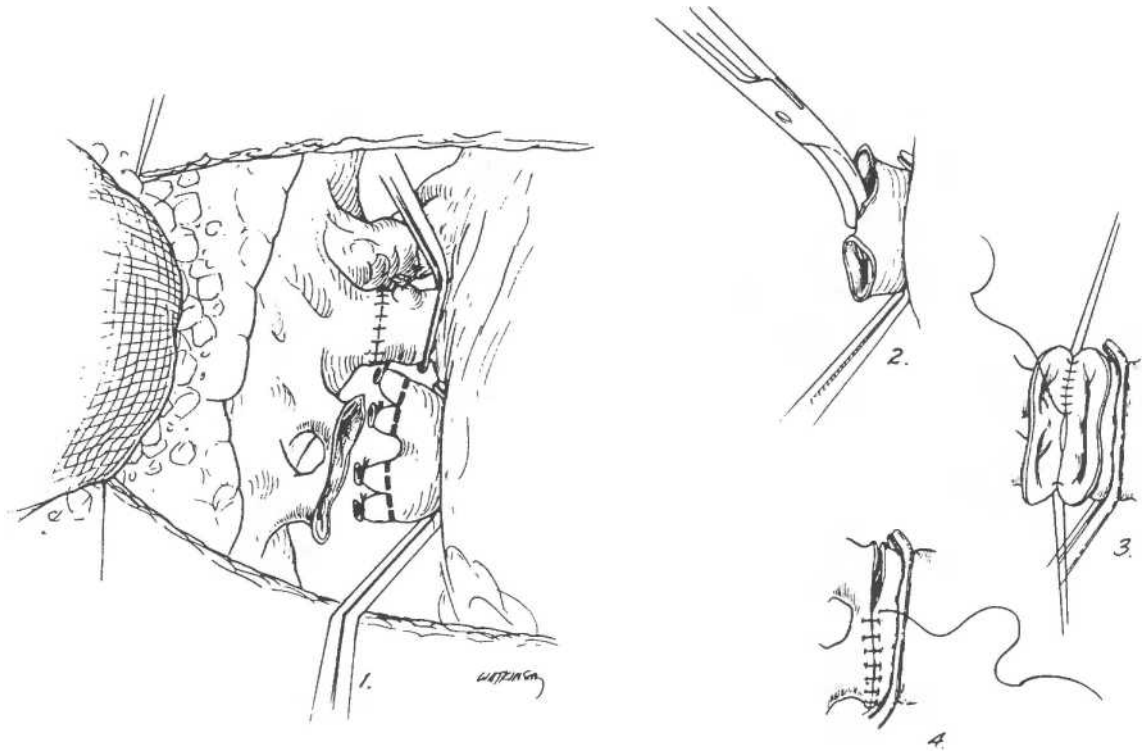

Figure 5 Implantation of donor right lung.The pulmonary artery anastomosis has been completed. A central left atrial (Satinsky) clamp is placed while the pulmonary venous stumps are amputated and the bridge of left atrial tissue is divided. A 4/0 polypropylene suture is used to complete the left atrial anastomosis

Closure

After hemostasis is secured, bilateral apical and basal chest tubes are inserted. The sternum is approximated with three no. 7 stainless-steel wires. Two short segments of heavy-gauge Kirschner wire may be used to prevent dislocation of the sternal ends. The K wire is vertically anchored in the marrow of upper and lower sternum before wire closure. Ribs are approximated with four to six no. 2 polypropylene pericostal sutures on each side. Subcutaneous tissues are approximated with running layers of absorbable suture material and the skin is closed with clips.

The double lumen endotracheal tube is exchanged to a single lumen tube and fiberoptic bronchoscopy is performed to confirm the integrity of both bronchial anastomoses and to remove residual secretions.

SINGLE LUNG TRANSPLANTATION

Choice of side

If possible, the lung with least function as determined by preoperative ventilation–perfusion scintigraphy is selected for transplantation. While a right lung graft was previously assumed to provide greater functional capacity, because of its larger size, it does not appear that right single lung transplant recipients have any functional advantage[11]. We therefore have no preference for a particular side in patients with emphysema and equal lung function. A right-sided approach does provide superior access for cannulation when cardiopulmonary bypass is planned, i.e. in patients with pulmonary vascular disease, pulmonary fibrosis with pulmonary hypertension, or for patients who require concomitant repair of PFO, ASD or VSD. For such patients with primary or secondary pulmonary vascular disease, a median sternotomy provides satisfactory exposure for cardiac repair and simultaneous right SLTx.

Exposure

For standard SLTx, a generous posterolateral thoracotomy through the fifth interspace, or through the bed of the resected fifth rib, is performed. Resection of the fourth rib instead may facilitate placement of an aortic cannula for CPB. Alternatively, a median sternotomy can be used if repair of intracardiac lesions for Eisenmenger's syndrome is to be combined with transplantation of the right lung. While we have previously used femoral access for bypass, we now prefer central cannulation through the thoracotomy. An additional incision in the groin and the required reconstruction of artery and vein are thus avoided. Nonetheless, we always include the ipsilateral groin in the operative field. For this purpose the leg is straightened and the hip is allowed to tilt

backwards once the patient is placed in the lateral thoracotomy position.

Recipient pneumonectomy and implantation

The technique of SLTx follows the procedure described for the initial side in the bilateral procedure. SLTx for emphysema is a very straightforward procedure facilitated by the absence of pleural adhesions and a large pleural space. However, for patients with pulmonary fibrosis of primary pulmonary hypertension, because of a reduced-sized pleural space, implantation can be tedious. A malleable retractor can be used to displace the ipsilateral diaphragm downward, thereby increasing the size of the pleural space and subsequent exposure. Another technical point is that superior exposure may be provided to the surgeon on the opposite side of the table in patients with a small pleural space. In this circumstance we have found it easier for this surgeon to conduct the implantation since he/she does not have to reach over the ice-covered allograft to the operative site.

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Lung Size and Impact on Transplantation

T.M. EGAN

INTRODUCTION

It is likely that organ size is a more important issue in lung transplantation than in transplantation of any other solid organ. Lungs must fit within the confines of the thoracic cage. This space has the capacity to change its size and shape with time; indeed, the very act of ventilation changes the volume of the thorax substantially by the amount of forced vital capacity. Because lungs are easily distensible, they have the ability to change their size and shape, and can frequently fill the space made available to them. In fact, the safe limits with respect to size mismatching between donors and recipients have not yet been established for lung transplantation.

Lung disease can affect the size of the thorax dramatically. Patients with chronic obstructive pulmonary disease have an increase in lung volumes manifested by flattening of the diaphragm and development of a so-called 'barrel-shaped' chest. Conversely, patients who develop restrictive lung disease frequently have contraction of the chest with a diminished anteroposterior diameter and elevation of the diaphragm. These factors may have an impact on appropriate size matching between recipients and prospective donors. What represents an acceptable size discrepancy between donor and recipient is an area of controversy. From a practical standpoint it is necessary to ascertain, for a given recipient listed for transplant, which donors have lungs that are an appropriate size.

Until recently, recipients listed with the United Network for Organ Sharing (UNOS) in the United States were listed with an acceptable donor weight range as a means of attempting to match donor size and recipient size. Lung size in healthy individuals, however, is a function of height, sex, age, and race, because these are the determinants of thoracic cage volume[1,2]. Often, patients with chronic lung diseases become debilitated and are underweight relative to population norms. Patients with cystic fibrosis (CF) suffer not only from end-stage lung disease but also from the effects of the CF abnormality on absorption, resulting in considerable imbalance between their weight and lung size. Principally for these reasons, UNOS policy has been changed to list potential lung recipients with acceptable height ranges for prospective donors, in an attempt to better size match donors and recipients.

Because prospective lung recipients may be substantially smaller than those in the potential donor pool, we have on several occasions performed lung resection to establish a better size match between donors and recipients at the time of transplant[3]. Perhaps the most dramatic example of 'down-sizing' for lung transplantation is the practice of living-related lobe transplant, where a lobe from one individual is transplanted into the hemithorax of a recipient and is expected to fill the space made available to it[4].

IMPACT OF SIZE MISMATCH IN LUNG TRANSPLANTATION

The amount of tolerable size discrepancy between lung donors and recipients is unknown. If donor lungs are too large for the chest in which they are placed, a form of tamponade physiology may become apparent at the time of chest closure. Subsequently, atelectasis and infection may pose a serious problem. A large size mismatch may result in impaired cough and ability to clear secretions. If donor lungs are too small for the intended recipient, then a pleural space problem may ensue. This might result in empyema, especially in recipients with infective end-stage lung disease. Overexpansion of the donor lung to fill the space may lead to parenchymal injury. A theoretical consideration is that overdistension of alveolar spaces might contribute to obstructive physiology by early closure of small airways, akin to the physiology of chronic obstructive lung disease. Despite these concerns, Lloyd *et al.*[5] demonstrated that organs from heart–lung donors larger than their recipients appeared to function well after transplant. Despite the potential for empyema and pleural space problems, this has not been a serious problem when bilateral lower lobe transplants have been performed for cystic fibrosis[6].

Many transplant programs have relied on radiographic measurements to assess the appropriate size of donors for prospective lung transplant recipients[7,8]. After analyzing 32 heart–lung recipients, Otulana *et al.*[8] determined that postoperative total lung capacity was more a function of *recipient* chest capacity than donor

lung size, implying that donor lungs adapted to the volume of the chest into which they were placed. Miyoshi *et al.*[9] recommended that a donor for double lung transplant should be chosen to approximate the predicted lung size of the intended recipient. However, an analysis of their 12 lung transplant recipients suggested that considerable size discrepancy between donor and recipients appeared to be well tolerated. In another analysis of 18 double lung transplant recipients, Massard *et al.*[10] argued that measurement of submammary thoracic perimeter was an appropriate way to match prospective donors with recipients, but they too had reported a wide range of donor-to-recipient total lung capacity, from 77% to 160%.

DONOR LUNG REDUCTION

In circumstances where donor lungs appeared at operation to be too large for the recipient chest hemithorax, we have performed some type of resection in 16 instances. On 15 occasions the recipient operation was double lung transplant or some variation, while on one occasion a 27-year-old female with pulmonary fibrosis and a predicted total lung capacity of 5.9 liters underwent right single lung transplant from a 32-year-old male donor with a predicted total lung capacity of 8.5 liters. Because of the size discrepancy between the donor lung and the recipient hemithorax, a right middle lobectomy was performed. The variety of resections performed to reduce the size of donor lungs for double lung transplant recipients and the calculated impact on lung capacity is depicted in Figure 1.

An analysis of this 'pneumoreduction' strategy for recipients of double lung transplants demonstrated the safety and long-term functional outcome to 12 months, which was equivalent to recipients of lungs that were not subjected to pneumoreduction[3]. Two double lung recipients who had a form of donor pneumoreduction were excluded from this analysis. One patient with bronchiectasis had had a previous left pneumonectomy and herniation of his native lung across the midline to occupy a substantial portion of the left hemithorax. This patient was transplanted with a right lung and left lower lobe to occupy the left pleural space. His operation necessitated cardiopulmonary bypass and was complicated by excessive postoperative blood loss due to coagulopathy. This probably contributed to the subsequent development of ARDS, and he succumbed from multiple organ failure 11 days post-transplant. The other patient, a 24-year-old female with post-infectious end-stage lung disease complicated by a pectus excavatum abnormality, was excluded from analysis because the recipient/donor size mismatch in her case was more a function of her altered chest size than a donor with a substantially larger lung capacity. Since publication of our experience with 11 pneumoreduction procedures in double lung transplant recipients, two additional patients with CF have been transplanted with lungs that were subsequently size reduced.

Because thoracic volume is related to height, age, sex, and race, but not weight, there is a much better relationship between predicted forced vital capacity (FVC) and total lung capacity (TLC) and height than between these predicted values and weight (Figures 2 and 3).

For patients undergoing pneumoreduction surgery after completion of double lung transplant, predicted total lung capacity can be recalculated by reducing predicted total lung capacity of the donor by a fraction representing an approximate number of segments removed with the pneumoreduction procedure (see Figure 1). The impact of pneumoreduction on the calculated size difference between donor TLC and recipient TLC is depicted in Figure 4. Corrected donor total lung capacity refers to the estimated impact of a pneumoreduction procedure on donor total lung capacity calculated using the fractions outlined in Figure 1, based on the estimate of number of segments resected. Table 1

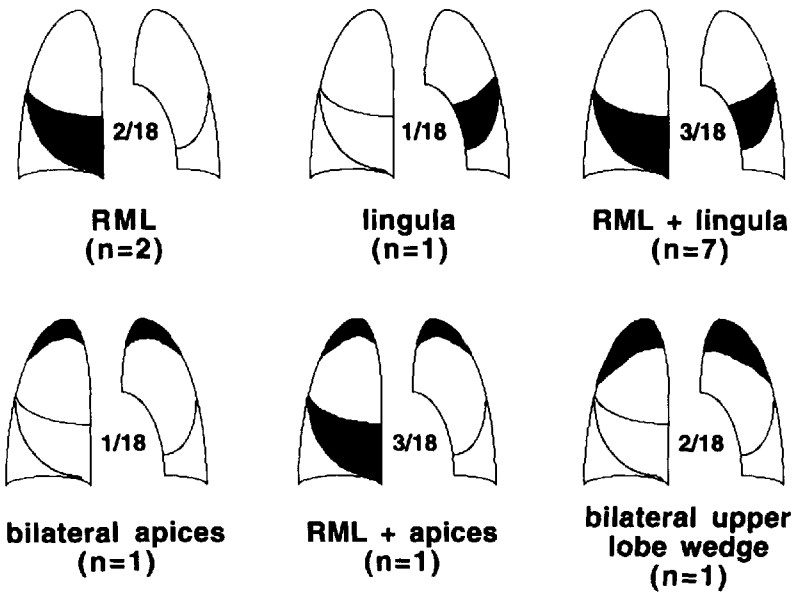

Figure 1 Type and number (*n*) of size reduction procedures performed on recipients of double lung transplant. The fraction represents the expected impact of each resection on predicted total lung capacity, based on an approximation of the total number of pulmonary segments resected

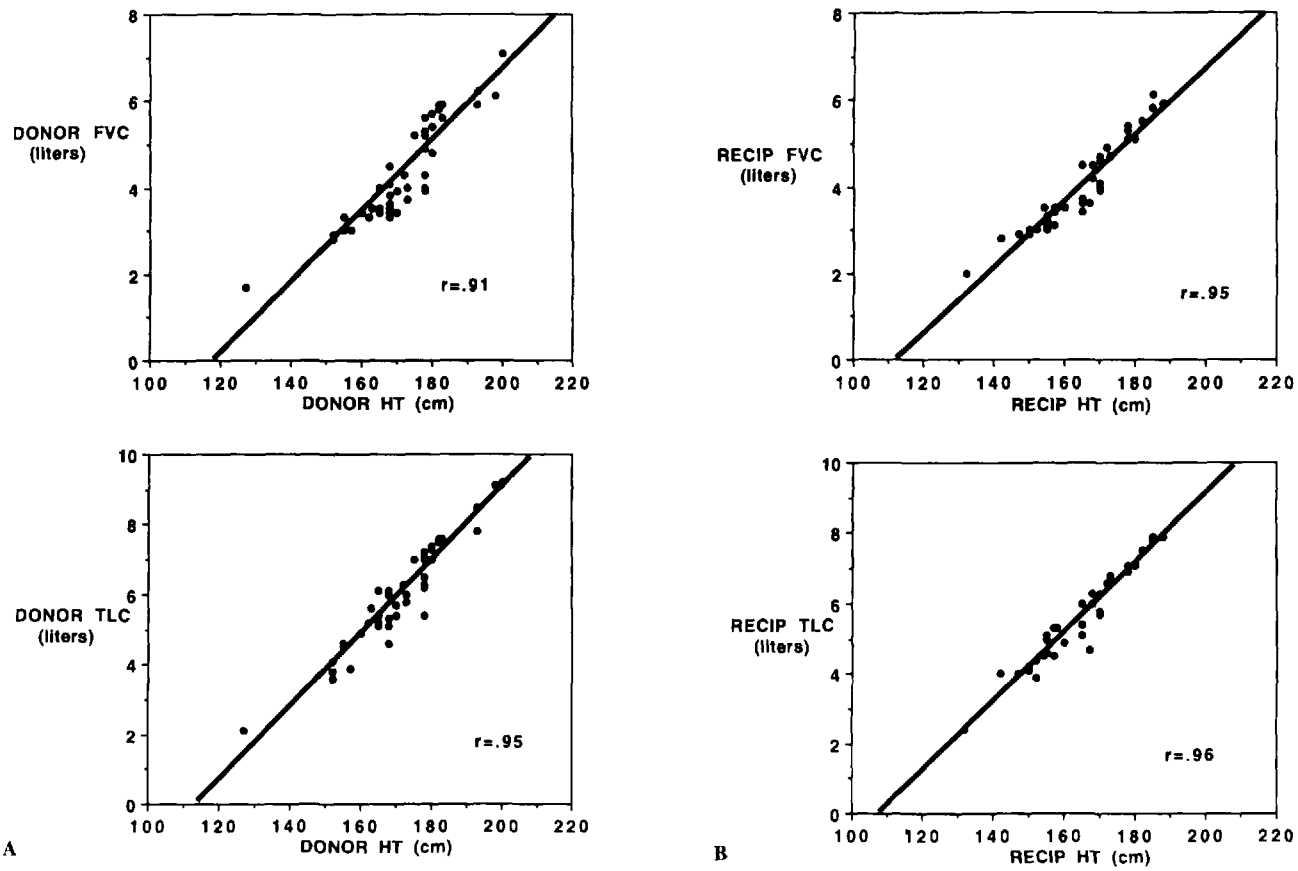

Figure 2 A: Donor FVC and TLC vs. height; B: recipient FVC and TLC vs. height. (Reproduced with permission, from ref. 3)

outlines the differences between recipients of double lung transplant and their prospective donors, according to whether the donor was size reduced. Four patients experienced catastrophic graft failure and were retransplanted; thus there are 69 donor/recipient pairs in this analysis of 65 patients with double lung transplants. Recipients who had donor lungs size reduced had a significantly greater discrepancy between calculated donor total lung capacity and recipient total lung capacity, regardless of whether this discrepancy was assessed by an absolute difference or a ratio of recipient-to-donor total lung capacity. After pneumoreduction and recalculation of a new donor total lung capacity (corrected total lung capacity), the differences between recipients whose lungs were size reduced and those whose lungs were not no longer reach statistical significance.

Although pneumoreduction improved the correlation between donor total lung capacity and recipient total lung capacity (Figure 4), from a correlation coefficient, r, of 0.46–0.56, there is still considerable variation between donor and recipient calculated total lung capacity. This discrepancy has had little impact on outcome, in our experience, although it is interesting to note that in two circumstances where donor predicted total lung capacity was substantially less than recipient total lung capacity, acute graft failure led to a requirement for urgent retransplantation. Nevertheless, in one instance in which bilateral lower lobe transplants were performed in a recipient with cystic fibrosis, no difficulty was encountered, despite a large size discrepancy between the predicted total lung capacity of the transplanted lobes compared with the predicted total lung capacity of the recipient.

Figure 5 depicts the impact of pneumoreduction procedures applied to double lung transplant recipients on outcome. Pneumoreduction procedures *per se* have not been associated with identifiable complications.

IMPACT OF SIZE DISCORDANCE

Despite concerns that transplanting a larger lung into a recipient with a smaller chest cavity might lead to atelectasis, in our experience size mismatch has not been associated with the development of untoward complications. Figure 4 suggests that an equivalent number of recipients have donor lungs that are larger than their predicted total lung capacity as lungs that are smaller. The limits of size discrepancy remain unknown, but our experience indicates that, in circumstances when lungs appear to be too large to safely close the chest, it is safe to resect portions of the lung to 'downsize' donor lungs.

What is the impact of implanting lungs that are substantially smaller than the recipient's explanted lungs? These issues have been addressed in several studies of lobar transplants in neonatal swine[11–14]. In an interesting series of experiments Kern *et al.*

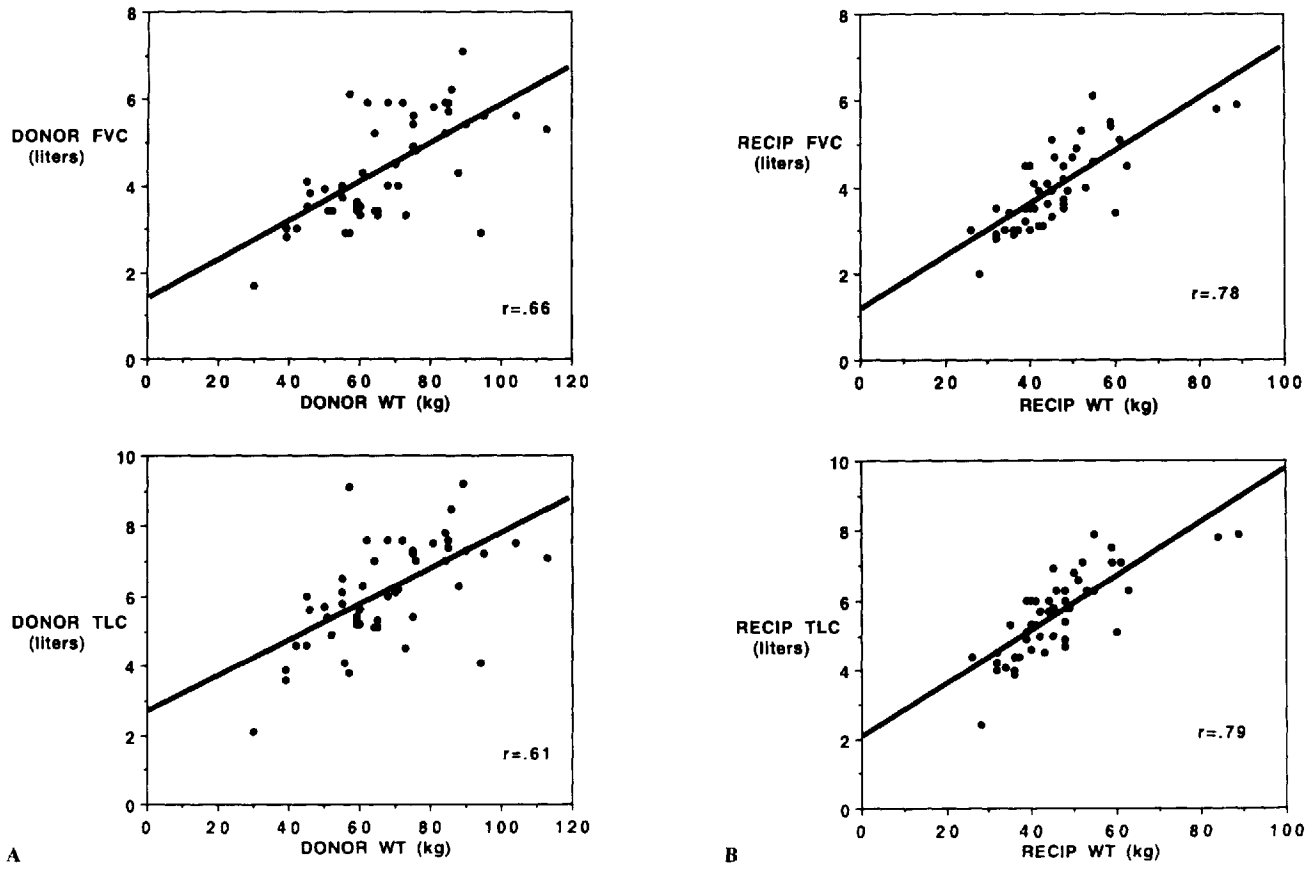

Figure 3 **A**: Donor FVC and TLC vs. weight; **B**: recipient FVC and TLC vs. weight. (Reproduced with permission, from ref. 3)

Table 1 Impact of size reduction on discrepancy of TLC between donors and recipients of double lung transplants

Parameter	*Reduced (n = 13)*	*Not reduced (n = 56)*	*p**
Recipient TLC (predicted) (L)	5.18 ± 0.26	5.82 ± 0.18	n.s.
Donor TLC (predicted) (L)	6.79 ± 0.32	5.91 ± 0.17	< 0.05
Recipient TLC/donor TLC	0.773 ± 0.04	1.0 ± 0.03	< 0.0005
Recipient TLC – donor TLC (L)	−1.61 ± 0.27	−0.09 ± 0.16	< 0.0001
Corrected donor TLC (L)	5.86 ± 0.29	5.91 ± 0.17	n.s.
Recipient TLC/corrected donor TLC	0.90 ± 0.04	1.0 ± 0.03	n.s.
Recipient TLC – corrected donor TLC (L)	−0.68 ± 0.23	−0.09 ± 0.16	n.s.

* by ANOVA

demonstrated an increase in fixed volume and total lobar weight of left lower lobes transplanted from mature pigs into 9-week-old piglets after a 12-week growth period in recipients. Despite this increase in fixed volume, no significant differences in total alveolar number or alveolar size, assessed by morphology, could be demonstrated between transplanted lower lobes subjected to a 12-week growth period compared with lower lobes retrieved from 6-month-old pigs that served as controls[14]. These authors speculated that transplanted lobes most likely grew through an increase in connective tissue and cellular components of the lung parenchyma, and not through an increase in alveolar number.

In humans the lung is generally considered to grow to fill the space available within the thoracic cage[15]. Compensatory growth of lungs following lung resection results in generation of tissue that is functional in gas exchange, although it is widely believed that the *number* of alveoli in the lung does not increase beyond childhood[16]. Compensatory lung growth is a hyperplastic response. Total tissue protein, RNA, and DNA increased in studies of post-pneumonectomy lung growth, indicating an increase in cell numbers[15]. This would suggest that substantial size discrepancy between potential donors and recipients may be well tolerated, and that compensatory lung growth may result in satisfactory long-term outcome. In humans these issues will be

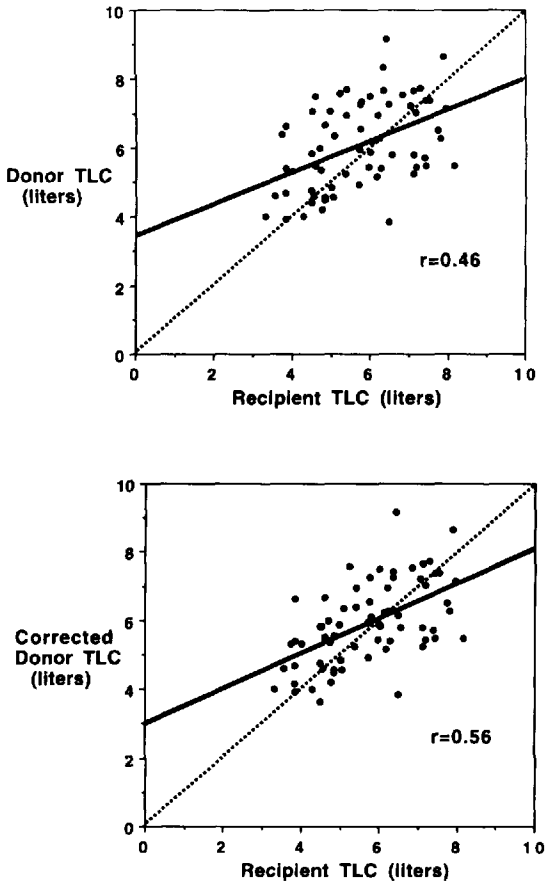

Figure 4 Relationship of recipient predicted TLC to donor predicted TLC. The regression line is solid; the line of identity is dashed. Before size reduction, more donors had larger TLC than recipients. After size reduction (lower panel), the correlation between recipient TLC and the new or corrected predicted donor TLC is better, and the scattergram appears more symmetric around the line of identity (dashed)

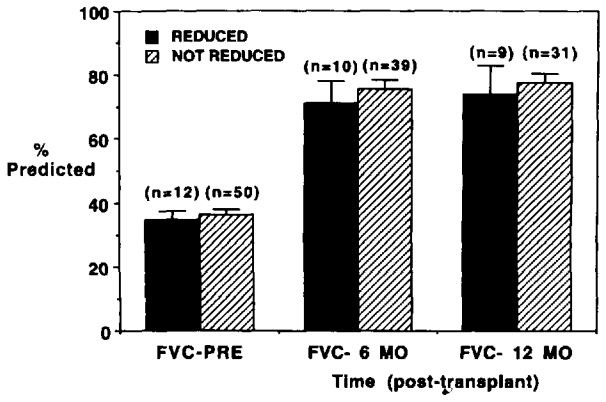

Figure 5 Measured FVC, preoperative and 6 and 12 months post-transplant, for reduced and non-reduced double lung transplant recipients. Note: one reduced patient did not have reliable PFT prior to transplant; he developed pneumonia and ARDS and was successfully transplanted off ventilatory support. Two patients with pulmonary hypertension, one primary and one Eisenmenger's, were excluded from pretransplant FVC calculations. (Mean ± SEM)

addressed by long-term follow-up of recipients of lobar transplants.

COMMENT

It is generally accepted that an appropriate size match between lung transplant recipients and prospective donors would provide a lung of a size approximate to the hemithorax into which it is transplanted. However, it is also clear that substantial latitude exists in size matching of donors and recipients. Donor lungs can be safely size reduced by a variety of procedures, with no detrimental impact on recipients. We believe the adage 'You can't make a small lung big, but you can always make a large lung smaller', and routinely apply it, particularly to recipients with cystic fibrosis whose growth may have been retarded due to their chronic illness. The limits of 'undersizing' donors with respect to prospective recipients have not been defined; presumably the risk of development of a pleural space increases with the size disparity.

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The Split-Lung Technique for Lobar Transplantation

J-P.A. COUETIL

INTRODUCTION

Pulmonary transplantation for the pediatric population, or for patients of small size, is particularly limited by the now well-described scarcity of organs available for transplantation[1,2]. Limitations of size mismatch and shortage of suitable donors make these groups of recipients especially difficult to accommodate. To circumvent these obstacles we have developed a technique of lung transplantation inspired from previous experience with liver bipartition.

Bismuth and Houssin[3] have shown that splitting the liver into its constituent lobes allows two children to be transplanted with one liver. Recent studies of transplantation of pulmonary lobes in animals have been successful at medium-term follow-up with respect to hemodynamics, adequacy of volume, and conformity of the lobes in the thorax of the recipient[4–8]. Satisfactory results of pulmonary reduction and lobar transplantation and liver bipartition, from either cadaveric[9,10] or living donors[11–13], are reported clinically. We postulated that bipartition of one large donor lung into its constituent lobes would allow bilateral pulmonary transplantation into a recipient of smaller thoracic size.

We first showed the feasibility of the procedure experimentally[14]. Using adult dogs as donors, single lungs (either right or left) were divided into separate lobes which were subsequently implanted unilaterally and bilaterally into young dogs. Follow-up of up to 21 weeks demonstrated satisfactory bronchial and vascular anastomoses, and perfect adaptation of the transplanted lobes to the morphology of the recipient thorax. Following the success of these animal experiments, the procedure was applied clinically for the first time in May 1993.

MATERIALS AND METHODS

Between May 1993 and November 1994 seven bilateral lobar transplantations using a bipartitioned left donor lung were performed at Broussais Hospital. There were five female and two male recipients. There were three children aged 13–17 years (median 14), and four adults aged 40–53 years (median 45). The etiology of the end-stage lung disease was cystic fibrosis in the case of the three children; two adults had primary pulmonary hypertension, one had bronchiectasis and one had idiopathic pulmonary fibrosis. All patients required continuous O_2 therapy, and had grade IV dyspnea. Preoperative lung function tests for cystic fibrosis patients revealed mean FEV_1 <25% of predicted values and FVC <30% of predicted. All were judged to have a life expectancy <12 months.

Table 1 Data regarding donor/recipient size discrepancy

(a) Adult recipients

	R1:D1	*R2:D2*	*R3:D3*	*R4:D4*
Height (cm)	150:180	158:190	163:180	160:188
TLC:LLC (L) predicted	4.10:3.26	5.57:3.06	5.10:3.26	5.40:3.46

(b) Child recipients

	R5:D5	*R6:D6*	*R7:D7*
Height (cm)	154:174	140:178	135:170
TLC:LLC (L) (predicted)	4.01:3.04	3.06:3.26	2.76:2.42

R = recipient; D = donor; TLC = total lung capacity (predicted TLC has been calculated using the European Community for Coal and Steel Formula); LLC = estimated left lung capacity of donor (as 45% predicted TLC of the donor).

The criteria were similar to those for single lung transplantation, except that bilateral lobe transplantations were performed when there was a discrepancy in height or weight of more than 20% between donor and recipient. In the present reported cases the weight discrepancy was 44–50% and the height discrepancy 12–17% (Table 1).

SURGICAL TECHNIQUE

Donor operation

A median sternotomy is performed and the pericardium and pleural cavities are opened. The lungs are inspected and particular attention is paid to the left oblique fissure to ensure that it is well defined. In most young adult donors the fissure is well defined and therefore does not present any difficulty in separation. The trachea, aorta, pulmonary artery and both venae cavae are dissected free. Heparin is administered in a central venous line, followed by prostacyclin (500 μg over 10 min) into the pulmonary artery. The heart is then excluded from the circulation by cross-clamping the aorta and the venae cavae. Cardioplegia is administered via the ascending aorta and, when the heart is arrested and the lungs still ventilated, pneumoplegia is infused via the pulmonary artery (Papworth solution 60 ml/kg). The heart is decompressed by incising the inferior vena cava and the left atrial appendage. Topical cardiac and lung cooling is applied.

The heart is then excised, taking care to leave enough atrial tissue surrounding the left and right pulmonary veins. After aspiration of bronchial secretions the lungs are inflated, the endobronchial tube withdrawn, and the trachea stapled and transected. The double lung block is then excised, leaving the esophagus and the descending aorta in the donor chest. If the decision is made to transplant one lung in two different institutions (twinning procedure), the pulmonary block is divided on a back table. The pericardium is split vertically midway between the two atrial cuffs, and the pulmonary artery is divided at its bifurcation. The dissection is then completed at the level of the carina, and the proximal left main bronchus is stapled and divided after inflation of the lungs. Each lung and the heart are placed separately in cold containers for transportation.

Preparation of the donor lung

Bench preparation of the donor left lung involves separation of the upper and lower lobes, and can be completed within 15 min. Initial inspection determines the direction and completeness of the fissure and the presence or not of anatomic variants. The two pulmonary veins are exposed on the mediastinal surface of the lung and divided, leaving a small cuff of atrial tissue. The two veins are separated from each other (Figure 1). The oblique fissure is dissected down to the pulmonary artery. Small vessels crossing the fissure are clipped and divided. Parenchymal bridges are divided after stapling. After completion of dissection of the fissure, the pulmonary artery is divided between the apical branch of the lower lobe and the lingular artery (Figure 2). The upper and lower lobe bronchi are dissected down to the level of the segmental branches, with minimal dissection to preserve retrograde vascularization (Figure 3). Both upper and lower lobe bronchi are transected at their origin (just before implantation of the donor lobes) (Figure 4).

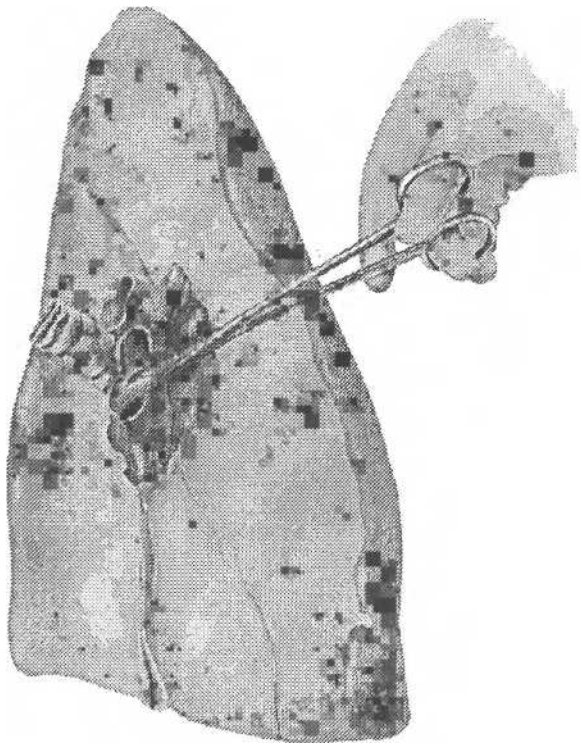

Figure 1 The left lung showing the hilum with the bronchus posteriorly, the pulmonary artery antero-superiorly and the confluence of the veins antero-inferiorly

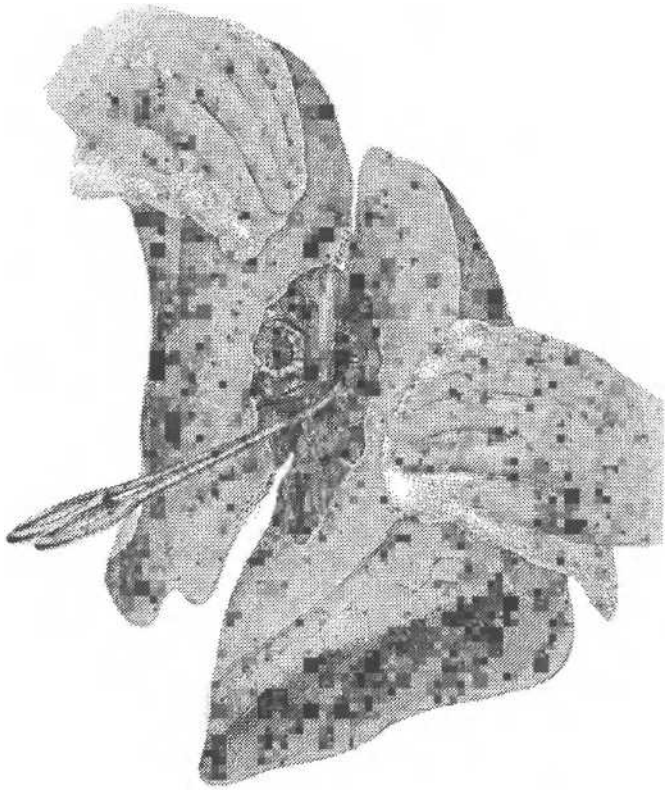

Figure 2 After completion of dissection of the fissure, the pulmonary artery is divided between the apical branch of the lower lobe and the lingular artery (dotted line)

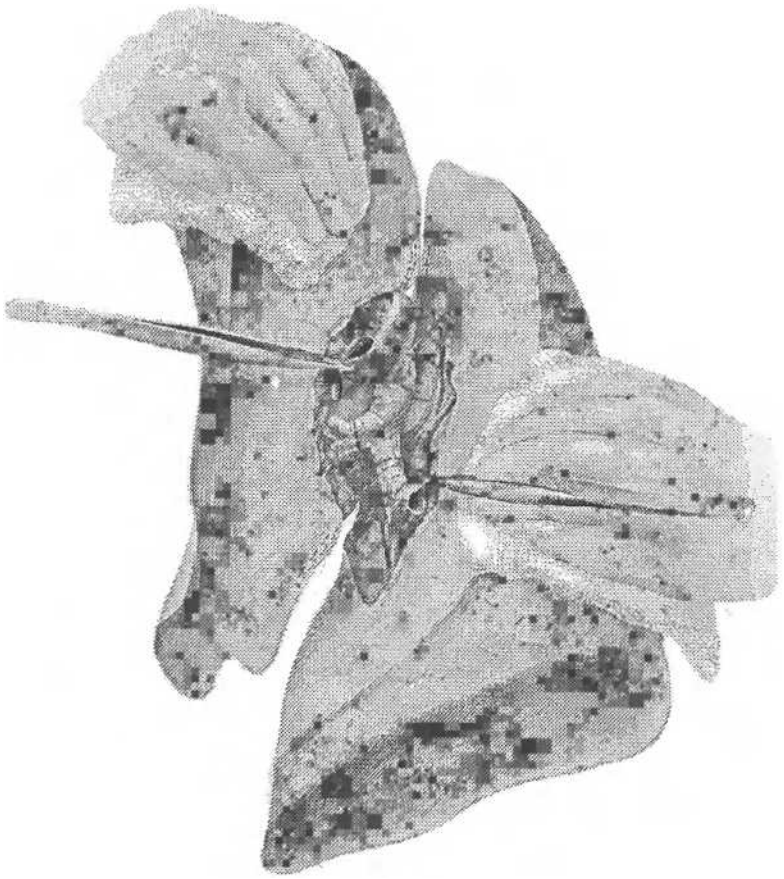

Figure 3 The upper and lower lobe bronchi are dissected down to the level of the segmental branches and are transected at their origins (dotted lines)

Recipient operation

The patient is anesthetized and monitored with standard single-lumen endotracheal tube, radial artery line, two central venous catheters, and a Swan–Ganz catheter. Surgical exposure is via bilateral thoracotomy and transverse sternotomy through the fourth or fifth intercostal space (the 'clam-shell' incision). The pulmonary ligaments and any pleural adhesions are divided with cautery. The pulmonary artery is dissected intra- and extrapericardially on both sides of the hilum, and dissected as distally as possible into the parenchyma of the lung, beyond the upper lobe branch, to have sufficient length for subsequent anastomosis. The pulmonary veins, both inferior and superior, are dissected free and a tape passed around the superior pulmonary vein on the right.

Excision of recipient's right lung

Full normothermic cardiopulmonary bypass (CPB) with a beating heart is established via the ascending aorta and the right atrium (using a two-stage venous cannula), and ventilation is discontinued. The recipient right lung is then excised in a standard fashion, but ensuring that as long a vascular pedicle as possible remains (Figure 5). The first branch of the pulmonary artery is ligated to achieve greater length. This is also useful to orientate the anastomosis. The inferior vein is oversewn in the adult, but in pediatric cases the recipient cuff is fashioned to incorporate both inferior and superior veins.

Implantation of the left upper lobe in the right hemithorax

The donor left upper lobe is then placed in the right hemithorax, having undergone a 180° vertical axis rotation for approximation of donor and recipient hila (Figure 6). Thus, in this situation, the posterior border of the donor left upper lobe becomes anterior, and its anterior border lies posteriorly along the spine. This has the effect of placing the membranous portion of the donor bronchus opposite the cartilaginous portion of the recipient bronchus and vice-versa. The donor pulmonary artery is postero-superior to the donor bronchus, and the recipient artery is anterior and slightly inferior to the recipient bronchus. The donor and recipient superior veins are well aligned.

The bronchial anastomosis is performed with continuous 4/0 prolene. Any size mismatch is overcome by using an end-to-end anastomotic technique, avoiding telescoping as far as is possible. They are sutured as they were aligned, cartilaginous portion of donor to membranous of recipient and vice-versa (Figure 7). Bronchial wrapping is not performed. This is followed by anastomosis of the donor pulmonary vein to the recipient superior pulmonary vein (continuous 5/0 prolene).

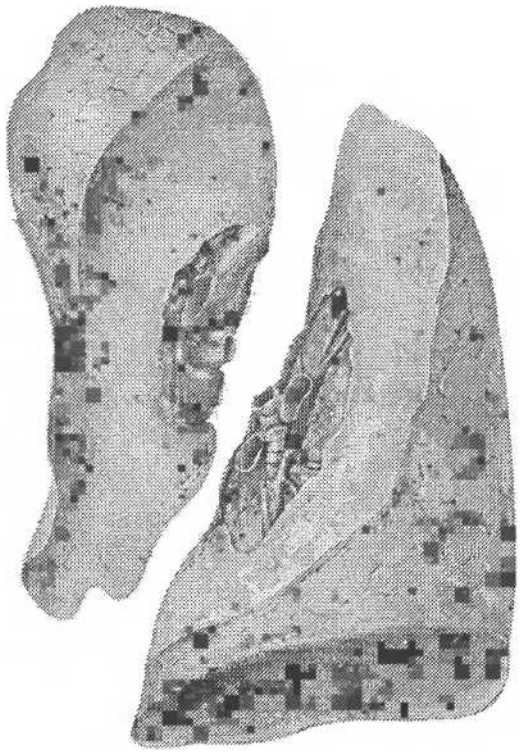

Figure 4 The upper and lower lobes aligned ready for implantation on the right and left sides respectively. Note the fissural opening of the pulmonary artery antero-superior to the bronchus

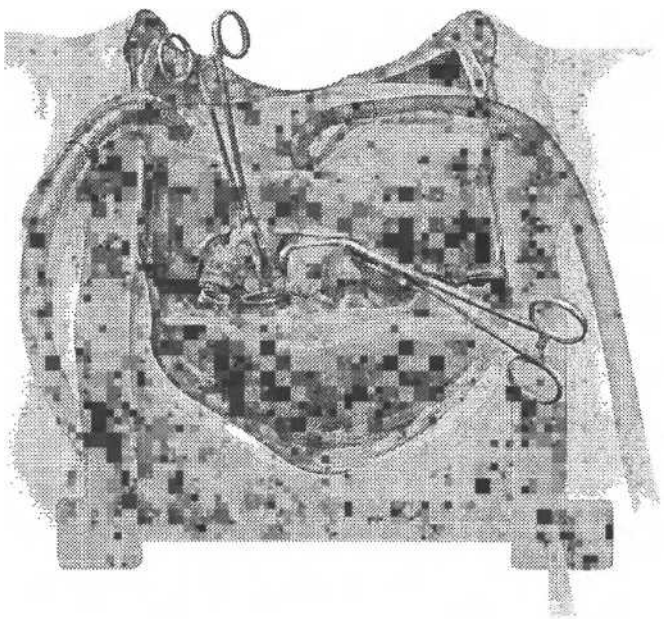

Figure 5 Excision of the recipient *right* lung showing intra- and extra-pericardial dissection of the pulmonary artery to allow as long a pedicle as possible

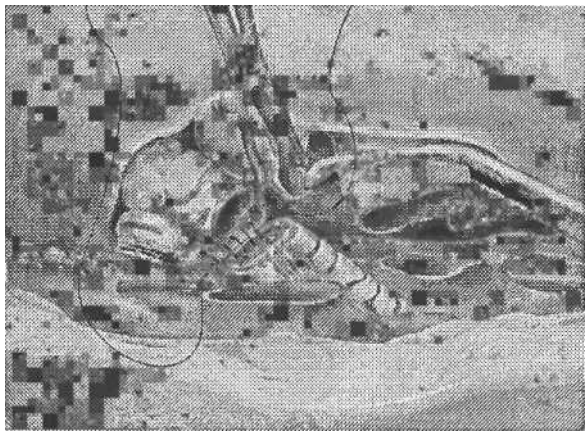

Figure 6 Alignment of the donor *left upper lobe* with the recipient *right* hilum. The end-to-end bronchial anastomosis is commenced, and is followed by the well-aligned pulmonary venous anastomosis

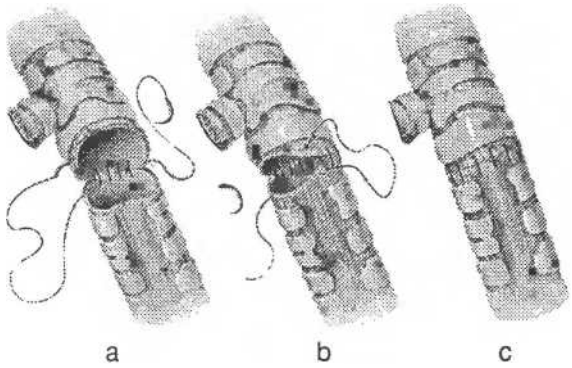

Figure 7 The technique of bronchial anastomosis; a continuous end-to-end stitch sutures cartilaginous portion to membranous portion and vice-versa

The proximal pulmonary artery of the donor is posterior to the bronchus, but leads forward to the fissural section which is anterior and in good alignment with the artery of the recipient. Therefore, the proximal portion is trimmed close to the upper segmental branches and oversewn, and the anastomosis is fashioned end-to-end with the recipient artery using 6/0 prolene (Figure 8). The pulmonary artery anastomosis is made possible by having dissected sufficient length of recipient artery to allow it to come forward in front of the bronchial anastomosis without tension or twist.

Retrograde de-airing is achieved by releasing the venous clamp and evacuating air through the pulmonary artery before securing that anastomosis. The right transplanted lobe is then ventilated gently with 50% F_iO_2. CPB is reduced to allow perfusion of the lobe.

Excision of the left lung

The recipient left lung is resected in a standard manner, but again leaving a long vascular pedicle. The endotracheal tube is placed into the right main bronchus, a procedure which can be directly assisted by the surgeon. This ensures continued ventilation of the transplanted lobe.

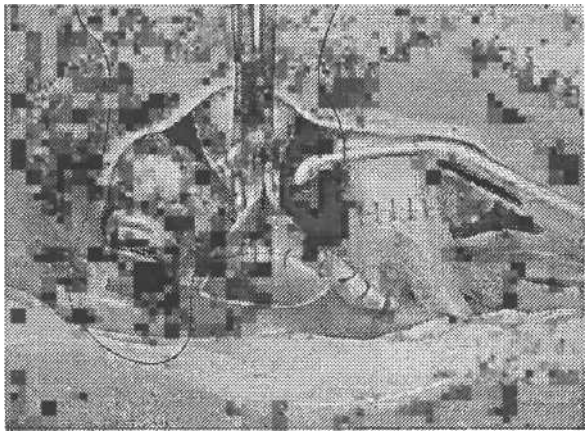

Figure 8 The long pulmonary artery pedicle of the recipient may be aligned with the fissural aspect of the donor pulmonary artery to form the anastomosis in front of the bronchial anastomosis

Implantation of the left lower lobe in the left hemithorax

The donor left lower lobe is placed in the anatomical position in the thorax of the recipient. Alignment of the bronchi and vessels is uncomplicated (Figure 9). Anastomoses are fashioned in the same order: (a) bronchus, followed by (b) vein, and (c) artery. The bronchial anastomosis involves the recipient main bronchus. Again, any size mismatch is overcome while suturing the bronchi end-to-end. The venous anastomosis differs slightly from the right in that the recipient cuff is fashioned incorporating both superior and inferior veins, as they are more closely aligned than on the right (Figures 10 and 11). Both recipient and donor arteries present anteriorly, and there are no special difficulties forming the anastomosis.

De-airing procedures are repeated before the vascular anastomoses are secured. CPB is gradually discontinued. (At this stage it may be apparent in some cases that the right graft in its new position is too long for the thorax. In such situations the lingula may be resected, using a linear stapler.)

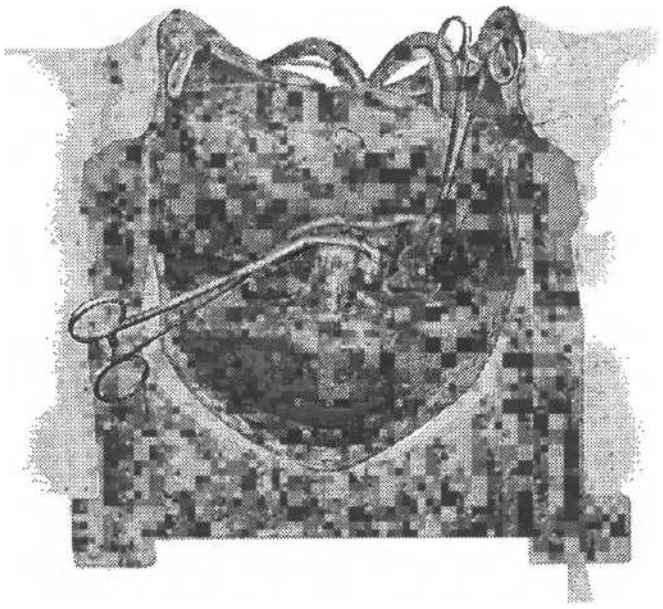

Figure 9 The donor *left lower lobe* implantation into the *left* hemithorax, showing the completed bronchial and pulmonary venous anastomoses and the pulmonary artery anastomosis in progress

Thereafter, bilateral drains are placed and the thoracotomy closed in layers. Postoperatively, patients are extubated as soon as oxygenation is adequate.

Transbronchial biopsies have not been performed in the initial 2 weeks; rejection episodes have been diagnosed on clinical grounds. Otherwise, bronchoscopy, transbronchial biopsy, and lavage have been performed on a routine basis and when clinically indicated by chest radiograph abnormalities, altered gas exchange, or unexplained fever. After discharge from hospital,

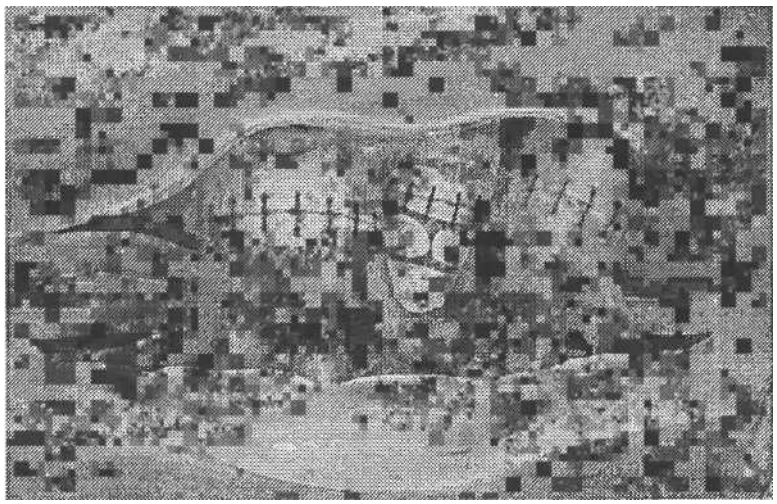

Figure 10 The donor pulmonary vein is fashioned incorporating both superior and inferior veins of the recipient

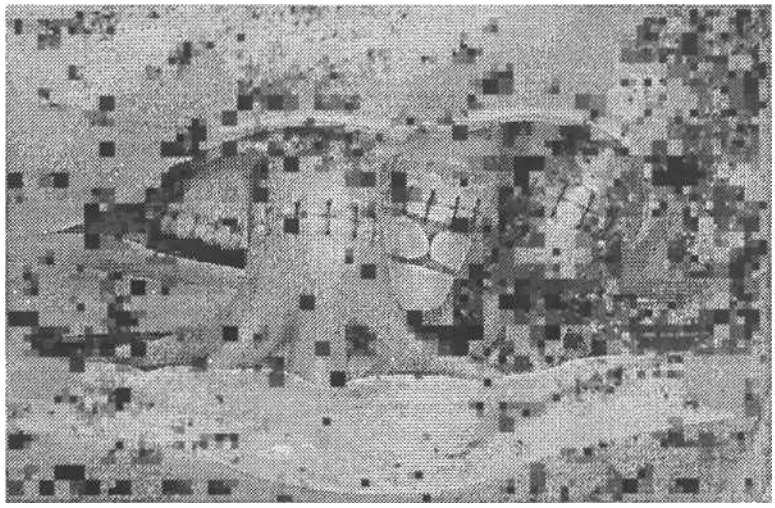

Figure 11 Alternative technique in which the donor pulmonary vein is anastomosed only to the recipient superior pulmonary vein. The inferior vein has been oversewn

patients have been enrolled in a physiotherapy and rehabilitation program.

RESULTS

Early results

There were no technical failures at the time of operation, and all patients were successfully weaned from CPB. The graft mean ischemic time was 150 min for the first lobe (range 90–172), and 210 min for the second lobe (range 145–305). Additional procedures performed included patent ductus arteriosus division and ligation, patent foramen ovale closure, and a reduced liver transplantation in a cystic fibrosis patient. The duration of ventilation postoperatively ranged from 12 to 105 h (median 30). There was one early death in a cystic fibrosis patient in whom widespread systemic aspergillosis could not be controlled, with death from cerebral hemorrhage on day 21. Morbidity was minimal. There were no bleeding complications, and neither bronchial stenosis nor dehiscence became apparent. There was a persistent air-leak in two patients, and three others were noted to have partial pneumothoraces. Hospital stay ranged from 21 to 70 days (median 42).

Late results

Follow-up of the six survivors now ranges from 3 to 9 months (median 7). All patients are subjectively very well with no major morbidity, and minimal problems of rejection or infection. Median values of respiratory function tests are FEV_1 of 1.83 l/min (81% predicted) and FVC of 2.53 l (71% predicted). Changes in the percentage of predicted normal values for the recipient demonstrate an improvement with time after transplantation. There has been only one bronchial stenosis, which has been successfully dilated. This occurred on the left. Bronchoscopy in all other patients has demonstrated patent lumina, free of ulceration or stricture. Follow-up CT scans have demonstrated disappearance of residual pneumothoraces and perfect adaptation of the transplanted lobes to the shape of the recipient thorax. Of note in the majority of CT scans is a mild shift of the mediastinum towards the right, probably due to the longitudinal orientation of the transplanted lobe on the right.

COMMENT

In conclusion, following the success of our animal experiments we have now shown that the technique of lobar separation of the left lung, followed by bilateral transplantation of the two lobes, is feasible and easily performed without an increase in mortality or morbidity. Good functional results have been obtained in carefully selected patients with a large size discrepancy with their donors. Discharged patients have returned to a normal lifestyle with adequate arterial gaseous exchange on exertion. There has been full adaptation of the lobes to the shape of the recipient thorax. Further long-term studies in animals and patients are awaited to assess the full potential of this technique in increasing the number of transplantations in children and adults of short stature. We are at present developing a technique for separation of the lobes of the right lung and their subsequent transplantation.

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Postoperative Management of the Single Lung Transplant Patient

R.C. DALY AND C.G.A. MCGREGOR

INTRODUCTION

This chapter will concentrate on the routine postoperative care of the single lung transplant recipient, and will not duplicate topics covered in other chapters, such as immunosuppressive therapy and postoperative complications, except where a specific alternative management philosophy exists.

Much of the postoperative management of patients undergoing bilateral lung transplantation is similar to that following heart–lung transplantation, and will not be duplicated here. The postoperative care of the patient who has undergone single lung transplantation (SLTx) is different in that: (a) left or right thoractomy, rather than median sternotomy, is performed; (b) cardiopulmonary bypass is generally not utilized; and (c) the contralateral native diseased lung remains *in situ*. Whatever the technique of lung transplantation used, however, there are the problems of lung preservation and the diagnosis of pulmonary rejection, infection and obliterative bronchiolitis. Some aspects of postoperative management, particularly early postoperatively, will be affected by the type of lung transplant performed, for example SLTx versus bilateral LTx. In this chapter, only those aspects peculiar to SLTx will be elaborated upon. Postoperative management will clearly be modified by the recipient's pulmonary disease leading to LTx (e.g. emphysema versus pulmonary hypertension) due to the effects (parenchymal or vascular) of the remaining lung.

Postoperative management can be conveniently divided into two phases: (a) the early intensive therapy period, and (b) the medium and pre-discharge period (stepdown and general ward). During the early experience with LTx, the mean stay in the intensive-care unit (ICU) was 7–9 days[1,2]. Current studies report an average ICU stay of 5.6 days (median 3 days)[3]. The mean hospital stay has decreased from 41 days[1,2] to 26–35 days[3,4]. Our own patients have been dismissed an average of 25 days after LTx for emphysema, and many can be dismissed in about 2 weeks[5]. Longer stays have been reported for patients with underlying pulmonary fibrosis (mean of 37 days)[6].

EARLY POSTOPERATIVE PERIOD

As for much of cardiothoracic surgery, the relative difficulty of immediate postoperative care is largely dictated by the events that occurred in the operating room. If a good donor lung is obtained and preserved well, and the operative procedure is technically uncomplicated, then the early management of the SLTx recipient is relatively straightforward.

No consensus exists regarding the effectiveness of reverse barrier nursing in the care of the transplant recipient, but many accept that reverse isolation in the early postoperative period is helpful in minimizing traffic of personnel, and impresses on both staff and visitors the risk of transmitting infection, and the importance of maintaining strict hygienic standards. We keep all hospitalized transplant recipients in single rooms with standard transplant isolation. All individuals entering the rooms must wash their hands for a strict 2 minutes, and must wear a mask. Preliminary comparison of this isolation policy with our historical use of strict reverse isolation indicates that the current, simpler procedure is acceptable. An individual nurse should not combine care of a transplant patient with that of an infected patient, and continuing strict adherence to hand washing, in particular, is essential[7]. Early postoperatively, LTx recipients are kept in rooms with filtered, positive-pressure ventilation systems.

In the immediate postoperative period, two nurses are generally required to perform the extensive duties that are necessary. Standard intensive-care monitoring of vital signs is carried out. Experienced resident medical presence is mandatory. Frequent clinical examination is important. Early signs of rejection or infection can be subtle but, if elicited, can allow timely investigation and therapy. It has been our practice for the senior medical team (surgeon, anesthesiologist, respiratory physician, infectious disease specialist) to meet at least twice daily to review in depth the patient's condition; this allows for the results of investigations to be noted, and management to be optimized, and provides continuity of care, as well as serving as a forum for communication and education of the staff involved. This proactive approach

results in the timely introduction of measures that prevent the development of complications and emergency problems.

Ventilatory management

A standard volume-cycled mechanical ventilator is adequate for ventilation of most single-lung recipients. In rare circumstances, high-frequency ventilation or use of individual ventilation of each lung may prove helpful.

The aim of mechanical ventilation is to achieve adequate oxygenation of the patient (Pa_{O_2} >80 mmHg) at the lowest possible inspired oxygen concentration (F_iO_2 <0.6) and the lowest possible peak airway pressure (<30 cmH_2O). Measures to achieve these ends include:

(1) Optimization of ventilatory rate, inspiratory time, and tidal volume, to achieve the same minute ventilation. Routine settings are respiratory rate of 8–10 breaths per minute and tidal volume 15 ml/kg.
(2) The use of positive end-expiratory pressure (PEEP) ≤4–6 cmH_2O. Greater levels of PEEP may be necessary to achieve adequate oxygenation in the presence of compromised lung function, but are clearly undesirable for prolonged periods in view of a potentially deleterious effect on bronchial healing. Patients with a retained, contralateral emphysematous lung may require lower levels of PEEP and longer expiratory time to minimize hyperexpansion of the native lung and consequent mediastinal shift. Occasionally, use of separate ventilators for each lung via a double-lumen endotracheal tube is helpful.
(3) Adequate pulmonary toilet. A soft endotracheal suction catheter is introduced into the lower trachea every 2 hours, or more frequently if secretions are copious. Aggressive endotracheal suction with a rigid catheter in the region of the bronchial endotracheal suction should be avoided to minimize stress on the anastomosis. If there are significant secretions that cannot be aspirated, or if lobar collapse develops, then fiberoptic bronchoscopy should be carried out; with care, it is possible to traverse the bronchial anastomosis. Throughout the period of mechanical ventilation, and after extubation, active respiratory therapy, including vibration and percussion with gravity drainage, is given 4-hourly.
(4) Maintenance of the lowest left atrial pressure compatible with satisfactory hemodynamics, preferably maintaining the pulmonary arterial diastolic pressure lower than 12 mmHg (see also Hemodynamic Management, below). This aims to minimize the propensity for pulmonary edema in the preserved, transplanted lung.
(5) Recipients of a SLTx with pulmonary hypertension (including some with a primary diagnosis of pulmonary fibrosis) will have a propensity to develop pulmonary edema in the graft, since the preponderance of the cardiac output will be directed through the transplanted lung (after an ischemic insult and lacking lymphatic drainage). It may be helpful to position these patients to keep the allograft above the native lung, to reduce pulmonary venous pressure on that side. Further, these patients should be kept well sedated, even paralyzed, in the initial postoperative phase to minimize pulmonary artery pressure in the allograft.
(6) Avoidance of pleural fluid collection by continuous suction (at –20 cmH_2O) of apical and basal chest tubes inserted at the time of transplantation.

Many SLTx recipients can be extubated within 1–3 days of operation, but some require more prolonged periods of ventilation. Before weaning, the following criteria should be met:

(1) The patient's mental status should be satisfactory. He or she should be easily rousable, and have satisfactory cough and gag reflexes.
(2) Blood gas parameters must include:
   (a) Pa_{O_2} >75 mmHg, with an F_iO_2 <0.5, a Pa_{CO_2} <40 mmHg, and PEEP <6 cmH_2O;
   (b) a vital capacity >10 ml/kg;
   (c) a peak inspiratory pressure force ≥25 cmH_2O;
   (d) the demonstrated ability of the patient to resume adequate spontaneous ventilation during a progressive decrease in assisted ventilation.
(3) Acceptable chest radiographic appearances. In the presence of diffuse opacification of the lung in a patient with marginal blood gases, ventilation should be continued until improvement is seen.
(4) Stable hemodynamics, including stable cardiac rhythm and acceptable hemodynamic parameters without heavy inotropic therapy.
(5) Good muscle strength, reflected by adequate chest excursion. The patient should be able to lift his/her head off the pillow. Pain may prevent satisfactory chest movement; the insertion of a lumbar epidural catheter for postoperative pain management is helpful, unless cardiopulmonary bypass has been used, in which case this technique for analgesia is probably contraindicated.
(6) Satisfactory acid–base status. Significant metabolic alkalosis should be corrected prior to weaning.
(7) Acceptable fluid balance. In particular, the patient should not be fluid overloaded; diuresis may be indicated prior to weaning (see Hemodynamic Management).

Once an adequate level of oxygenation with an F_iO_2 ≤0.5 is achieved, a standard weaning protocol can be used. For example, the ventilator is switched from assist control to an intermittent mandatory ventilation (IMV) of 8 breaths/minute. If the patient remains comfortable with acceptable blood gases, then the rate is further decreased to 4 breaths/minute, and the patient is subsequently extubated.

In patients with early impairment of donor lung function, from use of a suboptimal donor or inadequate pulmonary preservation (Figure 1), weaning first requires improvement in the patient's overall clinical status. This allows a gradual increase in spontaneous ventilatory work with concomitant decrease in ventilatory assistance. Intravenous nutrition may be required if this period is prolonged, as well as control of any volume overload and/or sepsis. Topical oral and gastrointestinal agents are used to minimize microbial colonization (see Infectious Disease Management, below). Even though the patient is ventilator dependent, suitable physical activity should be encouraged. The patient's psychological status should be maintained as near normal as possible, with avoidance of sleep deprivation and depression. The rehabilitation process is begun at this time, and is tailored to the general state of

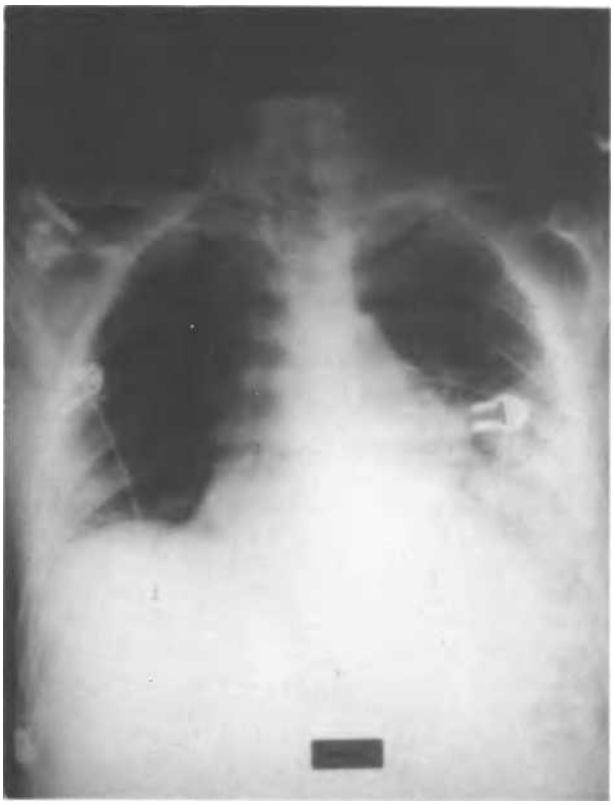

Figure 1 Anteroposterior chest radiograph of patient taken 48 h after single left lung transplantation. The left lung opacification was believed to result from inadequate donor lung preservation

the patient. Continuity of expert care is essential in such cases. The overall philosophy is to increase the periods of active respiratory movement by alternating these with periods of muscle rest, usually entailing an assist control mode of ventilation at night. Gradually, periods off the ventilator are increased until ventilation can be discontinued permanently.

In the early Toronto series of SLTx, the duration of ventilatory support was a mean of 5.5 days[1]. In our recent experience mean ventilatory time after SLTx for emphysema was 3.3 days[5]. Prolonged ventilation (>7 days) has been reported to be more common in recipients with pulmonary fibrosis and pulmonary hypertension (incidence of 25% and 21%, respectively) than in those with obstructive lung disease (7%)[6].

After extubation, a close-fitting mask delivering 40% or 70% O_2 is applied, and blood gases are monitored 30 minutes later, and then at hourly or 2-hourly intervals until the patient's respiratory status is clearly satisfactory. Continuous ear or finger oximetry for Sa_{O_2} is carried out. Chest radiographs are taken at 8-hourly intervals throughout the first postoperative week. Four-hourly chest physiotherapy, with breathing exercises and vibration or percussion therapy, is provided to help prevent atelectasis. Pulse oximetry and heart rate should be monitored during respiratory therapy, and any deterioration in these parameters reported to the physician. Bronchodilator therapy is not employed routinely. The patient is given guidance in the use of incentive spirometry with one of the many commercially available devices, and encouraged to perform this therapy for 2–5 minutes of every waking hour.

Hemodynamic management

Continuous invasive hemodynamic monitoring is essential until the patient is established off the ventilator. An arterial line, a urinary catheter, and a triple-lumen central venous catheter are utilized. Additionally, Swan–Ganz catheterization is helpful, particularly in patients with pulmonary hypertension who have a greater tendency to have hemodynamic instability; if anxiety exists regarding trauma to the pulmonary arterial anastomosis, the catheter can be passed into the contralateral lung under the surgeon's control in the operating room before the chest is closed.

The transplanted lung is at risk for developing pulmonary edema due to its recent ischemic injury and interruption of lymphatics. This is particularly troublesome in patients with pulmonary hypertension, since the majority of the cardiac output will pass through the allograft due to its lower vascular resistance. Furthermore, patients with pulmonary hypertension are likely to have required cardiopulmonary bypass during the transplant. Efforts should be made to minimize administration of intravenous fluids in all patients from the time they enter the operating room. The anesthesia team should treat the recipient like a patient undergoing pneumonectomy, and intravenous fluids should be certainly limited to less than 2 liters. Postoperatively, cardiac filling pressures (CVP and PCWP or PA diastolic pressure) should be kept at the lowest level possible to maintain the mean arterial pressure 60–65 mmHg. Maintenance of an adequate blood pressure may be important in ensuring satisfactory blood supply to the ischemic bronchus. To achieve these hemodynamic parameters, it is often necessary to use a dopamine infusion at 2–5 μg/kg per minute. Concomitant vasodilator therapy (sodium nitroprusside) may be used to optimize cardiac function. More powerful pulmonary vasodilator therapy (PGE_1 and PGI_2) may be helpful if pulmonary artery pressures remain high, with resultant right heart failure; this problem is most acutely faced, however, during the period of one-lung ventilation that is necessary as the donor lung is being implanted[8]. As indicated above, it may be helpful to position the patient with pulmonary hypertension so that the allograft is above the level of the native lung to minimize pulmonary venous pressures on the allograft side. It may also be necessary to keep patients with pulmonary hypertension sedated and even paralyzed for a few days after transplantation, to prevent pulmonary hypertensive crises and reduce allograft reperfusion injury.

Renal function must be monitored closely. At our center we do not use intravenous cyclosporin postoperatively (see Immunosuppressive Therapy, below) which may compromise renal function. Usually we strive for a negative fluid balance each day postoperatively, maintaining low cardiac filling pressures, and keeping the patient as 'dry' as possible. Judicious administration of diuretics is often necessary. Postoperative bleeding is usually minimal. Transfusion is indicated if the hematocrit falls below 30%.

Immunosuppressive therapy

Triple immunosuppressive drug therapy is employed. Our protocol for immunosuppression[5] is as follows:

Cyclosporine: Begun on postoperative day 2–4 depending on perioperative renal dysfunction. We no longer give a preopera-

tive loading dose. Thereafter, dosage adjusted to maintain trough serum level (cyclosporin + metabolites) at 200–300 ng/ml for 6 weeks and 75–150 ng/ml thereafter.

Methylprednisolone: 500 mg intravenously after organ perfusion, 125 mg intravenously every 8 h for 24 h.

Azathioprine: 4 mg/kg loading dose, 1–2.5 mg/kg per day to maintain white cell count at 4000–6000/μl.

OKT3: 2.5 mg/day, 14-day course. First dose given 30 min after methylprednisolone in the operating room.

Prednisone: 1 mg/kg per day beginning on day 15, 0.3 mg/kg per day from the first to sixth months, and 0.2 mg/kg per day thereafter.

We have evolved to the above dose of OKT3 after a trial of adjusting the dose each day according to an algorithm based on the total CD3 count and the CD3 percentage. We had no episodes of rejection during OKT3 administration, and reduced the total dose by almost 60%. Subsequently, we have used 2.5 mg/day for 14 days and have not had an incidence of rejection during the course of OKT3 therapy. The patients are often maintained on OKT3 as monotherapy for the initial days postoperatively. When hemodynamics and renal status are stable, and the patient is extubated and tolerating oral intake, cyclosporin is carefully started and the dose increased with the goal of having therapeutic levels for 2–3 days before the end of the course of OKT3. Prednisone is not begun until after the course of OKT3, 2 weeks postoperatively. If hepatic function is stable, azathioprine can be started early postoperatively, and given intravenously until the patient can take it orally.

The administration of antithymocyte (ATG) or antilymphocyte globulin (ALG), or OKT3, may result in an anaphylactic reaction, and so pretreatment with hydrocortisone, an antipyretic, and an antihistamine is given before the first three doses. Should a mild or moderate anaphylactic reaction occur, the infusion of the cytolytic agent should be stopped, the premedication repeated, and the infusion recommenced 30 min later at half the rate. If a severe reaction occurs, then standard resuscitation measures should be taken, including the administration of epinephrine. OKT3 is given as a push injection.

Acute rejection episodes are treated with intravenous methylprednisolone pulse therapy (10 mg/kg per day) for 3 days.

Anticoagulation

In the Toronto series an intravenous infusion of heparin and dipyridamole has been administered in the early postoperative period, in order to minimize the risk of anastomotic thrombosis[9]. We have not utilized any specific anticoagulation in our own small series, and believe it is unnecessary for the standard anastomoses. The use of subcutaneous heparin for prophylaxis of deep venous thrombosis seems appropriate for the patient who is unable to be mobilized in the early postoperative period. Any bed-bound patient has sequential compression devices used on the legs. Unless contraindicated, we give a dose of aspirin of 162 mg/day to patients who have had direct bronchial artery revascularization[10].

Infectious disease management (see also Chapter 57)

Invasive tubes and lines, with the exception of the central venous line, are removed as soon as possible; the chest drains and urinary catheter are usually removed 1–2 days after operation, and the arterial line 24 h after extubation. We routinely change central venous lines every 7 days, and more often if the patient has an unexplained fever. A central venous line is left *in situ* throughout a patient's stay, to allow for painless blood sampling and early management of cardiorespiratory instability. Central venous lines are treated with careful aseptic technique and are flushed with sodium metabisulfite solution after each use or once daily[11]. While intubated, all patients receive selective bowel decontamination (SBD), 30 ml four times daily, down the nasogastric tube, and the Orabase mixture of SBD is applied locally to the mouth four times daily.

Surveillance cultures of the blood, sputum and urine for bacteria and fungi are performed weekly while the patient is hospitalized. Sputum is cultured for fungi weekly for 6 months, then monthly for 6 months. Surveillance blood and urine cultures for cytomegalovirus (CMV) are performed weekly for 2 months beginning 3 weeks after transplantation, then monthly for 3 months. Positive surveillance cultures for *Aspergillus* or CMV are always treated.

The presence in the donor trachea of heavy bacterial or fungal growth, or of large numbers of neutrophils, increases morbidity and mortality after heart–lung transplantation[12]. Material obtained from the airways of the donor lung at the time of harvest, and from the recipient bronchus at the time of transplant, are routinely sent for bacteriological examination; when the results are known, routine antibiotic prophylaxis must be modified accordingly. Routine antimicrobial prophylaxis is as follows:

(1) Cefotaxime, 1 g i.v. every 8 h for 48 h. This is most often extended on the basis of cultures.
(2) Metronidazole, 500 mg i.v. every 8 h, for the first 2 weeks to minimize potential anaerobic infection around the 'ischemic' bronchial anastomosis. Converted to oral when able.
(3) Fluconazole, 200 mg i.v. daily, for one dose post-transplant. Therapy is extended if cultures of donor or recipient sputum grow yeast, generally for 2 weeks. Converted to oral when able.
(4) Trimethoprim/sulfamethoxazole, starting when cefotaxime is stopped, one double-strength tablet daily for 1 month, then one single-strength tablet daily. Dose may need to be reduced for renal insufficiency. Sulfa-allergic patients are treated with inhaled pentamidine monthly.
(5) Clotrimazole troche, one tablet three times daily, after extubation for 8 weeks. Nystatin swish-and-swallow or nystatin pastilles may be substituted. Female patients receive a terconazole vaginal suppository twice weekly for 8 weeks.
(6) Acyclovir, 200 mg orally three times daily, for 2 months for all recipients seropositive for herpesvirus. Discontinued if the patient is on ganciclovir.
(7) Ganciclovir, 5 mg/kg i.v. twice daily for 2 weeks then once daily for 10 additional weeks (reduced dosages for renal insufficiency), for all recipients of CMV-seropositive donors. Patients are dismissed with a percutaneous central catheter or other durable form of venous access.
(8) Cytogam CMV immunoglobulin prophylaxis for CMV mismatch patients (donor seropositive/recipient seronegative). An initial dose of 150 mg/kg i.v. is followed by 100 mg/kg i.v. every 2 weeks to day 56 post-transplant, then 50 mg/kg i.v. on

days 84 and 112 post-transplant. Passive immunization with CMV hyperimmune globulin has been shown to be effective in diminishing the frequency and severity of CMV disease in seronegative mismatched recipients of renal[13] and cardiac allografts[14].

(9) Itraconazole, beginning at a dose of 200 mg orally twice daily. H_2 blockers cannot be given at the same time as itraconazole. Serum levels are checked every 2 weeks initially and the dose modified accordingly. Drug interactions may occur with cyclosporin (necessitating a <50% reduction in cyclosporin dose), terfenadine (arrhythmias), and digoxin (increased serum levels of digoxin).

Experience with solid organ transplantation, including lung transplants, at our institution has shown a high incidence of lymphoproliferative disease in the presence of seromismatches (donor seropositive and recipient seronegative) for Epstein–Barr virus (EBV)[15]. Because of the high prevalence of previous exposure to EBV in the general population, we believe the potential recipients of lung allografts who are EBV-seronegative should be selected with care. For a time we considered EBV serological negativity a contraindication to LTx. More recently we have initiated a protocol of prophylaxis in those EBV-seronegative recipients who we do choose for LTx. The protocol involves prophylactic intravenous ganciclovir and gammaglobulin that has a high titer of activity against EBV. Our experience with this protocol is to-date limited. Aggressive prevention of infection is critical. In addition to adjusting antimicrobial coverage for intraoperative donor and recipient tracheal cultures, and performance of surveillance cultures, early and aggressive pursuit of any infective process to determine its microbiological cause is carried out; this often necessitates bronchoscopy and bronchoalveolar lavage.

Investigations

Chest radiography is performed twice a day for two weeks, and daily thereafter, as well as when clinically indicated. A baseline ventilation–perfusion (V/Q) scan is performed. We have not found V/Q scans helpful in the diagnosis of rejection. They do, however, provide interesting physiological information, such as a shift in ventilation to the transplanted lung with time (Figure 2). When the patient is able to visit the pulmonary laboratory, full pulmonary function tests are carried out weekly. Daily spirometry is performed from the time the patient is extubated, and is continued at home with a portable device.

Daily investigations include a complete blood count, T cell subsets (if OKT3 or ATG are being used), basic blood tests of renal function and metabolism (urea, creatinine, glucose and electrolytes), and a trough cyclosporin level. A general blood chemistry panel is monitored twice a week.

Routine surveillance specimens of urine, throat, sputum, and endotracheal secretions (if intubated) are cultured for bacteria and fungi twice a week. Blood and urine are cultured for CMV weekly. CMV serologies are monitored weekly in seronegative recipients.

Diagnosis of implantation response/rejection/infection

The nature of any new pulmonary opacification seen on the chest radiograph may be suggested by the time of its occurrence in relation to the transplantation procedure. Opacification developing in the first few days after transplantation is most likely related to an implantation response resulting from preservation–reperfusion injury and/or division of lymphatics (Figure 1). Rejection is possible at this early stage, but is more common after the first 4–5 days. However, we have not noted rejection during the 14-day course of OKT3. Thereafter, the clinical features and radiographic

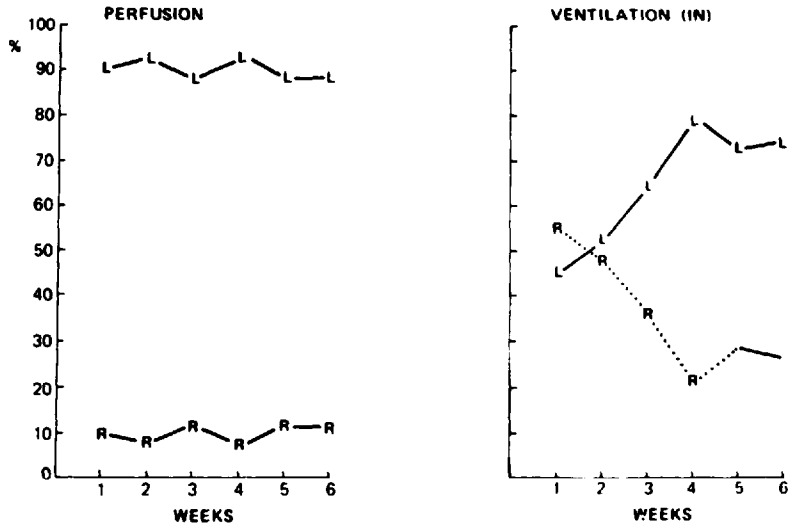

Figure 2 Ventilation and perfusion of left (L) and right (R) lungs after single left lung transplantation. Ventilation (and perfusion) of each lung, recorded at weekly intervals, is expressed as a percentage of the total

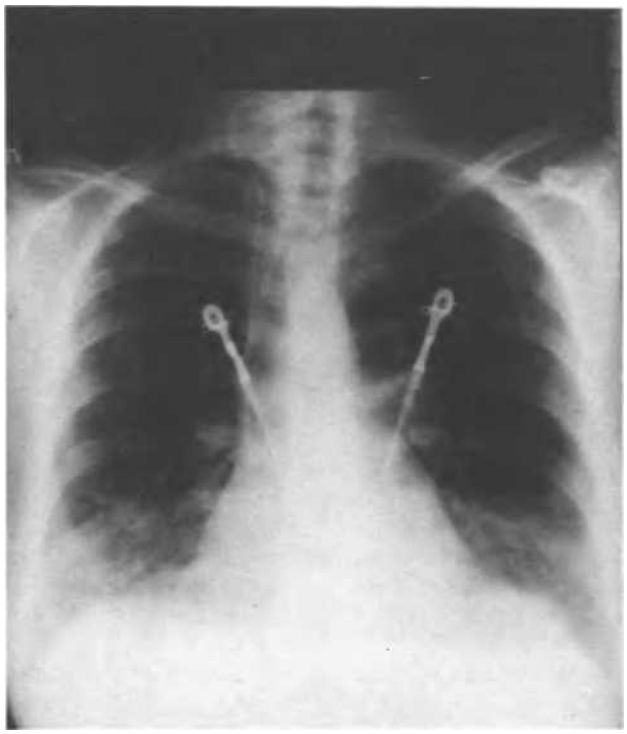

Figure 3A Posteroanterior chest radiograph taken (in the morning) 10 days after single left lung transplantation

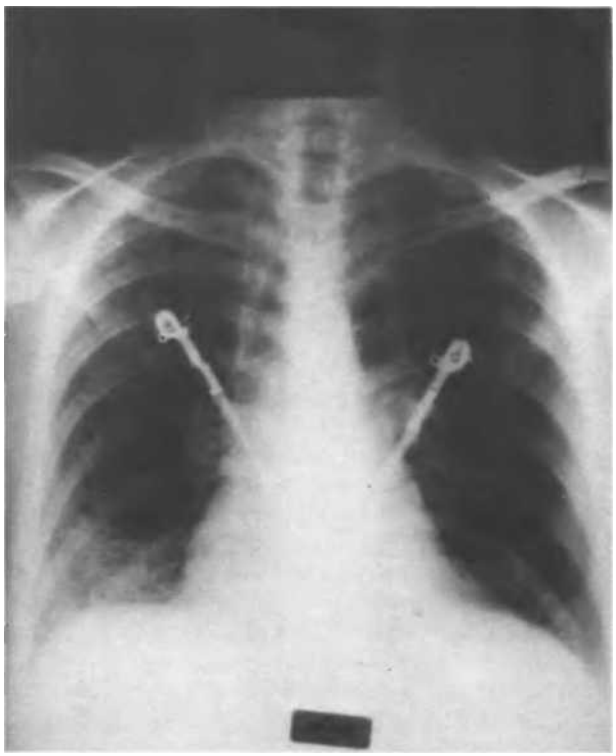

Figure 3C Chest radiograph of the same patient 6 h after initiating antirejection therapy, demonstrating marked clearing of the left lower zone opacification

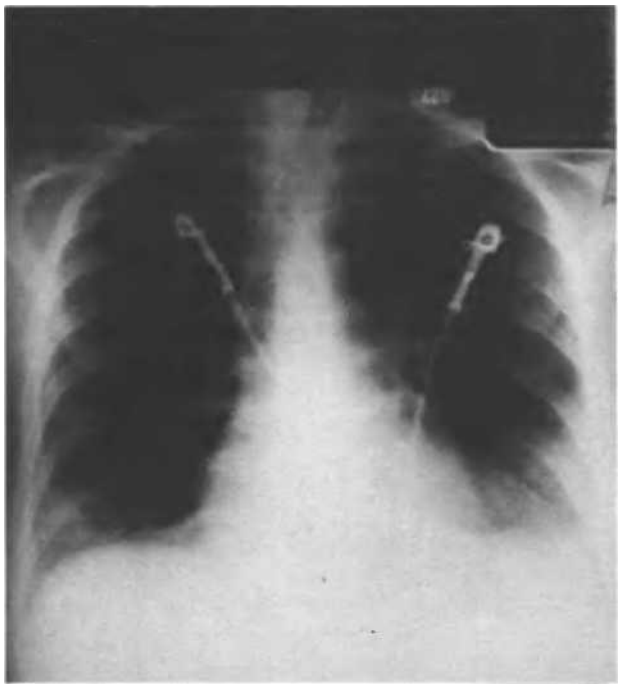

Figure 3B Chest radiograph (taken later the same day) showing a left basal pulmonary infiltrate and effusion, which was associated with fever, pleuritic pain, and a pleural rub

appearances of rejection and infection can be indistinguishable. Pulmonary rejection may develop over a short time period (hours) but, if diagnosed early, can resolve in an equally short period (Figures 3 A, B, and C).

In the investigation of any new respiratory sign or symptom, or any significant change in a physiological (including daily spirometry results) or radiographic parameter, we maintain an aggressive approach with regard to bronchoscopy and bronchoalveolar lavage (BAL). After BAL of the 'opacified' area of lung, four transbronchial biopsies are obtained by fiberoptic bronchoscopy using alligator forceps under local anesthesia (with an anesthesiologist in attendance) and fluoroscopic control (Figure 4). Alligator forceps provide a 3–4 mm^3 specimen. Ideally, biopsies should contain the three pulmonary anatomical elements of bronchiolar epithelium, alveolus, and blood vessel. Bronchoscopy also allows inspection of the bronchial anastomosis.

BAL specimens are stained and cultured according to a standard 'immunocompromised host protocol', which includes investigation for bacteria, *Legionella*, *Nocardia*, mycobacteria, fungi, viruses, and *Pneumocystis*. Biopsies undergo rapid histological processing.

The role of surveillance transbronchial biopsy remains uncertain[16,17]. We, like others, have abandoned routine serial surveillance biopsies but have adopted a policy of low threshold for proceeding to transbronchial biopsy. Transbronchial biopsy has proven a safe technique even in the very ill patient.

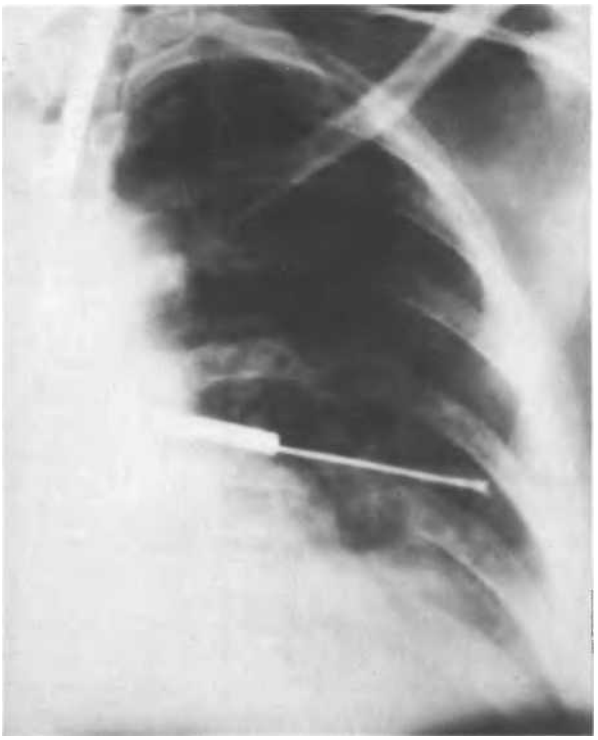

Figure 4 Fluoroscopic appearance of a transbronchial biopsy being taken, using alligator forceps directed through a fiberoptic bronchoscope

MEDIUM AND PREDISCHARGE PERIOD

Monitoring

The indications for transfer from the intensive-care unit (ICU) to a stepdown unit may vary between institutions. It is psychologically preferable for a patient to leave the ICU as soon as he/she no longer requires this level of care. The decision to transfer a patient may be influenced by the availability of monitoring facilities in the stepdown unit. Telemetric oximetry has allowed us to transfer patients from the ICU early and reduce nursing needs.

It has been our practice to monitor the patient continuously by electrocardiographic telemetry as well as by ear or finger pulse oximetry. Telemetric pulse oximetry is helpful in weaning oxygen from patients as they become more active after transplant. Noninvasive blood pressure monitoring is also performed several times per day, and orthostatic pressures are checked daily. At this stage of postoperative care the patient will be in the early phase of mobilization; most invasive monitoring lines will have been removed, but it has been our practice to maintain a central venous line to provide vascular access in an emergency and to allow blood sampling without needle stick. The disadvantages of this policy, however, are the risks of line sepsis and air embolism. Careful nursing practice should avoid the latter complication. In our experience, the risk of line infection is minimized by three measures: (a) meticulous daily change of dressing, (b) the line is changed to a separate site each week, and (c) the line is flushed with sodium metabisulfite solution (0.05%) after any venous sample has been taken[11].

Rehabilitation

A preoperative rehabilitation program is extremely important, not only in optimizing the condition of the potential SLTx recipient, but also in preparing the patient for postoperative rehabilitation, which is integral to a successful outcome of the transplant. The goals of a postoperative rehabilitation program include: (a) improvement and maintenance of efficient breathing; (b) musculoskeletal reconditioning; (c) improvement in the maintenance of aerobic capacity, and of body posture and neuromuscular relaxation.

By promoting physical self-reliance, attainment of the goals results in decreased anxiety and depression, and also assists in the process of retraining for an independent existence. As patients vary considerably in their physical status at the time of transplantation, it is necessary to provide an accelerated as well as a standard program. A standard program is necessary in the patient who has lost considerable muscle mass; this may result from enforced inactivity or represent the cachexia that develops secondary to advanced pulmonary disease. An accelerated program is indicated in the patient who is relatively fit.

The patient is treated twice a day, and is allowed to progress in a sequential fashion to the next stage of exercise when he or she has satisfactorily completed two consecutive treatments at the previous level. Continued measurement of oxygen saturation is essential during exercise; oxygen therapy should be adjusted to maintain the oxygen saturation of the blood >90% (Figure 5).

In the event of a rejection or infectious episode, activity is modified accordingly. In the presence of severe rejection, only gentle passive movements are continued. With moderate rejection, activity can be maintained, but not increased. With mild rejection, progress can be continued as per protocol.

Our own protocol includes five levels of activity, with documentation of changes in heart rate, respiratory rate, blood pressure, and oxygen saturation, and of the development of any symptoms. Indications for cessation of a specific activity include: (a) a heart rate response >30–40 beats above the resting rate, (b) a respiratory rate >30/min, or (c) an oxygen saturation persistently <90%.

Education

As with all solid organ transplants, a successful long-term result will be more likely if each patient takes a degree of personal responsibility for his or her own health care. This can be achieved only by the methodical education of the patient. This should include relevant information and guidelines on: (a) transplantation in general, (b) lung transplantation in particular, (c) medications, (d) nutrition, (e) exercise and activity, (f) personal health surveillance, and (g) medical follow-up care. Information should also be provided on how the patient can obtain the appropriate medical care should problems arise. Basic knowledge of (h) the immune system, (i) the problems of pulmonary rejection and infection, and (j) the potential long-term complications of LTx should be discussed. The patient should be made aware of the symptoms and signs of complications so that he or she can refer himself or herself early for medical care, which may be crucial if the process is to be fully reversed.

Figure 5 A recipient of a single lung transplant, shown 2 weeks after surgery, participating in a postoperative exercise program. Continuous monitoring of oxygen saturation is provided by finger oximetry

Detailed advice regarding discharge medications should include: (a) dose of drug, (b) method of administration, (c) potential side-effects, and (d) the reason it is prescribed. Medications discussed should include cyclosporin, azathioprine, corticosteroids, antihypertensive agents, and diuretics.

Practical nutritional advice is essential as most patients will be receiving corticosteroid therapy, which can act as an appetite stimulant as well as lead to metabolic changes that predispose to obesity. Such advice should include: (a) appropriate caloric intake, (b) food preferences, and (c) food intake patterns (Chapter 16). Basic principles include: (a) modification of the fat content of the diet, and (b) calorie control, to achieve and maintain desired body weight. Patients with low bone density (at or below the fracture threshold) are also seen by endocrine specialists, to consider calcium and hormonal supplements.

After discharge from hospital the patient will continue to participate in a medically supervised and monitored pulmonary rehabilitation program, but specific guidelines are required for the patient to exercise on his or her own. The importance of a continuing exercise program, and of the promotion of mental and physical well-being, cannot be over-emphasized. A regular schedule of moderate exercise will increase stamina, strength, and endurance, will assist in the handling of stress, and lead to improved relaxation and easier weight control. Patients should recognize that, when they accepted the transplantation option, they made a long-term commitment to rehabilitation. Guidance to the patient regarding personal, recreational and sexual pursuits should also be proffered.

The transplant recipient should be given an outline of future outpatient care. When the recipient resides a long distance from the transplant center, arrangements should be made with a local physician to share the long-term care. Close cooperation and communication between patient, family, home physician, and transplant team are essential, both to achieve full rehabilitation and to detect potential complications at the earliest opportunity.

A record book is given to the patient in which he or she is instructed to record daily the medications taken, temperature, pulse rate, weight, and incentive spirometry performance, and any new symptoms the patient has developed. When the transplant physician sees the patient, the physician will record the results of examination and investigations in another part of the record book.

It is helpful to record results of daily spirometry on a graph, condensing several months on a single page. Development of progressive deterioration in these pulmonary flow tests is suggestive of chronic rejection and demands aggressive evaluation. In the absence of obvious causes, a 20% decline in FEV1 and FVC is diagnostic of obliterative bronchiolitis syndrome[18] (Chapter 59). If infection has been ruled out by aggressive evaluation, including bronchoscopy, transbronchial lung biopsy and BAL, augmentation of immunosuppression may be indicated[19].

The patient should be instructed to avoid individuals with any infection, crowded theaters, stores, and restaurants, particularly in the early postoperative weeks. Avoiding construction sites or other dust-laden atmospheres is also prudent.

Clinic visits are arranged initially twice a week, subsequently once a week, and after 6 months reduced to a frequency of once a month. The patient is advised to report to the physician any new symptom or sign, e.g. fever, breathlessness, or a decrease in spirometry performance. Specific instructions regarding preparation for transbronchial biopsy are necessary. Finally, it is important for a member of the transplant team to be available to the patient or the local physician at all times for discussion, reassurance, and advice.

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Physiology and Pharmacology of the Transplanted Lung

R.E. GIRGIS, R. FISHMAN AND J. THEODORE

INTRODUCTION

The rapid growth of clinical pulmonary transplantation during the past decade has provided a wealth of information on the physiology of the lung allograft in humans. Routine measures of pulmonary function are remarkably normal many years after transplantation in uncomplicated cases[1]. Maximal exercise capacity, while reduced, is not limited by ventilatory factors[2]. While the overall physiology of the healthy transplanted lung is compatible with a normal functional status, numerous subtle abnormalities have been described (Table 1).

The mechanisms for many of these changes have not been fully elucidated. Loss of sensory afferents readily explains impaired cough reflex[3], and pulmonary denervation has also been postulated as the basis for bronchial hyperresponsiveness to methacholine[4] and altered ventilatory response to hypercapnia observed in some studies[5]. Changes in lung volumes have been ascribed to mechanical factors as a result of surgery and the underlying disease[6,7]. Special considerations in single lung transplantation (SLTx) include the impact of the native lung on respiratory mechanics[8], expiratory flow limitation[9] and ventilation and perfusion relationships[10]. An understanding of these physiologic changes in the healthy lung allograft allows the detection of abnormal function and the anticipation of potentially altered responses to respiratory insults.

Since many of the physiologic alterations after lung transplantation have been ascribed to pulmonary denervation, we will begin with a brief overview of the nerve supply to the normal lung, followed by the effects of denervation in various models including experimental transplantation.

Table 1 Physiologic abnormalities in lung transplant recipients

Mild restrictive ventilatory defect in some patients
Blunted ventilatory response to hypercapnia in restricted patients
Impaired cough and mucociliary clearance
Decreased maximal exercise capacity
Non-specific bronchial hyperresponsiveness in some patients
Loss of bronchodilator response to deep inspiration during induced bronchoconstriction
Unique aspects of single lung transplantation

INNERVATION OF NORMAL LUNG

Afferent nerves

Three types of receptors convey sensory information to the central nervous system forming the afferent limb of vagal sensory reflexes (Table 2). Almost all afferent fibers travel in the vagus nerve with cell bodies located in the nodose ganglia that terminate in the vagal nuclei[11].

Cholinergic mechanisms

Preganglionic fibers from the vagal nuclei in the brainstem descend in the vagus nerve to terminate on ganglia located around airways and blood vessels. Postganglionic fibers innervate airway smooth muscle, mucous glands and pulmonary vessels. Muscarinic receptors are activated by the release of acetylcholine, resulting in bronchoconstriction, mucus secretion and pulmonary vasodilatation, although the latter effect is probably not physiologically important[12].

Adrenergic mechanisms

Preganglionic sympathetic nerve fibers arise from the upper six thoracic spinal segments and synapse in the upper four thoracic paravertebral ganglia. Postganglionic fibers enter at the hilum and intermingle with cholinergic nerves forming a dense plexus around airway and blood vessels. Adrenergic nerve fibers can be found close to submucosal glands and bronchial arteries. In contrast to a rich parasympathetic nerve supply there is sparse sympathetic innervation of airway smooth muscle. Despite this, there is a high density of beta-adrenoreceptors in airway smooth muscle and on airway epithelial cells. Beta-agonists cause bronchodilatation and may increase mucus and water secretion into airways, thereby enhancing mucociliary clearance. Relatively few

Table 2 Characteristics of the three pulmonary vagal sensory reflexes

Receptor	*Location*	*Fiber type*	*Stimulus*	*Response*
Pulmonary stretch, slowly adapting	Associated with smooth muscle of intrapulmonary airways	Medullated	1. Lung inflation 2. Increased transpulmonary pressure	1. Hering–Breuer inflation reflex 2. Bronchodilatation 3. Increased heart rate 4. Decreased peripheral vascular resistance
Irritant, rapidly adapting	Epithelium of (mainly) extrapulmonary airways	Medullated	1. Irritants 2. Mechanical stimulation 3. Anaphylaxis 4. Lung inflation or deflation 5. Hyperpnea 6. Pulmonary congestion	1. Bronchoconstriction 2. Hyperpnea 3. Expiratory constriction of larynx 4. Cough 5. Mucus secretion
C-fibers pulmonary (type J) bronchial	 Alveolar wall Airways and blood vessels	Non-medullated	1. Increased interstitial volume (congestion) 2. Chemical injury 3. Microembolism	1. Rapid shallow breathing 2. Laryngeal and tracheobronchial constriction 3. Bradycardia 4. Spinal reflex inhibition 5. Mucus secretion

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alpha-receptors are present in the lung. They may be involved in the regulation of bronchial blood flow[11].

Non-adrenergic, non-cholinergic mechanisms

In addition to classic parasympathetic and sympathetic pathways, a third mechanism, involving regulatory peptides as neurotransmitters, has been described in the lung, analogous to that established in the gut. This non-adrenergic, non-cholinergic system appears to consist of both pre- and postganglionic nerves, and may be important in the regulation of airway smooth muscle. Vasoactive intestinal peptide (VIP) immunoreactive nerves are often distributed with cholinergic nerves and VIP may be cotransmitted with acetylcholine. Since VIP is a potent bronchodilator it may function as a neuromodulator of airway smooth muscle tone[11].

Substance P (SP) is a sensory neuropeptide localized to afferent nerve endings in airway epithelium, around blood vessels and within airway smooth muscle. It is synthesized in the nodose ganglion and transported to the lung via the vagus nerve. SP can cause bronchoconstriction, increased microvascular permeability and mucus secretion[11].

EFFECTS OF DENERVATION

Regulation of breathing

Loss of vagal afferents in subprimate mammals produces a low-frequency, high-tidal-volume, irregular breathing pattern[13,14]. A similar pattern is observed after heart–lung transplantation (HLTx) that eventually progresses to apnea[15]. In addition, transplanted dogs respond to hypercapnia by augmentation in tidal volume only with unaltered respiratory rate[16]. These effects are attributed to loss of the Hering–Breuer inflation reflex that normally terminates inspiratory effort in response to lung inflation, allowing exhalation to occur. Afferent impulses decrease as lung deflation proceeds, resulting in repetition of the respiratory cycle. In humans, vagal afferent blockade does not alter the resting ventilatory pattern, suggesting the lack of an important control loop between the lung and respiratory centers[17]. This control loop may assume importance during stimulated breathing, such as with hypercapnia[18], with exercise[19] and in cardiopulmonary disease[20].

Respiratory mechanics

It is well recognized that the vagus nerve exerts resting bronchomotor tone. Intravenous administration of atropine in humans reduces airway resistance by 50%. This is associated with a small but significant increase in lung compliance, presumably as a result of smooth muscle relaxation in the terminal bronchioles and alveolar ducts[21]. There is no tonic sympathetic influence on airway smooth muscle as evidenced by the absence of bronchoconstriction to inhaled propranolol in normal individuals[22].

Airway hysteresis

The airway property of hysteresis is modified by vagal tone. As the lung inflates from residual volume to total lung capacity (TLC), airway caliber increases due to radial traction by surrounding parenchyma. At a given transpulmonary pressure, airway diameter is less during inflation compared with deflation. Vagal blockade abolishes this effect while vagal stimulation enhances it[21,23,24]. Inflation hysteresis is responsible for the transient bronchodilatation observed after a deep inspiration in normal subjects. Atropine reduces the effect of volume history on airway resistance, suggesting a vagally mediated phenomenon[25]. Alternatively, hysteresis may be related to intrinsic mechanical properties of contracted airway smooth muscle[26] and could be modified by the degree of airway–parenchymal interdependence[23,27].

Reinnervation, neurotransmitters and receptors in transplanted lungs

After autotransplantation in animals, bronchial arteries and lymphatics rapidly regain continuity[28,29], while nerves regenerate slowly, if at all[30]. Edmonds demonstrated vagal efferent reinnervation in dogs 3–6 months after lung reimplantation, but no evidence of functional afferent or sympathetic reinnervation[31]. Whether this also occurs in humans is not known. No reinnervation is observed after human heart transplantation[32]. Springall and colleagues, using immunohistochemical techniques, demonstrated the absence of sensory nerve fibers in bronchial mucosal epithelium below the tracheal anastomosis up to 42 months after human heart–lung transplantation[33]. SP was depleted in transplanted lungs, and this finding has also been observed in bronchoalveolar lavage fluid of lung transplant recipients[34]. Markers for adrenergic nerves were decreased compared with controls. However, staining for VIP, which is present largely in cholinergic nerves, was similar to that found in non-transplanted lungs, implying the persistence of postganglionic parasympathetic innervation. Stretton and co-workers supported this observation by demonstrating a normal contractile response in transplanted human bronchi *in vitro* to electrical field stimulation, which is mediated by postganglionic excitatory cholinergic nerves. Responses to isoproterenol were also similar in this model[35]. Additionally, there are no alterations in the density or binding affinity of muscarinic receptors in transplanted lungs[35,36].

PULMONARY FUNCTION AT REST

Lung volumes

Heart–lung and bilateral lung transplantation (HLTx, BLTx)

Early after transplantation there is a restrictive ventilatory impairment that gradually improves with time, so that most patients with healthy grafts achieve predicted total lung capacity (TLC) by 1 year after surgery[6,37–40] (Figure 1). This occurs despite discrepancies between donor and recipient predicted TLC of over 1 liter, suggesting that the major determinant of post-transplant TLC is the mechanical characteristics of the recipient's thoracic cavity[37,41,42]. The restriction observed during the early postoperative period is accounted for by a reduction in inspiratory capacity with preserved end-expiratory lung volume[6], implying an impairment in the respiratory bellows. Glanville *et al.* reported normal lung compliance in restricted patients and found a correlation between maximal transpulmonary pressures and TLC (Figure 2), again implicating abnormal thoracic cage expansion[7]. Maximal inspiratory pressure also correlated with TLC, suggesting respiratory muscle weakness as a factor in some patients. Muscle weakness could be related to pretransplant illness, malnutrition, corticosteroids or phrenic nerve injury. Alternatively, reduced motion of the spine and rib cage, as demonstrated after cardiac surgery[43], could account for decreased dynamic lung volumes. Some patients remain mildly restricted several years after transplantation[1].

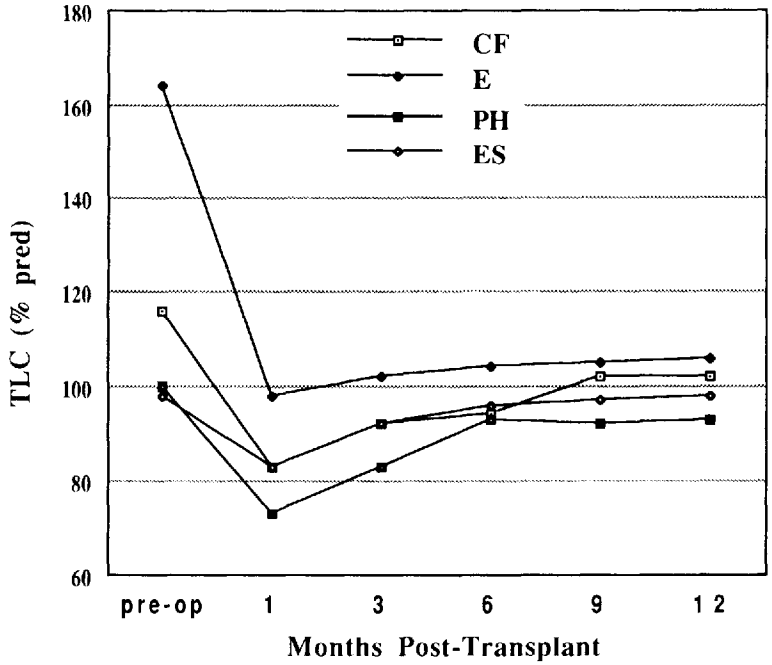

Figure 1 Percentage predicted total lung capacity (TLC) after heart–lung transplantation for various disorders. Data points represent mean values. CF = cystic fibrosis, n = 23; E = emphysema, n = 6; PH = primary pulmonary hypertension, n = 10; ES = Eisenmenger's syndrome, n = 18; Data from ref. 37

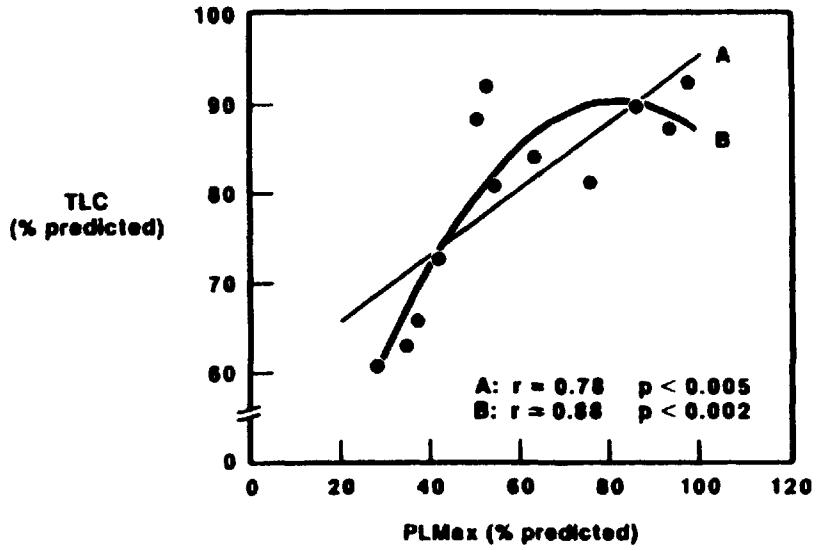

Figure 2 Relationship between maximal transpulmonary pressure ($P_{l,max}$) and total lung capacity (TLC) in 12 heart–lung transplant recipients. Curve B represents a second-order polynomial curve. (Reproduced from ref. 7 with permission)

The nature of the underlying lung disease may also modify lung volumes. Some patients with cystic fibrosis retain high functional residual capacity and residual volume after transplantation due to expansion of the rib cage along the anteroposterior diameter[38]. This lung volume pattern was not observed in patients transplanted for chronic obstructive pulmonary disease (COPD). The authors of this study speculated that hyperinflation in COPD is accommodated primarily by flattening of the diaphragm rather than elevation of the rib cage, whereas in cystic fibrosis the disease is present since childhood at a time when the bony rib cage is growing and can be remodeled.

Single lung transplantation (SLTx)

Respiratory mechanics following SLTx are clearly influenced by the remaining native lung. Restrictive lung diseases (RLD) are ideally suited for SLTx, since the stiff native lung does not interfere with expansion of the normally compliant transplanted lung[44]. Shortly after surgery such patients typically demonstrate a dramatic improvement in lung volumes, being left with only a mild to moderate restrictive defect[45] (Figure 3).

Historically, patients with emphysema were not felt to be good candidates for SLTx because of concern over compression of the

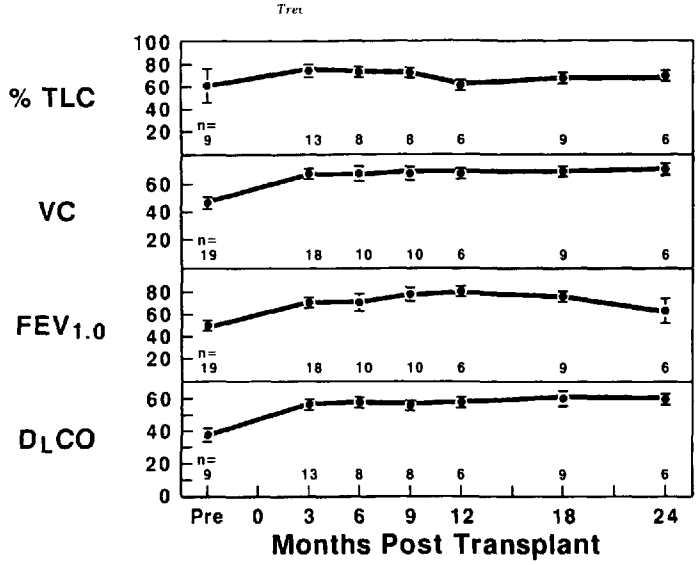

Figure 3 Lung function in 19 single-lung transplant recipients surviving at least 1 month (16 with interstitial lung disease, three with emphysema). Values are percentages of predicted normal and expressed as mean ± SEM. TLC = total lung capacity, VC = vital capacity, FEV_1 = forced expiratory volume in 1 second, DLCO = diffusion capacity for carbon monoxide. (Reproduced from ref. 45 with permission)

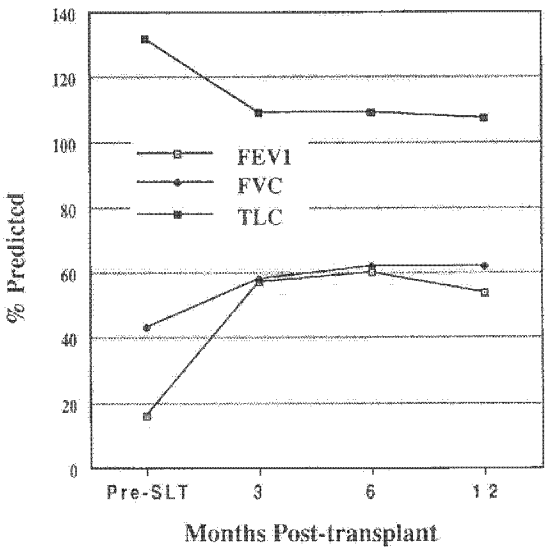

Figure 4 Lung function in 22 subjects after single-lung transplantation for COPD. Values are mean percentages of predicted. Abbreviations as in Figure 3. (Data from ref. 50)

transplanted lung by the hyperinflated, highly compliant native lung[46] and the potential for severe ventilation–perfusion ($\dot{V}/\dot{Q}$) mismatch[47]. Veith and co-workers, in 1973, demonstrated that SLTx in dogs with drug-induced emphysema ventilated the transplanted lung as well as or better than the native side, and that severe $\dot{V}/\dot{Q}$ imbalance did not occur in the absence of complications in the transplanted lung[48]. Numerous centers have now reported good medium-term success with SLTx for patients with COPD[49–51]. Patients experience a marked improvement in forced expiratory volume in 1 second (FEV_1) with less improvement in forced vital capacity (FVC) (Figure 4). TLC falls towards predicted values, but remains mildly elevated in some patients[8]. Although most patients have radiographic evidence of mediastinal shift[52], intervention to relieve the hyperinflation is rarely required[52–54].

The relative contributions of the native and transplanted lung to the TLC after SLTx for obstructive lung disease (SLTx-OLD) has been quantified using planimetry by Cheriyan *et al.*[8]. They found the transplanted lung TLC to be only 35% of predicted. This was associated with low maximal negative pleural pressures at TLC. The reduced distending pressures were attributed to chronic hyperinflation with decreased diaphragmatic length and subsequent impairment of maximal transpulmonary pressure generation.

The contribution of each lung to dynamic lung volumes can be estimated by radiospirometry, in which the relative distribution of inhaled xenon-133 gas is used[55] to calculate transplant lung FVC and FEV_1. This technique confirms that the transplanted lung accounts for the bulk of dynamic lung volumes in patients with restrictive or obstructive disease in the native lung. Patients with pulmonary vascular disease (PVD), in whom lung mechanics are normal, have equal distribution of dynamic lung volumes after SLTx.

Airway resistance and expiratory flow patterns

There are sparse data on airway resistance (R_{aw}) measures in lung transplant recipients. In a small group of patients with pulmonary vascular disease, specific conductance (sG_{aw}, the inverse of R_{aw} corrected for lung volume), increased significantly after heart–lung transplantation[6]. This was associated with an increase in FEV_1/FVC ratio. These findings may be related to the effects of vagotomy on airway smooth muscle tone or the presence of mild airways disease pretransplant[56]. Despite resolution of restriction, HLTx and BLTx recipients tend to retain a slightly elevated FEV_1/FVC ratio[1,37,52]; again, this could reflect the loss of parasympathetic tone. Alternatively, because airflow is dependent on lung elastic recoil in addition to airway caliber, high flow rates could be due to relatively higher lung elastance in donor lungs that tend to be harvested from younger individuals.

Maximal expiratory flow volume (MEFV) curves in HLTx recipients generally have a normal configuration or a slight concavity towards the volume axis, reflecting high flow rates (Figure 5). A plateau is occasionally observed on the expiratory limb. Two mechanisms could account for this phenomenon in the absence of large airway obstruction.

According to the wave speed theory of expiratory flow limitation, airflow velocity cannot exceed the wave speed of the disturbance in the airway wall. Wave speed is slower with decreasing cross-sectional area and more compliant walls[57]. When airflow velocity approaches wave speed, a choke point is created, such that further increases in driving pressure only narrow downstream (mouthward) airways without increasing flow. At high lung volumes the choke point is at the carina. With tracheomalacia at the level of the anastomosis, wave speed is slow and the compliant segment collapses under positive pleural pressure

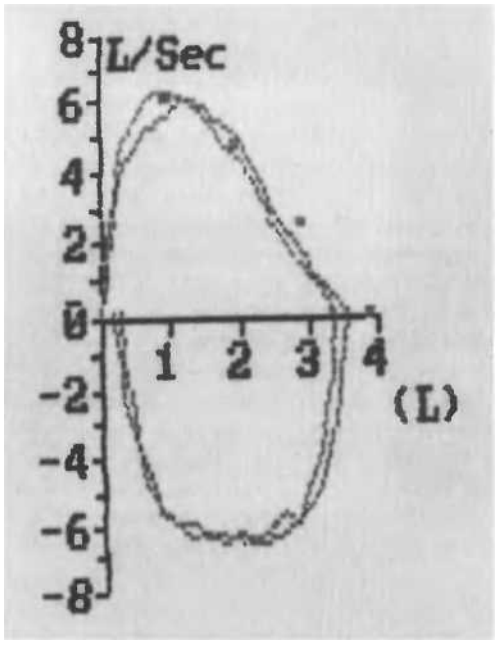

Figure 5 Maximal expiratory and inspiratory flow–volume curve in a 34-year-old female 5 years after heart–lung transplantation for Eisenmenger's syndrome

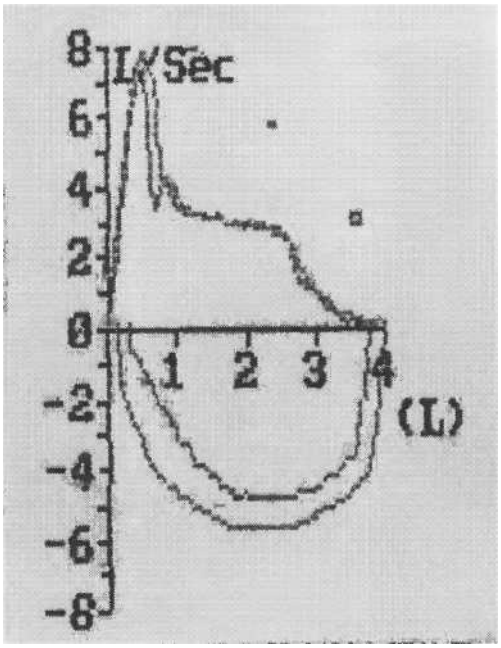

Figure 6 Maximal expiratory and inspiratory flow–volume curve in a 33-year-old male 11 years after heart–lung transplantation for primary pulmonary hypertension, demonstrating a plateau on the expiratory limb. Expiratory collapse of the trachea at the level of the anastomosis was noted at bronchoscopy

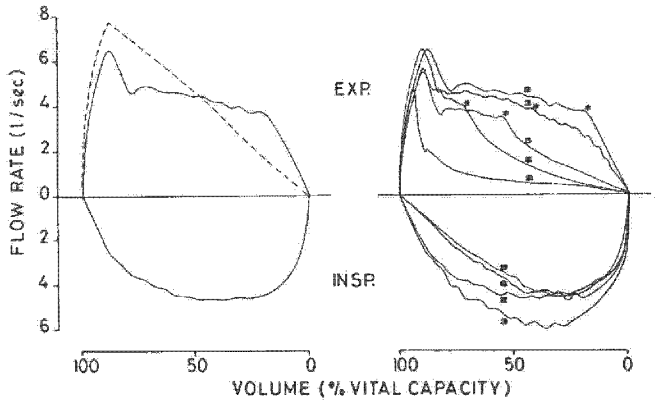

Figure 7 Maximal expiratory and inspiratory flow–volume curves of a 26-year-old woman who received a heart–lung transplant for lymphangioomyomatosis. Left panel: curve obtained 2 months after transplant (solid line) showing a plateau on the expiratory limb compared with the predicted curve (dashed line). Right panel: sequential curves 2–9 months after transplant. As peripheral airways disease progresses due to obliterative bronchiolitis, the plateau shortens and the knee (asterisk) shifts to the left, eventually becoming absorbed into the flow–volume loop. (Reproduced from ref. 58 with permission)

(Figure 6). Alternatively, the loss of vagal smooth muscle tone could likewise reduce the rigidity of the trachea and create a similar plateau[58]. As deflation occurs, radial traction on intrapulmonary airways decreases, resulting in reduced cross-sectional area and stiffness. This results in slower wave speed in distal airways and the choke point moves progressively upstream with exhalation. The 'knee' at the end of the plateau reflects the shift of the choke point from the trachea to more peripheral airways. With increased peripheral airways resistance, as occurs in obliterative bronchiolitis, the choke point and knee move up to higher lung volumes and are eventually absorbed into the flow–volume loop[58] (Figure 7).

A plateau on the MEFV curve is often observed after SLTx for obstructive lung disease (SLTx-OLD) (Figure 8). Using individual lung radiospirometry and fluoroscopy, Herlihy and colleagues concluded that the site of flow limitation is the native bronchus immediately downstream to the anastomosis[9]. This area would be expected to have a compliant wall compared with the rigid telescoped anastomosis. A biphasic pattern (defined as flow >3.5 l/s at some point early in expiration, followed by flow <0.5 l/s over at least the last 30% of the FVC) was also described on all MEFV curves in SLTx-OLD patients. This was attributed to differential elastic recoil of the two lungs with the slower flow rate reflecting emptying of the native lung. These investigators subsequently described an expiratory plateau after SLTx for RLD and implicated the same mechanism[59]. However, two other reports failed to detect an expiratory plateau in SLTx recipients with RLD[60] and PVD[61], while confirming its presence in chronic obstructive pulmonary disease (COPD). These groups contend that the expiratory plateau originates from the emphysematous lung following complete emptying of the donor lung, and not from flow limitation at the bronchial anastomosis.

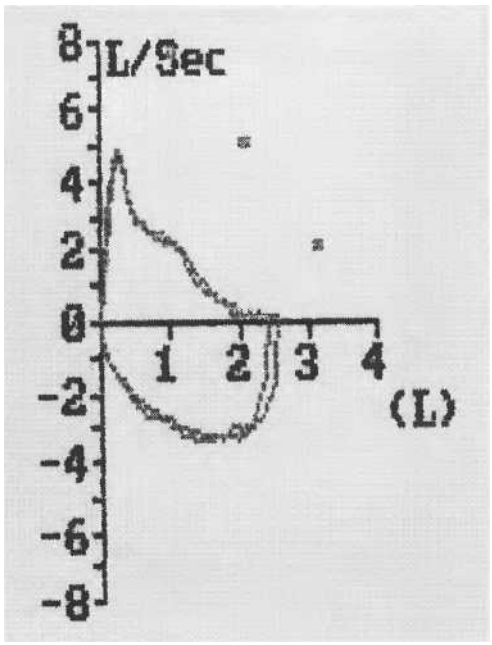

Figure 8 Maximal expiratory and inspiratory flow–volume curve in a 56-year-old male 2 years after single-lung transplantation for COPD, demonstrating plateau on the expiratory limb

Variability of spirometric measurements

Spirometric data are heavily relied upon to detect complications in the lung allograft. Thus, awareness of the expected intra-

individual variability of these measures is important in clinical decision-making. The coefficients of variation (CV) are only slightly higher than normal volunteers[62]. In HLTx and BLTx recipients, CV for FVC is 2.45–3.8%, and 3.07–4.4% for FEV_1. Comparable values[63] for SLTx recipients are 4.2% for FVC and 5.6% for FEV_1.

Pulmonary circulation

Although increased pulmonary vascular resistance (PVR) has been observed in animal models of lung transplantation[64,65], no hemodynamically evident pulmonary vascular changes are noted in the long-term follow-up of uncomplicated HLTx recipients[66,67]. Replacement of one lung results in an immediate and marked fall in PVR in patients with pre-existing pulmonary hypertension[68–70]. In a recent series, PVR index fell from a mean of 852 dyne-s/cm^5 per square meter preoperatively to 139 after SLTx[71]. This is accompanied by a dramatic recovery of right ventricular function, as indicated by improved ejection fraction, decreased cavity size and diminution of tricuspid regurgitation[72]. While cyclosporin is known to increase systemic vascular resistance, no such effect was demonstrated on the pulmonary circulation in heart transplant recipients[73]. We have not observed significant increases in PVR up to 5 years after SLTx, even in a patient with primary pulmonary hypertension (Girgis, Theodore, unpublished data).

Pulmonary arterial anatomy and blood flow after SLTx have been characterized by magnetic resonance imaging in patients with restrictive and obstructive lung disease[74]. Luminal diameters proximal and distal to the anastomosis were similar, and not different when compared with control subjects. A small pressure gradient was detected across the suture line, although in one patient the calculated gradient was 25 mmHg. As expected, blood flow in the transplanted lung was higher than in the native lung, with a mean ratio of 2.8. The flow pattern was also qualitatively different in the transplanted lung. The artery to the transplanted side showed continuous forward flow throughout systole and most of diastole. In contrast, the native side had a narrow forward flow during early systole only, with a reverse flow during late systole and diastole. Reversed flow from native to transplanted lung was observed in four of nine subjects.

Regional distribution of blood flow has been evaluated in HLTx recipients, using single-photon emission computed tomographic imaging of pulmonary perfusion[75]. This study demonstrated a normal horizontally layered distribution characterized by preferential perfusion in the central region of the lungs, with a gradual decrease towards the periphery. The normal vertical distribution was also preserved according to the zones of West *et al.*[76]. Thus, pulmonary denervation has no effect on the pattern of blood flow at rest.

Diffusion capacity of carbon monoxide (DLCO)

No consistent changes in DLCO have been observed after lung transplantation. When corrected for alveolar volume (DL/VA), transfer of CO is normal after HLTx[67,77] and BLTx[78]. Total DLCO is decreased after SLTx, as would be expected, given the reduced surface area available for diffusion[45,69,78]. Reductions in DL/VA have also been observed after SLTx[44,79], probably reflecting the altered alveolo-capillary membrane in the native lung. In one report, DLCO was significantly lower in SLTx recipients with interstitial lung disease (ILD) compared with OLD[79]. DL/VA also tended to be lower in the ILD group. We have noted higher than normal DL/VA in several patients with OLD after SLTx. This may reflect the compressed transplanted lung, with relatively small alveolar volumes as measured by single-breath helium dilution, receiving most of the pulmonary blood flow, and thus enhanced transfer of CO per unit volume. A small decline in DLCO has been reported after heart transplantation that correlated with cyclosporin levels[80–82]. A similar finding in lung transplant recipients has not been described.

Hypoxic pulmonary vasoconstriction (HPVC)

It is generally agreed that local mechanisms, rather than neural, mediate HPVC[83]. Robin *et al.* demonstrated the preservation of HPVC in HLTx recipients[84], suggesting that autonomic innervation is not required for this response. In dogs after single lung autotransplantation, HPVC was similar to controls[65]. On the other hand, monkeys had no response to hypoxia 5 years after heart–lung auto- and allotransplantation[64].

Ventilation, ventilation–perfusion ($\dot{V}/\dot{Q}$) relationships and gas exchange

The distribution of ventilation in the peripheral airways is normal in uncomplicated HLTx recipients, as determined by inert gas single-breath washout[85]. As indicated previously, these patients also have normal distribution of perfusion; thus HLTx and BLTx recipients with healthy allografts have normal $\dot{V}/\dot{Q}$ relationships. This is reflected by the normal to slightly elevated alveolar–arterial oxygen difference [$P\ (A–a)\ O_2$] observed in these patients[39,67]. The $P\ (A–a)\ O_2$ is widened immediately after transplantation due to ischemic-reperfusion injury, but falls rapidly to near normal values within 24 hours[86]. Similarly, the arterial to end-tidal CO_2 gradient is increased immediately after BLTx, reflecting high alveolar dead space ventilation, but normalizes within 24 hours[87]. Dead space to tidal volume ratio at rest is normal to slightly elevated in HLTx recipients[88,89]; therefore, arterial blood gases should be near normal soon after HLTx and BLTx in the absence of complications[39,88,89]. Some patients have mild alveolar hyperventilation with hypocapnia and chronic respiratory alkalosis[88,89], possibly due to a restrictive ventilatory impairment.

Nuclear ventilation scanning techniques commonly employ either radioactive inert gas (^{133}Xe or ^{81m}Kr), or the technetium-labeled (^{99m}Tc) radioactive aerosol of diethyleneamine pentacetic acid (DTPA). The latter method, in addition to demonstrating the distribution of ventilation, can assess the permeability of the alveolo-capillary membrane since it is cleared via the bloodstream. Clearance of ^{99m}Tc-DTPA is normal after HLTx, but is markedly faster during rejection[90].

The distribution of ventilation and perfusion after SLTx is affected by the ventilatory impedance and PVR, respectively, of the native lung. In patients with RLD and OLD most of both ventilation and perfusion is diverted to the transplanted lung immediately postoperatively. The percentage gradually increases, mostly during the first month, reaching maximal values ranging from 68% to 99% for $\dot{Q}$ and 61% to 85% for $\dot{V}$ at 3 months[8,10,44,50,54,79,91]. Most studies report roughly equal proportions of $\dot{V}$ and $\dot{Q}$ to the transplanted lung, while some groups have found slightly less

ventilation[91]. One series demonstrated a significantly smaller proportion of ventilation distributed to the transplanted lung in ILD compared with OLD patients[79]. The *P (A–a)* O_2 is similar in these two groups, in the range of 14–21 mmHg, and mild hypocapnia is also observed[10,50,79].

Patients with pulmonary vascular disease have relatively normal ventilatory mechanics; thus, SLTx results in only a minor shift of ventilation (44–65% to the transplanted lung), while nearly the entire cardiac output (95–99%) is directed to the allograft[10,92]. Despite the low $\dot{V}/\dot{Q}$ ratio, the *P (A–a)* O_2 is still sufficiently narrow (18–37 mmHg) to allow adequate gas exchange. However, with acute rejection or OB, ventilation shifts away from the allograft. Perfusion cannot shift as readily to the native lung because of the high PVR, resulting in severe hypoxemia. This situation contrasts with that observed in RLD and OLD, in which acute rejection affects perfusion more than ventilation[10,44,92].

The ability of transplanted lungs to shift perfusion away from poorly ventilated units may be impaired. In a study of 13 SLTx recipients with various underlying disorders, Kuni and colleagues demonstrated a significantly greater degree of $\dot{V}/\dot{Q}$ mismatching (ventilation worse than perfusion) in the allograft compared with the native lung. Most of the scans were obtained at a time when chest radiographs and lung biopsies were abnormal[93]. Similarly, a case of OB in a BLTx recipient had widespread ventilation defects with only mildly abnormal distribution of perfusion[94]. Thus, while hypoxic pulmonary vasoconstriction is preserved in healthy lung allografts, these reports suggest that this mechanism may be impaired during pathologic processes.

Cough and mucociliary clearance

The lack of cough upon stimulation with a bronchoscope below the anastomosis has long been recognized in lung transplant recipients, and taken as evidence of sensory denervation[95]. In a group of HLTx recipients studied 1–36 months after surgery, the cough response to ultrasonically nebulized distilled water (USNDW) was markedly reduced[3]. All patients, however, coughed in response to direct instillation of distilled water onto the larynx. The failure of USNDW to stimulate rapidly adapting (irritant) receptors mediating the cough reflex in the larynx could be explained by the fact that only 10% of the aerosolized particles were deposited in the laryngeal/tracheal region. Alternatively, loss of pulmonary slowly adapting stretch receptors, which has been shown to blunt cough in response to chemical laryngeal stimulation in rabbits[96], could account for this observation[3].

Several reports have documented decreased mucociliary clearance (MCC) after HLTx and SLTx as assessed by the clearance of radiolabeled sulfur colloid or albumin[97–99]. Ciliary beat frequency was slightly diminished in one study[97] and normal in another[98]. Thus, the decreased MCC could reflect decreased mucus secretion or altered rheologic properties of mucus as a result of autonomic denervation. Impaired MCC could partly explain the high frequency of lower respiratory tract infections observed in these patients[100].

Regulation of breathing

Resting minute ventilation ($\dot{V}_E$) is similar in HLTx recipients and normal individuals[5,77,101]. There is a trend, however, towards mild hyperventilation[5,101]. When corrected for body surface area, Duncan *et al.* found a significantly higher $\dot{V}_E$ in HLTx recipients (5.3 ± 0.3 vs 4.6 ± 0.2 l/min per square meter in normals). This was achieved by a small increase in tidal volume (V_t) with no difference in respiratory frequency (f). An identical pattern was noted in heart transplant recipients[102]. Restricted HLTx recipients, while having a similar degree of hyperventilation, exhibit shallow breathing, with a significantly higher f value than controls (15.3 ± 3.3 vs 11.8 ± 2.0 breaths/min)[101].

The shorter total respiratory cycle time (T_{tot}) noted in restricted patients is associated with a reduced inspiratory time (T_i) and a smaller duty cycle (T_i/T_{tot})[103]. Tachypnea is a recognized manifestation of restrictive lung diseases and is related to decreased lung compliance[104]. The modified breathing pattern noted in restricted HLTx recipients suggests that afferent neural signals from the lungs are not the only mediators of tachypnea in pulmonary restriction. Animal studies have demonstrated the importance of chest wall receptors in modifying breathing pattern[105]. The reduction in T_i/T_{tot}, however, is not typical of restrictive lung disease, in which it is generally unchanged[106]. The failure of expiratory time (T_e) to shorten in proportion to T_i may partially blunt the frequency response of these patients[103]. This hypothesis is supported by work showing a reduction in resting f after vagal blockade in pulmonary restriction, but not in normals[20].

Ventilatory response to hypoxia and hypercapnia

The ventilatory response to isocapnic hypoxia is normal in HLTx recipients[5]. In non-restricted HLTx and BLTx patients the minute ventilation increases appropriately in response to progressive hypercapnia[101,102,107]. When comparing only male HLTx recipients with male heart transplant (HTx) recipients and control males, Duncan and co-workers found the increment in V_t adjusted for FVC to be significantly higher and the frequency response blunted in HLTx compared with HTx. Control males had an intermediate response in both V_t and f (Figure 9)[102]. Thus, a rapid, shallow breathing pattern in response to hypercapnia was most prominent in HTx and least prominent in HLTx. Although the mean FVC of the transplanted subjects was in the normal range, it was lower than the control group (88%, 95% and 105% predicted for HLTx, HTx and controls, respectively). TLC in both transplant groups was 88% predicted. Similar to other forms of pulmonary restriction, the HTx patients responded to hypercapnia with rapid, shallow breathing[108]. The blunted frequency and augmented V_t response in the HLTx patients with similar pulmonary function probably represents the sequelae of pulmonary vagotomy with loss of inflation inhibition. This response has also been observed after BLTx[107]. The counteractive effects of restriction and vagal denervation have been shown in dogs in which experimentally induced interstitial lung disease obscured the slow, deep breathing pattern observed with exercise after vagotomy[109].

Grossly restricted HLTx recipients (FVC <80% predicted) have a limited or diminished V_t response to hypercapnia expressed as $\Delta V_t/\Delta P_{CO_2}$, as is the case in vagally intact subjects with reduced lung volumes. The latter respond to hypercapnia with supranormal respiratory rates and are able to mount a normal increase in minute ventilation. As a result of a relatively blunted frequency response, restricted HLTx subjects exhibit a reduced overall ventilatory response to hypercapnia[5,101]. This is accompanied by a de-

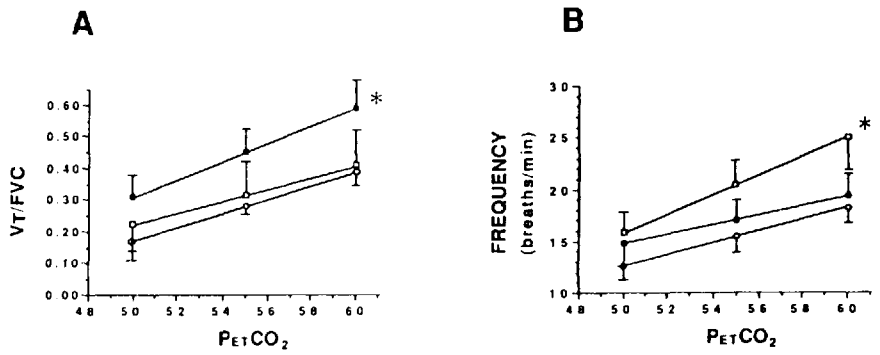

Figure 9 Breathing patterns during CO_2 rebreathing in male subjects. Closed circles denote heart lung transplant recipients; open circles represent normal control subjects; open squares denote heart transplant recipients. Points shown are mean ∓ SEM. **A:** Adjusted tidal volume (V_t/FVC) response to end-tidal CO_2 was significantly greater in HLTx than the other groups (asterisk indicates $p = 0.02$). **B:** Frequency response was significantly greater in HTx than the other groups (asterisk indicates $p<0.03$). (Reproduced from ref. 102 with permission)

creased ventilatory drive response, as indicated by a reduced inspiratory flow–CO_2 relationship ($\Delta V_t/T_i/\Delta P_{CO_2}$) and $\Delta P_{m_{0.1}}$ (mouth pressure 100 ms after the beginning of an occluded inspiration[101])/ΔP_{CO_2}. The mechanism of these abnormalities is not clear; they may indicate diminished neuromuscular output or mechanical alterations that interfere with the relationship between pressure generation and respiratory neural efferent activity[101]. Sanders *et al.* postulated that the loss of afferent signals from CO_2-sensitive receptors in the lungs, which have been detected in animals[110], may be responsible for these observations[5]. Whatever the mechanism, these physiologic derangements could predispose restricted lung transplant recipients to respiratory failure when lung function is further compromised or when ventilatory demand increases.

Breathing during sleep

Normal sleep architecture is preserved after HLTx[103]. Breathing pattern was generally normal with no evidence of sleep apnea or desaturation in two small series of HLTx subjects[77,103]. There was perhaps more variation in V_t and T_e during stage 2 sleep[77], and restricted patients maintained their mild tachypnea and respiratory alkalosis throughout all sleep stages[103]. No large cross-sectional studies are available to determine the prevalence of sleep-disordered breathing after lung transplantation. A high prevalence of sleep apnea (8%) has been reported in HTx patients[111]. Weight gain due to corticosteroids and pulmonary function abnormalities could predispose lung transplant recipients to sleep-disordered breathing.

PHARMACOLOGY OF THE TRANSPLANTED LUNG

Airway pharmacology and bronchial reactivity

Response to inhaled bronchodilators

The response to inhalation of albuterol (a β_2-agonist) and ipratropium bromide (an anticholinergic agent) was assessed in eight recent (1–4.5-month) HLTx recipients without obstruction, by Glanville and co-workers[4]. Neither agent led to changes in FEV_1; however, albuterol produced up to a 100% increase in sG_{aw}, considerably higher than the 24% increase observed in normal subjects[112]. In contrast, only a small increment in sG_{aw} was noted after ipratropium, compared with the doubling seen in normals[21]. This observation is consistent with the absence of basal vagal bronchomotor tone after transplantation.

Longitudinal responses of spirometric indices to inhaled β-agonists were studied by the Pittsburgh group in pediatric HLTx and BLTx recipients[113]. Half of the subjects studied more than 8 weeks after transplant exhibited a significant improvement in expiratory flow rates, while 10% experienced a paradoxical decrease. In contrast, 50% of subjects studied within 8 weeks of surgery demonstrated a negative response. Thus, the acutely denervated and ischemic airway may be predisposed to collapse during forced exhalation, resulting from the smooth muscle relaxant effects of β-agonists.

Bronchodilator responsiveness may reflect underlying airway inflammation. The Toronto group reported that 11 of 14 BLTx patients with bronchiolitis obliterans syndrome (BOS)[114] had a bronchodilatory response to β-agonist inhalation preceding the clinical onset of BOS by a mean of 4.4 months[115]. Six of the 11 responders had OB on biopsy. None of 10 subjects who did not develop BOS had a bronchodilator response. The relationship between reactive airways and inflammation after lung transplantation is also supported by the correlation of diurnal variation in FEV_1, with acute rejection and airway cellular infiltration and epithelial damage[116].

Bronchoprovocation studies

The issue of bronchial hyperresponsiveness (BHR) to methacholine (MCh) inhalation after lung transplantation is controversial. Glanville *et al.* demonstrated BHR to MCh in nine of 10 HLTx recipients 1.5–28 months after surgery. The mean $PD_{20}FEV_1$ (the dose of MCh required to induce a 20% fall in FEV_1) was 1.7 mg compared with 11.6 mg in controls[117]. In a subsequent study that separately evaluated recently transplanted subjects and long-term survivors (>1 year), similar results were obtained[4]. The Harefield group supported this observation by showing BHR to MCh in 10 of 12 HLTx patients with a mean $PC_{20}FEV_1$ (concentration of MCh required to induce a 20% fall in FEV_1) of 8 mg/ml compared with 64 in HTx subjects and >64 in controls[118]. Likewise, the Papworth group found BHR to MCh in 11 of 16

HLTx patients[119]. Concomitant transbronchial biopsies revealed no correlation between rejection or airway inflammation and BHR; nor was there a relationship between baseline FEV_1 or time since transplant and PC_{20}. Six patients had a bronchomotor response to ultrasonic nebulized distilled water (USNDW) that was associated with acute rejection, but not airway inflammation. There was no relation between BHR to MCh and USNDW[119]. Maurer and colleagues observed BHR to MCh in all of three HLTx and three BLTx recipients[120]. Histamine challenge produced similar results in all but one patient tested. Interestingly, only five of eight and four of seven SLTx patients were reactive to MCh and histamine, respectively. Furthermore, the PC_{20} values of both agents were significantly higher in the SLTx vs the HLTx/BLTx groups. Bronchial mucosal biopsies showed minimal inflammatory changes in only two SLTx and one HLTx patients. These results suggest that intact innervation of the native lung may modify BHR in SLTx recipients[120]. In contrast, Herve and co-investigators detected BHR to MCh in only one of 13 HLTx and BLTx subjects with normal lung histology[121]. Ernst *et al.* found BHR in two of four BLTx patients early after transplantation, who subsequently became normal responders when restudied at 9–12 months[122].

Clearly some patients do have BHR to MCh. The possible mechanism(s) is unclear. In asthma, BHR is related to airway inflammation[123]. This does not appear to be the case after lung transplantation, as indicated above. Further differentiating the BHR after transplantation from asthma is the limited maximal airway narrowing that occurs in the former[4]. Asthmatics often fail to demonstrate a plateau, with a progressive fall in FEV_1 as the dose of MCh is increased. Lungs from asthmatic or atopic donors could be responsible in some cases[124]. Pulmonary denervation with hypersensitivity of muscarinic receptors has been implicated[4,118,119]. Up-regulation of muscarinic receptors has been noted after denervation[125] and rebound hyperresponsiveness to MCh occurs after cessation of long-term treatment with inhaled ipratropium bromide[126]. However, as discussed previously, the contractile response of transplanted airways to MCh *in vitro* is normal, and there is no evidence of increased density or binding affinity of muscarinic receptors[35,36]. Cyclosporine, while having recognized effects on vascular smooth muscle, does not alter bronchial smooth muscle contraction in isolated rat airways[127]. Another possibility is the loss of bronchodilatory impulses from the NANC nervous system[117,119]. This seems unlikely given the finding of normal amounts of VIP-ergic nerves in transplanted lungs[33]. Alteration in bronchial blood flow is an attractive theory for the hyperresponsiveness to USNDW, especially in the setting of rejection[119]. Decreased mucociliary clearance and alterations in the rheologic properties of mucus could increase the amount of MCh reaching its receptor[117]. Finally, subtle epithelial damage or low grade inflammation not evident in mucosal biopsies could explain the non-specific BHR of lung transplant recipients.

Effect of inhaled capsaicin

Capsaicin, the pungent extract of red pepper, stimulates unmyelinated C-fiber afferents in airway epithelium, causing reflex bronchoconstriction in dogs. This effect may be mediated by the collateral release of SP and calcitonin gene-related peptide (CGRP) as part of an axon reflex[11]. In normal humans, inhaled capsaicin induces reflex cough[128], with no effect on FEV_1; after adrenergic and cholinergic blockade it leads to bronchodilatation[129]. Asthmatics often respond with bronchoconstriction. In HLTx patients, inhalation of capsaicin failed to induce cough, but eight of 15 subjects responded with bronchodilatation[128]. In this study there was no relationship between capsaicin responsiveness and rejection or BHR to MCh which was detected in six subjects. This observation can be explained by the depletion of the bronchoconstrictor sensory neuropeptides SP and CGRP, and the persistence of the bronchodilatory VIP after transplantation[33].

Effect of deep inspiration

Deep inspiration (DI) in asthmatics is followed by an immediate and transient increase in airway resistance[130]. This effect is not observed in HLTx patients, despite the presence of BHR[131]. In normal individuals, DI transiently abolishes or attenuates experimentally induced bronchoconstriction. Absence of this effect in asthmatics correlates with increased severity of disease[132]. In hyperresponsive HLTx recipients, Glanville *et al.* demonstrated no consistent effect of DI on sG_{aw} during MCh-induced bronchoconstriction (Figure 10)[131]. In contrast, Banner and colleagues found a preserved response in four of six HLTx patients as assessed by the ratio of maximal and partial expiratory flow volume curves[118]. The mechanism of DI-induced bronchodilatation during induced bronchoconstriction is controversial. Some investigators have attributed it to a neural reflex[131], since airway hysteresis is abolished by atropine[25]. However, inhaled ipratropium did not block bronchodilatation after DI during bronchoconstiction induced by prostaglandin $F_2\alpha$[133]. Based on studies in isolated trachea, Sasaki and Hoppin[26] postulated that tension in contracted airway smooth muscle increases with stretch until a critical level is reached, which leads to disruption of myofilament cross-links. In severe asthma, peribronchial edema could interfere with airway–parenchymal interdependence during lung inflation, and airways would thus remain narrow after deep inspiration. This concept is supported by the finding of a greater bronchodilatory effect of DI during MCh-induced than during antigen-induced bronchoconstriction in hay-fever subjects[27]. Alterations in bronchial cartilage, consisting of ossification, calcification and fibrovascular ingrowth observed in transplanted lungs[134], may have a similar effect and could explain the impaired inflation hysteresis of airways.

Vascular pharmacology

Few data exist regarding the reactivity of the pulmonary vasculature after lung transplantation. Nilson and colleagues studied the response of isolated pulmonary artery vascular rings from dog lung allografts 8 days after transplantation to a variety of vasoactive agents[135]. Contractile responses to phenylephrine were similar to controls, indicating no alterations in α_1-adrenergic receptor responsiveness after denervation. However, in vascular rings with intact endothelium, norepinephrine (NE) caused enhanced vasoconstriction in the transplanted group. No difference was observed in vascular rings devoid of endothelium. Stimulation of α_2 adrenoreceptors has been shown to cause endothelium-dependent relaxation of pulmonary as well as systemic

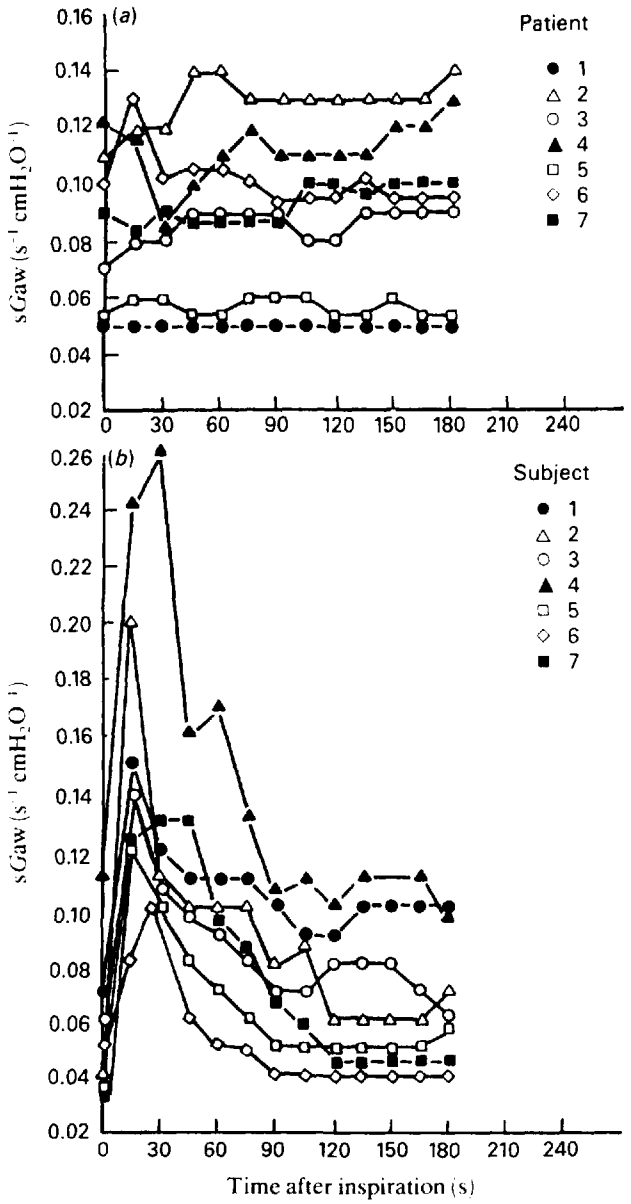

Figure 10 Effect of a deep inspiration on sG_{aw} in **(a)** seven heart–lung transplant recipients and **(b)** seven normal controls after methacholine-induced bronchoconstriction. (Reproduced from ref. 131 with permission)

vessels[136]. The enhanced vasoconstriction to NE, an agonist of α_1, α_2 and β_1 receptors, in endothelialized rings is consistent with impaired release of endothelium-derived relaxant factor after denervation[137].

The contractile responses to endothelin were depressed in the transplanted group[135]. Serum endothelin levels were not elevated after transplantation in this study. Shennib and co-workers reported increased plasma endothelin levels 1 week after lung allo- and autotransplantation in dogs, and elevated BAL endothelin levels during the first 24 hours after allotransplantation[138]. There was also decreased sensitivity to angiotensin I in vascular rings without endothelium from the transplanted dogs[135]. No differences were observed in vasodilatation induced by nitric oxide, ADP, histamine, bradykinin, and the calcium ionophore, A23187, despite the use of cyclosporin in these animals, which is known to attenuate nitroprusside-induced vascular relaxation in systemic arteries[139]. This is consistent with the absence of elevated PVR in long-term survivors of HLTx[66,67] and the lack of correlation between cyclosporin levels and PVR in HTx[73] and HLTx recipients[66].

Pharmacokinetic considerations of cyclosporin in cystic fibrosis (CF)

It is well recognized that oral dosage requirements of cyclosporin in CF patients are higher than those of other lung transplant recipients. The lipophilic nature of the drug accounts for decreased bioavailability in this population, due to biliary and pancreatic exocrine insufficiency. Administering the drug concomitantly with pancreatic enzyme replacement improves bioavailability (from 0.11 to 0.17), but it remains well below normal (0.33)[140]. Mancel-Grosso *et al.* reported enhanced total clearance of cyclosporin after intravenous administration in CF subjects awaiting transplantation[140]. This may be related to enhanced hepatic microsomal cytochrome P-450 metabolism, as has been shown for theophylline in these patients[141]. Tan and colleagues, on the other hand, did not demonstrate enhanced biotransformation in CF patients[142]. In this study, CF subjects awaiting transplantation had normal total clearance, while those with Eisenmenger's syndrome had reduced clearance, probably reflecting an elevated hematocrit. Oral clearance in CF patients was increased, as indicated by a decreased area under the time–concentration curve, decreased maximal concentration (C_{max}) and prolonged time to C_{max}. These authors recommended that initial oral dosages of 1.5–2 times normal be used in these patients, and given in small divided doses (three or four a day). Additional factors that could alter pharmacokinetics of cyclosporin in CF patients include low serum lipoproteins and enhanced prehepatic metabolism[142].

CARDIOPULMONARY EXERCISE TESTING AFTER LUNG TRANSPLANTATION

The exercise capacity of lung transplant recipients is more than adequate to support activities of daily living. Formal cardiopulmonary exercise testing, however, uniformly reveals subnormal maximal work capacity and oxygen consumption, despite normal resting values of pulmonary and cardiac function. Reported values of maximal oxygen consumption (max $\dot{V}O_2$) consistently range between 40% and 60% of predicted, with no significant difference among recipients of SLTx, BLTx or HLTx (Figure 11)[2,79,89,143–147]. Exercise capacity is also similar to that of HTx recipients[88,147,148]. Max $\dot{V}O_2$ peaks at roughly 6 months post-transplant without further significant improvement[89,143]. Most subjects stop exercise because of leg fatigue rather than dyspnea[79,143,147]. Exercise is not limited by ventilatory factors or gas exchange, but is associated with early onset of the anaerobic threshold (Figure 11). The latter observation indicates impaired oxygen delivery to exercising muscles or inefficient utilization of O_2[149]. As will be discussed below, cardiac limitation *per se* has not been

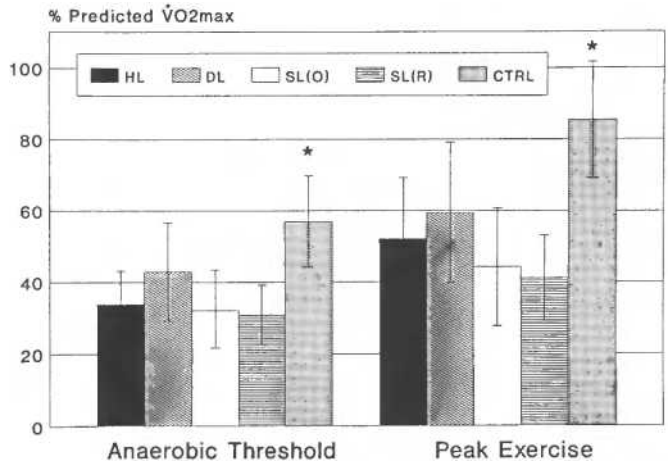

Figure 11 Mean values of oxygen uptake ($\dot{V}O_2$) at the anaerobic threshold and peak exercise. Values are similar among different groups of lung transplant recipients, but significantly less than controls. HL = heart–lung, DL = double lung, SL(O) = single lung for obstructive disease, SL(R) = single lung for restrictive disease, CTRL = normal controls. (Reproduced from ref. 2 with permission)

demonstrated. Recent theories of exercise limitation after lung transplantation have focused on metabolic derangements in skeletal muscle as a result of deconditioning or immunosuppressive medications[148,150].

Ventilatory responses to exercise

Breathing reserve [BR: 1 – the ratio of $\dot{V}_E$ at peak exercise to maximum voluntary ventilation (MVV)] is well above 0.3 (0.53–0.67) in HLTx and BLTx patients[88,144,145,147,151]. SLTx subjects achieve a higher $\dot{V}_E$/MVV (BR = 0.3–0.53) due to increased dead space ventilation[79,143–146]. Peak $\dot{V}_E$/MVV tends to be higher in SLTx-RLD compared with other SLTx patients due to the additional influence of restrictive physiology in the native lung[2,79].

The ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$, respectively) are elevated in conditions with increased V_d/V_t or hyperventilation (low end-tidal or arterial CO_2)[152]. Most studies report an appropriate fall in V_d/V_t with exercise in HLTx and BLTx recipients, but the ratio remains slightly above age- and sex-matched normals, in the range of 0.22–0.34 at maximal exercise[89,144,151]. One series of female HLTx recipients achieved a normal mean V_d/V_t of 0.11 at peak exercise[88]. Elevated dead-space ventilation could be a consequence of increased airway caliber as a result of denervation[151] or subtle pulmonary vascular disease[153]. Alternatively, a relatively small tidal volume response due to restriction can result in an elevated V_d/V_t ratio during exercise.

As previously mentioned, mild hyperventilation is often present at rest. This persists during exercise, as indicated by low arterial or end-tidal CO_2[88,144,145,151]. One group speculated that hyperventilation could be due to the loss of inhibitory sympathetic afferents[151]. Elevated dead-space and hyperventilation combine to produce an increased $\dot{V}_E/\dot{V}O_2$ in the range of 45 and $\dot{V}_E/\dot{V}CO_2$ around 40 at the anaerobic threshold[2]. Dead-space fraction is elevated at rest in SLTx patients (0.4–0.5) and falls only to 0.37–0.4 at maximal exercise[144,154,155]. Despite the higher V_d/V_t during exercise, SLTx-OLD and SLTx-PVD subjects have ventilatory equivalents similar to those observed in HLTx and BLTx patients[2,79,145,146]. On the other hand, SLTx-RLD patients tend to have higher ventilatory equivalents, probably reflecting a greater degree of hyperventilation due to the presence of the stiff native lung[2,79].

The pattern of breathing has been of interest to investigators studying the role of pulmonary innervation in modifying the tidal volume and frequency response to exercise. The V_t/FVC% at maximal exercise is normal and ranges from 44 to 57, with no significant difference among transplant groups. Maximal respiratory rate ranges from 28 to 40/min, with the higher values observed in SLTx-RLD subjects[2,79,145]. Normally, increases in minute ventilation during exercise are initially achieved predominantly by an increase in V_t with little change in f, until a plateau is reached, at which point V_t stabilizes and further increments in $\dot{V}_E$ are brought about by an increase in f. Sciurba and colleagues observed a blunted frequency response in HLTx subjects compared with HTx recipients, and attributed this to loss of afferent signals from intrapulmonary stretch receptors[88]. However, two other groups demonstrated a frequency response similar to normals[147,151]. Chronic pulmonary congestion with resultant low lung compliance probably accounts for the rapid, shallow breathing observed in the heart transplant group[151]. Patients with OB demonstrate rapid, shallow breathing with exercise similar to nontransplanted subjects with lung disease. Arterial PCO_2 is appropriately regulated in OB patients, indicating that pulmonary innervation is not essential to the regulation of ventilation or breathing pattern in lung disease[156]. The perception of dyspnea in lung transplant recipients is not different from controls at similar levels of ventilation as is the inspiratory drive[151] as indicated by $P_{0.1}$. This is consistent with work demonstrating normal detection of inspiratory resistive loads[157].

Cardiovascular response to exercise

Heart rate (HR) at maximal exercise is well below predicted values after lung transplantation, ranging from 68% to 90% of predicted[2,79,89,143–147,151]. No significant differences are observed among the different groups of lung transplant recipients[2,145,146]. The $\dot{V}O_2$/HR relationship, an indirect indicator of stroke volume, is low at peak exercise, but increases linearly with HR without reaching a plateau, indicating the absence of a cardiac limitation[144]. As a result of cardiac denervation, the HR response at the onset of exercise is blunted in HLTx recipients, increases briskly with higher workloads and falls slowly after stopping[88]. This pattern is similar to that observed after HTx, and is explained by the reliance of chronotropy on intrinsic regulation and circulating catecholamines[158]. Heart transplant recipients also demonstrate an impaired stroke volume response due to diastolic dysfunction[159,160], which, combined with chronotropic incompetence, results in an inadequate cardiac output. However, arteriovenous oxygen difference was found by Kao *et al.* to be lower than normal, suggesting an additional peripheral abnormality in oxygen uptake or utilization[159]. Cardiac output responses are also blunted in HLTx recipients[161,162], although diastolic function is better than in HTx patients as a result of augmented left atrial contractility[163]. Ross and coworkers obtained invasive hemodynamic measurements during exercise in seven SLTx and one BLTx recipients[155]. Although

pulmonary hemodynamic responses were abnormal in most of the subjects, cardiac output (CO) was not limiting. In fact, the ratio of $\Delta CO/\Delta \dot{V}O_2$ was increased 2.5-fold above normal, again implying a peripheral defect in oxygen utilization.

Gas exchange during exercise

As indicated previously, hyperventilation with low $PaCO_2$ levels is observed during exercise. Arterial PO_2 demonstrates the normal slight increase during exercise in HLTx and BLTx patients[89,144]. The *P (A–a)* O_2 also remains normal or minimally elevated. In contrast, arterial oxygen saturation (SaO_2) falls in SLTx recipients, particularly those with ILD and PVD. Orens *et al.* reported a downward trend in mean peak exercise SaO_2 of 3% and 3.8% in SLTx-PVD and SLTx-RLD patients, respectively, compared with 0.7% in SLTx-OLD and an increase of 0.9% in BLTx recipients[146]. The Barnes Hospital group reported resting *P (A–a)* O_2 values ranging from 18 to 25 mmHg that increased slightly to 25–32 in SLTx-OLD patients compared with a peak value of 34–38 in SLTx-PVD subjects[144]. No changes in the relative distribution of ventilation and perfusion between the transplanted and native lungs occur during exercise[164]. The normal increase in apical perfusion with exercise was absent in both lungs in this study. This finding suggests either more maximal apical recruitment at rest or an abnormality in the apical vascular bed. Nevertheless, most patients maintain SaO_2 >90%, so that hypoxemia is not a limiting factor.

Causes of exercise limitation

The results discussed above indicate that exercise in lung transplant recipients is not limited by ventilation, cardiac output or hypoxemia. The early onset of anaerobic metabolism implies either impaired flow of O_2 to muscles or inefficient O_2 utilization. While this patient population tends to be slightly anemic, the degree of limitation is out of proportion to the decrease in hemoglobin. Increased affinity of hemoglobin for oxygen does not appear to be a factor[155]. Metabolic acidosis is not present in most subjects, and in any case should cause a ventilatory limitation[152]. Peripheral vascular disease as a result of cyclosporin-induced arterial hypertension could account for these findings, but patients do not complain of claudications. Muscle atrophy resulting from a sedentary lifestyle pretransplant, malnutrition and steroid use can limit maximal work rate and oxygen consumption[165]. Gibbons and colleagues[79] reported the $\Delta \dot{V}O_2/\Delta$ work rate relationship in SLTx patients to be 7.1 ± 1.9 ml min^{-1} W^{-1}, well below the normal value of 10.2 ± 1.0[152]. This observation, combined with the high $\Delta CO/\Delta \dot{V}O_2$ reported by Ross *et al.*[155], points to the possibility of a defect in skeletal muscle bioenergetics.

Recent studies support the hypothesis that inefficient transformation of oxygen into power output exists after transplantation. Changes in arterial plasma potassium concentration during exercise were evaluated in a group of HTx, HLTx and SLTx recipients by Hall and co-investigators[148]. Transplanted subjects demonstrated higher values for $\Delta[K^+]/\Delta W$ and the ratio of $\Delta[K^+]$ to time of exercise compared with normals. Efflux of intracellular potassium could lead to hyperpolarization of myocyte membranes and early muscle fatigue. Alternatively, the findings may reflect reduced Na^+-K^+-ATPase activity or a reduction in myocyte pump concentration. These observations were attributed to muscle deconditioning. Biochemical and histologic changes in skeletal muscle are observed in congestive heart failure[166] that mimic those found in deconditioning[167]. Metabolic abnormalities during exercise persist after heart transplantation[168], as do skeletal muscle weakness[169] and diminished respiratory muscle endurance[170]. Training improves exercise performance after HTx[171,172] and is also likely to benefit lung transplant recipients[155].

Cyclosporine has recently been incriminated in the poor exercise performance in transplant patients. Similar to its effects on kidney mitochondria, 14 days of cyclosporin administration in rats resulted in decreased skeletal muscle mitochondrial electron chain capacity[150]. This was accompanied by decreased endurance in the cyclosporin-treated animals. Other potential detrimental effects of cyclosporin include microvascular alterations with reduction in regional muscle blood flow and sympathetic hyperactivity with shift of carbohydrate metabolism towards increased glycogenolysis and glycolysis[150].

SPECIAL CONSIDERATIONS IN PEDIATRIC AND LOBAR TRANSPLANTATION

Lung transplantation has been recently extended to pediatric patients with end-stage lung disease. Routine pulmonary function measurements have been comparable to those of adult recipients[173–175]. Long-term follow-up data to assess the growth of transplanted lungs are not available. In a rat model, immature donor lungs transplanted into immature recipients continued to grow by an increase in alveolar number and increase in airway and alveolar size[176]. Similarly, immature lungs transplanted into adult animals fulfilled their growth potential[177]. However, vascular and small airway function may be abnormal after transplantation of immature lungs[178,179], and denervation has been shown to impair small airway growth and development[180]. A comparison of immature whole lung with mature lobar transplantation in a porcine model demonstrated elevated dynamic airway resistance and reduced lung compliance in the former[181]. Living related lobar transplantation in patients with cystic fibrosis has resulted in mean FVC and FEV_1 values of 76% and 70% predicted, respectively, and *P (A–a)* O_2 of 15 mmHg at 1 year post-transplant[182]. The donors in these cases, who undergo a lower lobectomy, experience a 17–20% decrease in FVC and FEV_1[183].

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Histopathology of Lung Transplantation

N.P. OHORI AND S.A. YOUSEM

INTRODUCTION

The increasing number of transplant centers has resulted in providing lung transplantation as a therapeutic option for many patients with end-stage pulmonary diseases. However, despite improvements in immunosuppression, surgical techniques, and diagnostic accuracy, post-transplant complications remain problematic. One of the key elements to patient survival is the prompt and appropriate intervention of allograft dysfunction[1]. While there are a number of ways to monitor the recipient, tissue examination still remains the mainstay in assessing allograft alterations[2–5]. Perhaps it is important to distinguish between rejection and non-rejection processes such as infection, since treatment is often opposite. Graft syndromes typically occur in their particular context, and it is the understanding of the adaptation of the lung allograft to the host environment which is critical in arriving at the correct diagnosis. The intent of this chapter is to review the histopathology and pathophysiology of lung allograft rejection and other non-rejection processes which may also contribute to graft dysfunction. The efficacy of types of biopsies in specific situations will also be discussed.

EARLY POST-TRANSPLANT ALLOGRAFT COMPLICATIONS

During the first week post-transplant, virtually all allografts are subject to the so-called 're-implantation response' characterized by bilateral opacification on chest radiograph and histologic demonstration of interstitial and alveolar edema and margination of neutrophils (Figure 1)[6]. The process is thought to be related to fluid overload secondary to disruption of the hilar lymphatics, organ ischemia during harvesting and transport, and division of nerves and bronchial arteries[7]. It usually resolves by the end of the first week after transplantation, before acute cellular rejection generally takes place.

Following the immediate post-transplant period a variety of other complications are encountered, many of which are related to the donor organs. Preservation (harvest) injury manifests pathologically as diffuse alveolar damage (DAD) with interstitial edema, hyaline membranes, and granulation tissue (Figure 2)[8,9]. While the process is thought to be secondary to organ ischemia, we have seen DAD in cases with minimal ischemic times in living-related transplants, thus implicating other etiologic factors. In contrast to the usual DAD is the occasional development of a temporally homogeneous patchy (as opposed to diffuse)

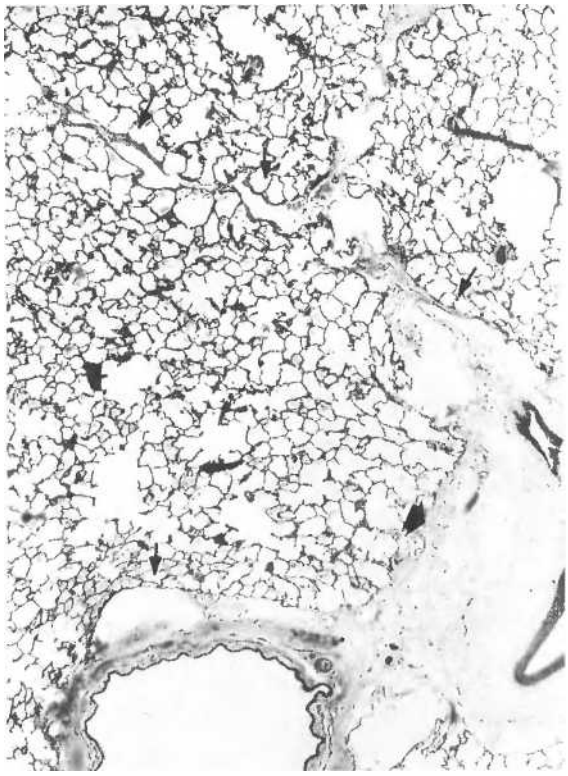

Figure 1 Reimplantation response. The pulmonary interstitium is edematous, as reflected in the perivascular pallor (large arrow) and marked dilatation of lymphatic channels (small arrows)

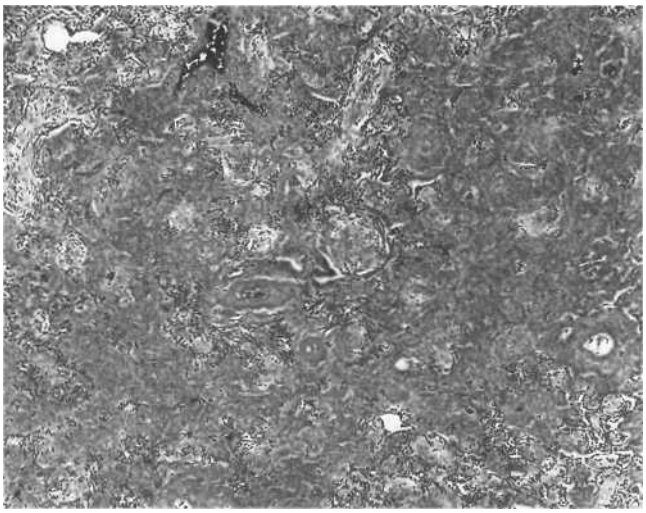

Figure 2 Acute harvest injury manifesting as organizing diffuse alveolar damage. Plugs of myxoid granulation tissue are seen diffusely in the airspaces as well as the airways

Figure 3 Thromboembolic disease. Massive thromboembolus seen adjacent to a large area of infarct resulted in organ failure in this case

process[10]. Clinically, its distinction from acute cellular rejection is the main differential diagnosis. This is not difficult in most cases with mild to moderate degrees of reversible DAD. However, in severe or prolonged cases, uncertainty in the clinical impression often necessitates a biopsy. Pathologically, severe DAD demonstrates extensive injury, to involve not only the interstitium but also the airways to produce acute bronchitis and bronchiolitis with luminal ingrowth of loose granulation tissue[10,11]. Although some cases may demonstrate concurrent DAD and rejection, attempts should be made to distinguish features of DAD from alveolar damage secondary to severe acute cellular rejection (see below) and chronic airway rejection. While the intraluminal granulation tissue of DAD has often been referred to as 'bronchiolitis obliterans', it differs from the chronic rejection-related bronchiolitis obliterans, which exhibits dense eosinophilic collagen characteristic of irreversible intraluminal scar[10,12].

Early in the history of heart–lung transplantation, tracheal dehiscence was a relatively common complication[1,13,14]. Due to improved surgical techniques this complication is now a rarity. While the acute complications of tracheal dehiscence are now under control, chronic bronchomalacia, involving the main stem bronchi and their branches due to the sacrificed bronchial artery circulation, is still a problem[15–18].

Other causes for early post-transplant complications include donor organ infection and thromboembolic disease. Sources of the embolic material include the brain, bone marrow, cartilage, and deep venous thrombi[19]. The consequences of embolic disease are probably as varied as in the non-transplant setting. Reports of rapidly fatal embolic diseases are noted at one end of the spectrum, while small incidental thromboemboli are not uncommonly found in biopsy specimens (Figure 3). Finally, a progressively downhill respiratory course lacking a demonstrable etiology is classified as primary graft failure[9]. At our institution the incidence of primary graft failure has been approximately 6% since 1982.

ACUTE LUNG REJECTION

In solid organ allografts, rejection may take the form of hyperacute, acute or chronic rejection. Hyperacute rejection is an immediate rejection response following implantation, and results in graft failure. While it has been reported in the animal lung transplant model[20], rigorous documentation in human lung transplants has not been made. Morphologic findings by themselves are not specific and therefore an integrated approach with clinical findings, histology, serology, and immunofluorescence is required. Specifically, the following are the considered criteria for diagnosis: (a) early graft failure without alternative etiology; (b) consistent gross, histologic, and immunofluorescence findings; (c) a high percentage of panel-reactive antibodies prior to transplantation; and (d) demonstration of donor-specific antibodies in the eluate of the failed allograft[21].

Acute cellular rejection (ACR) typically manifests after a week post-transplant and is one of the main clinical differential diagnoses of graft dysfunction along with harvest injury and infection. It should be noted, however, that ACR may occur any time post-transplant, especially when there is an alteration in the effectiveness of immunosuppression. ACR is mediated by an immunologic mechanism targeting the donor histocompatibility antigens expressed on bronchial-associated lymphoid tissue (BALT), bronchial epithelium, and vascular endothelium[22–25]. The relationship between the infiltrating cellular population and MHC class II antigen expression is somewhat unclear. HLA-DR and DQ expression is found in the transplanted bronchial epithelium[26,27], but there is no correlation between the level of expression and episodes of rejection. Furthermore, normal pulmonary epithelium and endothelium may also express MHC class II antigens[28]. The major infiltrative cell population consists of T lymphocytes with occasional B cells[29] of recipient origin as demonstrated by Y chromosomal probe analysis[30,31]. In early ACR, most of the infiltrating T lymphocytes belong to $CD4^+$ (helper) phenotype whereas, later, the population of $CD8^+$ (suppressor/cytotoxic) T cells increases[29,32]. Recently the role of B cells in persistent and immunosuppression-resistant ACR has

been appreciated. When comparing rejection episodes responding and not responding to solumedrol in the early transplant period, the number of infiltrating B cells was significantly larger in the non-responder group than in the responder group[33]. Furthermore, another study has documented the formation of nodular B cell aggregates reminiscent of lymphoid follicles in early bronchiolitis obliterans[34]. Since the number of episodes of ACR has been correlated with the subsequent development of chronic rejection (bronchiolitis obliterans), the involvement of a humoral mechanism in ACR may implicate another pathway for long-term graft compromise.

ACR is characterized by a perivascular mononuclear cell (lymphocyte and plasma cell) infiltrate primarily surrounding pulmonary veins, but also involving arteries and lymphatics, depending on the severity (Figure 4)[8,35,36]. The cuff of infiltrating mononuclear cells undermines the endothelium to produce reactive changes in the endothelial cells ('endothelialitis') (Figure 5). The airway mucosa, particularly the BALT, is also targeted early in acute rejection. The resulting depletion of the donor BALT has been postulated to play a role in the increased susceptibility to graft infection due to the loss of mucosal immunity[22]. With increasing airway inflammation the infiltrate insinuates into the overlying airway mucosa, inducing cytotoxic effects on bronchial epithelial cells (apoptosis). Over time the peribronchiolar and perivascular mononuclear cell cuffs result in disruptions of the laminin and type IV collagen basement membrane components, as demonstrated immunohistochemically[37]. These alterations probably contribute to irreversible remodeling in the long-term allograft.

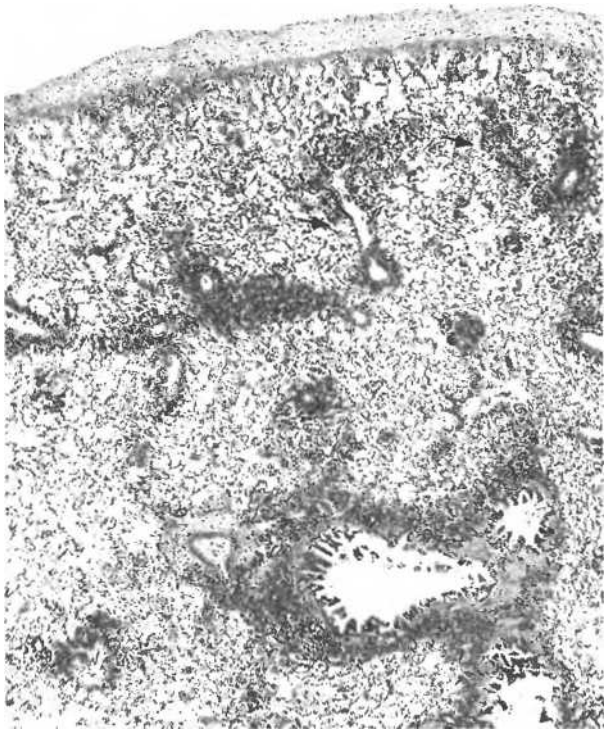

Figure 4 Acute lung rejection. A marked inflammatory infiltrate cuffs the pulmonary veins running in the pleura and interlobular septa (arrows). Concentric cuffing of bronchioles and arterioles is seen at lower right

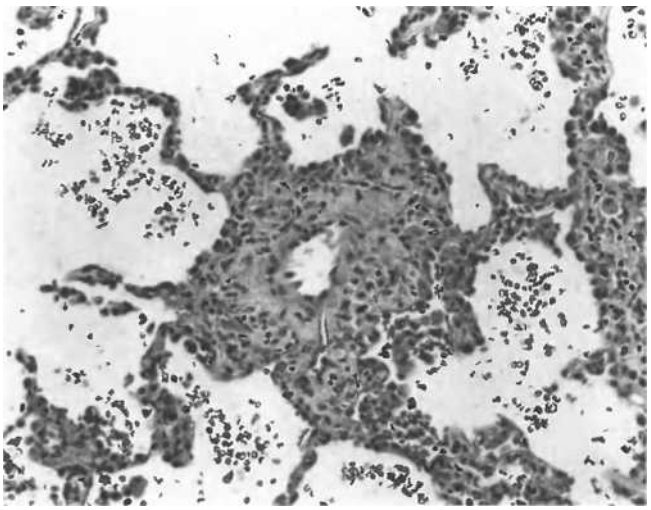

Figure 5 Acute cellular rejection. Concentric perivascular cuffing by mononuclear cells (lymphocytes, plasma cells, and macrophages) with endothelialitis

Grading of ACR by the Working Formulation for the Diagnosis of Lung Rejection[38] is based on the intensity, distribution, and quantity of the mononuclear cells. The lowest degree of rejection response is characterized by the subtle, two-to-three-cell-layer cuffing of small vessels by small, round, plasmacytoid, and transformed lymphocytes (minimal ACR, grade A1). Bronchial and bronchiolar involvement by mononuclear cells is not commonly seen in this grade. In mild ACR (grade A2) there is a significant, five-to-seven-cell-layer perivascular cuffing, which is obvious at low-power examination. The infiltrate commonly also involves the peribronchial/bronchiolar areas. Extension of the infiltrate into the interstitium and air spaces qualifies for moderate ACR (grade A3). With this degree of rejection, airway involvement is seen in most cases and additional histologic features of eosinophilia, neutrophilia, and airspace collections of lymphocytes and macrophages are common (Figure 6). With severe ACR (grade A4), the infiltrate diffusely permeates the lung parenchyma as it involves vascular, airspace and interstitial components, and produces parenchymal damage manifested by alveolar damage, necrosis, hyaline membrane formation and neutrophilic and macrophage infiltrates[39,40]. Localization of the mononuclear infiltrates to the perivascular and peribronchial/bronchiolar areas is lost and other inflammatory cell types, including large numbers of neutrophils and macrophages, are attracted. The resulting injury produces a picture similar to diffuse alveolar damage, and its distinction from other processes such as preservation (harvest), infectious, chemical, drug, and physical injuries is important.

Evaluation of airway alterations is a difficult task in TBB (transbronchial biopsy) interpretation, since inflammation involving the airways is less specific than perivascular inflammation when considering rejection as a diagnosis. In contrast to other solid-organ transplants the lung is constantly exposed to the external environment so low-level chronic inflammation involving the large airways often represents non-specific inflammation. Some long-term patients have airway inflammation due to large airway

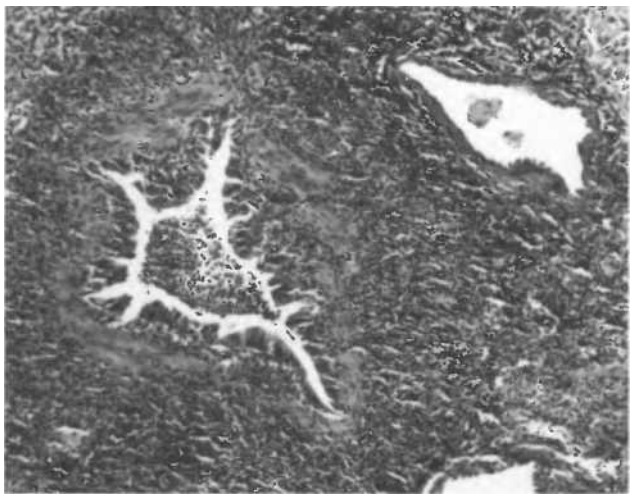

Figure 6 Moderate acute cellular rejection with intense mononuclear cell infiltrate involving the arteries and the bronchioles. The intervening interstitium and airspaces are also involved

alterations such as bronchiectasis, bronchomalacia, and persistent bacterial colonization (e.g. *Pseudomonas* species in cystic fibrosis patients). Small airway inflammation, particularly when involved primarily by a mononuclear cell population, may indicate rejection. However, one should keep in mind that similar appearances may be produced by infections; therefore attributing airway inflammation to rejection is a diagnosis of exclusion[41]. In most instances of ACR the vessels as well as the airways are involved, but there are situations when the biopsies only demonstrate airway inflammation with activated mononuclear cells typical of rejection. The term 'lymphocytic bronchitis/bronchiolitis (LBB)' (grade B) is used to describe this type of inflammation involving the airways exclusively (Figure 7)[41]. It should be recognized that the diagnosis of LBB lacks specificity, and an infectious etiology should be considered as well as a rejection process. When infection is ruled out, the possible reasons for LBB include: (a) treatment of ACR with resolution of the perivascular but not the airway inflammation, (b) inadequate sampling of the perivascular component, (c) bronchocentric ACR, or (d) chronic airway inflammation of unknown significance[41,42]. The decision to treat for rejection would depend more on the clinical parameters.

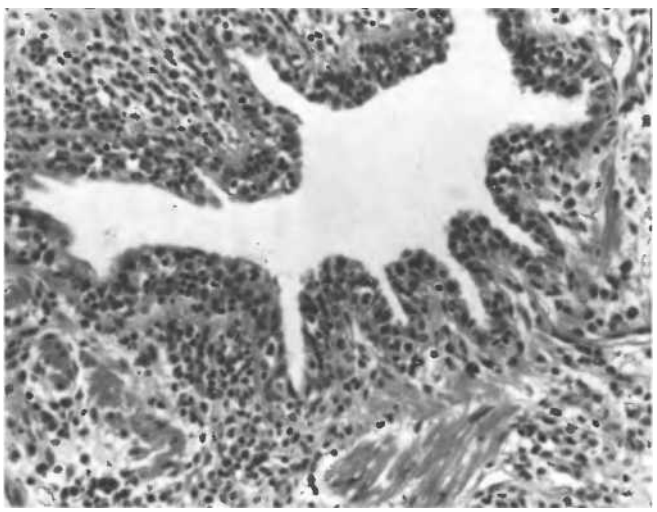

Figure 7 Lymphocytic bronchiolitis. An active lymphocytic cell infiltrate in the submucosa extends into the overlying respiratory epithelium, resulting in focal areas of necrosis

Histopathologic assessment is the most informative diagnostic method in assessing rejection. While thoracoscopic or open lung wedge biopsies are considered the gold standard, the associated morbidity and the intensive labor to obtain the tissue preclude routine use. As an alternative, transbronchial biopsies are commonly utilized. Perhaps the most important point in evaluating transbronchial biopsies is the assessment of adequacy. Since rejection and other allograft syndromes tend to be patchy and focal in nature, transbronchial biopsies should sample multiple areas to obtain alveolated parenchyma with small airways (terminal and respiratory bronchioles). Furthermore, since the features of ACR (such as perivascular and airway inflammation) are not entirely specific, adequate sampling must be obtained to identify histologic features indicating non-rejection processes, particularly infection and lymphoproliferative disorders[43,44].

It is generally agreed that five or more pieces of alveolated lung tissue provide adequate sampling[5,38,45]. Fragments of large airway wall representing the entry point of the biopsy forceps should not be counted in the assessment for adequacy, since they are not as diagnostically informative. In situations in which the transbronchial biopsy findings do not correlate with the clinical presentation, a thoracoscopic or open lung wedge biopsy may be necessary for histopathologic assessment.

Once the diagnosis of rejection is made, enhanced immunosuppression (e.g. bolus doses of solumedrol) is administered. Histologic response is initially seen with the diminution of perivascular infiltrates while the peribronchiolar and interstitial infiltrates may persist. Clinical response often precedes histologic resolution, which may take up to 4 weeks and, even after complete resolution, biopsies may show evidence of previous injury (e.g. interstitial scarring)[46,47].

CHRONIC REJECTION

Chronic rejection represents the development of an irreversible injury to the allograft with permanent functional compromise. In lung allografts, chronic rejection manifests as small airway scarring (bronchiolitis obliterans, OB), large airways bronchiectasis and graft atherosclerosis[48–50]. Injury to the small airways begins with a mucosal mononuclear cell infiltrate which, over time, produces luminal occlusion with granulation tissue and dense hyalinized scar (Figure 8). Like ACR, OB appears to be immunologically mediated and is associated with a $CD8^+$ T cell infiltrate in the peribronchial areas with heightened expression of MHC class I and II antigens in the airway of the allograft[51,52]. Recent studies have also demonstrated the possible role of humoral immunity with B cell aggregates recognized in developing OB[34]. ACR is often seen concurrently with OB, and the recognition of a B cell component in refractory ACR, as well as developing OB, leads one to speculate whether humoral immunity is a common denomi-

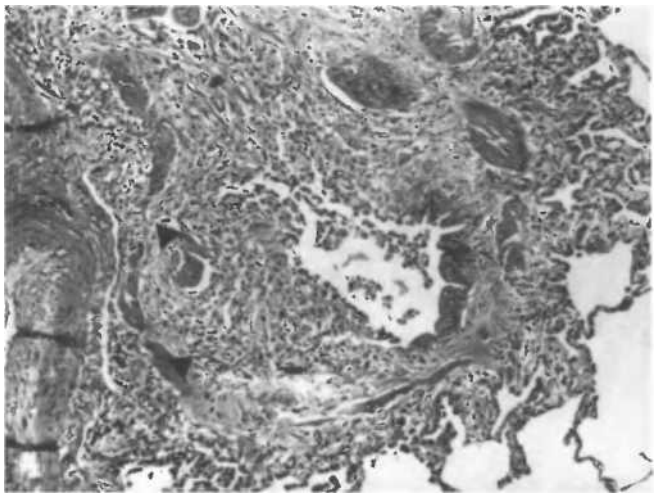

Figure 8 Subtotal active bronchiolitis obliterans. An intraluminal plug of granulation tissue (arrows) entraps metaplastic epithelial cells

nator in ACR and this form of progressive airway injury. The patchy and segmental distribution of OB also suggests a link to ACR[8,53]. Regardless of the precise mechanism, repeated insults to the airway mucosa contribute to disruption of the bronchiolar basement membrane, epithelial cell necrosis, myofibroblastic ingrowth, loss of smooth muscle, and eventual scarring[37,49,50,53–55]. In the final phase of OB the bronchiolar lumen is replaced by a dense hypocellular scar (Figure 9). Since OB proceeds in a temporally heterogeneous manner[8,56], obstructed bronchioles are often seen adjacent to actively inflamed, as well as relatively normal, airways. Although OB requires the exclusion of other causes of airway fibrosis, including infection, aspiration, and ischemia, this patchy, predominantly bronchocentric injury and scarring are highly characteristic of immunologically related airway rejection process. Clinically, the pulmonary function abnormalities are obstructive early in the course of OB, and later become restrictive. In contrast to chronic rejection of the liver, the diagnosis of OB does not portend imminent organ failure, and the rate of functional deterioration is variable.

While the small airways scar are obliterated as a consequence of chronic airway rejection, the inflamed large airways scar and paradoxically develop bronchiectatic changes. This alteration may be seen in non-rejection processes such as chronic infection and aspiration, and therefore lack the specificity to be attributed solely to an airway rejection process[8,53].

In addition to the airway damage, many long-term survivors show graft arteriosclerosis (GAS) characterized by a myofibrointimal proliferation and collagen deposition[48]. These vascular lesions are patchy, segmental and circumferential, although asymmetry is occasionally noted. The degree of proliferation corresponds to grade 2 in the Heath–Edwards classification of pulmonary hypertension (Figure 10). However, the clinical significance of these vascular lesions is unclear, since these patients rarely develop pulmonary hypertension and the development of GAS does not necessarily correlate with the onset of OB[53].

Since the clinical significance of GAS is uncertain, and the large airway alterations are non-specific, the diagnosis of chronic rejection depends largely on the identification of OB. This can been a challenge to both the clinician and the pathologist. The histologic diagnosis of OB requires the demonstration of dense submucosal scarring of the small airways that may be eccentric, concentric, or associated with total obliteration of the bronchiolar lumen[38]. The trichrome stain is particularly helpful in this assessment. Transbronchial biopsy may establish the diagnosis of OB, and the sensitivity and specificity are 87% and 99%, respectively[57]. Nevertheless, the bronchoscopist occasionally encounters a patient with scarred and fibrotic lungs, which are difficult to biopsy due to the lack of compliance. In these cases, despite multiple biopsies, the pieces obtained tend to be minute and small airways are not often sampled. This may further necessitate an open lung or thoracoscopic wedge biopsy to assess the possibility of OB.

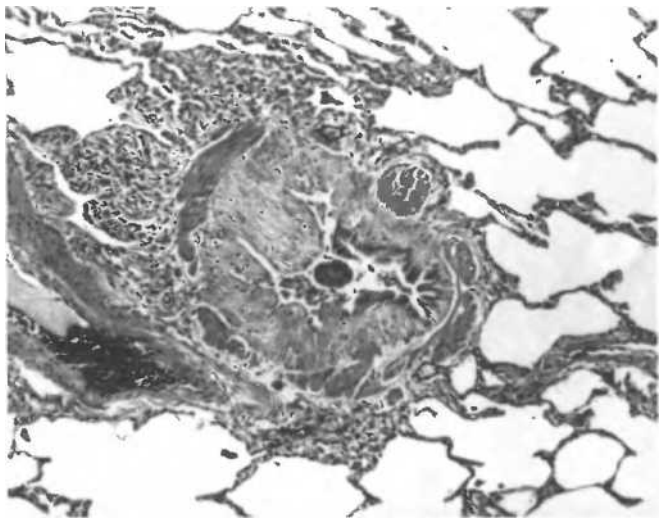

Figure 9 Subtotal inactive bronchiolitis obliterans. Diminution of the mononuclear cell infiltrate leaves an eccentric old scar tissue in the bronchiolar lumen (arrows)

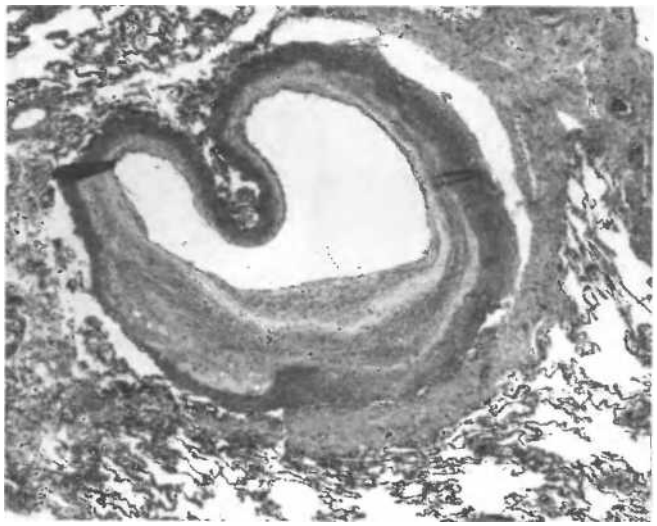

Figure 10 Graft atherosclerosis. Pulmonary artery branch with an eccentric fibromyxoid plaque and a mild mononuclear cell infiltrate produces an endovasculitis

Due to these difficulties, diagnostic terms have been defined to describe the manifestations of OB. The term bronchiolitis obliterans (OB) is reserved for histologically proven lesions either by biopsy (transbronchial or wedge) or at autopsy. Bronchiolitis obliterans syndrome (BOS) is a clinically defined entity of allograft deterioration secondary to progressive airway disease with no other known cause[58]. A pulmonary function test measuring the forced expiratory volume in one second (FEV_1) is utilized in grading the severity of the airway lesion. BOS does not require histologic confirmation, but patients must demonstrate less than 80% of baseline FEV_1 value for this diagnosis to be made. Following the diagnosis of OB, patients are treated with enhanced immunosuppression in an attempt to quell the active cellular component of OB to recover some of the pulmonary function deficits.

INFECTION

The allograft environment is ideal for the proliferation of opportunistic microorganisms. In addition to enhanced immunosuppression there are a multitude of reasons for the susceptibility, some of which are unique to the lung allograft. During the terminal course of the donor, aspiration resulting in bacterial and fungal contamination contributes to a lower 1-year survival of 35% (in contrast to 67% for those without early infection)[59]. The lung transplantation procedure involves anastomoses of the major airways and pulmonary arteries, but not the bronchial arteries and the peripheral nerves, which are sacrificed. Consequently, the vascular supply to the large airways is dependent on the collaterals from the pulmonary arteries. With the denervation there is loss of mucociliary clearance and cough reflex[60,61]. Another reason for early infectious susceptibility is the loss of the bronchial-associated lymphoid tissue (BALT) secondary to ACR targeting the MHC class II antigens on the donor BALT lymphocytes. BALT normally provides secretory IgA-mediated humoral defense along the airway mucosa, and its compromise and constant bombardment by external pathogens through the airways increase the chances of early allograft infection[22]. During the mid and late post-transplant course, additional factors contribute to graft susceptibility. In single lung transplants the remaining native lung may become a nidus of infection and seed the allograft. Patients with the primary diagnosis of cystic fibrosis are known to have their upper airways and sinuses colonized by *Pseudomonas* species (*aeruginosa* and/or *cepacia*), which subsequently infects the allograft lung downstream[62–64]. Unfortunately, these *Pseudomonas* species are often resistant to currently available antibiotics and therefore difficult to control. Finally, the parenchymal alterations following chronic rejection result in remodeling, manifesting as interstitial, septal and subpleural scarring and cylindrical bronchiectasis which alter air flow and decrease mucus clearance[8,53]. These airways are readily colonized by Gram-negative rods, particularly *Pseudomonas*. Under these compromised circumstances, acute bronchitis and pneumonia is not uncommon.

Specific types of infections are often encountered in the typical clinical context mentioned above. Bacterial pneumonia is the most common infection in lung transplant recipients, manifesting early (within the first 2 months) or late in the post-transplant course[59,65,66]. The common types of bacteria include *Pseudomonas*, *Staphylococcus*, *Enterobacter*, *Enterococcus*, *Streptococcus pneumoniae*, *Acinetobacter*, *Hemophilus*, and *Klebsiella*[67]. The early infections are related to aspiration by the donor, whereas the later infections are due to parenchymal remodeling, bronchiectasis, mucus inspissation and primary disease such as cystic fibrosis.

Bronchoalveolar lavage (BAL) is the most efficacious method for isolating and speciating bacteria as well as fungal and viral organisms. Biopsies are less sensitive and specific, and speciation is not possible. Nevertheless, histologic identification of bronchopneumonia may be made before culture results are available, allowing empiric therapy to be instituted. When considering infectious processes, determination of the significance of isolated microorganisms is an important issue. This depends on multiple factors including type of species isolated, colony count, and clinical manifestation. The diagnosis of bacterial pneumonia depends on the documentation of new fevers, infiltrates on chest radiograph, and isolation of significant numbers of the organism (generally greater than 100 000)[68].

Nocardiosis is less common, but is nevertheless an important bacterial infection in the transplant population. These Gram-positive aerobic, filamentous rods infect the immunocompromised or others with underlying medical conditions[69]. Eighty-five percent of nocardiosis is by *N. asteroides* and the manifestations include bronchopneumonia, abscess formation, cavitation, and empyema (Figure 11). Furthermore, the infection may metastasize to the brain, bone, skin, and subcutaneous tissue. The irregularly branching, thin, beaded, filamentous rods are characteristic of *Nocardia* although *Actinomyces* and *Streptomyces* should also be considered in the differential diagnosis (Figure 12). *Nocardia* may be seen on Gram and Grocott, as well as Fites (modified Ziehl–Nielsen) stain, which has been shown to be particularly useful. Since *Nocardia* abscesses often manifest as localized lesions, TBB may be ineffective in obtaining diagnostic tissue. Under such circumstances, fine-needle aspiration biopsy is often

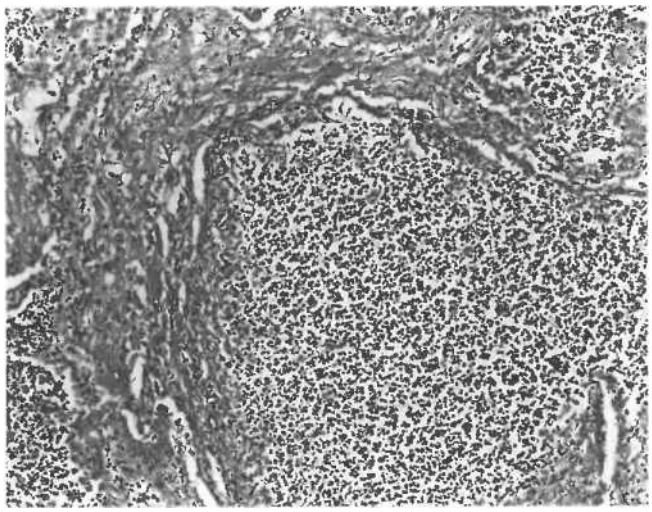

Figure 11 *Nocardia* abscess. Along with bronchopneumonia and empyema, abscess formation is one of the common manifestations of nocardiosis.

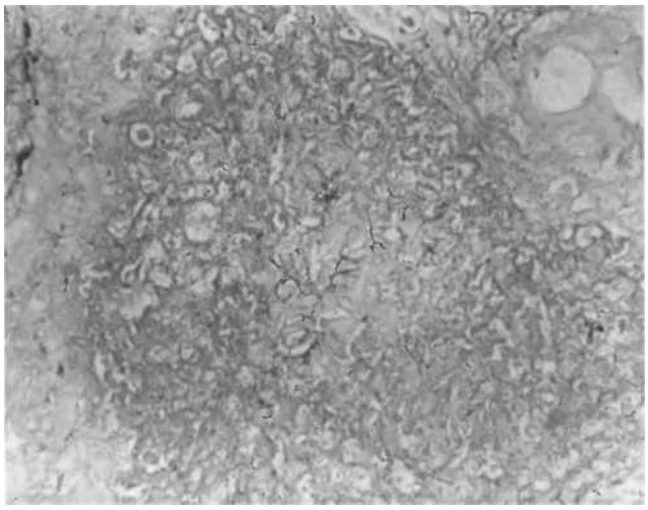

Figure 12 Grocott stain of a *Nocardia* abscess. Thin, irregularly branching, beaded filaments are noted. These organisms are also Gram-positive and stain with the Fite's modification of the acid-fast stain

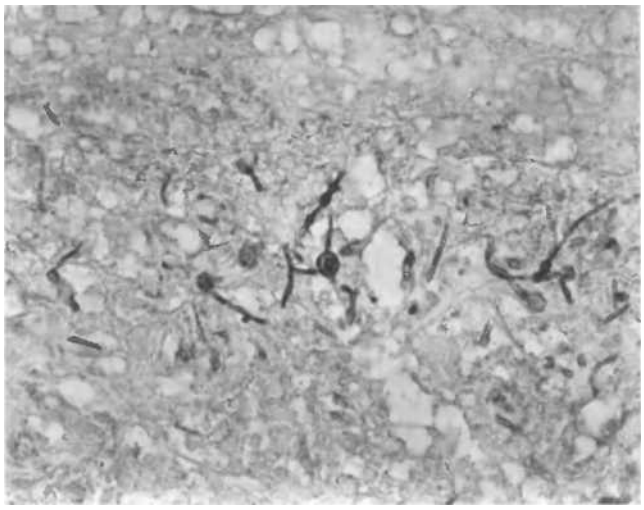

Figure 13 *Pseudallescheria boydii* may colonize cavities or produce invasive pneumonia and empyema. Although they resemble *Aspergillus* species, the identification of thin-walled vesicles and less-acute-angle branching are helpful features in recognizing *P. boydii*

more effective in sampling the centrally necrotic material which harbors the organisms.

Fungal infections are also most common in the early post-transplant period, but may occur any time afterwards. *Candida* and *Aspergillus* are common offenders and their identification must be taken in the context of their invasiveness[67,70]. *Candida* mainly infests the upper tracheobronchial tree with less chance of dissemination. However, the isolation of *Aspergillus* may represent colonization, allergic fungal response, or invasive disease involving the deep parenchyma. Although highly sensitive and specific, the BAL culture has a low predictive value[68] and in consideration of the high fatality from invasive aspergillosis, many cases representing contaminant and colonization are probably overtreated. Nonetheless, the current antifungal regimen has been effective in decreasing the morbidity and mortality from fungal disease.

Pseudallescheria boydii is ubiquitous in the environment and produces an opportunistic infection which mimics aspergillosis both clinically and pathologically[69,71]. Like *Aspergillus* infections, the isolation of *P. boydii* needs to be correlated with the setting where it is found. Colonization commonly occurs in the remodeled pulmonary parenchyma and cavities. On the other hand, invasive necrotizing pneumonia with abscess formation and pleural involvement with empyema may be associated with hematogenous dissemination to the brain, kidney, heart, and thyroid. Manifestation as an allergic bronchopulmonary fungal disease has been also recently reported. Morphologically, *P. boydii* and *Aspergillus* are similar, with both showing narrow (2–5 μm) septate hyphae with acute angle branching. The hyphae of *P. boydii* may show thin-walled vesicles and terminal conidia and these features are helpful in distinguishing it from *Aspergillus* (Figure 13). This distinction has clinical importance as amphotericin which is usually used for aspergillosis is not effective in pseudallerscheriasis, whereas miconazole or ketoconazole may be effective.

Among the viral infections, cytomegalovirus (CMV) is the most common and important[72–74]. Unfortunately, due to the various clinical presentations and methods to detect CMV, identification must be correlated with disease presentation. CMV-related illnesses may be subdivided into CMV infection, recognizing only the presence of the virus with or without associated clinical/pathological manifestations, and CMV disease, with recognizable pulmonary manifestations (i.e. pneumonitis) due to the virus[68].

To assess the appropriate risk, both the recipient (R) and donor (D) are tested for circulating CMV antibodies[68]. The risk for CMV infection, disease, and related deaths varies depending on the combination of the R/D serologic status. The highest risk for significant disease and death occurs in R–D+ patients and requires the most aggressive anti-CMV prophylactic regimen. While the risk for significant CMV infection and disease is lowest in R–D– patients, the risk of death is approximately 8%. This is in contrast to the R+D– and R+D+ patients who may have a higher incidence of infection and disease but whose risk for CMV-related death is lowest, approximately 1–2%, perhaps due to acquired immunity[68]. Significant CMV disease occurs most commonly in the first 2–3 months post-transplant, although occasional presentation may occur afterwards. Histologically, the manifestation of CMV pneumonitis ranges from a subtle patchy interstitial mononuclear cell infiltrate with rare inclusions to diffuse interstitial and perivascular neutrophilic and mononuclear cell infiltrates with alveolar damage and numerous CMV inclusions[75,76]. The identification of CMV in biopsies should be placed in context of the patient's risk of developing significant disease, as discussed above. The inflammatory background may be distributed in a perivascular pattern, mimicking ACR[75,77]. This reiterates the importance of obtaining adequate sampling to demonstrate the diagnostic inclusions. When an isolated CMV is found in a background lacking inflammation, the interpretation depends on the clinical context. It may represent the earliest manifestation of a developing pneumonitis or the detection of a latent virus; close follow-up is warranted. The detection of CMV by

culture or Shell-vial assay alone, without clinical disease or histologic confirmation, indicates CMV infection without disease. On such occasions the decision for treatment would depend on the clinical situation[68]. With the current antiviral regimen, mortality from CMV pneumonitis has markedly decreased. CMV involvement has also been associated with an increased risk for the development of chronic airway rejection (bronchiolitis obliterans)[78]. The up-regulation of HLA class II antigens following CMV infection has been postulated as a mechanism for its development. Such associations cloud the distinction between rejection and infection.

Due to prophylactic acyclovir, the incidence of and morbidity from herpes simplex pneumonia have diminished. Nevertheless, those susceptible present commonly in the first post-transplant month, and the lung may be the only site of infection[79]. An association with herpes tracheitis and prolonged intubation has been noted. The histologic findings of HSV pneumonia are similar to those occurring in other immunocompromised patients[80,81]. The pneumonia tends to be florid with extensive necrosis and presence of infected cells with intranuclear ground glass inclusions and occasional Cowdry type A inclusions. Multinucleated giant cells with similar nuclear changes are also common features. Rapid treatment following its detection is critical as the disease may be rapidly fatal if left unchecked.

Adenovirus (ADV) infections have been reported sporadically in the lung transplant literature[67,82]. The manifestations range from an acute bronchitis/bronchiolitis to diffuse alveolar damage. Even in cases of DAD a bronchocentric accentuation of severe necrosis is often noted (Figure 14). In our series most of the patients belonged to the pediatric age group[83]. They acquired the infection within the first $1\frac{1}{2}$ months post-transplant, and experienced a rapidly fatal course. Smudgy basophilic nuclear inclusions are characteristic of ADV infections and, in cases which are equivocal, the use of immunohistochemical stain or *in-situ* hybridization probe for ADV may be helpful (Figure 15). An indeterminate number of patients may carry ADV subclinically without ever developing disease. The relatively high incidence in the pediatric population, in contrast to the adult population, suggests that ADV pneumonia represents a primary infection rather than a reactivation. Those who develop antibodies may acquire lasting immunity.

The depressed cellular immunity also provides an opportune setting for *Pneumocystis* infection and, early in the history of lung transplantation, *Pneumocystis carinii* pneumonia (PCP) was a common problem[84,85]. However, with the institution of PCP prophylaxis (sulfonamides), the incidence of PCP has markedly diminished[86,87]. Nevertheless, some patients are allergic to sulfonamides and in rare instances prophylaxis may not prevent the infection.

The pattern of PCP in the lung transplant recipient is similar to that of other immunosuppressed settings. The gross appearance of the lung appears as bronchopneumonia or diffuse consolidation. Histologically there is a range of tissue responses from minimal alterations to granulomatous response to florid diffuse alveolar damage. Foamy alveolar exudates are characteristic findings in H&E sections, although this appearance may be mimicked by alveolar fibrin, macrophages and other cellular debris. Therefore, the Grocott stain is indispensable in assessing the possibility of PCP, and should be a component of every BAL cytology and lung biopsy work-up. The typical Grocott morphology shows cup-shaped cysts with central intracystic bodies. The differential diagnoses include *Candida*, *Torulopsis*, *Coccidioides*, *Histoplasma*, and *Cryptosporidia*.

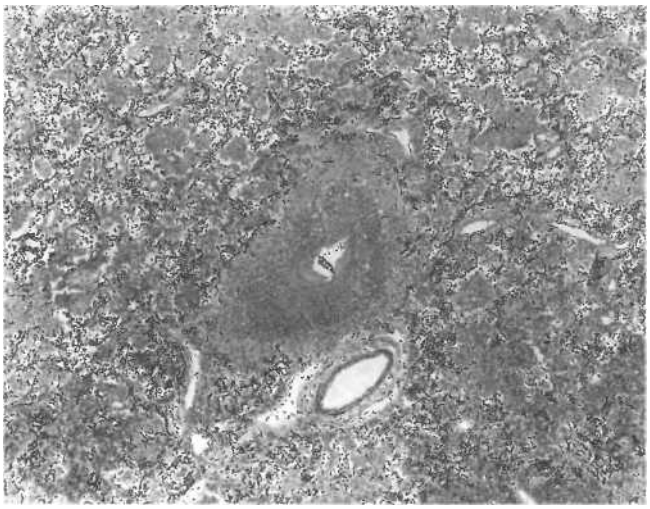

Figure 14 Adenovirus pneumonia typically manifests as a necrotizing bronchocentric pneumonia. In this severe case the background shows diffuse alveolar damage

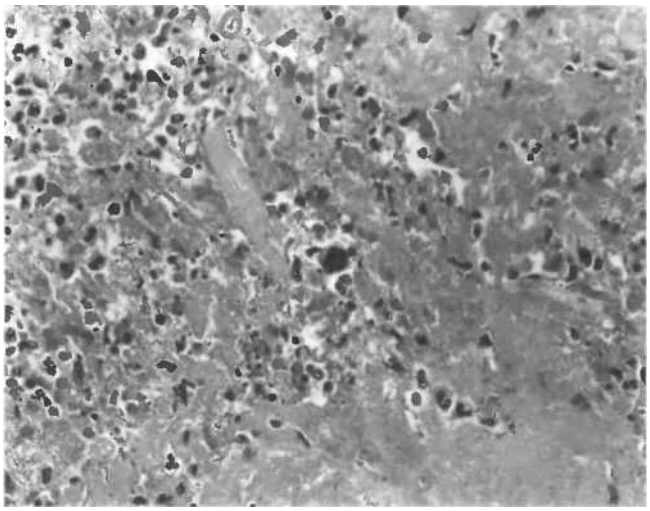

Figure 15 Adenovirus-infected cell with smudgy basophilic nuclear inclusions. In contrast to CMV, cytomegalic changes and intranuclear inclusions are not seen

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD)

PTLD arising in lung transplant patients is morphologically similar to those found in other solid organ transplants[88]. It consists of a proliferation of atypical lymphocytes (usually of B cell origin) arising in the background of overimmunosuppression, and has a strong association with primary Epstein–Barr virus infection, not reactivation. PTLD occurs early in the post-transplant course, generally in the first 3 months. Lung transplant patients

have a relatively high incidence of PTLD occurring in the allograft. At our institution, PTLD developed in approximately 7% of lung transplant recipients and, of these, approximately 60% occurred in the allografted lung[89,90]. This may be due to: (a) the allograft lung being the primary site of EBV infection, (b) the high level of immunosuppression as compared to other organ transplants, and (c) donor BALT acting as 'homing' sites for EBV-infected host B cells.

Morphologically, PTLD produces a mass-like lesion with some cases showing angioinvasion. Histological classification into monomorphous (uniform population of transformed large cells and immunoblasts) and polymorphous (representing the entire spectrum of B cell differentiation with small lymphocytes, plasma cells, large lymphoid cells and immunoblasts) subtypes has some correlation with monoclonality in the former and polyclonality in the latter (Figure 16). With expansion of the mass, foci of necrosis appear, leaving viable lymphoid cells at the periphery (Figure 17). When these areas are biopsied transbronchially, distinction from acute cellular rejection may be difficult. In these instances, demonstrating the presence of Epstein–Barr virus latent membrane protein (EBV-LMP) by immunohistochemistry or Epstein–Barr virus encoded RNA (EBER) by *in-situ* hybridization has been shown to be useful in establishing the diagnosis of PTLD. Specifically, perivascular lymphocytes marking with EBV-LMP are found at the peripheral edges of PTLD, whereas the perivascular lymphocytes of acute cellular rejection are negative[91]. While EBER *in-situ* hybridization studies are also informative, it should be cautioned that, due to the high sensitivity of the study, positive interpretation should be made only when EBER positivity is found in large atypical lymphocytes. Similar principles apply to the interpretation of polymerase chain reaction studies, which may detect very small quantities of EBV genomes in patients without evidence of PTLD[92].

DISEASE RECURRENCE

In contrast to most lung transplants, for disease primarily limited to the lungs, transplants for systemic diseases are at risk for recurrence. Of these, sarcoidosis and lymphangioleiomyomatosis (LAM) have been documented to recur[93–95]. In sarcoidosis, the diagnosis of recurrence is first suspected by the identification of non-caseating granulomas, negative for infectious organisms by special stains. Other etiologies for granulomas must be ruled out clinically. The granulomas found on the transbronchial biopsies tend to be very small and focal; often they may not be present on deeper levels of histologic sections. The significance of these recurrent granulomas is at present uncertain, since functional compromise attributable to recurrent disease has not been shown. Recurrent LAM was seen in a female recipient who had received an allograft from a male donor[95]. Interestingly, *in-situ* hybridization Y-probe analysis demonstrated the donor origin of the recurrent smooth muscle proliferation, thus suggesting the possibility of a circulating factor promoting the growth of myocytes in the pathogenesis of LAM. Due to its rarity, the clinical significance of recurrent LAM is also uncertain.

Early recurrence of diffuse panbronchiolitis (DPB) 10 weeks after transplantation has also been reported[96]. Clinical deterioration was attributed to the recurrent DPB, and the patient was treated with erythromycin, which resulted in resolution of symptoms over a few weeks. Rare case reports of giant cell interstitial pneumonia (GIP) have been documented in single-lung transplant recipients[97,98]. Since GIP is now thought to be a form of pneumoconiosis secondary to occupational hard metal exposure, recurrence suggests the possibility of residual hard metal in the remaining recipient lung 'seeding' the donor lung or the hard metal precipitating a persistent autoimmune reaction in recipient lymphocytes/monocytes.

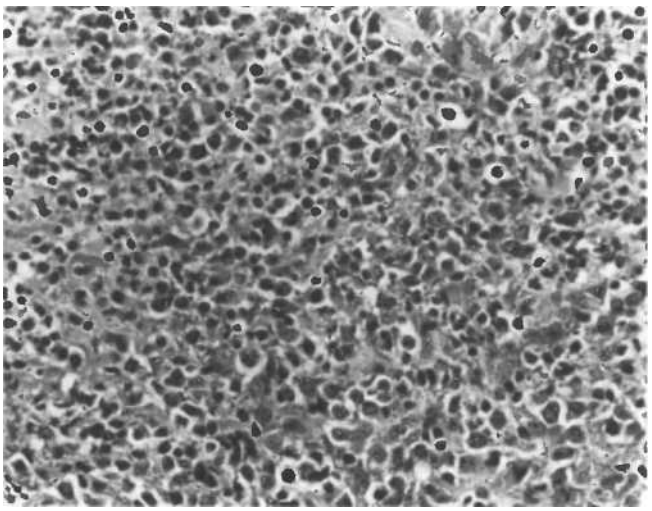

Figure 16 Polymorphous PTLD with a mixed population of small round, plasmacytoid, large, and occasional immunoblastic lymphocytes

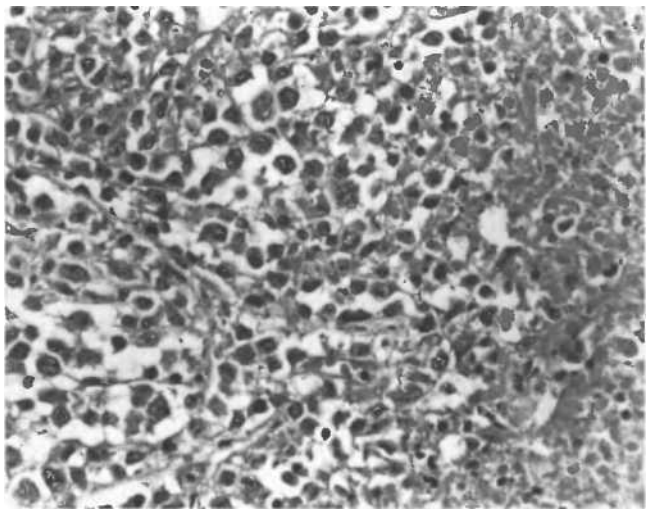

Figure 17 Monomorphous PTLD adjacent to area of necrosis. The proliferating cell population is uniformly large with a complex chromatin pattern. Nucleoli are also readily identified

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Diagnosis and Management of Acute Rejection

F.M. WAGNER AND H. SHENNIB

INTRODUCTION

Lung transplantation is currently offered as treatment to a variety of end-stage pulmonary diseases such as emphysema, interstitial pulmonary fibrosis, cystic fibrosis, and pulmonary hypertension. The most recent Registry report of the International Society for Heart and Lung Transplantation reveals that more than 4000 lung transplants have been performed worldwide, with a 1-year and 5-year survival of 75% and 50% respectively[1].

Although early graft failure and technical problems are frequently responsible for deaths during the perioperative period, mortality during the first 6 months is primarily caused by pulmonary infection or acute pulmonary rejection (APR) of the allograft. Considering the fact that the lung parenchyma with its large surface area is directly exposed to airborne pathogens, and that its immune defense mechanisms are impaired, a high rate of pulmonary infections can be expected. Repetitive local inflammation from infection or other reasons might lead to up-regulation of MHC class II antigen expression in the donor airway and perivascular dendritic cells, thereby increasing its allogenicity, host alloreactivity, and the likelihood of graft rejection[2]. Griffith *et al.* observed within their group of lung transplant recipients that none escaped at least one episode of acute rejection within the first 90 postoperative days, with an average of 2.1 to 3.1 episodes for all patients during 20-month follow-up[3]. Similarily, Kriett *et al.* reported 1.5 episodes of APR per 100 patient-days during the first 2 postoperative months. Rejection-related mortality rates, however, vary from 5% to 30%, indicating its clinical relevance and the need for adequate management[4].

This chapter reviews issues of diagnosis and management of acute rejection after lung transplantation. It summarizes the relevance of possible diagnostic tests, including transbronchial biopsies, the current histological classification, and the impact of various therapeutic options on the outcome of acute rejection.

HISTOLOGICAL CLASSIFICATION AND GRADING OF ACUTE PULMONARY REJECTION

The histological process of lung rejection was extensively studied and analysed in animal models by Prop and colleagues[5-7]. These studies were used as the basis for developing a histological grading system of the severity of APR. They found that the initial immunological response to implantation of a pulmonary allograft was an inflammatory reaction localized within the perivascular area. If not treated, such infiltrate progressed to expand into the pulmonary parenchyma and airways, eventually resulting in extensive vasculitis and hemorrhagic infarction of the lung. When lung transplantation became widely accepted for treatment of chronic pulmonary disorders, a relatively simple classification scheme was required that could easily be taught and reproduced, thereby allowing inter-institutional collaborations and comparisons. Therefore, a Lung Rejection Study Group was organized to elaborate a working formulation for classification and grading of histopathology found during pulmonary rejection[8]. A summary of this classification is given in Table 1.

Traditionally, graft rejection had been divided into three forms, i.e. hyperacute, acute, and chronic.

Hyperacute lung rejection, such as necrotizing vasculitis and hemorrhage shortly after reperfusion of the graft, has never been convincingly reported in the literature. Early graft failure with concomitant anti-HLA and or anti-endothelial antibodies has been described, and might be due to a similar mechanism[9,10]. Diffuse alveolar damage from ischemic injury with neutrophil margination and migration can resemble forms of subtle, early cases of hyperacute rejection and might be confused with this form of rejection[11].

Acute rejection usually does not occur earlier than 5 days after transplantation. It inflicts a dual injury to both vessels and airways caused by migrating cells, mostly neutrophils and lymphocytes, but involvement of eosinophils and macrophages has also been recognized[8]. Depending on its intensity, these infiltrates are initially limited to the perivascular space (grade 1), and can expand into alveolar septa along with the larger airways, leading to parenchymal necrosis (grade 4) (Table 1).

Chronic pulmonary rejection is defined as a fibrosing process primarily affecting the conducting airways and the vasculature, loosely termed obliterative bronchiolitis (OB) and graft atherosclerosis, respectively. The latter corresponds to progressive myointimal thickening of pulmonary arteries and veins, resulting in a strong correlation with the atherosclerosis seen in the coro-

Table 1 Working formulation for classification and grading of pulmonary rejection*

Grading	*Classification*	*Description*
A.	**Acute rejection**	
A.0	No significant abnormality	
A.1	Minimal acute rejection	Infrequent perivascular infiltrates
A.2	Mild acute rejection	Frequent perivascular infiltrates around venules and arterioles
A.3	Moderate acute rejection	Dense perivascular infiltrate with extension into alveolar septa
	Severe acute rejection	Diffuse perivascular, interstitial, and airspace infiltrates; alveolar pneumocyte damage; possible parenchymal necrosis, infarction, or necrotizing vasculitis
	Subclass for all A grades	
	(a) With bronchiolar inflammation	
	(b) No bronchiolar inflammation	
	(c) With large airway inflammation	
	(d) No bronchioles to evaluate	
B.	**Active airway damage without scarring**	
B.1	Lymphocytic bronchitis	
B.2	Lymphocytic bronchiolitis	Inflammatory infiltrate only peribronchially/peribronchiolally
C.	**Chronic airway rejection**	
C.1	Bronchiolitis obliterans, subtotal	
C.2	Bronchiolitis obliterans, total	Subtotal or total occlusion of bronchiolar lumen with fibrous scar
	Subclass for all C grades	
	(a) Active	
	(b) Inactive	High cellularity of fibrosed area. Low cellularity of fibrosed area
D.	**Chronic vascular rejection**	
		Fibrointimal thickening of arteries and veins

* Adapted from the working formulation of the Lung Rejection Study Group[8]

nary arteries of a cardiac allograft. However, its impact on graft function seems to be minimal[12].

OB, on the other hand, is the most important limiting factor of long-term survival, affecting at least 30–40% of all patients 3 years after transplantation[13]. Histopathologically it represents dense, irreversible eosinophilic scarring of the terminal and respiratory bronchioles, with partial to total obliteration of the lumen[14]. The underlying etiology of this process seems to be multifactorial, and to discuss details of current hypotheses would go beyond the scope of this chapter. In brief, it is believed that a repetitive inflammatory process of the bronchiolar wall leads to epithelial injury with focal denudation and/or fibropurulent exudates within the airway spaces. Despite partial epithelial regeneration, narrowing or complete occlusion of the airway lumen occurs, due to proliferation and granulation of the submucosal myxoid tisssue. Over time this tissue may convert into irreversible scarring or be reabsorbed to some extent. Factors that allow for the reabsorption of initial granulation are as yet unclear[15]. A correlation between the development of OB, however, and the number, frequency and intensity of acute rejection episodes has been postulated[16]. Non-rejection-related factors, such as previous pulmonary infections and, in particular, cytomegalovirus infection, as well as early ischemic damage, are believed to increase allogenicity of the graft through an up-regulation of MHC class II antigen by various inflammatory cells, thereby predisposing it to development of chronic graft rejection[17,18].

Another phenomenon often found in biopsy specimens is a lymphocytic bronchitis or bronchiolitis. It represents an airway inflammatory process unassociated with airway scarring and unassociated with perivascular mononuclear infiltrates. In the pediatric population it is believed to represent active acute immunologic injury to the pulmonary parenchyma and airways[19]. In other cases it is probably a smoldering form of low-grade rejection which eventually progresses to a less active variation of OB. It can certainly also be found as a result of chronic infection or due to denervation of the graft and reduced clearance of inhaled pathogens. Its interpretation is therefore difficult, and therapeutic consequences have to be related to the overall clinical picture and will therefore be discussed in the following section.

DIAGNOSIS OF ACUTE PULMONARY REJECTION

Despite progress in our knowledge of mechanisms leading to cellular rejection of a pulmonary allograft, prompt and accurate diagnosis remains a challenge, particularly since no diagnostic 'gold standard' has been agreed upon, such as is the case for heart transplantation[20]. Reports on the validity of different diagnostic methods remain controversial[21–23].

As in most other solid-organ transplants, clinical suspicion of acute rejection is prompted by non-specific manifestations, such as a subjective feeling of being unwell, shortness of breath, fatigue and pyrexia, abnormal breath sounds on auscultation, fluid retention, hypoxia, and other signs of cardiopulmonary dysfunction. All these symptoms are non-specific and usually neccessitate investigations to differentiate acute rejection from infection.

Pulmonary function tests (PFT) can be easily obtained, and their diagnostic reliability has been extensively studied in the heart–lung transplant (HLTx) population. Decreases in FEV_1, vital capacity, and diffusion capacity were found to have a sensitivity of over 80% in detecting acute rejection episodes, especially early after transplantation[24]. However, a decline in FEV_1 has also been observed during infection, resulting in a low specificity[25]. Recently, a prospective study was performed to

evaluate the validity of PFT in single-lung transplant recipients. Considering a decrease of more than 15% as relevant, the sensitivity and specificity of spirometry as a predictor of acute pulmonary illness were found to be significantly lower than those previously reported for HLTx. On the other hand, after single lung transplantation the underlying disease of the native lung can have significant influence on the diagnostic reliability of spirometric tests. A drop in FEV_1 was a better predictor in patients with pulmonary vascular disease than in those with obstructive lung disease[26]. An inherent advantage of the FEV_1 test is the ability to perform it at home using portable flowmeters. Currently, through telemetry, it is possible to transfer flow–volume curves directly via the patient's own telephone to the transplant center. Changes in FEV_1 can then be easily monitored in non-hospitalized patients, and at any index of suspicion the patient can be called for further investigation. This method is currently advocated as a screening test and might allow early detection of pulmonary dysfunction.

Chest radiography remains an essential element of the diagnostic armamentarium, whether for surveillance or prompted by clinical indications. It is still one of the most sensitive tests to pick up early stages of any pulmonary pathology; occasionally radiologic changes can be detected before the development of symptoms, or before a decline in pulmonary function is noted[27,28]. The observed radiological changes, such as honeycomb structures seen mainly in peripheral fields of the lung, pleural effusion, and diffuse reticular interstitial markings, show low specificity for the diagnosis of acute rejection, and do not permit reliable differentiation from pulmonary infections[29].

It was hoped that computerized tomography of the chest would allow more accurate diagnosis of APR. Medina and colleagues examined CT scan findings, such as interlobular septal thickening, air-space consolidation, ground-glass opacities, nodules, airway changes, and decreased vascularity, during histologically or clinically proven pulmonary rejection or infection. They concluded that none of these findings was specific enough to allow differentiation between rejection and infection[30]. Similarily, Loubeyre and colleagues observed that ground-glass opacities had a sensitivity of 65% in detecting lung rejection, and were specific only to detect acute forms of pulmonary pathology. They concluded that detection of ground-glass opacities could at best be used as an aid to decide when and where to perform transbronchial biopsies[31].

Other methods, such as quantitative perfusion/ventilation scanning, have also been examined for their reliability in the diagnosis of APR[32]. After single lung transplantation, quantitative ventilation–perfusion scanning was reported to show significant changes during APR[33]. Again, the pattern of changes seems to be largely influenced by the underlying disease in the remaining native lung. In patients who receive a single lung for interstitial pulmonary disease, acute rejection will primarily reduce the perfusion to the lung graft, whereas in patients with primary pulmonary hypertension APR seems to reduce ventilation rather than perfusion of the graft[33,34]. However, diagnosis of rejection based on changed perfusion or ventilation pattern depends largely on availability of previous studies for comparison. Differentiation from infection is difficult, since changes found on the scan can be very similar to those during rejection. In a study from the transplant group at the University of Munich, reduction in perfusion of the allograft was found in only 43% of single-lung transplant recipients during rejection episodes, but this finding reached a specificity for rejection of 84%[35]. It might therefore be a valid tool for long-term follow-up in this particular patient group.

Reliable differentiation between rejection and infection therefore requires more definitive and invasive tests. Most studies concentrate on the use of bronchoscopy, bronchoalveolar lavage (BAL) and transbronchial biopsy (TBB). BAL allows collection of secretions directly from the relevant pulmonary surface, and is considered a major tool to diagnose or exclude pulmonary infection[23,36,37]. Its usefulness to diagnose rejection is controversial. Many studies have attempted to isolate single or combined factors within BAL fluid that allow for the differentiation of rejection from infection episodes. Morphological analysis of BAL cells demonstrated a steady rise in the absolute number of lymphocytes, together with a persistent but less drastic polymorphonuclear leukocytosis, during acute cellular pulmonary rejection[38,39]. Unfortunately, other pulmonary pathologies, such as reimplantation response, pneumonia, and atelectasis, can cause similar alterations in the lavage cellular profile, making it impossible to differentiate between rejection and infection based on conventional cell counts[23,40].

Even more sophisticated immunologic methods, such as phenotypic and functional analysis of BAL cells, have failed to reveal any specific markers for APR. Zeevi and colleagues examined quantity and dynamics of T-lymphocyte subsets in BAL cells in lung allograft recipients. Although they noticed increases in $CD8^+$ cells and a parallel increased CD4/CD8 ratio during acute rejection, similar changes were noted during infective episodes, especially during *Pneumocystis carinii* and CMV infections[41,42]. The same dilemma of non-specificity was found for assays that quantify cytotoxicity of lymphocytic lavage cells against donor-specific antigens[43,44]. Similarily, efforts failed to correlate increased levels of hyaluronic acid in bronchoalveolar fluid specifically with episodes of APR[45]. Levels of soluble interleukin-2 receptor subunits within BAL in single-lung transplant recipients were found to be strongly elevated during APR, but did not allow differentiation from similar peaks during bacterial and viral pneumonia[46]. Therefore, the identification of one or more factors that can be easily isolated within the bronchoalveolar lavage fluid as a specific marker for acute rejection remains elusive. Perhaps the most important task accomplished by BAL is to rule out the presence of infection when adequate deep BAL samples are free of pathogens. However, the presence of virus in the BAL fluid alone is not diagnostic for interstitial pneumonitis and, particularly if observed for the first time since transplantation, requires further diagnostic steps.

More positive results have been published on the validity of transbronchial biopsies (TBB) for detection of APR. Although some researchers observed low detection rates of APR by TBB[22,23,35], centers with more experience reported sensitivity and specificity to be as high as >90%[47,48]. It appears that failure to diagnose by TBB was most often related to the absence of representative or sufficient pulmonary parenchyma gained through the biopsy[23,35]. To obtain optimal quality and quantity of tissue, it has been recommended that large alligator bioptomes should be used, the number of biopsy samples should be more than nine and biopsies should be obtained from at least two different biopsy sites[47]. It is important to keep in mind that, even then, potential pitfalls

remain. Although perivascular inflammation with or without lymphocytic bronchitis is often considered as pathognomonic for acute cellular rejection, similar histological pictures have been described during CMV and *Pneumocystis carinii* pneumonias[37]. The presence of either one of these infections necessitates very cautious interpretation of the histopathology of the biopsy, and requires close cooperation between pathologist and clinician. Limitations for TBB are found within its invasive nature, which can lead to complications such as pneumothorax or secondary pulmonary infection, as well as bronchial bleeding. These complications are reported to occur in less than 10% of cases and are rarely related to any mortality[22].

Open lung biopsy (OLB) via thoracoscopy or mini-thoracotomy has been used as a last resort in patients when all other tests have failed to determine the diagnosis, or a given diagnosis is doubted[21,49]. It offers the advantage of obtaining larger quantities of pulmonary parenchyma than through TBB, thus reducing the sampling error in disease processes with patchy distribution, for example in OB[50]. Recently, the Toronto group published their experience with OLB in lung transplant patients during the past decade[50]. They found that it was of little value within the early postoperative period, but yielded useful information that resulted in changed treatment strategy in approximately 30% of patients when performed more than 45 days after transplantation. Furthermore, it confirmed a suspected diagnosis in another 32%, which is also important since it reassures the medical team in the use of ongoing therapy and may prevent the use of 'shotgun' expensive and unneccessary presumptive therapy. The overall complication rate is approximately 10%, and morbidity from such complications as hemothorax or prolonged air leak with resulting empyema can be significant[49,50]. Therefore, this procedure continues to be useful, but cannot be applied as a routine measure to diagnose APR.

In summary, if pulmonary infection or rejection is suspected in lung transplant recipients on the basis of clinical manifestations, a new infiltrate on chest radiograph, or a deterioration in pulmonary function, bronchoscopy should be performed to obtain samples of lung parenchyma for histological examination and bronchoalveolar lavage fluid to exclude infection. If a biopsy is contraindicated due to coagulation abnormalities, severe respiratory distress, or other reason, or histology is inconclusive, additional investigations as described above become important. Concomitant use of selected tests usually allows accurate diagnosis of an acute rejection episode, or at least allows exclusion of infection[35]. Ultimately, a useful clinical measure for the diagnosis of APR remains the response to treatment such as pulsed high doses of i.v. methylprednisolone, but one has to keep in mind that a viral pneumonia can also improve initially following such therapy, due to reduction of its inflammatory process.

MAINTENANCE IMMUNOSUPPRESSION AND PREVENTION OF ACUTE PULMONARY REJECTION

Recurrent or persistent acute rejection carries a poor prognosis if inadequately controlled, and indicates failure of maintenance immunosuppression. It is for this reason that some comment must be made about the standard maintenance regimen and possible adjunctive salvage immunosuppression.

Successful organ transplantation was made possible by the introduction of drugs that were able to modify the recipient's immune response. These drugs suppress reactivity to the allograft but, being non-specific, they also suppress other defense mechanisms, predisposing the recipient to infection. Immunosuppressive therapy is regulated by drug toxicity and the presence of rejection. The modes of action and toxicity of the most commonly used agents are outlined in Table 2.

Currently, most protocols for maintenance immunosuppression consist of a triple drug regimen with cyclosporin, azathioprine, and corticosteroids. Most transplant programs begin treatment pre- or intraoperatively as induction therapy. The protocol used in our program is illustrated in Table 3.

Cyclosporin was clinically introduced in 1981 and has since become the cornerstone of maintenance immunosuppression. Its pharmacological actions are complex and cannot be fully discussed in this chapter. In brief, cyclosporin acts at an intracellular level where, by binding to calcineurin, it inhibits transcription of the IL-2 gene. Thereby it reduces production of this cytokine, which acts as an important cellular messenger leading to activation and proliferation of T lymphocytes directed against the allograft. Through this regulatory mechanism ongoing cellular rejection is efficiently suppressed[51]. Since direct measurement of the level of immunosuppression is as yet not possible, blood or serum levels of cyclosporin have been correlated with their relative clinical effectiveness. Based on these data the therapeutic

Table 2 Immunosuppressive drugs: mechanisms of action and toxicities

Drug	*Mechanism of action*	*Toxicity*
Cyclosporine	Inhibits transcription of IL-2 gene; diminishes IL-2 production and release; blunts activation and proliferation of lymphocytes	Nephrotoxicity; hypertension; neurotoxicity (tremors, paresthesia, depression); hepatotoxicity; hypertrichosis; gingival hyperplasia
Azathioprine	Inhibits nucleic acid synthesis; blocks proliferation of lymphocytes	Leukopenia; pancreatitis; hepatitis; cholestatic jaundice
Corticosteroids	Decreases inflammatory reaction by lysing T lymphocytes and by blocking cytokine production and secretion by mononuclear phagocytes	Hyperglycemia; hypercholesterolemia; osteoporosis; cataracts; myopathy; peptic ulcer
ATG or ALG	Opsonizes and depletes lymphocytes	Leuko/thrombocytopenia; fever; arthralgia; serum sickness
OKT3	Opsonizes or lyses lymphocytes with CD3 receptors that are present on all mature T lymphocytes	Leukopenia; hypotension; pulmonary edema; aseptic meningitis; fever; chills; nausea; vomiting; diarrhea; serum sickness

Table 3 Immunosuppressive drug regimen (used by the Montreal Lung Transplant Program)

Indication	*Immunosuppressant agent*	*Dosage*
Induction and maintenance	Cyclosporine	Preoperative bolus of 2 mg/kg i.v.; postoperative continuous i.v. infusion for first 7 days (1–3 mg/kg per day), then switch to oral/enteral route (p.o.); maintain blood level for first 6 weeks at 350–500 ng/ml (monoclonal testing), thereafter at 200–250 ng/ml
	Azathioprine	Preoperative bolus of 2 mg/kg i.v.; postoperative 1–3 mg/kg per day i.v., switch to p.o. as soon as GI tract working; adjust dose to maintain WBC around 5000/mm^3
	Corticosteroids	Methylprednisolone i.v.: intraoperative 500 mg; postoperative 125 mg q 8 hours, then day 1: 100 mg; day 2: 75 mg; day 3: 50 mg. Prednisolone p.o. from day 4 or as soon as GI tract is working: 1 mg/kg per day tapered over 2 months to maintenance dose of 0.1 mg/kg per day
Primary episode or APR	Methylprednisolone (MP)	Bolus of 500 mg/day i.v. for 3 days; followed by increased oral prednisolone (1 mg/kg per day) tapered to maintenance dose over 2–4 weeks
Steroid-resistant APR (after > 2 courses of MP)	ATG	10–15 mg/kg per day i.v. for 10 days, keep WBC > 2500/mm^3
	OKT3	5 mg/day i.v. for 10 days
Persistent low-grade APR	Methotrexate	10 mg p.o. two or three times a week in addition to maintenance immunosuppressive regimen

APR = acute pulmonary rejection; i.v. = intravenous; WBC = white blood cell count; GI = gastrointestinal

range of serum level has been defined as the range allocated between therapeutic extremes, with drug toxicity at one end and insufficient immunosuppression with ongoing cellular rejection at the other end. Because of individual variability in cyclosporin pharmacokinetics, i.e. absorption and metabolic rate, continued monitoring of its level is essential to optimize therapeutic efficacy[52].

Azathioprine has been used as an immunosuppressant after solid-organ transplantation since the early 1960s. Data collected by the Stanford group from their population of heart–lung transplant recipients seem to confirm its role in pulmonary transplantation. Patients who had received a dual immunosuppressive regimen with cyclosporin and prednisone showed a significantly higher prevalence of chronic graft dysfunction than those who received augmented immunosuppression with azathioprine[53,54]. Since this was a comparison between historical groups, it remains unclear if other factors, such as closer monitoring and surveillance, or more aggressive treatment of acute rejection, may have contributed to this decline in OB.

Corticosteroids have played an important role as one of the first immunosuppressants known to modern medicine. Their use in lung transplantation has been controversial, and has changed considerably over the past decade. The first attempts of human lung transplantation (between 1963 and 1973) failed due to airway dehiscence. During the following period of research, Joel Cooper and his colleagues showed, in a canine model of single lung transplantation, that corticosteroids exerted a negative influence on healing of the bronchial anastomosis[55]. This observation led to a recommendation to omit steroids from maintenance immunosuppression during the peri- and early postoperative period. With growing international experience it became obvious that technical surgical aspects are just as important for successful healing of the bronchial anastomosis, and that the use of steroids for induction and maintenance therapy does not interfere with successful pulmonary transplantation[56,57]. Although in most transplant centers steroids are currently a standard part of perioperative and maintenance immunosuppression, dosages and taper schedule vary significantly, leaving the question of the optimal regimen unanswered.

In heart transplantation, additional cytolytic induction therapy with OKT3, ALT or ATG in combination with standard triple-drug therapy has been reported to provide excellent intermediate survival and delayed onset of the first episode of rejection[58]. Barr *et al.*, however, found that such cytolytic therapy does not reduce the overall frequency of rejection episodes compared with patients receiving triple-drug therapy alone[58]. Prolonged administration of such prophylaxis with OKT3 was observed to cause sensitization, with formation of human antimouse antibody that led to a higher incidence of vascular rejection and reduced survival[59]. Induction cytolytic therapy in pulmonary transplantation has been successfully used in many centers, with similar results to those observed in the heart transplant population[3]; recently, however, it has been discussed controversially[4]. Using a standard immunosuppressive triple-drug protocol without cytolytic induction, Kriett *et al.* not only reported excellent survival within their lung transplant recipients, but also observed a reduced number of viral infections without any significant change in the clinical prevalence of rejection[4]. This confirmed results of another study that reported a similar positive experience in pulmonary transplant recipients after omission of such rejection prophylaxis[60].

Some patients, however, seem to need higher basic immunosuppression, since they experience recurrent or ongoing low-grade rejection. This form of graft rejection has been described as a smoldering, continuous process without any clinical symptoms, but is often associated with slowly progressive loss of function[16]. In these patients, often the only pathology found on pulmonary biopsy is a lymphocytic bronchitis or bronchiolitis[61,62]. In cardiac transplant recipients with low-to-mild grade persistent or recurrent rejection episodes, low-dose methotrexate therapy (0.1–0.2 mg/kg three times per week) has been described as a useful adjunct to baseline maintenance immunosuppression[63]. No results have been officially reported for this regimen in lung transplant recipients. In the authors' own experience, however, methotrexate has been used with a similar regimen (0.1 mg/kg every other day) in five cases with persistent grade II pulmonary rejection despite previous pulsed steroid therapy. In four of these patients, reversal of the process with partial regain of function was achieved. In one complicated case of single lung retransplantation (for OB in a

previous heart–lung graft) ongoing rejection could not be stopped and the patient died 3 months after initiation of methotrexate therapy (unpublished data). Side-effects observed during treatment were bone marrow depression, impairment of liver function, and nausea, all of which were reversible after cessation of treatment. Only future trials with a larger number of patients will allow us to determine the efficacy and role of methotrexate therapy in acute pulmonary rejection.

To date there has been no randomized, controlled prospective study that compares the results achieved by various immunosuppressive protocols within otherwise identically treated recipient populations. Therefore, any decision with regard to the immunosuppressive regimen to be used for maintenance therapy has to be based upon individual experience and conviction.

CLINICAL SIGNIFICANCE AND TREATMENT OF ACUTE PULMONARY REJECTION

Despite prophylactic and maintenance immunosuppression, pulmonary rejection following lung transplantation has been common, and most recipients experience at least one episode that requires treatment[3,22]. Acute rejection has been observed as early as 3 days and as late as several years after transplantation. The highest incidence, however, is during the first 3 weeks[64]. The clinical features have been described above. Sometimes, especially late (>3 months) after transplantation, acute rejection is not accompanied by any clinical symptoms and can be easily overlooked[65]. This is probably of even greater importance when one considers that late rejection episodes seem more difficult to reverse than those developing within the first weeks post-transplantation[66]. Close monitoring of lung transplant patients, as discussed above, is therefore essential, since short- and long-term adverse effects of undiagnosed rejection include not only acute dysfunction but also irreversible loss of functional pulmonary parenchyma[4,15]. Indeed, many studies postulate a correlation between persistent or recurrent acute rejection episodes and the development of chronic graft dysfunction or OB[12,13,15,16,18,54,83]. Aggressive therapy is therefore essential.

Several options are available for treatment of acute rejection. The first line of treatment is usually based on increased immunosuppression with high-dose corticosteroids. The regimen used in most centers is 10–15 mg/kg per day of methylprednisolone given intravenously as a single dose on three to five consecutive days, followed by an increase of oral prednisone to 1–2 mg/kg per day, which is tapered to baseline maintenance dosage. Rapidity of taper varies from one center to another, and is based on individual bias rather than solid data. Slow tapering of steroids over several weeks is often applied for higher-grade or recurrent episodes of rejection, since it prolongs the period of augmented immunosuppression and may provide a certain protection against early rebound of rejection. However, this may have the drawback of an increased risk for infection, in particular of viral origin, as well as an increase in the stigmata of long-term steroid usage (see Table 2). The latter is of particular importance in lung transplant recipients, as many of them have been exposed to long-term use of oral steroids even before transplantation. The currently used regimen in our program is detailed in Table 3. Most centers report a primary success rate with this kind of treatment that ranges from 90% to 95%.

Acute rejection episodes that are refractory to treatment with 15 mg/kg methylprednisolone are considered to be steroid-resistant. This form of rejection, and another (which is recurrence of rejection during tapering of the oral steroid dosage) are perceived by many clinicians as having a more ominous prognosis[66]. Various adjuvants have been proposed to treat such severe forms of rejection. These include cytolytic therapy, methotrexate therapy, and total lymphoid irradiation[67,68].

Cytolytic therapy is based on the use of antibodies that are directed against lymphocytes, thereby interfering with recruitment of these cells for rejection of the graft. The most commonly used and commercially available agents are polyclonal, non-selective antithymocyte globulins (ATG) and OKT3, a monoclonal antibody directed against CD3-carrying lymphocytes. Both forms are found to be effective immunosuppressants, and reversal rates of steroid-resistant acute rejection episodes are reported to be 60–90%[69]. Neutropenia and thrombocytopenia are often-described side-effects, which are an expression of profound immunosuppression and can result in life-threatening infections, particulary of viral origin. A risk of bleeding and increased costs are also associated with the use of these drugs[68]. Cell count-based dosing (maintaining a peripheral CD3 T cell level of 50–100/μl) rather than standardized dosing, has therefore been proposed and proven to be similarly effective while reducing treatment-related morbidity[70]. Since all of these drugs are of rabbit, murine, or equine origin, humoral or allergic reactions are often observed during administration, and are attributed either directly to the drug or to release of cytokines as a result of T cell lysis. Such first-dose-related side-effects are mostly limited to pyrexia, nausea, vomiting, diarrhea, hypotension, and bronchospasm, and are usually transient and seldom life-threatening[68]. However, cardiopulmonary shock, pulmonary edema, and aseptic meningitis have been observed with the use of OKT3, which led to the recommendation to use premedication with intravenous steroids, antihistamines, and diuretics during the first days of therapy[67,68]. It is important to note that successful OKT3 rescue therapy can be followed by a rebound of acute rejection in up to 40% of patients, requiring further treatment[67,69]. This can represent a significant therapeutic problem, since sensitization with formation of anti-OKT3 antibody occurs in up to 40% of the patients, precluding repeated use of the same agent or rendering it ineffective[68]. In view of these potential risks involved with their use, it seems clear that cytolytic agents should be reserved for treatment of steroid-resistant rejection episodes.

Information about cytolytic therapy for recurrent or persistent rejection in lung transplant recipients is still minimal and often anecdotal. The only published study about rejection therapy with OKT3 in pulmonary transplantation observed an 89% reversal rate in rejection episodes occurring within the first 6 months after transplantation. Thereafter, only 30% of the cases responded to OKT3 therapy[66]. Early in our program we used a non-specific, polyclonal antilymphocyte globulin of equine origin produced at the University of Minnesota (mALG). Its use was subsequently abandoned due to problems with its availability and a perceived batch-to-batch variation in its strength.

We and the Pittsburgh group currently use ATG preparations (ATGAM i.v. 10–15 mg/kg/per day for 14 days or RATG i.m. 5 mg/kg/per day for 5 days) for treatment of steroid-resistant, re-

current, or severe (grades III and IV) rejection. No published data, however, are available with regard to effectiveness or success rates of these treatments. Since any extra therapy for acute rejection enhances the overall level of immunosuppression, an increased prevalence of infection has to be expected. Bacterial, fungal and opportunistic infections consequent to augmented immunosuppression have been reported. Those of viral origin, especially from herpes simplex and cytomegalovirus, seem to predominate[3,4]. To reduce this risk, some authors recommend antimicrobial prophylaxis for the duration of rejection treatment[66].

Total lymphoid irradiation (TLI) has been reported as another possible therapy for steroid-resistant or low-grade smoldering acute cardiac rejection with continuous loss of graft function. Low-dose radiotherapy (with a total of 8 Gy) is targeted at major lymph-node-bearing areas, including the cervical, axillary, mediastinal, periaortic, and iliofemoral nodes, as well as spleen and thymus, with shielding of non-lymphoid tissue[71]. It is usually administered over a 5–8-week period and seems to be well tolerated, even in the pediatric population, with minimal or no increase in infection episodes[72,73]. The reported success rate in reversing rejection varies between centers, with a range of 50–70%. Another important observation is that the majority of these patients had a drastically reduced rate of further rejection episodes[73]. However, experience is still very limited and based on anecdotal reports. The group from Newcastle in England applied TLI in 11 patients with recalcitrant acute rejection and chronic loss of pulmonary graft function resistant to any other form of treatment. In seven of these patients the process came to a standstill, but only in four of these seven was a gain of previously lost pulmonary function observed (P.A. Corris, personal communication). To determine the role of TLI as treatment for resistant pulmonary rejection, further results have to be awaited.

With successful therapy of acute rejection, clinical signs such as fever, shortness of breath, adventitial lung sounds, etc., usually disappear rapidly. It is important to note, however, that radiological and histological signs tend to persist longer than clinical symptoms. This time period varies, but seems to correlate with the severity of the histological rejection grade. In severe cases, residual fibrosis of alveolar spaces and terminal airways can be observed.

NEW IMMUNOSUPPRESSANTS AND THEIR POTENTIAL ROLE

As discussed above, the main disadvantage of cyclosporin in its classical formulation is the individual variation in its absorption and metabolism. Furthermore, blood concentration levels do not neccessarily reflect the adequacy of immunosuppression, which could be partially responsible for an increased risk of acute and chronic rejection[74]. A new microemulsion formulation of cyclosporin, called 'Neoral', might overcome some of these shortcomings. Absorption is increased by 20–30%, and maximum concentration by 30–60%, and day-to-day variability is almost halved[75,76]. An international randomized prospective study in renal transplant recipients with this substance not only confirmed these data, but also found a reduced graft rejection rate when compared to patients treated with classical cyclosporin[77,78]. The incidences of hypertension, malignancies, and infection, on the other hand, were not increased.

In cystic fibrosis patients with potential malabsorption syndrome, this drug might be of particular advantage. In our initial experience in this transplant population we have been impressed with the responses to Neoral in patients who hitherto had difficulty in maintaining adequate blood cyclosporin levels. Currently, first clinical trials have been initiated in various lung transplant centers, but long-term data are not available as yet.

Tacrolimus (FK506) is another immunosuppressive drug that has been introduced clinically recently. Its mode of action, efficacy, and toxicity profile are similar to cyclosporin. In a US multicenter trial in liver transplant recipients, similar graft and patient survival was observed when compared to those achieved with cyclosporin. The incidence of acute, resistant, and refractory rejection, however, was significantly lower[79]. It also seems to have the potential to reverse cyclosporin-resistant rejection with concurrent reduction in dosage of prednisone[80]. Experience with it in lung transplantation is limited to very few institutions. Preliminary reports are mainly from the Pittsburgh group, where FK506 has been used in combination with prednisone and azathioprine since 1991 in selected patient groups[3] (Chapter 10). Their results indicate that these patients tend to have a lower incidence of acute rejection with a similar spectrum of side-effects[3,38,62].

Hopes that this drug will lead to a reduction in the prevalence of OB or chronic graft dysfunction have not yet been confirmed. Follow-up, however, averages less than 12 months and does not allow a definite answer to this specific problem. Therapeutic drug monitoring can be achieved mainly through measurement of whole blood levels. Correlation between these levels and clinical events seems not as clear as with cyclosporin, since significant overlap of toxicity and rejection can occur. Large international multicenter trials are currently under way in heart and lung transplant recipients, and will hopefully provide further understanding of efficacy, toxicity, and monitoring of this agent.

A third immunosuppressant that has recently reached clinical phase II trials is mycophenolate mofetil. Its mode of action resembles that of azathioprine rather than cyclosporin. It inhibits the enzyme that synthesizes guanosine monophosphate (GMP), which is required for the production of nucleic acids[82]. This being an essential step for lymphocyte metabolism, it causes a profound inhibition of all strains in these cells. Since most other cells possess a salvage pathway for GMP synthesis, this inhibition is relatively specific[82].

The only clinical data available to date derive from renal, hepatic, and cardiac transplantation. In most protocols, mycophenolate has been used as a substitute for azathioprine in combination with cyclosporin and prednisone. A decrease in incidence and severity of rejection episodes, as well as a decreased requirement for anti-rejection therapy, have been reported in all studies[83,84]. It also showed potential for reversal of renal graft rejection that had been found resistant to corticosteroids and antilymphocytic products[85]. In pulmonary transplantation, however, this drug has not yet been tested. Due to observed *in-vitro* antiproliferative properties, it might be of particular benefit to lung transplant patients with OB[86].

Two other agents, namely rapamycin and leflunomide, with recognized immunosuppressive action, are currently being tested in various experimental studies to evaluate their potential role in transplantation, but are as yet not available for clinical trials (Chapter 70).

COMMENT

Acute pulmonary rejection is one of the most important causes of morbidity following lung transplantation. Hardly any lung transplant recipient seems to escape at least one episode of rejection. Accurate and prompt diagnosis is still a clinical challenge, in particular since pulmonary infections often mimic clinical and even histological features of rejection. Use of all diagnostic tools, including bronchoscopy with bronchoalveolar lavage and transbronchial biopsy, allows differentiation between rejection and infection in most cases. Thoracoscopic or open-lung biopsy can be a useful and safe intervention in cases in which the diagnosis remains obscure despite all other tests, or when rapid deterioration of the patient allows insufficient time for routine examinations.

Close surveillance of lung transplant patients is indicated not only because untreated acute rejection leads to sudden organ dysfunction, but also because data are increasing to indicate that the development of chronic pulmonary graft dysfunction is correlated to the number and severity of acute rejection episodes[87]. Since acute rejection can occur without any detectable clinical symptoms, the important question about the role of TBB as a tool of surveillance has to be answered. In experienced hands, and following the above-mentioned strategy, TBB continues to be a safe procedure and a reasonably specific means to diagnose acute pulmonary rejection[22,47,48]. Most recent data from the largest series of pulmonary transplants published by the Pittsburgh group indicate that surveillance TBB might lead to a reduction in late morbidity and mortality. This group observed that TBB helped to pick up acute and chronic forms of rejection at an earlier stage, which reduced the severity of the disease process and significantly improved the results of treatment. Although previous studies from smaller patient groups did not confirm this view, it has been shown that a more aggressive diagnostic approach leads to detection of acute rejection episodes that might have been otherwise missed, or detected only at a later stage. Inasmuch as increased frequency and severity of APR episodes lead to chronic graft dysfunction, it seems logical that better monitoring of acute rejection by surveillance biopsies (with, consequently, earlier and improved treatment) should result in a reduction in late mortality.

What remains to be defined is the optimal therapy for asymptomatic patients who have histological evidence of low-grade acute rejection. In other transplant patients, such as heart transplant recipients, evidence is accumulating to indicate that untreated minimal or mild rejection may lead to long-term loss of graft function[88]. Based on these data, and on the observation that severity of acute rejection and development of OB may be correlated, we and others[89] believe that every documented episode of rejection should be treated with therapy adjusted to its severity. Augmentation of maintenance immunsuppression may be adequate for treatment of minimal, clinically inapparent, or recurrent low-grade rejection. This can be achieved by increasing oral steroids, adding inhaled steroids or low-dose methotrexate, or altering immunosuppression altogether with FK506 or mycophenolic acid. However, intravenous pulsed high-dose steroid therapy or antilymphocytic therapy should continue to be the standard for treatment of severe or recurrent rejection. Recognizing that more aggressive therapy of rejection might lead to increased problems with infection, which is in itself a risk factor for early and late mortality, more aggressive monitoring and infection prophylaxis become necessary.

The development of better immunosuppressive agents that selectively suppress allogeneic influences while allowing intact humoral and cellular immunity to fight invading microorganisms will help to reduce this problem. Induction of a chimeric state between donor and recipient is another promising approach, since it could accomplish at least a partial tolerance of the graft, which might help to decrease severity and prevalence of rejection episodes[90].

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Infection After Lung Transplantation

I.L. PARADIS

INTRODUCTION

Infection remains the most common cause of morbidity and mortality after lung transplantation (LTx)[1–3]. Of the 402 lung allograft procedures that were performed in 386 recipients at the University of Pittsburgh between 1982 and 1 July 1995, 187 allografts (47%) in 180 recipients (47%) failed (Table 1). Because some infections were due to more than one type of organism (e.g. bacteria plus *Aspergillus*), 115 infectious organisms were responsible for the failure of 97 (52%) allografts in 86 (48%) recipients that failed primarily due to infection. However, since 1989, infection has declined significantly from 46% to 32% as an etiology, from 80% to 53% as a cause of allograft failure, and from 85% to 54% as a cause of recipient death. This occurred because of the cumulative effect of a small decline in the prevalence of each type of infectious organism as a cause of allograft failure. Because infection has been the primary cause of failure in 53–54% of the grafts that have failed since 1989, it still remains the principal and unacceptable cause of allograft failure and death after LTx.

Infection has been the principal etiology (35%) of allograft failure (58%) and death (60%) in the first year after LTx at the University of Pittsburgh (Table 2) and at other centers[4–7]. Infection has also been the principal etiology (40%) of allograft failure (74%) and death (70%) more than 1 year post-transplant (Table 2). Thus, infection has been the primary cause of allograft failure at all times after LTx.

Infectious complications in LTx recipients at the University of Pittsburgh have occurred twice as frequently as in cardiac, hepatic

Table 1 Causes of lung allograft failure before and after 1 January 1989 at the University of Pittsburgh

	< 1989 *n (%)*	*≥ 1989* *n (%)*	*Total* *n (%)*
Infection due to:	47	68*	115
Bacteria	19 (17)	32 (16)	51 (16)
Fungus	9 (9)	13 (6)	22 (7)
Virus	13 (12)	21 (10)	34 (11)
Other	6 (6)	2 (1)	8 (3)
ARDS/DAD	9	36	45
Ischemic lung injury	6	12	18
Ischemic airway injury	3	5	8
Hemorrhage	12	16	28
Acute rejection	1	9	10
Obliterative bronchiolitis	12	25	37
Primary graft failure	3	10	13
Unknown	0	10	10
Other	10	19	29
Total	103	210	313
Infection/etiology	47/103 (46)	68/210 (32)*	115/313 (37)
Infection/recipient death	47/55 (85)	68/125 (54)*	115/180 (64)
Infection/graft failure	47/59 (80)	68/128 (53)*	115/187 (61)

* $p < 0.05$ compared to before 1989 by chi-square analysis.
ARDS/DAD = adult respiratory distress syndrome/diffuse alveolar damage.

Table 2 Causes of lung allograft failure in the first year and later than 1 year post-transplant at the University of Pittsburgh

	< 1 year *n*	*≥ 1 year* *n (%)*	*Total* *n*
Infection due to:	78	37	115
Bacteria	35	16	51
Fungus	14	8	22
Virus	25	9	34
Other	4	4	8
ARDS/DAD	42	3	45
Ischemic lung injury	18	0	18
Ischemic airway injury	7	1	8
Hemorrhage	25	3	28
Acute rejection	9	1	10
Obliterative bronchiolitis	4	33 (35)	37
Primary graft failure	13	0	13
Unknown	4	6	10
Other	20	9	29
Total	220	93	313
Infection/etiology	78/220 (35)	37/93 (40)	115/313 (37)
Infection/recipient death	78/130 (60)	37/50 (74)	115/180 (64)
Infection/allograft failure	78/134 (58)	37/53 (70)	115/187 (61)

Abbreviations as for Table 1.

or renal allograft recipients who have received nearly the same immune suppression[4-7]. While the first 14 heart–lung transplant recipients experienced an average of 3.0 infections/recipient, the figure for cardiac, hepatic or renal recipients was 1.41, 1.83 or 0.98 infections/recipient, respectively[4,5]. At Stanford University, lung recipients experienced an average of 2.4 infections/recipient, while heart recipients experienced 0.47 infections/recipients[6,7]. While infection has been the primary cause of death in <20% of long-term cardiac recipients, it has been the primary cause of death in 74% of long-term lung recipients (Table 2)[8]. Possible reasons for these differences include the fact that the lung allograft (a) is continuously exposed to the external environment, (b) has impaired mucociliary clearance[9-13], and (c) provides an HLA-incompatible microenvironment where the alveolar macrophages and lymphocytes of the recipient live in the alveoli of the donor[14].

A total of 1173 significant infections have occurred in 367 'at-risk' lung recipients (survival ≥2 days) who have been followed for 278 270 days post-transplant between 1982 and 1 July 1995, at the University of Pittsburgh (Table 3). This is an average of 3.23 infections/recipient, 0.43 infections/100 days of observation, or 0.0012 infections/100 days of observation/recipient. The lungs, mediastinum and pleural spaces have been the initial and/or only site for 70% of all infections (data not shown). Bacteria have been the most common organism, with pneumonia, bronchitis or sepsis having caused 62% of all infections.

When the infectious complications of the 64 recipients who received allografts prior to 1989 were compared to those of the 303 recipients who received allografts later, the number of infections/100 days of observation did not change (0.38 vs 0.45) but the number of infections/recipient (5.05 vs 2.85) and the number of infections/100 days of observation/recipient (0.0059 vs 0.0015) both decreased. Since 1989 the proportion of infections due to bacterial pneumonia, bronchiectasis and pneumocystis (PCP) decreased significantly (42% vs 22%) while those due to cytomegalovirus (CMV), bacterial bronchitis and *Clostridium difficile* increased significantly (23% vs 42%). Bacterial bronchitis and CMV infection, however, have not changed over time when examined as a proportion of at-risk recipients (63% vs 52% and 52% vs 45%, respectively). Only infection due to *C. difficile* appears to have truly increased in frequency over time. This has most probably been due to the interval recognition of this agent as a pathogen, and also to the increasing use of antibiotics as prophylaxis of bacterial infections. The most significant finding is that the rate of infection (as defined by days of observation post-transplant) has not changed over time, but that defined by the number of infections/recipient has decreased, primarily because of a decrease in the number of episodes of bacterial pneumonia.

The risk of infection has been much higher in the first year post-transplant and 72% (859/1173) of all infections have occurred during that time (Table 4). This is not surprising since fresh surgical wounds are present, more intense invasive monitoring is required, and immune suppression is maximal during the first year post-transplant. The infections that occur primarily in the first year are CMV, bacterial sepsis, *C. difficile* and infections located in the mediastinum and pleural spaces. The infections that occur primarily later than 1 year post-transplant are bacterial bronchitis and bronchiectasis. All other infections are equally likely to occur at any time. The prevalence of late infection in lung recipients at 1.48 infections/recipient is 7.8 times greater than that reported for cardiac recipients, who experienced 0.19 late infections/recipient[8]. Thus, whether comparing (a) early or recent experience or (b) early or late post-transplant periods, infection remains the most common cause of morbidity (Tables 3 and 4) and mortality (Tables 1 and 2) after LTx, despite a decrease in the prevalence of pneumonia due to bacteria and PCP.

Table 3 Types of infectious complication after lung transplantation before and after 1989 at the University of Pittsburgh

	< 1989 n (%)	*≥ 1989 n (%)*	*Total n (%)*
Bacterial pneumonia	97 (30)	170 (20)*	267 (22)
Bacterial bronchitis	40 (12)	160 (19)*	200 (17)
Bronchiectasis	13 (4)	6 (< 1)	19 (2)
Fungal infection	18 (7)	50 (6)	68 (6)
Cytomegalovirus	33 (10)	136 (16)*	169 (14)
Pneumocystis	24 (7)	11 (1)*	35 (3)
Lymphoproliferative disease	6 (2)	16 (3)	22 (3)
Herpes simplex	12 (4)	43 (5)	55 (5)
Viral hepatitis	2 (< 1)	9 (1)	11 (1)
Other viruses	5 (2)	39 (5)	44 (4)
Mediastinitis	11 (3)	3 (< 1)	14 (1)
Empyema	4 (1)	8 (1)	12 (1)
Bacterial sepsis	34 (10)	100 (9)	134 (11)
Sinusitis	7 (2)	23 (3)	30 (3)
C. difficile colitis	2 (4)	59 (7)*	61 (5)
Other	12	20	32 (3)
Total infections	320	853	1173
At-risk recipients	64	303	367
Days of observation	85 146	193 124	278 270
Infections/recipient	5.05	2.85	3.23
Infections/100 days of observation	0.38	0.45	0.43
Infections/recipient/100 days of observation	0.0059	0.0015	0.0012

* $p < 0.05$ compared to before 1989 by chi-square.

Table 4 Types of infectious complication in the first year and later than 1 year post-lung transplantation at the University of Pittsburgh

	< 1 year n (%)	*≥ 1 year n (%)*	*Total n*
Bacterial pneumonia	192 (22)	75 (23)	267
Bacterial bronchitis	111 (13)	89 (27)	200
Bronchiectasis	6 (4)	13 (4)	19
Fungal infection	49 (6)	19 (6)	68
Cytomegalovirus	150 (18)	19 (6)	169
Pneumocystis	24 (3)	11 (3)	35
Lymphoproliferative disease	17 (3)	5 (2)	22
Herpes simplex	46 (5)	9 (3)	55
Viral hepatitis	9 (1)	2 (< 1)	11
Other viruses	25 (3)	19 (6)	44
Mediastinitis	14 (2)	0 (0)	14
Empyema	12 (1)	0 (0)	12
Bacterial sepsis	103 (12)	31 (9)	134
Sinusitis	17 (2)	13 (4)	30
C. difficile colitis	51 (6)	10 (3)	61
Other	20	12	32
Total infections	846	327	1173
At-risk recipients	367	222	367
Days of observation	16 276	261 994	278 270
Infections/recipient	2.34	1.48	3.23
Infections/100 days of observation	5.28	0.13	0.43
Infections/recipient/100 days of observation	0.1438	0.0006	0.0012

SURVEILLANCE FOR INFECTION

Prior to transplantation (in the recipient) and at the time of transplantation (in the donor), the serologic status of recipient and donor for CMV; Epstein–Barr virus (EBV); varicella virus; herpes simplex virus (HSV); *Toxoplasma*; hepatitis A, B and C; and human immunodeficiency (HIV) type 1 virus are determined. Sputum for cultures and stains for bacteria and fungus are obtained every 3 months from candidates with septic lung disease. A transplant procedure can be performed when *Pseudomonas* species are sensitive to more than one class of anti-pseudomonal antibiotics, or if *Aspergillus* species are not present in respiratory tract secretions.

At the time of transplantation, specimens for cultures and stains for fungi and bacteria are obtained from airways of both donor and recipient[15,16]. This information is useful in assessing the susceptibility of the recipient to develop infection with bacteria or fungus posttransplantation. Another aerobic and fungal sputum culture is obtained prior to extubation. Chest radiographs are obtained daily, with the frequency decreasing to once or twice a week by the end of the initial hospital stay. Fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial lung biopsy (TBBx) is performed at least once, and as often as weekly, during the initial hospital stay, every 3 months during the first year, every 4 months during the second year, twice a year thereafter, and whenever infection or rejection in the allograft is suspected[17]. Recipients record their spirometry at home and report any consistent, unexplained >10% decrease in the FEV_1 over a 1–2-week period[18]. If a decrease in home spirometry is confirmed by spirometry in a pulmonary function laboratory, a re-evaluation, which includes a bronchoscopy with BAL and TBBx, is performed at the transplant center.

BACTERIAL PNEUMONIA

Definition

The diagnosis of bacterial pneumonia has been made by: (a) the presence of a new or predominant organism by Gram stain of the sputum in association with fever or new or increased radiographic infiltrates, (b) histologic criteria, or (c) the presence of $>10^4$ cfu/ml of cultured BAL fluid and/or >5% of BAL cells containing intracellular bacteria[19–29].

'Prophylactic' antibiotic regimens

Although still the most common infectious complication after LTx, there has been a significant decrease since 1989 in the prevalence of bacterial pneumonia (a) as a proportion of all infectious complications (38% vs 20%; Table 3), (b) as a proportion of at-risk recipients (78% vs 33%; Table 5) and (c) in regard to the number of episodes/at-risk recipient (1.50 vs 0.56; Table 5). This coincides with the introduction of an antibiotic regimen tailored to the results of aerobic cultures of the donor and recipient airways obtained at the time of transplantation[15,16] and with the introduction of TBBx to distinguish between diffuse alveolar damage, infection and/or rejection[17,30–36]. Prior to 1989, recipients received only cefamandole 1 g every 8 h for 72 h post-transplant. Since that time, recipients without pretransplant septic lung disease have received clindamycin and ceftazidine immediately post-transplant. If the recipient and donor airway cultures are sterile, these antibiotics are stopped at 72 h post-transplant. If they contain oral flora organisms, clindamycin is continued for 10 days. If they contain *Staphylococcus*, clindamycin is continued, and vancomycin is added to complete a 10-day course if the

Table 5 Morbidity and mortality of bacterial pneumonia at the University of Pittsburgh before and after 1989

	< 1989	*≥ 1989*	*Total*
Affected recipients/at-risk recipients	50/64 (78%)	101/303 (33%)*	151/367 (41%)
Episodes/at-risk recipient	97/64 (1.50)	170/303 (0.56)*	267/367 (0.73)
Episodes/affected recipient	97/50 (1.94)	170/101 (1.70)	267/151 (1.78)
Mortality/episode	10/97 (10%)	23/170 (13%)	33/269 (12%)
Mortality/at-risk recipient	10/64 (17%)	23/303 (8%)	33/367 (9%)
Mortality/affected recipient	10/50 (20%)	23/101 (23%)	33/151 (22%)

* $p < 0.0001$ compared to before 1989 by chi-square analysis.

Staphylococcus is methicillin-resistant. If these cultures contain Gram-negative organisms, clindamycin is discontinued, ceftazidime is continued, and another antibiotic with Gram-negative coverage is added to the regimen. Alternatively, the ceftazidime and clindamycin may both be discontinued and two new culture-directed antibiotics with Gram-negative coverage are administered for 10 days.

For recipients with septic lung disease, three or four antibiotics active against *Pseudomonas aeruginosa* and/or *Burkholderia cepacia* are begun immediately pretransplant and continued for two full weeks or longer until a positive clinical outcome is certain. The choice of antibiotics is based on the antibiotic sensitivities of the organism(s) present in the sputum of the recipient pretransplant. These recipients also receive less immune suppression. The level of cyclosporin or tacrolimus is maintained at approximately half of that in the non-infected recipient. Azathioprine and corticosteroids are withheld unless more than two episodes of histologically documented acute rejection occur, or unless corticosteroids are necessary to avoid corticosteroid-induced adrenal suppression (secondary to pretransplant use of corticosteroids). With this approach the prevalence of bacterial pneumonia in the first 2 weeks post-transplant has decreased significantly from 47% to 9% (Table 6).

Prevalence

The prevalence of bacterial pneumonia has also decreased significantly between postoperative days (POD) 15 and 180 after LTx. This is beyond the perioperative period (i.e. POD 1–14) where an effect due to the antibiotic regimen employed (according to the results of cultures obtained from the airways of the donor and recipient at the time of transplantation) would not be expected.

This reduction in the prevalence of bacterial pneumonia between POD 15 and 180 is most probably due to the use of bronchoscopy with BAL and TBBx to distinguish between diffuse alveolar damage, infection and/or acute rejection. Information gained from bronchoscopy has avoided empiric pulses of augmented immune suppression to treat clinical rejection, and has allowed for lower levels of maintenance immune suppression when rejection has not been present. Less immune suppression reduces the risk of bacterial pneumonia.

Table 6 Change in the prevalence of bacterial pneumonia before and after 1989 at different time periods after lung transplantation at the University of Pittsburgh

Post-transplant time intervals	*< 1989 n (%)*	*≥ 1989 n (%)*	*P-value*
≤ 14 days	30/64 (47)*	28/303 (9)	< 0.0001
15–90 days	21/57 (37)	50/302 (17)	0.0008
91–180 days	12/40 (30)	20/253 (8)	0.0001
181–365 days	6/36 (17)	23/219 (8)	n.s.
1–2 years	7/33 (21)	34/189 (18)	n.s.
2–3 years	9/31 (29)	7/128 (5)	0.0003
3–4 years	5/29 (17)	4/66 (6)	n.s.
> 4 years	7/26 (27)	3/27 (11)	n.s.

* Data expressed as number of episodes/at-risk recipients.

Relationship with obliterative bronchiolitis (chronic lung rejection)

Between 6 months and 2 years post-transplant the prevalence of bacterial pneumonia has been between 8% and 21%, and this has been unaffected by advances in the care of recipients. This is the period of time, however, when lung recipients are most at risk of developing obliterative bronchiolitis (OB)[37,38]. This complication is a significant risk factor for developing bacterial pneumonia or bronchitis >90 days post-transplant (Table 7). In the 291 recipients who survived >90 days post-transplant, pneumonia occurred twice as frequently in recipients with OB compared to those without OB (1.03 vs 0.53 episodes/recipient). Bronchitis occurred five times more frequently in recipients with (as compared to those without) OB (1.12 vs 0.21 episodes/recipient). In the 155 recipients who survived >2 years post-transplant, pneumonia occurred twice as frequently in recipients with OB (1.01 vs 0.45 episodes/recipient), and bronchitis occurred three times more frequently in those with OB (1.15 vs 0.38 episodes/recipient). Thus, both early and late after LTx, the majority of the episodes of pneumonia and bronchitis due to bacteria have occurred in recipients with chronic rejection.

Additionally, the number of recipients who developed pneumonia or bronchitis due to bacteria was affected by the presence of chronic rejection. For the 291 recipients who survived >90 days post-transplant, pneumonia developed in 50% of the recipients with OB, but in only 30% of recipients without OB (p=0.001) (Table 7). Bronchitis occurred in 54% of recipients with OB, but in only 21% of those without OB (p<0.0001). For those recipients who survived >730 days post-transplant, pneumonia developed in 50% of those with OB, but in only 32% of those without OB (p=0.02). Similarly, bronchitis developed in 56% who developed OB but in only 29% without OB (p=0.0008). Thus, both early and late after LTx, significantly more episodes occurred and more re-

cipients experienced bacterial pneumonia and/or bronchitis when chronic rejection was present.

It is also true, however, that many recipients with OB do not develop pneumonia or bronchitis and, conversely, many recipients without OB do develop these infections. For recipients who survived >90 days post-transplant, 50% of those with OB have never developed pneumonia and 30% of those without OB have developed pneumonia. Similarly, for recipients who survived >90 days post-transplant, 46% of those with OB never developed bronchitis and 21% of those without OB have experienced at least one episode of bronchitis (Table 7).

Table 7 Relationship of bacterial pneumonia (BP) and bacterial bronchitis (BB) to chronic rejection (CR) in recipients who survived > 90 days or > 2 years post-transplant at the University of Pittsburgh

	Episodes/recipient	
Survival	*> 90 days*	*> 2 years*
Bacterial pneumonia		
CR+	110/107 = 1.03	79/78 = 1.01
CR–	98/184 = 0.53	35/77 = 0.45
Total	208/291 = 0.71	114/155 = 0.74
Bacterial bronchitis		
CR+	120/107 = 1.12	90/78 = 1.15
CR–	38/184 = 0.21	29/77 = 0.38
Total	158/291 = 0.54	119/155 = 0.77

	Affected recipients (%)	
	> 90 days	*> 2 years*
Bacterial pneumonia		
CR+, BP+	54 (19)*	39 (25)*
CR+, BP-	53 (18)	39 (25)
CR–, BP+	56 (19)	25 (16)
CR–, BP–	128 (44)	52 (34)
Total	291 (100)	155 (100)
Bacterial bronchitis		
CR+, BB+	58 (20)*	44 (28)*
CR+, BB–	49 (17)	34 (22)
CR–, BB+	38 (13)	22 (14)
CR–, BB–	146 (50)	55 (36)
Total	291 (100)	155 (100)

* $p < 0.05$ by chi-square analysis.

Specific bacterial infections

Pseudomonas aeruginosa and *Staphylococcus aureus*, either alone or in combination with other organisms, have been responsible for the majority of the episodes of pneumonia and bronchitis that have occurred at all time points post-transplant (Table 8). *P. aeruginosa* was the agent responsible for 35% and 38% of the episodes of pneumonia and bronchitis, respectively, that occurred in recipients who survived >90 days post-transplant (Table 8). *P. aeruginosa* was the agent responsible for 37% of the episodes of pneumonia and 37% of the episodes of bronchitis that occurred in recipients who survived >730 days post-transplant. *S. aureus* was responsible for 20% and 22% of the episodes of pneumonia and bronchitis, respectively, that occurred in recipients who survived >90 days post-transplant. This organism caused 18% and 19% of the episodes of pneumonia and bronchitis, respectively, that occurred in recipients who survived >730 days post-transplant. These two organisms have been responsible for 55–60% of all of the episodes of pneumonia and bronchitis that have occurred in these recipients.

The likelihood that *P. aeruginosa* was the agent responsible for an episode of pneumonia or bronchitis, however, was unaffected by the presence of chronic rejection (Table 8). Pneumonia or bronchitis due to *P. aeruginosa* occurred in 33–35% of the episodes when OB was present, and in 39–47% when it was not

Table 8 Episodes of pneumonia or bronchitis due to *Pseudomonas aeruginosa* and *Staphylococcus aureus* in recipients with or without chronic rejection (CR) who survived > 90 or > 730 days post-transplant at the University of Pittsburgh

	Pseudomonas aeruginosa *n (%)*	*Staphylococcus aureus* *n (%)*	*Total* *n (%)*
Pneumonia in recipients who survived > 90 days			
CR+	29 (33)	20 (22)	49/89 (55)
CR–	17 (39)	6 (14)	23/43 (53)
Total	46 (35)	26 (20)	72/132 (55)
Pneumonia in recipients who survived > 730 days			
CR+	20 (34)	13 (22)	33/59 (56)
CR–	8 (47)	1 (6)	9/17 (53)
Total	28 (37)	14 (18)	42/76 (55)
Bronchitis in recipients who survived > 90 days			
CR+	37 (35)	27 (25)	64/106 (60)
CR–	17 (45)	5 (13)	22/38 (58)
Total	54 (38)	32 (22)	86/144 (60)
Bronchitis in recipients who survived > 730 days			
CR+	28 (35)	19 (24)*	47/80 (59)
CR–	11 (44)	1 (4)	12/25 (48)
Total	39 (37)	20 (19)	59/105 (56)

* $p = 0.05$ compared to no chronic rejection by chi-square.

present. For unclear reasons, however, *S. aureus* was more clearly associated with OB. Pneumonia or bronchitis due to this organism occurred in 22–25% of the episodes in which OB was present, but it was the causative organism in only 4–14% of the episodes in which OB was not present. Thus, although *P. aeruginosa* has been the most common organism responsible for episodes of pneumonia or bronchitis, it is equally likely to occur in recipients with or without chronic rejection. *S. aureus* has been the second most common cause of pneumonia or bronchitis, and it has been more frequently observed in recipients with OB. Thus, any bacterial infection, particularly that due to *S. aureus*, occurring >90 days post-transplant, suggests the co-presence of chronic rejection. An evaluation to detect this process should be performed *after* the infection has been treated (because the inflammation caused by infection can result in pulmonary function and histologic changes that make OB difficult to identify clinically and histologically).

Because of the association of OB with recurrent airway and parenchymal bacterial infection, bacteria in the airways of recipients with OB have been treated with 'prophylactic' antibiotics. Recipients with oral flora or methicillin-sensitive *Staphylococcus* organisms receive oral cephalexin 500 mg four times daily, and those with sensitive *P. aeruginosa* receive oral ciprofloxacin 500 mg twice daily and aerosolized tobramycin 80 mg three times a day for 10 days/month. Aerosol colistin (30–75 mg two or three times a day) is substituted for tobramycin when the *Pseudomonas* organism(s) is (are) resistant to tobramycin. Occasional recipients receive intravenous (i.v.) antibiotics 10 days/month to suppress infection/colonization by organisms resistant to the above measures. The reduction in the prevalence of bacterial pneumonia more than 2 years post-transplant from 17–29% per year before 1989 to 5–11% per year since 1989 (which is statistically significant between 2 and 3 years post-transplant) is most probably due to these measures (Table 6).

Multiple antibiotic-resistant strains of *P. aeruginosa* and/or *Burkholderia cepacia* have been defined as organisms sensitive to ≤ 1 class of antipseudomonal antibiotics (such as an aminoglycoside, third-generation cephalosporin, fluoroquinolones, antipseudomonal penicillin, carbapenems or monobactams). These multiply antibiotic-resistant bacteria are thought to be markers of increased morbidity/mortality. In the Toronto series, 46% of recipients with *B. cepacia* in their pretransplant sputum died, whereas none of the recipients with only *P. aeruginosa* in their pretransplant sputum died of infection due to this organism post-transplant[39]. One uncontrolled study has suggested an improved outcome for recipients with cystic fibrosis who undergo pretransplant maxillary sinus antrostomy with repeated sinus lavage with tobramycin[40]. Other perform such procedures plus ethmoidectomy only if clinically significant sinus infections occur in the post-transplant period.

Although the prevalence of early and late episodes of bacterial pneumonia has decreased significantly since 1989, bacterial pneumonia still occurs in 33% of lung recipients, each affected recipient averages 1.7 episodes, and each at-risk recipient averages 0.56 episodes of bacterial pneumonia (Table 5). Additionally, there is a relationship between the use of 'prophylactic' antibiotics and the development of antibiotic resistance[41–48]. The use of clindamycin has also been implicated in the subsequent development of enterocolitis due to *C. difficile*[49]. Bacterial pneumonia has been treated with culture-specific antibiotics for at least 2 and sometimes up to 4 weeks, depending on the rate of clinical resolution. Additionally, the levels of immune suppression are reduced modestly. Usually, the target blood level of cyclosporin or tacrolimus is reduced by about 33%, azathioprine is held until clinical recovery is apparent, and the dose of corticosteroids is reduced to levels necessary to prevent a hypoadrenal state.

The results from this treatment approach as assessed by mortality have not changed over time. The mortality per episode (10–13%) and per affected recipient (20–23%) before and after 1989 have not changed significantly (Table 5). Bacterial pneumonia remains a fairly lethal disease with a mortality of 12% per episode, 9% per at-risk recipient, and 22% per affected recipient. Thus, while this infection can usually be avoided, accurately diagnosed and reliably treated in most recipients, there is room for new strategies both in prevention and in treatment.

CYTOMEGALOVIRUS (CMV)

Definition

As in any host, CMV in LTx recipients is a pathogen with protean manifestations[50–57]. Nevertheless, clinical *illness* due to CMV can be defined by the presence of an infection, syndrome or disease, which may or may not be symptomatic. CMV *infection* has been defined by the presence of CMV in cultures obtained from any body site in the absence of symptoms and cytologic or histologic changes typical of CMV. CMV *syndrome* has been defined as a positive culture for CMV from any body site, plus symptoms typical of CMV infection, usually associated with leukopenia and >3% atypical lymphocytes in the peripheral blood smear, but without cytologic or histologic changes typical of CMV. CMV *disease* has been defined by the presence of a positive culture of CMV obtained from any body site plus the presence of intracellular inclusions typical of CMV in cells or tissue obtained from any body site. Symptomatic CMV disease has included the findings associated with a CMV syndrome. In the following discussion, the few episodes (n=5) of CMV syndrome in the University of Pittsburgh experience have been included with the group with CMV disease, because these occurrences were treated as though disease was present.

Prevalence

Using these definitions the prevalence of CMV illness has been 47%, with 169 episodes in 359 allografts in 350 recipients who survived ≥14 days post-transplant at the University of Pittsburgh (Table 9). It has accounted for 14% of all infectious complications, and has been the next most common pathogen encountered after bacteria (Table 3). CMV has been recovered as early as the first day post-transplant and has caused disease as early as 16 days post-transplant. Mean and median occurrences were 48 and 41 days post-transplant, respectively, before the use of ganciclovir for prophylaxis. Because of the effectiveness of ganciclovir prophylaxis, CMV illness now occurs significantly later (p<0.001 by Mann–Whitney) with a mean and median occurrence at 141 and 131 days post-transplant, respectively, which is almost always after the prophylaxis has stopped. Nevertheless, CMV

Table 9 Effect of prophylaxis with ganciclovir or CMV-negative blood products on the prevalence of CMV illness after lung transplantation at the University of Pittsburgh

	Prophylaxis		
	No	*Yes*	*Total*
Allografts at risk	94	265	359
Infection	16	59	75
Disease			
Pneumonia only	24	41	65
Gastrointestinal only	3	5	8
Disseminated	6	10	16
Syndrome	0	5	5
Illness (total)	49	120	169
Infection/allograft	17%	22%	21%
Disease/allograft	35%	23%*	26%
Illness/allograft	52%	45%	47%

* $p = 0.03$ compared to no prophylaxis by chi-square.

illness is still usually an early infection, as 89% of all episodes have occurred in the first year post-transplant (Table 4). *De-novo* CMV pneumonia, however, has occurred as late as 2338 days post-transplant, and CMV infection has occurred as late as 1533 days post-transplant. As it accounts for 6% (Table 4) of all infections that have occurred >1 year post-transplant, CMV can never be excluded from the differential diagnosis, especially since treatment with ganciclovir is very safe and effective.

Of the 169 episodes of CMV illness, 75 (44%) have been due to infection, 94 (46%) have been due to disease, and 15 resulted in death (Table 10A). The majority (84%) of the episodes of infection have occurred in seropositive recipients (R+). The majority (62%) of the episodes of disease have occurred when the donor was seropositive (D+). The majority (73%) of deaths have occurred in seronegative recipients (R–), especially when the allograft came from a seropositive donor (R–D+) (47%).

Seronegative recipients who received lungs from seronegative donors (R–D–) have had the lowest risk of infection (6%), disease (16%) and illness (22%), but their risk of death from CMV has been significant (5%) (Table 10B). R–D+ recipients have had the lowest risk of infection (4%) but the highest risk of disease (47%) and death from CMV (9%). R+D– recipients have had the highest risk of infection (38%) and the lowest risk of disease (17%) and death from CMV (<1%). R+D+ recipients have had an intermediate risk of infection (29%) and disease (31%), but their risk of death from CMV has been low (1.3%).

Episodes of CMV illness most likely represented disease when recipients were seronegative (R–) (at 72% for R–D–, and at 90% for R–D+ recipients) (Table 10C). These episodes were also more likely to result in death (19%) as compared to when the recipient was seropositive (R+) (2%). The majority (69%) of the episodes of CMV illness in R+D– recipients represented infection, whereas the likelihood of infection or disease was equal in R+D+ recipients. This information is similar to that previously reported[51,57]. The sites of occurrence of CMV disease and infection have been biased by a tendency to evaluate the lung allograft more frequently and intensively compared to other organs. Nevertheless, the allograft has been the overwhelming primary site of CMV illness, with 85% of the episodes of CMV disease arising in the

Table 10 Prevalence and severity of CMV illness by pretransplant recipient (R) and donor (D) serologic status for CMV at the University of Pittsburgh

A: Number of at-risk recipients and episodes of CMV infection, disease, illness and deaths due to CMV

Serologic status	*Recipients at risk*	*Infection*	*Disease*	*Illness*	*Deaths*
R–D–	82	5	13	18	4
R–D+	75	4	35	39	7
R+D–	107	41	18	59	1
R+D+	75	22	23	45	1
Unknown	20	3	5	8	2
Total	359	75	94	169	15

B: Proportion of at-risk recipients who experienced an episode of, or died as a result of, CMV illness

Serologic status	*Recipients at risk*	*Infection (%)*	*Disease (%)*	*Illness (%)*	*Deaths (%)*
R–D–	82	6	16	22	5
R–D+	75	5	47	52	9
R+D–	107	38	17	55	1
R+D+	75	29	31	60	1
Unknown	20	15	25	40	10
Total	359	21	26	47	4

C: Proportion of episodes of CMV illness that resulted in infection, disease or death

Serologic status	*Infection (%)*	*Disease (%)*	*Deaths (%)*
R–D–	5/18 (28)	13/18 (72)	4/18 (22)
R–D+	4/39 (10)	35/39 (90)	7/39 (18)
R+D–	41/59 (69)	18/59 (31)	1/59 (2)
R+D+	22/45 (49)	23/45 (51)	1/45 (2)

allograft either as isolated CMV pneumonitis (n=65) or as part of a disseminated process (n=16) (Table 9).

Clinical features

Signs and symptoms of lung disease have ranged from none (with normal oxygenation and a normal chest radiograph) to fever, dyspnea, hypoxemia and diffuse radiographic infiltrates. Because the clinical presentation of CMV pneumonitis can be similar to that of acute rejection, these entities can be reliably distinguished from each other only by TBBx and BAL. In two instances, CMV pneumonitis has resulted in severe alveolar hemorrhage with hemoptysis.

The organ next most likely to be involved has been the gastrointestinal tract. In 8% of affected recipients the only site of disease has been in the colon and/or stomach. Another 17% of affected recipients also developed gastrointestinal disease as part of a disseminated illness. Symptoms associated with gastrointestinal disease have included anorexia, nausea, vomiting, weight loss, abdominal pain and/or diarrhea.

In the vast majority, CMV disease has been a single event that appears to have established or re-established long-lasting immunity. However, recurrent CMV disease has occurred in six recipients, one of which was due to ganciclovir resistance. In the other instances the recipient's immune system appears to have been unable to generate an effective immune response against this organism[58].

Detection and diagnosis

Advances in detection and diagnosis have improved the prognosis of CMV infection after transplantation. The greater use of endoscopy with biopsy of the allograft or the gastrointestinal tract has increased the rate of detection of CMV disease with minimal morbidity and no mortality to the recipient[17]. Whereas conventional cultures on foreskin fibroblasts often required weeks to become positive, the shell vial assay and culture have allowed detection of virus antigen in 48 hours in the majority of specimens where a significant virus burden has been present[59,60]. Cytologic examination of BAL cells for the detection of the typical cytopathic effects of CMV has been a rapid and specific (but not sensitive) assay to detect CMV disease in cancer patients and in lung and bone marrow recipients[61–63].

The significance of detecting CMV-specific pp65 protein by immunofluorescence with monoclonal antibodies on peripheral blood neutrophils and monocytes is being evaluated clinically[64,65]. This protein appears to be associated with active virus replication, which would indicate active CMV infection[66]. The advantages of this assay are that it is quantitative, rapid, easy to perform, and sensitive and specific for CMV. In fact it appears to be more sensitive than the shell vial method for the detection of CMV in blood neutrophils[67]. The clinical characteristics of the assay, however, are not quite as reliable (when compared to conventional culture) with a sensitivity of 87%, specificity of 92%, negative predictive value of 98%, and positive predictive value of 65%. Additionally, events in the allograft do not always mirror those in peripheral blood. CMV pneumonitis has been observed when the CMV pp65 antigen assay performed on peripheral blood leukocytes was negative (personal experience). Nevertheless, the assay has merit, and the correlation of its clinical characteristics (for the detection of CMV pp65 protein in BAL cells of lung recipients) with the presence of CMV infection and disease by conventional methods should be evaluated.

The polymerase chain reaction to detect CMV has not gained wide acceptance because it is more complicated, expensive, and time-consuming compared to the methods discussed above[68,69]. Additionally, the assay is probably too sensitive to be useful clinically because it appears to detect latent virus, which is usually not a concern clinically.

Treatment

Advances in treatment have also improved the prognosis of CMV disease after LTx. CMV disease has been treated with ganciclovir (5 mg/kg twice a day i.v.) for 2 full weeks with the dose adjusted for renal function. Compared to historical controls at the University of Pittsburgh, who received no therapy or only acyclovir, this regimen has significantly reduced the mortality of CMV disease from 45% to 7%.

Prevention

Advances in prevention have been due to the use of: (a) CMV-negative blood products for R–D– recipients and of (b) ganciclovir in all other combinations of seropositive donors/recipients. These efforts occurred because CMV illness and disease had been a major source of morbidity post-LTx with a prevalence of 52% and 35%, respectively, in the absence of prophylaxis (Table 9) and because CMV appeared to increase the risk of allograft rejection[56,70–72]. Gene products of CMV appear to block the ability of cyclosporin to inhibit interleukin-2 (IL-2) transcription[73]. Restoration of IL-2 production despite the presence of cyclosporin could result in normal T cell function with resultant allograft rejection. With prophylaxis the prevalence of CMV disease has decreased significantly from 35% to 23% (Table 9).

In R–D– recipients the use of CMV-negative blood products has significantly decreased the prevalence of CMV disease from 75% to 9% (Table 11). Some CMV illness has continued to occur in this group, possibly because of false-negative CMV antibody titers in some allograft donors/recipients and/or blood donors. Three episodes of CMV disease occurred after retransplantation where large amounts of CMV-negative blood products were used.

Table 11 Effect of CMV-negative blood products on risk of primary CMV illness in seronegative donors and recipients at the University of Pittsburgh

	Blood product	
	Unknown	*Negative*
R–D–Recipients	8	74
Infection	0	5
Disease	6 (75%)	7 (9%)*†
Illness	6 (75%)	12 (16%)†

* Includes three re-transplants.
† $p < 0.002$ compared to CMV unknown blood products by chi-square.

It is likely that these episodes were transmitted from blood products that were not obtained from truly CMV-negative donors. Perhaps ganciclovir prophylaxis should also be used in retransplant R–D– situations.

Since ganciclovir has been so effective in treating CMV disease, a variety of mostly ganciclovir-based regimens have been administered for variable periods of time post-transplant to at-risk recipients (i.e. seropositive donor and/or recipient) to try to prevent CMV disease[74–79]. Acyclovir (800 mg three times daily) from POD 7 to 90 in 11 recipients was compared to a short course of ganciclovir (5 mg/kg twice a day) from POD 5 to 19, followed by 5 mg/kg daily from POD 20 to 26, followed by acyclovir 800 mg three times daily until POD 90 in 13 recipients[74]. By 140 days post-transplant the prevalence of CMV illness with acyclovir (91%) was similar to that of historical controls who received no prophylaxis. The ganciclovir-treated group, however, experienced significantly fewer episodes of CMV illness (38%) compared to the acyclovir-treated group. In a second study, ganciclovir (as administered in the previous study[74]) was compared to a longer course of 5 mg/kg twice a day from POD 5 to 19 followed by 5 mg/kg a day until POD 90[75]. A significant advantage for the longer course of ganciclovir was observed for up to 1 year post-transplant (50% vs 15%). However, when recipients were followed for 2 years post-transplant, no significant differences were observed between shorter and longer durations of ganciclovir prophylaxis due to additional episodes of CMV illness that occurred later in the longer-duration prophylaxis group (58% vs 42%).

The effect of prophylaxis with ganciclovir as assessed by level of risk (defined by donor and recipient pretransplant serologic status for CMV) has also been evaluated[76–79]. The highest-risk group of recipients are those at risk for primary infection (R–D+), and they have received the most intensive prophylaxis regimens (Table 12). The most aggressive regimen has been a 90-day course of ganciclovir at the University of Pittsburgh. The only effect of this regimen was to significantly delay the onset of CMV illness from a mean and median of 60 and 51 days post-transplant to 135 and 126 days post-transplant, respectively. It did not affect the location or severity of the disease that developed once the prophylaxis was stopped. When the data from all of the prophylaxis regimens from different centers are combined, the prevalence of CMV disease was not significantly different between the group that received some kind of prophylaxis (67%) compared to the group that received no prophylaxis (87%). Hence, no regimen has yet been demonstrated to adequately protect R–D+ recipients from CMV disease.

In recipients at risk of CMV reactivation (R+), the following observations regarding prevalence and effect of prophylaxis can be made from the combined data from several centers[76,79] (Table 13). The overall prevalence of disease has been 25%, and this was significantly higher in R+D+ recipients (at 36%) compared to R+D– recipients (at 20%). The prevalence of CMV disease was: (a) 38% in the absence of prophylaxis and not significantly different in R+D+ recipients (46%) compared to R+D– recipients (21%); (b) significantly lower (22%) with any type of prophylaxis compared to those who received no prophylaxis (38%); and (c) significantly higher in R+D+ recipients (34%) compared to R+D– recipients (20%) despite prophylaxis.

At the University of Pittsburgh the prevalence of CMV disease in the 2 years following completion of a course of prophylaxis was 25% in R+ recipients who received one of three regimens that contained at least 2 weeks of ganciclovir (5 mg/kg twice daily) from POD 7 to 21. This was not significantly different from the prevalence of 31% in historical control recipients who received no prophylaxis. Compared to controls, all three regimens modestly decreased the prevalence of CMV disease in R+D+ recipients, and this achieved statistical significance for the R+D+ recipients who received ganciclovir for 90 days post-transplant (10% vs 63%). All regimens except acyclovir for 90 days in R+D– recipients significantly ($p<0.05$ by Mann–Whitney) delayed the emergence of CMV illness from a mean and median of 44 and 39 days without prophylaxis to 214 and 154 days post-transplant with prophylaxis, respectively (data not shown). Thus,

Table 12 Prevalence of CMV disease in R–D+ recipients with different prophylaxis regimens

Regimen	*St Louis*[76]	*Toronto*[77]	*Newcastle*[78]	*Seattle*[79]	*Pittsburgh*	*Total Rx*	*Pittsburgh*	*St Louis*[76]	*Total No. Rx*
Ganciclovir	POD 0 → 14: 5 mg/kg b.i.d.	*n* = 5 POD 0 → 14: 10 mg/kg q.d. POD 15 → 90: 5 mg/kg three times a week	None	POD 0 → 14: 5 mg/kg b.i.d. POD 15 → 43: 5 mg/kg 5 days/week	POD 7 → 21: 5 mg/kg b.i.d. POD 22 → 90: 5 mg/kg q.d.		None	None	
Acyclovir	POD 15 → Disease onset: 600–2400 mg q.d.	*n* = 2 POD 0 → 90: 2400 mg q.d.	POD 0 → 90: 600 mg q.d.	None	None		None	None	
Immunoglobulin	200–400 mg/kg per week for 2–4 weeks	*n* = 11 POD 7, 14: 150 mg/kg POD 30, 45, 60, 75, 90: 100 mg/kg	POD 0, 7, 14, 21, 28, 49: 100 mg/kg	None	None		None	None	
R–D+ recipients	8	11	9	6	22	54	10	5	15
Disease	8	6	5	3	15	36	8	5	13
Prevalence	100%	55%	56%	50%	68%	67%	80%	100%	87%

any type of prophylaxis appears to confer some resistance to CMV disease, especially in R+D+ recipients. These recipients do have a significant risk of CMV disease (36%), although it is intermediate to that of R–D+ recipients (87%) and R+D– recipients (20%). The primary effect of prophylaxis in R+ recipients is to significantly delay the emergence of CMV illness.

In summary, the use of CMV-negative blood products in R–D– recipients is mandatory, and is the only prophylaxis necessary in this group (Table 11). In recipients at risk of reactivation infection (R+) and especially of primary infection (R–D+), no prophylaxis regimen has conclusively demonstrated a benefit in CMV disease prevention. All ganciclovir-based regimens, however, delay the emergence of CMV disease until after the greatest risk of acute rejection has passed. This is beneficial because acute rejection can be treated more aggressively when coexistent or emergent CMV disease is not a concern. Recipients can also cope better with the effects of CMV when they have more fully recovered from the non-specific effects of the transplant procedure (i.e. weakness, fatigue, anorexia, etc.). Ganciclovir as a single agent at a dose of 5 mg/kg twice a day for 2 weeks followed by 5 mg/kg a day is remarkably effective at preventing CMV disease as long as it is administered daily. The problem arises when ganciclovir stops. Recently, oral ganciclovir has been demonstrated to be as efficacious as i.v. ganciclovir in preventing progression of CMV retinitis in AIDS patients[80]. Perhaps R–D+ recipients should receive lifelong prophylaxis with oral ganciclovir.

FUNGAL INFECTION

The prevalence of fungal infection in lung recipients has been 15% at Stanford University[81], 13% at the University of Toronto[2], and 22% at Loyola University[82]. At the University of Pittsburgh the prevalence of fungal infection has been 19%, with 68 infections caused by 71 fungal organisms in 359 allografts in 350 recipients who survived ≥14 days post-transplant (Tables 14 and 15). Three infections have been due to two different types of fungi. A little more than one-third (38%) of these infections have been due to *Candida* species, and nearly half (45%) have been due to species of *Aspergillus*. Fungal infection has accounted for 6% (68/1173) of all infectious complications and has been equally likely to occur within the first year or >1 year post-transplant (Table 4). It has caused the failure of 22 allografts (11%); 14 failures (64%) occurred within the first year post-transplant (Table 2). These infections have occurred as early as 6 days and as late as 2917 days (6.5 years) post-transplant. As the median occurrence is only 52 days post-transplant, the majority of these infections have occurred early after transplantation.

Prevalence

The prevalence of fungal infection before the availability of fluconazole, itraconazole, or aerosol amphotericin for prophylaxis was 20% (with 37 infections in 185 at-risk recipients) (Table 15).

Table 13 Prevalence of CMV disease in R+ recipients with different prophylaxis regimens

Regimen	*St Louis*[76]	*Toronto*[77]	*Pittsburgh*	*Total No. Rx*	*Toronto*[77]	*Newcastle*[78]	*Seattle*[79]	*Pittsburgh*	*Pittsburgh*	*Pittsburgh*	*Total Rx*	*Total All*
Ganciclovir	None	None		None	n = 6 R+D+ 6 R+D– POD 0 → 14: 10 mg/kg q.d. POD 15 → 90: 5 mg/kg three times a week	None	POD 0 → 14: 5 mg/kg b.i.d. POD 15 → 43: 5 mg/kg 5 days/week	POD 7 → 21: 5 mg/kg b.i.d.	POD 7 → 21: 5 mg/kg b.i.d. POD 22 → 50: 5 mg/kg q.d.	POD 7 → 21: 5 mg/kg b.i.d. POD 22 → 90: 5 mg/kg q.d.		
Acyclovir	None	None	None	None	n = 4 R+D+ 6 R+D– POD 0 → 90: 2400 mg q.d.	POD 0 → 90: 600 mg q.d.	None	POD 22 → 90: 2400 mg q.d.	None	None		
Immunoglobulin	None	None	None	None	n = 32 POD 7, 14: 150 mg/kg POD 30, 45, 60, 75, 90: 100 mg/kg		None	None	None	None		
R+D+ recipients		5	8	13	10	16		7	25	10	68**	81**
Disease (%)		1 (20)	5 (63)	6 (46)	6 (60)	4 (25)		2 (29)	10 (40)	1 (10)*	23 (34)	29 (36)
R+D– recipients			24	24	22	19		13	22	19	95	119
Disease (%)			5 (21)	5 (21)	7 (32)	1 (5)		2 (15)	5 (23)	4 (21)	19 (20)	24 (20)
R+ recipients	8	5	32	45	32	35	15	20	47	29	178*	223
Disease (%)	6 (60)	1 (20)	10 (31)	17 (38)	13 (41)	5 (14)	5 (33)	4 (20)	15 (32)	5 (17)	39 (22)	56 (25)

* $p < 0.05$ compared to none by chi-square.
** $p < 0.05$ compared to R+D– recipients by chi-square.

Table 14 Etiologic agents and time of occurrence of fungal infections after lung transplantation at the University of Pittsburgh

	n (%)
Etiologic isolates	
Candida species	27 (38)
Aspergillus species	32 (45)
Mucor	1
Cryptococcus	1
Phialophora repens	1
Pseudallescheria species	4
Torulopsis	3
Trichophytin	1
Dactylaria gallopava	1
Total	71
Time of occurrence in days post-transplant	
Mean ± 1 SD	371 ± 695
Median	52
Range	6–2917

Table 15 Prevalence, site and mortality of fungus infections with or without prophylaxis at the University of Pittsburgh

	Prophylaxis		*Total (%)*
	No (%)	*Yes (%)*	
Recipients	185	165	350
Infection sites			
Disseminated	13 (35)	1 (3)*	14
Lung	13	10	23
Airway	3	9	12
GI	0	2	2
Brain	0	3	3
Skin incision	1	1	2
Mediastinum	1	1	2
Pleura	2	2	4
Aorta, pulmonary artery anastomosis	3	0	3
Skin	0	1	1
Breast	0	1	1
Blood	1	0	1
	37 (20)	31 (19)	68 (19)
Mortality	18 (10)	7 (4)*	25 (7)

* $p < 0.05$ compared to no prophylaxis by chi-square.

All of these infections, except one episode of septicemia and one infection in an incision, originated in the allograft. By the time of diagnosis, 35% of these infections had disseminated to other body sites, particularly within the thorax, such as the mediastinum (one), pleural space (two) and aortic anastomosis (three)[83]. Fungal infection was highly lethal (49% mortality). Eleven (30%) of these infections were recognized only at autopsy.

'Prophylaxis' and treatment

It was noted, however, that the fungus culture of respiratory tract secretions (sputum or BAL) obtained within 2 weeks before a diagnosis of a fungal infection was always positive whenever true infection was present (n=8), and was always negative when infection was absent (Table 16). Because of this observation, any fungal isolate from respiratory tract secretions has since been promptly treated with anti-fungal agents. If a culture from the airways of the donor or recipient contains *Candida*, fluconazole (400 mg orally or i.v. daily) has been administered until 4 weeks have elapsed since the last positive culture. If *Aspergillus* has been recovered, aerosol amphotericin (5–15 mg three times daily) and/or oral itraconazole (400–600 mg daily) have been administered. When the itraconazole blood level drawn 2–4 hours after a dose had risen ≥8 μg/ml, which was usually within 7–10 days, the amphotericin was usually discontinued. Itraconazole is continued for 6–12 months after the last positive culture. When the risk of *Aspergillus* infection was high (i.e. symptoms present, abnormal chest radiograph, <POD 90 or chronic rejection present) or it was not possible to use itraconazole, these isolates have additionally usually been treated with i.v. amphotericin (<0.6 mg/kg per day) until the itraconazole blood level was therapeutic and/or the clinical outcome was improving.

Since the introduction of this regimen, 31 infections due to 31 fungal isolates have occurred in 165 at-risk recipients for a prevalence of 19% (Table 15). The majority of these infections (68%) originated in the allograft, all but one infection was detected antemortem, and only one infection (3%) disseminated to other body sites by the time of diagnosis. Seven (23%) were fatal. The nine airway infections included five episodes of ulcerative tracheobronchitis due to *Aspergillus*, and all were treated successfully[81].

As compared to the era before prophylaxis, the prevalence of fungal infections has not changed (20% vs 19%), but the likelihood of dissemination has decreased significantly from 35% to 3%, and the risk of death has decreased significantly from 10% to 4%. This is particularly significant since the frequency of isolation of fungal organisms from the respiratory tract has increased, most probably due to the increasing use of 'prophylactic' antibacterial antibiotics. Treating all fungal isolates from the allograft has resulted in many instances of treating contaminants, insignificant infection, and/or 'colonization' because the predictive value of a positive culture was only 16% (Table 16). Nevertheless, this approach appears to be justifiable because the mortality from this infection has decreased significantly, and this is most likely a consequence of this intervention.

Relationship with obliterative bronchiolitis (chronic rejection)

Just as with bacterial pneumonia and bronchitis that occur late after transplantation (Table 7), there also appears to be a relationship between late fungal infection in the allograft and chronic allograft rejection (Table 17). Luckily, only 8% of the recipients

Table 16 Reliability of a fungus culture of respiratory secretions to detect fungus infections in lung recipients at the University of Pittsburgh in the pre-prophylaxis era

	Infection	
Culture	*Present*	*Absent*
Positive	8	41
Negative	0	398
Sensitivity	100%	
Specificity	92%	
Positive predictive value	16%	
Negative predictive value	100%	

Table 17 Relationship of fungal infection in the allograft to chronic rejection (CR) in recipients who survived > 90 days or > 2 years post-transplant at the University of Pittsburgh

Fungus infection	*Episode/recipient*	
	Survival > 90 days	*Survival > 2 years*
CR+	16/107 = 0.15	6/78 = 0.08
CR–	8/134 = 0.04	2/75 = 0.03
Total	24/291 = 0.08	8/155 = 0.05

who survived >90 days and 5% of the recipients who survived >2 years have developed fungus infection in the allograft. However, 66% of the fungal infections that developed >90 days post-transplant and 75% of those that developed >2 years post-transplant occurred in recipients with OB. The infection rate was three to four times greater in recipients with OB (0.08–0.15 for OB+ versus 0.03–0.04 for OB– recipients). This relationship between OB and increased risk of fungal infection may be due to: (a) a direct effect of an inability of airways damaged by OB to clear inhaled fungal spores, (b) an indirect effect of the increased use of antibacterial antibiotics used to treat the airway bacterial infection that is also associated with OB, or (c) the use of augmented immunosuppression to treat OB. Thus, any fungal infection in the allograft >90 days post-transplant should raise a suspicion that OB is also present.

PNEUMOCYSTIS INFECTION

The overall prevalence of this infection in patients who survived >2 weeks post-LTx has been low at 8% (Table 18). Its prevalence before prophylaxis was employed was 71%[84]. Its prevalence with prophylaxis with either (a) one single-strength trimethoprim-sulfamethoxazole tablet twice or three times per week or (b) dapsone 100 mg orally three times per week for recipients who are allergic to or intolerant of sulfonamides has decreased significantly to 4%. An episode of infection has almost always been associated with non-compliance with prophylaxis.

The majority (69%) of these infections have been detected by surveillance BAL procedures in asymptomatic recipients. Clinical pneumonia occurred in seven cases (20%), and the infection was subclinical in four (11%). This infection has occurred as early as POD 13 and at any time thereafter. With mean and median occurrences of 433 and 166 days post-transplant, respectively, most infections (n=25) have occurred within the first year post-transplant.

Table 18 Prevalence of *Pneumocystis* (PCP) infection with and without prophylaxis after lung transplantation at the University of Pittsburgh

	Prophylaxis		
	No	*Yes*	*Total*
Recipients at risk	21	338	359
Recipients with PCP	15 (71%)	14 (4%)*	29 (8%)
Infections	19	16	35

* $p < 0.0001$ compared to no prophylaxis by chi-square.

EPSTEIN–BARR VIRUS (EBV)-INDUCED LYMPHOPROLIFERATIVE DISEASE (PTLD)

The prevalence of this infection has been 4% at Papworth Hospital with three infections in 67 heart–lung transplant recipients[85]. It has been 7% at the University of Pittsburgh with 22 infections in 325 recipients who survived >30 days post-transplant[86–88]. Eleven episodes (50%) occurred 43–120 days post-transplant and 17 (77%) within the first year. Five recipients developed PTLD after the first year. Where serology for EBV from both donor and recipient was available, six infections occurred in R–D+ recipients (27%), two infections occurred in R+D+ recipients (9%), and one infection occurred on POD 554 in a R–D– recipient. Three R–D+ recipients have never developed PTLD. The initial site of involvement was the allograft in 16 (73%) recipients, lymph nodes in two recipients, the gastrointestinal tract in three, and the brain in one. PTLD disseminated to multiple organs in six recipients.

The first affected recipient was treated with chemotherapy and died 45 days later from bacterial sepsis from a bowel perforation secondary to a regressing focus of PTLD. The autopsy revealed persistent disseminated PTLD and disseminated *Cryptococcus* infection. The initial treatment for all other affected recipients has been a major reduction in immunosuppression (by withdrawal of corticosteroids and azathioprine and an approximate 75% reduction in the dose of cyclosporin or tacrolimus) until clear evidence of regression was present[89]. Thirteen patients (59%) achieved a remission of all clinically evident disease although, of these, one subsequently developed recurrent PTLD, one died of bacterial pneumonia, and six developed OB which was fatal in four. Thus, there are five remaining recipients (24%) treated only with a temporary reduction in immune suppression, who are without PTLD or other complications.

The PTLD did not remit with decreased immunosuppression in eight recipients. One received no further therapy and died of bacterial pneumonia and disseminated PTLD. A second died immediately after retransplantation. Three were treated with alpha-interferon[90]. The PTLD remitted in two, but one of these developed severe acute rejection which necessitated retransplantation, and OB developed in the second allograft. One recipient did not respond to alpha-interferon and was retransplanted without further sequelae. Three recipients received radiotherapy for what was thought to be localized disease. These died from (a) disseminated PTLD and bacterial sepsis, (b) disseminated *Aspergillus* infection after chemotherapy for disseminated PTLD (PTLD was not found at autopsy), and (c) OB (PTLD was localized and did remit after radiation therapy) respectively. Overall, the mortality has been 50% but that due directly to PTLD has been 23%.

Thus, PTLD occurs in about 4–7% of LTx recipients usually (a) in the first 120 days post-transplant, (b) in recipients at risk of primary infection, and (c) with the allograft being the initial site of involvement. While remission can be achieved in most with decreased immunosuppression alone, morbidity and mortality are significant.

OTHER VIRUSES

Lung recipients are at risk for significant infection with herpes simplex virus (HSV). The prevalence of this infection at

Papworth Hospital has been 18% with six episodes of pneumonitis, one of which was fatal[91]. However, acyclovir effectively prevents serious HSV infection. At the University of Pittsburgh no HSV infections have occurred in seronegative recipients who received an allograft from a seropositive donor (R–D+) when treated with acyclovir (400 mg orally three times daily for the first 3 months). Some episodes of mucocutaneous infection due to HSV have occurred after prophylaxis has stopped.

At the University of Minnesota the prevalence of infection with the paramyxoviruses, parainfluenza (PIV), and respiratory syncytial virus (RSV), has been 21%, with 19 infections in 18 of 85 at-risk recipients[92]. All were associated with signs and symptoms of lower respiratory tract involvement, and nine were also associated with signs and symptoms of upper respiratory tract infection. Nine infections (11%) were due to RSV. Only one RSV infection was associated with a transient but significant decline in spirometry, and one untreated RSV infection was fatal. RSV infections were seasonal – all occurred between January and June with the peak incidence in February. Ten infections (12%) were due to PIV, five of which were associated with a significant decline in spirometry. There was no seasonality associated with this infection. Fourteen infections were treated with ribavirin, which was tolerated by all and efficacious in most patients.

A few episodes of infection due to adenovirus, influenza, and Coxsackie virus[93] have been reported. At the University of Pittsburgh, four of five infections with adenovirus were fatal. All other infections of these types have resolved without specific treatment.

MYCOBACTERIA

Candidates for LTx should receive intradermal skin tests to PPD, *Candida* and mumps, but the appropriate response to a positive PPD pretransplant is not clear. The practice at the University of Pittsburgh has been to treat those who react to PPD with isoniazid (INH) for 1 year before and 1 year after LTx. Infections due to *Mycobacterium tuberculosis* have been rare at all centers. Only eight cases have been reported[94–96] and all have involved the allograft between 2 and 20 months post-transplantation. Infections isolated to the allograft have all been treated successfully, but two disseminated infections were fatal. The prevalence of *M. tuberculosis* infection at the University of Pittsburgh has been <1%, with only one infection in 299 recipients who survived >60 days post-transplant. This single episode occurred in a single-lung recipient who developed pulmonary and pleural tuberculosis from a pan-sensitive isolate 64 days post-transplant, while receiving INH prophylaxis for a positive pretransplant intradermal PPD skin test. The diagnosis was made by recovery of the organism in two sequential BAL specimens separated in time by 2 months. Remission was achieved by eliminating azathioprine, lowering the doses of corticosteroids and tacrolimus, and treating the infection with INH, pyrazinamide (PZA), and ethambutol for 1 year. (Rifampin was not used, because of the difficulty of maintaining adequate blood levels of cyclosporin or tacrolimus with its concurrent use.) Remission has continued for >1 year without antituberculosis therapy. One further double-lung recipient with unsuspected *M. tuberculosis* in the native lungs had no recurrence post-transplant after treatment with INH and PZA (for 4 months) followed by INH and ethambutol (for 8 additional months).

Atypical mycobacteria have been isolated in several instances from BAL specimens in asymptomatic recipients at the University of Pittsburgh. Treatment has been withheld and no recipient has developed disease. *M. chelonae* infection, however, has occurred in a heart–lung recipient about 6 months post-transplant[97]. This recipient developed OB 2 months after the organism was isolated, and died 2 months later from OB and persistence of infection, despite the aggressive use of appropriate antibiotics. Thus, infection with atypical mycobacteria is a rare but possible event.

INFECTION IN THE NATIVE LUNG

After single LTx a diseased native lung is left in proximity to the allograft. When this lung becomes infected, the infection can be transmitted to the allograft or systemically. Infections in the remaining native lung due to *Pseudomonas*, *Nocardia* and especially *Aspergillus* have occurred and some have been transmitted to the allograft. The principles of treatment of these infections include a reduction in immunosuppression, administration of appropriate antibacterial or antifungal therapy, and consideration of surgical resection of the infected native lung[98,99]. With the increasing popularity of single LTx, infection arising in the native lung and transmitted to the allograft will be a more frequent occurrence.

INFECTION TRANSMITTED FROM THE DONOR LUNG

The observation that the presence of bacteria in the airways of the donor lung at the time of transplantation was significantly associated with early bacterial infection in the allograft led to the concept that the donor could transmit infection to the recipient[15]. This was subsequently demonstrated in a canine model[16]. While inocula into the lung of 10^4 colony-forming units (cfu) of *Streptococcus pneumoniae* did not cause pneumonia in normal or even immunosuppressed dogs, inocula of only 10^2 cfu given to donors resulted in pneumonia in all recipients. Thus, a minimally infected donor lung appears to be very susceptible to the subsequent development of bacterial pneumonia in the immunosuppressed recipient. In the canine model, treatment of infected donors with antibiotics prevented pneumonia from occurring in the recipients. In the human situation, broad-spectrum antibiotics are begun preoperatively and adjusted postoperatively according to the results of cultures taken from the airways of the donor at the time of transplantation. This is the most likely reason for the marked decrease in the prevalence of early bacterial pneumonia at the University of Pittsburgh (Table 6) and for the low rate of early infection at Papworth Hospital, where only two of 19 early infections in 125 heart–lung recipients (2%) were felt to be donor-acquired[100]. It is also suspected that fungi in the airways of the donor carry a high risk of causing fungal infection in the recipient. Hence, such isolates are commonly 'prophylactically' treated.

In this era where CMV-negative blood products are employed with any seronegative recipient (R–), it is evident that allografts from CMV-seropositive donors (D+) very effectively transmit CMV disease to the recipient (Table 12). In fact, prevention of this transfer from the donor is a challenge yet to be solved. It also appears that the majority of the episodes of EBV-induced PTLD

that occur as a consequence of primary infection result from transmission of EBV virus from the donor lung to the recipient. Preventing or ameliorating this transfer is another challenge for the future. As has been demonstrated for tuberculosis[94], the donor is probably capable of transmitting any type of infection to the recipient, including hepatitis C and the human immunodeficiency virus[101-103].

COMMENT

Although infection as a cause of morbidity (Table 3) and mortality (Table 1) has decreased significantly, it remains the primary cause of morbidity and mortality after LTx. The advances that are responsible for the decline in the toll exacted by infection include the use of: (a) antibacterial and antifungal agents to treat organisms in the airway of the donor at the time of transplantation and when OB is present, (b) CMV-negative blood products when the donor and recipient are seronegative (R–D–), (c) ganciclovir to treat CMV disease, (d) ganciclovir for at least 2 weeks post-transplant when the recipient is seropositive (R+), (e) antifungal agents when fungi are recovered from the allograft, (f) trimethoprim-sulfamethoxazole or other agents to prevent *Pneumocystis* infection, (g) frequent bronchoscopy with BAL and TBBx to assess the allograft[17,33], and (h) fewer empiric pulses of augmented immunosuppression to treat clinical rejection. The improvement in survival after LTx over the past decade has been largely due to the advances made in the control of infection.

Challenges still remain! As the current trend is of increasing immunosuppression to try to prevent or ameliorate acute rejection, and thus try to prevent the subsequent development of OB[72], our challenge will be to prevent an upsurge in post-transplant infections. The optimum regimens (a) to prevent and treat bacterial and fungal infections, (b) to prevent CMV disease in R–D+ recipients, and (c) to prevent EBV-induced PTLD are still to be discovered. The current approach of treating so many bacterial and fungal isolates is not cost-effective in today's environment of cost containment, and may well promote the development of antibiotic resistance. Thus, although much has been accomplished, much remains to be done to minimize the morbidity and mortality related to infection after lung transplantation.

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Management of Complications of the Airway

H. DATE AND G.A. PATTERSON

INTRODUCTION

In the first 15 years after the first human lung transplantation by Hardy, in 1963, there were no long-term survivors despite some 45 such procedures. Of those patients who survived more than 2 weeks, the majority died as a result of bronchial dehiscence[1]. Lung transplantation (LTx) is unique among solid-organ transplants in that the systemic arterial blood supply is not routinely re-established at the time of implantation. Without reconnection of the bronchial arterial circulation, airway viability is exclusively dependent on a vascular supply by retrograde collateral flow from pulmonary to bronchial circulation. Parenchymal pulmonary pathology due to poor graft preservation, pulmonary edema, infection and rejection may impair pulmonary microvascular circulation and reduce this retrograde flow during the critical early postoperative period.

Based on a series of laboratory investigations by J.D. Cooper and his colleagues, routine use of omentopexy[2] and avoidance of high-dose perioperative corticosteroids[3] were thought to be key strategies for the first successful human LTx in 1983[4]. However, recent studies have demonstrated that omentopexy is not essential and early postoperative corticosteroids do not impair airway healing. In 1991 the San Antonio group demonstrated that acceptable airway healing could be expected without omentopexy, with the use of a telescoping technique, in a group of patients receiving routine perioperative corticosteroids[5]. Improvements in patient selection, preservation methods, surgical technique, and postoperative care including better immunosuppression, have reduced the incidence of airway complications. However, airway complications remain a significant cause of morbidity. Experienced centers have recently reported the incidence to be in the range of 7–14%[6–8].

TECHNIQUE OF BRONCHIAL ANASTOMOSIS

Donor and recipient bronchial preparation

Great care should be taken to avoid unnecessary mobilization of the donor and recipient main bronchi. Peribronchial nodal tissue is left intact. The main bronchus of the donor is shortened to two rings proximal to the upper lobe take-off. The recipient's main bronchus is transected just proximal to its bifurcation.

Bronchial anastomosis

The technique of bronchial anastomosis is undoubtedly important. The ideal technique of bronchial anastomosis is controversial and varies from one center to another, yet results appear similar even when different techniques are employed.

Our experience did not show a significant difference in the rate of airway complications between end-to-end and telescoped anastomoses. Similarly, there was no difference in complication rate whether it was the donor or recipient bronchus that was intussuscepted. We recommend the use of end-to-end or telescoping technique depending on the difference in bronchial size between donor and recipient. It is critical to maintain the natural configuration of these bronchi. When the bronchial sizes are equivalent we use an end-to-end anastomosis by either simple interrupted or figure-of-eight sutures. For a small left bronchial anastomosis we recommend simple interrupted sutures. When the discrepancy in bronchial size is obvious, we use a telescoping technique employing figure-of-eight sutures.

Bronchial wrapping

Coverage of the bronchial anastomosis with healthy tissue may improve donor bronchial circulation and contain a bronchial leak, should a dehiscence occur. However, no difference has been observed in the airway complication rate when various types of bronchial wrapping are compared. A prospective randomized study recently reported by Khaghani *et al.*[8a] has demonstrated that the incidence of bronchial anastomotic complication after single LTx is not affected by wrapping the anastomosis with either omentum or an internal mammary artery pedicle. Our current standard is to cover the anastomosis with peribronchial nodal tissue or mediastinal fat. This is very easy to accomplish in most patients. Furthermore, this technique interposes healthy tissue between the bronchial and pulmonary artery anastomoses.

Bronchial artery revascularization

The Harefield[9] and Bordeaux[10] groups have both reported successful bronchial artery revascularization for *en-bloc* double LTx with tracheal anastomosis. We remain unconvinced regarding its benefit in patients undergoing single and indeed bilateral sequential single LTx[11]. Daly and his colleagues from the Mayo Clinic[12] have reported a small series of patients undergoing revascularization following single LTx. We await with interest the long-term follow-up of these patients, to determine whether or not such revascularization will have any impact on the development of obliterative bronchiolitis, as has been suggested.

PREDICTORS OF AIRWAY COMPLICATION

We have recently completed a detailed review of our LTx experience with respect to airway complications. We evaluated 32 various clinical factors to identify predictors of airway complication of 348 bronchial anastomoses performed in 229 single (SLTx) and bilateral (BLTx) recipients[13]. Factors evaluated were: (a) recipient factors (age, sex, diagnosis, preoperative steroid history, preoperative steroid use); (b) donor factors (age, sex, Pao_2, ischemic time); (c) operative factors (type of transplant, side of transplant, requirement for cardiopulmonary bypass, type of bronchial anastomosis, suture material, type of wrapping); (d) postoperative factors (Pao_2, mean systemic pressure, mean pulmonary artery pressure, cardiac output, peak airway pressure – all measured on arrival in the intensive care unit – use of prostaglandin E1, percentage of allograft perfusion measured by quantitative VQ nuclear scintigraphy, duration of mechanical ventilation, early maintenance steroid use, number of 3-day courses of bolus methylprednisolone therapy, total dose of bolus methylprednisolone, the first day of bolus methylprednisolone, type of cytolytic therapy, transbronchial biopsy-proven acute rejection of grade A2 or greater, cytomegalovirus status, biopsy-proven cytomegalovirus pneumonia).

Airway complications occurred more often following SLTx than after BLTx (14.4% versus 7.1%, $p < 0.05$). Modified mattress sutures (13.7%) were associated with more frequent complications than were simple interrupted sutures (6.6%) or figure-of-eight sutures (5.5%) ($p < 0.05$). Airway complications were less commonly encountered in patients ventilated <7 days (7.6%) in comparison to patients ventilated >7 days (18.6%) ($p < 0.01$).

Patients receiving SLTx for pulmonary fibrosis and pulmonary hypertension have a longer requirement for mechanical ventilation as a result of allograft dysfunction[14]. This may compromise collateral bronchial flow more seriously than occurs in bilateral grafts in which allograft dysfunction is less commonly encountered. This may explain why complications were more common in SLTx bronchial anastomoses than in BLTx anastomoses. The relationship between allograft dysfunction and airway complication is further suggested by the fact that airway complications increased as the period of mechanical ventilation was prolonged. We do not believe that mechanical ventilation in and of itself predisposes to increased incidence of airway complications. Indeed, Yokomise and his colleagues[15] have previously demonstrated that positive end-expiratory pressure augments retrograde collateral bronchial mucosal flow.

A modified mattress suture technique more readily achieves the desired intussusception and avoids potential for an obstructive flange of invaginated cartilage that may occur when a standard horizontal mattress suture is employed[7]. However, no strength is provided to maintain apposition of the overlapped cartilages (Figure 1A). On the other hand, a figure-of-eight suture provides firm strength to maintain apposition between the overlapped cartilages (Figure 1B), although it is somewhat more difficult to achieve complete telescoping.

Perioperative steroid use did not affect airway integrity in our experience. It appears that immunosuppression with corticosteroids may actually enhance donor bronchial viability. The Hannover group reported improved bronchial blood flow in porcine lung allografts when prednisolone was added postoperatively[16]. Whether this is due to better control of rejection leading to improved microvascular pulmonary collateral flow or a direct effect to enhance local angiogenesis is not known. We currently accept for LTx patients receiving prednisone <20 mg/day preoperatively, and administer methylprednisolone 0.5 mg/kg per day immediately after LTx.

CHRONOLOGIC INCIDENCE OF AIRWAY COMPLICATIONS

Three experienced centers have recently reported the incidence of airway complication to be in the range 7–14%[6–8]. The chronologic

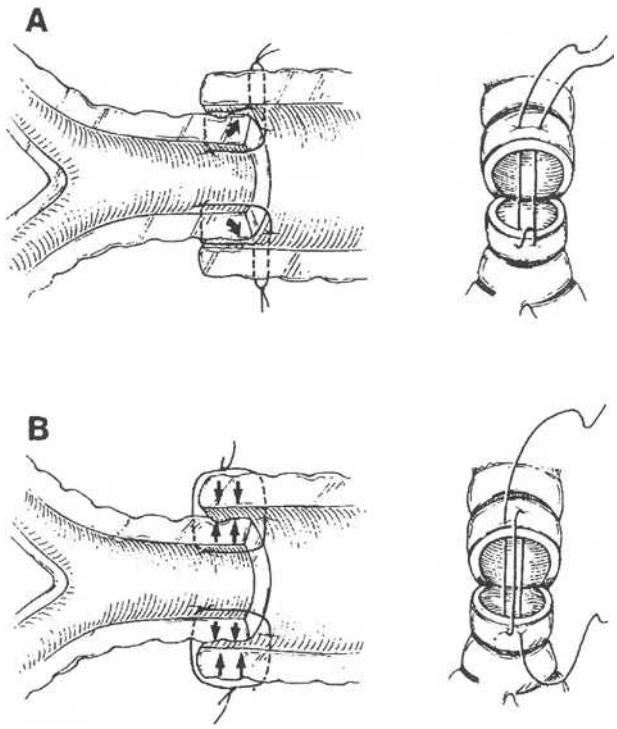

Figure 1 **A**: Modified mattress suture. This technique holds the rings of the smaller airway to the mucosal surface of the larger airway to avoid potential for an obstructing flange of invaginated cartilage. However, no strength is given between the two overlapped cartilages. **B**: Figure-of-eight suture. Firm strength is given between the two overlapped cartilages although it is more difficult to achieve telescoping. (From ref. 13)

incidence of airway complications at our center was evaluated by separating the recent 229 LTx procedures into three groups: phase I, the first 77 transplants; phase II, the next 76 transplants, and phase III, the most recent 76 transplants. The airway complication rate was significantly reduced to 4.0% in phase III from 10.9% in phase I and 14.3% in phase II. No significant technical change was made between phases II and III, but postoperative immunosuppressive therapy and rejection monitoring differed significantly between these two phases.

In phase III, low-dose corticosteroid administration from day 1, along with induction cytolytic therapy, was routinely employed. Rejection therapy by bolus injection of methylprednisolone (MP) was mainly based on histologic evidence (transbronchial lung biopsy) rather than on clinical diagnosis (such as fever, cough, shortness of breath, decrease of arterial oxygen tension). These results suggest that the significant improvement in airway healing in phase III is likely due to better maintenance of immunosuppression and rejection surveillance. Fujimura *et al.*[17] have reported that healing of the bronchial anastomosis in canine LTx correlates closely with the incidence or degree of allograft rejection. We believe it is important to give timely but not excessive MP for the treatment of acute rejection, since pulmonary-to-bronchial collateral flow will be impaired by infection as well as rejection.

DIAGNOSIS OF AIRWAY COMPLICATIONS

The majority of airway complications are first identified by bronchoscopic assessment. Patients routinely undergo initial bronchoscopic evaluation before leaving the operating room and again immediately prior to extubation. In our program, routine surveillance bronchoscopies are performed at 2–3 weeks, 2, 3, 6, and 12 months and annually thereafter. It is common to observe a ring of whitish slough at the anastomosis at 1–3 weeks. This usually heals without requiring clinical intervention. Significant dehiscence may result in massive air leak (if a chest tube is still *in situ*), pneumothorax, pneumomediastinum, and/or atelectasis with obstructive pneumonia. New symptoms of stridor or wheeze should initiate bronchoscopic evaluation, which may reveal a late stricture or the development of a malacic segment.

MANAGEMENT OF AIRWAY COMPLICATIONS

Bronchial dehiscence

Patchy areas of superficial necrosis of donor bronchial epithelium are commonly observed. These areas usually heal without complication. Full-thickness necrosis may be more problematic. Our experience suggests that membranous wall defects generally heal without requiring clinical intervention, whereas cartilaginous defects often result in some degree of late stricture.

Most major airway dehiscences are satisfactorily drained into the airway at the time of first presentation. Significant dehiscence associated with massive air lead, pneumothorax, atelectasis with obstructive pneumonia, or pneumomediastinum should be managed expectantly. The first treatment is adequate drainage by a pleural or mediastinal tube. If the lung remains completely expanded with adequate drainage, the leak will automatically seal and the airway may heal without significant stenosis. Surgical revision of an early anastomotic dehiscence should be undertaken only if conservative measures fail, and an adequate length of donor airway is available for resuturing[18].

For BLTx recipients with a unilateral dehiscence, ipsilateral pneumonectomy is an option and has been employed successfully. Retransplantation has been carried out but, in view of the donor shortage, is rarely a practical option. It is important to emphasize that even major areas of bronchial dehiscence will heal with adequate drainage.

We have observed significant dehiscence in 15 anastomoses in 12 patients. Five patients, including two who underwent retransplantation, died of this complication. In another five patients chest and/or mediastinal tube placement permitted satisfactory closure of the leak. In a further recipient a silastic stent was placed 26 days after transplantation with a satisfactory result. In one, sudden death from unknown cause occurred 3 days after chest tube placement.

Bronchial stenosis

Chronic airway stenosis should be treated when it causes sputum retention, cough, obstructive pneumonia, or deterioration in pulmonary function. A bronchial stricture is generally managed by dilatation with serial rigid bronchoscopes. Under general anesthesia the tip of the rigid bronchoscope is passed through the stenosis. One should feel a 'pop-through' sensation. Proximal right main bronchial strictures can easily be managed in this way, as there is usually a length of donor airway into which the tip of the bronchoscope can be passed. However, more distal strictures or distal left main strictures can be very difficult to dilate using rigid instruments. In these situations we have used dilating balloon catheters which can easily be passed through the suction channel of a 5.8 mm fiberoptic bronchoscope. Repeated and frequent dilatation (every 7–10 days) will often maintain patency until a granulating stricture is covered by mucosa, resulting in permanent patency. Web-like stenoses can be managed by laser, but great care needs to be taken not to injure the vital normal distal donor airway.

When repeated dilatation fails to maintain adequate patency we use silastic endobronchial stents preferentially[19]. The stent is mounted on the tip of a rigid bronchoscope together with a pushing tube[20] (Figure 2). With the patient under general anesthesia the bronchoscope is maneuvered into the stenotic segment. With the pushing tube to hold the stent in place the bronchoscope is then withdrawn. Proper placement of the stent is confirmed by fiberoptic bronchoscopy. DeHoyos and Maurer[21] reported that insertion of stents has resulted in dramatic improvement in pulmonary function. Although endobronchial stents are usually well tolerated, a few complications may occur after their insertion. Proximal displacement is relatively common and requires repositioning. Mucus plugging is avoided by daily use of *N*-acetylcysteine inhalations. Granulation tissue growing around the end of the stent may also cause airway obstruction. Stents placed for stricture are usually required only temporarily. After several months the airway seems to obtain a degree of rigidity without the stent in place. Wire mesh stents (Gianturco) are useful for malacic strictures completely lined by epithelium[22]. They can be placed

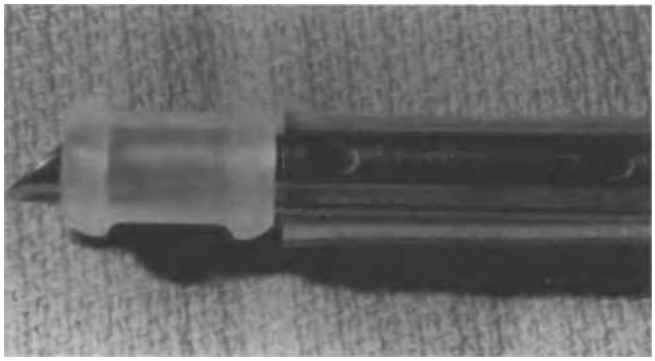

Figure 2 Silicone stent mounted on the tip of a rigid bronchoscope. A segment from an endotracheal tube has been used as a sheath around the bronchoscope proximal to the prosthesis. This sheath is held in place while the bronchoscope is withdrawn, preventing withdrawal of the stent with the bronchoscope. (From ref. 20)

across the upper lobe orifice without causing occlusion. However, they should not be used in the presence of active granulation, as the growth of granulation through the interstices causes a stricture within the stent.

Schafers *et al.*[23] have reported surgical treatment for airway complications. Sleeve resection of a stenotic segment was performed in selected cases.

We have observed significant stenosis in 18 anastomoses in 17 patients after LTx. All but one patient were successfully managed by conservative therapy, such as dilatation (17 patients), stent insertion (11 patients), and/or laser therapy (four patients). One BLTx recipient developed complete obstruction in a bronchus intermedius which could not be adequately treated by endoscopic means. Nonetheless, even in this circumstance the middle and lower lobes remain inflated, presumably by collateral ventilation.

COMMENT

Meticulous surgical technique and rigorous postoperative care have significantly reduced the incidence of airway complication following LTx. At our center the incidence is now 4%. The majority of airway complications can be successfully treated and are rarely fatal.

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Diagnosis and Management of Bronchiolitis Obliterans

J.M. KRIETT AND S.W. JAMIESON

INTRODUCTION

The most common cause of late death after lung transplantation has been a progressive and unrelenting deterioration in pulmonary allograft function related to the development of obliterative airway disease. In the transplantation literature this entity has been referred to as bronchiolitis obliterans, obliterative bronchiolitis, chronic rejection and, most recently, bronchiolitis obliterans syndrome. Although the etiology of bronchiolitis obliterans remains unclear, it most likely represents a manifestation of chronic lung rejection. The current approaches to the diagnosis and management of bronchiolitis obliterans are reviewed below.

HISTORICAL PERSPECTIVE

The problem of bronchiolitis obliterans was not recognized during the initial clinical experience with single lung transplantation prior to the 1980s. The reason for this was that the vast majority of patients had died within the first 2 months after lung transplantation due to pulmonary infection or acute rejection[1]. However, the clinical course of the lone patient who did survive more than 3 months does appear consistent with a diagnosis of bronchiolitis obliterans[2]. This patient developed a gradual decline in lung function and gas exchange, and subsequently died of *Pseudomonas* and fungal sepsis 10 months after single lung transplantation. Post-mortem examination showed no evidence of acute rejection, but revealed abnormalities in the airways with fibrosis.

The clinical, functional, radiographic, and histologic features of obliterative airway disease after lung transplantation were first recognized during the early experience in combined heart–lung transplantation at Stanford University[3–9]. Five of the initial 14 survivors developed progressive deterioration in lung function with evidence of bronchiolitis obliterans on lung biopsy[3]. Although case reports indicated that augmented immunosuppression may slow progression of pulmonary dysfunction[10,11], the prognosis for long-term survival in the patients with bronchiolitis obliterans was quite poor. In 1987 the Stanford group reported a 50% incidence of obstructive airway disease following heart–lung transplantation[9].

Bronchiolitis obliterans was the indication for the first heart–lung retransplantation, performed by the senior author at Stanford University in 1984[8]. In this particular case the early postoperative course after heart–lung transplantation in 1981 had been complicated by a systemic cytomegaloviral infection. However, the patient recovered and remained well until an episode of bacterial pneumonia occurred 36 months after transplantation.

Subsequently, progressive dyspnea on exertion and deterioration in pulmonary function ensued. The diagnosis of bronchiolitis obliterans was confirmed by open lung biopsy. Further studies demonstrated abnormalities of the pulmonary and coronary vasculature with small-vessel occlusion, peripheral pruning of pulmonary arterioles and diffuse triple vessel coronary artery disease. Despite treatment with high-dose steroids, bronchodilators, and antibiotics the patient's condition rapidly deteriorated. After 2 months of mechanical ventilatory support successful heart–lung retransplantation was performed.

As the worldwide experience with heart–lung and lung transplantation accumulated from the later half of the 1980s to the present time, the significance of bronchiolitis obliterans in limiting the long-term survival of lung recipients became even more apparent. Early reports suggested a lower incidence of late allograft dysfunction due to obliterative airway disease after single and double lung transplantation as compared to the experience with heart–lung transplantation; however, this initial optimism has been proven unfounded. In 1993 an *ad-hoc* committee of the International Society for Heart and Lung Transplantation recommended a standardized nomenclature of bronchiolitis obliterans syndrome and a severity classification based on degree of deterioration in pulmonary function with or without histologic evidence of bronchiolitis obliterans[12].

CURRENT INCIDENCE

Clinical and/or histologic evidence of bronchiolitis obliterans has been reported in 11–54% of lung recipients surviving more than 6 months after transplantation[13–22]. Differences in patient survival

intervals and in the definition of bronchiolitis obliterans in reports from individual transplant centers account for the variability in the reported incidence of bronchiolitis obliterans.

The relationship between survival interval after transplantation and the frequency of significant allograft dysfunction (defined as a greater than 20% reduction from baseline FEV_1) in our series of 85 lung transplant procedures at the University of California, San Diego, is shown in Figure 1. As survival interval after transplantation lengthens, the percentage of surviving lung recipients with evidence of a significant deterioration in lung function increases. The percentage of our lung recipients with bronchiolitis obliterans syndrome stage 1 or greater was 14% at 1 year and 32% at 2 years after transplantation. At 4 years after lung transplantation over 50% of surviving recipients demonstrated a greater than 20% reduction from peak FEV_1 with 43% of all survivors showing a reduction in FEV_1 of greater than 40% (includes BOS stage 2 or 3).

A recent review of the entire Stanford experience for heart–lung transplantation reported actuarial rates of freedom from bronchiolitis obliterans of 71% at 1 year, 51% at 5 years, and 42% at 10 years[21]. The Papworth experience with heart–lung transplantation in children suggested a higher incidence with an actuarial freedom from bronchiolitis obliterans of 37% at 3 years after transplantation[20]. In a series of 44 double lung transplant procedures in patients with cystic fibrosis actuarial freedom from bronchiolitis obliterans was 59% at 2 years[22].

Bronchiolitis obliterans has been the most common cause of late death in heart–lung and lung transplant recipients surviving more than 6 months after transplantation[23–25]. The International Society for Heart and Lung Transplantation Registry data indicate that over 50% of all late deaths were related to bronchiolitis obliterans[23]. In our series at the University of California, San Diego, 10 of 14 late deaths (71%) have been directly related to the development of bronchiolitis obliterans syndrome.

PATHOGENESIS

Bronchiolitis obliterans is a histopathologic term which describes a non-specific pattern of airway injury characterized by fibrosis and obliteration of the distal bronchioles. In the non-transplant population, bronchiolitis obliterans has been associated with toxic fume inhalation, drug reactions, a variety of pulmonary infections including viral and *Mycoplasma* infections, and collagen vascular diseases[26,27]. In many cases the etiology of the obliterative airway disease is not clearly defined.

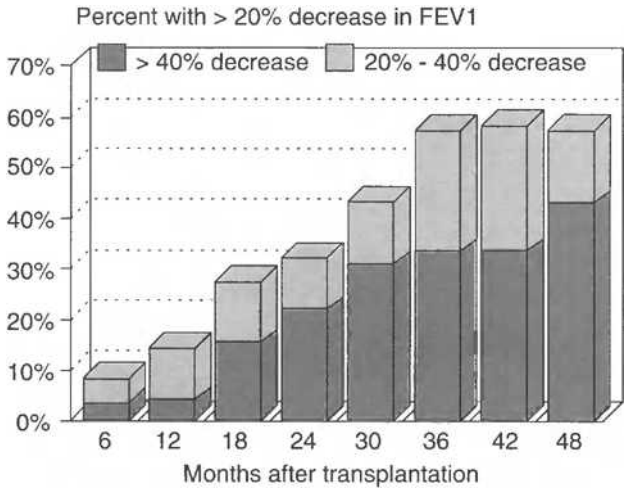

Figure 1 Percentage of recipients with bronchiolitis obliterans syndrome by survival interval after lung transplantation

In the transplant population obliterative airway disease has been reported after bone marrow allotransplantation and after lung transplantation. In bone marrow recipients bronchiolitis obliterans, a common manifestation of graft-versus-host disease, has been reported in 3–18% of recipients[28]. However, graft-versus-host disease as the basis for bronchiolitis obliterans in lung recipients appears unlikely, as all detectable lymphocytes are of recipient phenotype within 3 months after transplantation[29].

A number of other factors may be eliminated as major contributors to the development of bronchiolitis obliterans in lung recipients. Although cyclosporin has been associated with myocardial fibrosis[30], long-term survivors of cardiac transplantation do not develop obliterative airway disease. Thus, it appears unlikely that cyclosporin toxicity is a significant factor in the development of bronchiolitis obliterans in lung transplant recipients. The absence of obliterative airway disease in experimental autotransplantation models indicates that denervation, bronchial artery ligation, and lymphatic division are probably not major contributors to the development of bronchiolitis obliterans[31–33].

The significance of donor lung ischemic injury due to inadequate and/or prolonged organ preservation on late fibrosis and obliterative airway disease is less clear. Pleural fibrosis and obstructive and emphysematous changes have been noted in canine lung autografts more than 6 years after transplantation[34] and obliterative airway disease has been identified in canine autografts after 24-hour preservation[35]. However, in clinical lung transplantation the experience with donor organ ischemic times beyond 6 hours has been limited, and an association between preservation time and the risk of bronchiolitis obliterans has not been reported.

The preponderance of both experimental and clinical evidence has pointed to an immunologically mediated basis for bronchiolitis obliterans in lung recipients. The pulmonary and coronary vascular involvement commonly associated with bronchiolitis obliterans in heart–lung recipients appears similar to chronic rejection patterns with other solid-organ transplantation such as accelerated graft atherosclerosis in hearts, sclerosing endarteritis in kidneys, and sclerosing cholangitis in livers.

An immunologic response directed against the donor airway epithelium appears responsible for the development of bronchiolitis obliterans. Airway ulceration and obliterative airway disease have been uniform findings in experimental models of lung allotransplantation[36]. Increased expression of MHC class II antigens on bronchiolar epithelium and lymphocytes of pulmonary allografts has been reported[37–39]. Immunocytofluorometric analysis of peripheral blood lymphocytes has indicated a distinct phenotypic profile in patients with bronchiolitis obliterans syndrome characterized by the disappearance of $CD19^+$ B cells, a decrease in $CD4^+/CD8^+$ ratio, and an increase in cytotoxic effector T cells[40].

The development of graft tolerance may be the mechanism which accounts for the absence of bronchiolitis obliterans in some heart–lung and lung recipients at follow-up intervals beyond 5–10 years after transplantation. Recent studies have indicated that microchimerism, the presence of donor-derived cells in various

tissues and blood of the recipient, is associated with a low risk for bronchiolitis obliterans. Lung recipients with bronchiolitis obliterans demonstrated less microchimerism, as well as greater donor-specific alloreactivity, than recipients without bronchiolitis obliterans[38,41]. In addition, donor antigen-specific hyporeactivity has been correlated with improved long-term allograft outcome[42]. In a series of 23 lung recipients surviving at least 1 year after transplantation, none of eight recipients showing hyporeactivity developed obliterative bronchiolitis, whereas six of the 15 recipients who remained responsive to donor antigens developed bronchiolitis obliterans.

RISK FACTORS ASSOCIATED WITH BRONCHIOLITIS OBLITERANS

Frequent or persistent acute lung rejection and infection have been identified as the most significant risk factors associated with subsequent development of bronchiolitis obliterans after lung transplantation[18]. Three or more episodes of clinically and histologically defined acute rejection within the first 3 months and acute lung rejection episodes occurring after the first month have been more frequent in the patients who developed bronchiolitis obliterans syndrome[18,20,43,44]. The occurrence of organizing pneumonia with acute lung rejection, which may indicate a more severe acute rejection episode, has been associated with a six-fold increase in the risk for development of bronchiolitis obliterans[44].

Experimental and clinical evidence supports a synergistic role for infection in the development of chronic airway disease[45–47]. The majority of lung recipients who develop bronchiolitis obliterans syndrome have had an episode of lower respiratory tract infection within the preceding weeks or months[3,10]. Factors which may contribute to progressive airway injury in lung recipients with infection include an impaired immunologic response to infection related to chronic immunosuppression, an altered cough reflex due to denervation and abnormalities in mucus clearance which may increase susceptibility of infection, and bronchiectasis resulting in an increased risk of recurrent infection in patients with bronchiolitis obliterans.

An association between bronchiolitis obliterans and cytomegalovirus (CMV) infection, specifically CMV pneumonitis, has been reported[18,46]. One year after lung transplantation CMV-negative recipients showed significantly better pulmonary function than the CMV-positive recipients. In addition, within the CMV-positive group a significantly higher incidence of bronchiolitis obliterans was evident in those patients with CMV pneumonitis[46]. Immunologic studies have demonstrated up-regulation of donor-specific antigen alloreactivity associated with CMV infection.[47]

DIAGNOSIS

Clinical presentation

The clinical presentation in lung recipients with bronchiolitis obliterans syndrome is quite similar to that of patients with chronic obstructive pulmonary disease; however, the time scale may be telescoped into a period of months rather than years. In almost all cases a deterioration in pulmonary function is evident prior to the onset of symptoms. The common symptoms include a persistent cough, which may be productive of mucopurulent sputum, and worsening dyspnea with exertion. Increasing exertional dyspnea may rapidly progress to the need for assisted ventilation within a period of a few weeks to months. Recurrent pulmonary infections are very common in patients with bronchiolitis obliterans. In the patients with marginal pulmonary function such infections may result in a rapid deterioration in clinical status with acute pulmonary failure and death.

Functional assessment

The changes which occur in pulmonary function after heart–lung and lung transplantation have been well described. A gradual improvement in pulmonary function is typical during the first 6–12 months after heart–lung and lung transplantation[48–50]. A mild-to-moderate restrictive ventilatory defect is evident early after transplantation, with reduced total lung capacity (TLC) and forced vital capacity (FVC), probably related to decreased chest wall compliance rather than intrinsic lung dysfunction. This restrictive defect improves during the initial 6 months to 1 year following transplantation.

Although a transient acute deterioration in pulmonary function may occur with acute rejection, infection, and airway anastomotic stenosis, appropriate treatment of these complications generally reverses the functional abnormalities immediately. In contrast, serial pulmonary function testing in lung recipients who develop bronchiolitis obliterans syndrome reveals a significant and progressive reduction in lung function.

The great variability in time interval from transplantation to onset, as well as the rate of deterioration in pulmonary function for lung recipients with bronchiolitis obliterans syndrome, is demonstrated in Figure 2. In some patients a relentless and rapid decline in function occurs. Other patients with clinical evidence of bronchiolitis obliterans may remain relatively clinically stable and show a more gradual decline in pulmonary function.

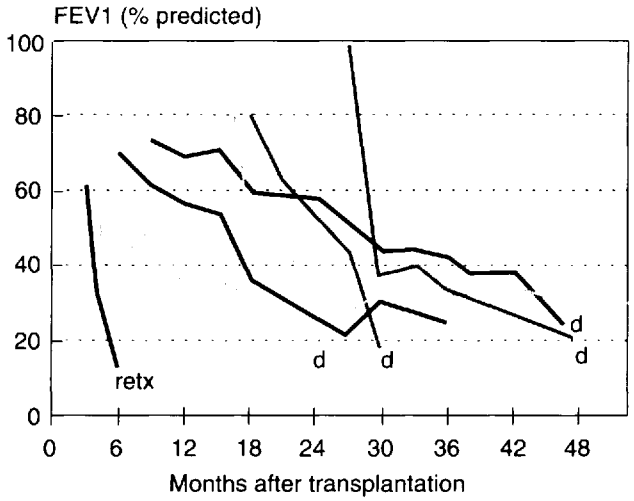

Figure 2 Variability in survival interval to onset and rate of deterioration in FEV_1 in recipients with bronchiolitis obliterans syndrome: retx, retransplantation; d, death

Table 1 Clinical staging system for bronchiolitis obliterans syndrome (OB)

	FEV_1 results	*Biopsy results*
Stage 0	FEV_1 more than 80% of baseline value*	(a) Without pathological evidence of OB (b) With pathological evidence of OB
Stage 1	FEV_1 = 66–80% of baseline value	(a) Without pathological evidence of OB (b) With pathological evidence of OB
Stage 2	FEV_1 = 51–65% of baseline value	(a) Without pathological evidence of OB (b) With pathological evidence of OB
Stage 3	FEV_1 less than 50% of baseline value	(a) Without pathological evidence of OB (b) With pathological evidence of OB

* Baseline value defined as the average of two previous highest consecutive measurements of FEV_1.

The standardized system for the classification of bronchiolitis obliterans syndrome based on serial measurements of forced expiratory volume in 1 second (FEV_1) and histological evidence of bronchiolitis obliterans, as developed by the International Society for Heart and Lung Transplantation[12], is shown in Table 1. The expected baseline level of peak improvement in lung function is variable, depending on both the type of transplant procedure and the primary lung disease. For heart–lung and double lung recipients pulmonary function generally returns to normal levels. In single-lung recipients with emphysema the expected peak in FEV_1 ranges between 50% and 70% of normal.

Characteristically, the development of bronchiolitis obliterans is associated with combined obstructive and restrictive changes in pulmonary function[5,48,49]. Forced vital capacity (FVC) and FEV_1 fall in parallel, with the fall in the flow-dependent FEV_1 being more pronounced. Total lung capacity (TLC) falls rather than increases, indicating that air trapping does not occur. Indices of flow, including the ratio of forced expiratory flow at 50% of FVC (FEF_{50}/FVC), the mean expiratory flow rate between 25% and 75% of vital capacity (FEF_{25-75}), and specific airway conductance (sG_{aw}) may be more sensitive than lung volumes in the detection of bronchiolitis obliterans syndrome.

Gas exchange generally remains normal until the later stages of obliterative bronchiolitis[48]. With progression of the obliterative airway disease arterial hypoxemia and depressed alveolar–arterial oxygen gradients are found uniformly. Hypocapnia is characteristic until the final stage, when carbon dioxide retention occurs.

Hemodynamic evaluation in long-term survivors has demonstrated that normal pulmonary arterial pressure, pulmonary vascular resistance, cardiac output, and ventricular function are possible after heart–lung and lung transplantation[4,50]. In parallel with obliterative airway disease, pulmonary arterial involvement may be detected from increasing pulmonary hypertension and pulmonary vascular resistance.

Radiographic features

The great variability of the chest radiographic findings in lung recipients with bronchiolitis obliterans syndrome is demonstrated in Figure 3. Common radiographic findings include interstitial infiltrates, micronodular opacities primarily involving the lower lung zones, and peripheral patchy air-space disease. However, in some patients with severe deterioration in lung function the chest radiograph may not show significant changes until infection supervenes[51].

High-resolution CT scanning may be a more sensitive indicator of the early parenchymal and airway changes related to bronchiolitis obliterans[52–54]. Examples of serial CT scans in patients with and without bronchiolitis obliterans syndrome are shown in Figure 4. Common indicators of bronchiolitis obliterans on high-resolution CT scan include peribronchial and interstitial infiltrates, pleural and interlobar septal thickening, and evidence of central and/or peripheral bronchiectasis. However, these CT scan findings do not appear specific in differentiating between the various parenchymal complications including acute rejection, infection, and bronchiolitis obliterans[53].

Pulmonary arteriography may show pruning of the distal pulmonary vessels, consistent with obliterative changes of the pulmonary arterial tree[4]. The development of bronchiolitis obliterans may also be detected on radionuclide lung scans in single-lung recipients with pulmonary hypertension by an increase in ventilation–perfusion mismatch. In the heart–lung recipients, similar to the chronic rejection seen in heart recipients, coronary angiography may reveal evidence of diffuse coronary artery disease.

Histologic and immunologic studies

Bronchiolitis obliterans is characterized histologically by a fibrosing inflammatory process involving the terminal and respiratory bronchioles[6]. The earliest histologic abnormalities consist of ulcerations of the bronchiolar epithelium with granulation tissue containing necrotic epithelial and polymorphonuclear cells within the airway lumen. More organized mucopolysaccharide-laden 'Mason bodies' may form onion-skin-appearing plugs within the airway lumen, associated with epithelial thinning and squamous metaplasia.

Progression of these abnormalities results in submucosal fibrosis with replacement of the bronchiolar smooth muscle wall, interruption of the elastic lamina, and eventual total obliteration of the bronchioles by scar tissue. In association with the histologic changes in the airways a concentric fibroelastosis of the intimal surface and muscular hypertrophy of the pulmonary arterioles has been described. Intimal thickening may also be evident in the pulmonary venules.

Lung biopsy, by transbronchial or open techniques, for a histologic confirmation of bronchiolitis obliterans, is not routinely performed or necessary. However, biopsy may prove useful in the identification of other potentially reversible causes of late allograft dysfunction, including acute rejection, infection, recurrence of the primary lung disease, or post-transplant lymphoproliferative disease.

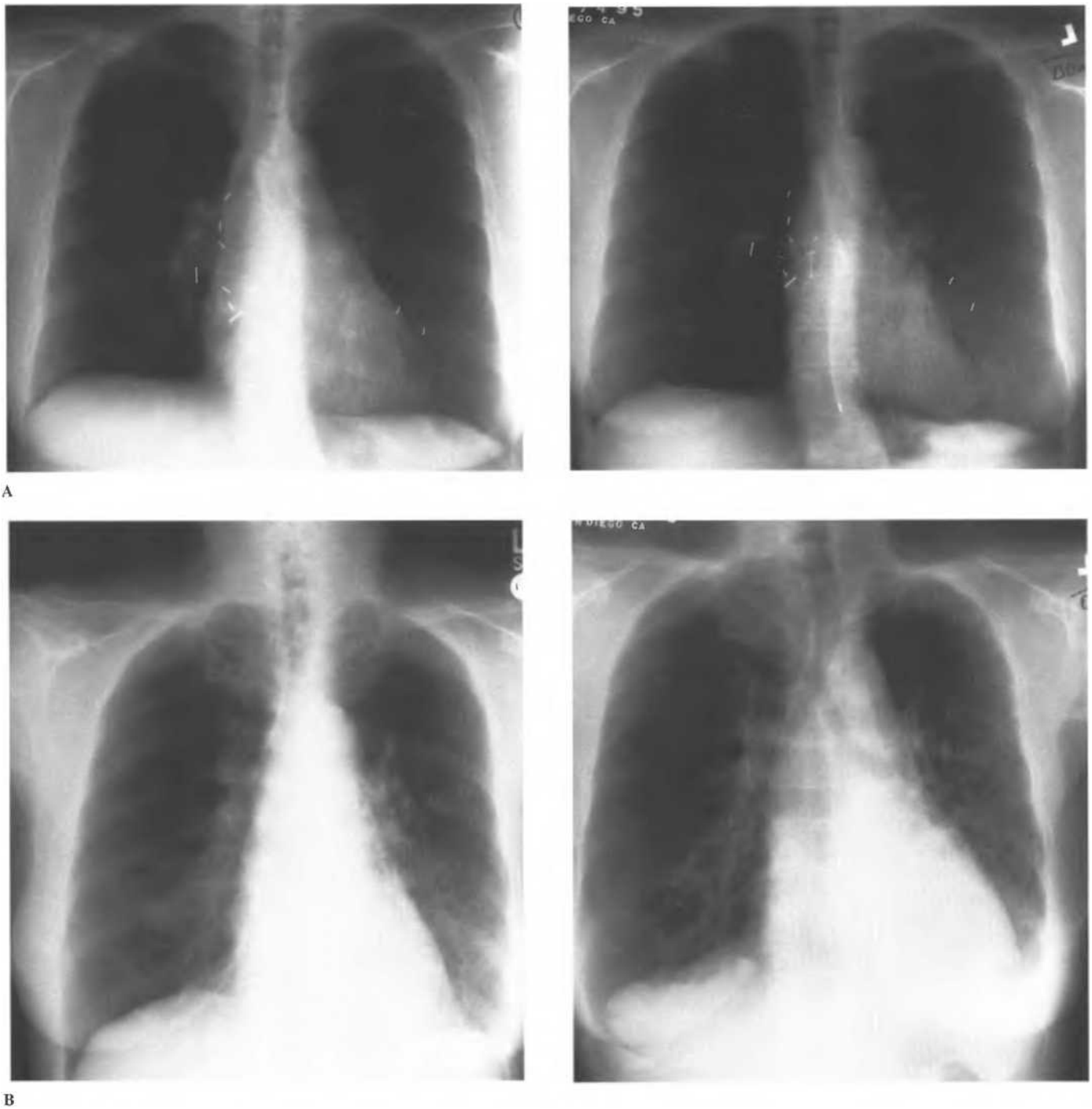

Figure 3 Spectrum of chest radiographic findings with bronchiolitis obliterans syndrome. **A**: Double-lung recipient at 1 year and 4 years after transplantation. Radiographs show no change in lung fields with development of slight hyperinflation. **B**: Left-single-lung recipient with emphysema at 1 year and 4 years after transplantation. Radiographs show progressive air-space disease in left lower lung field

The reported sensitivity of the transbronchial biopsy technique in the diagnosis of obliterative bronchiolitis has been highly variable. A high false-negative rate has been reported[55], although, the University of Pittsburgh reported histologic confirmation of obliterative bronchiolitis by transbronchial biopsy in 87% of cases[56]. In addition, acute bronchitis/bronchiolitis with submucosal granulation tissue and a predominance of lymphocytic and plasma cell infiltrates on biopsy may be of predictive value for later development of bronchiolitis obliterans[57].

Analysis of the cellular content and function from bronchoalveolar lavage has not proved significantly helpful in the diagnosis of bronchiolitis obliterans[58]. A diagnosis of acute rejection or infec-

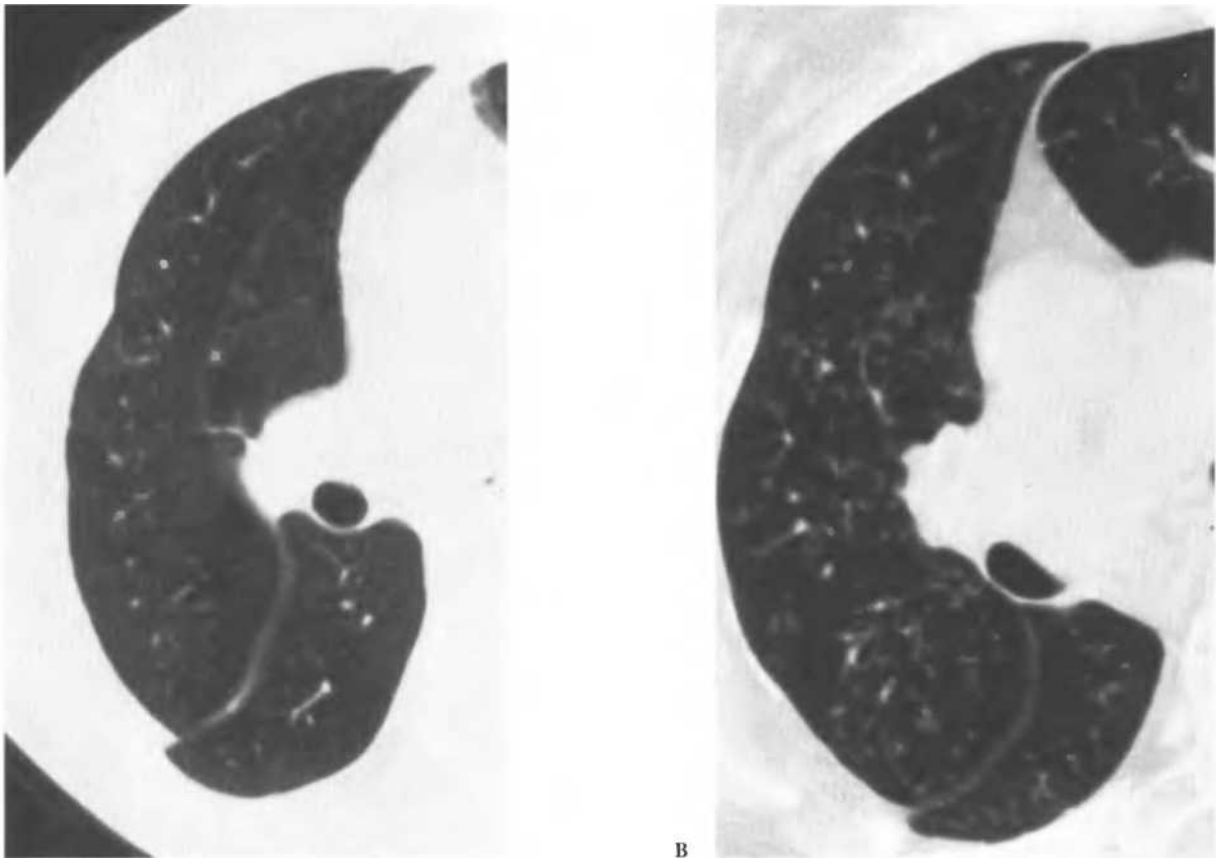

Figure 4 Serial CT scans in a single-lung recipient with bronchiolitis obliterans syndrome. **A**: Normal CT scan 4 months after transplantation. **B**: CT scan 1 year later, demonstrating increasing bronchiectasis and parenchymal changes

tion may be suggested by an increase in neutrophils and a decrease in pulmonary macrophages. T lymphocytes activated to respond to donor-specific HLA antigens may be demonstrated; however, their presence is not necessarily indicative of bronchiolitis obliterans. Recent data suggest that assessment of donor-specific antigen reactivity may identify the subgroup of lung recipients at highest risk for the development of bronchiolitis obliterans, and be predictive of response to augmented immunosuppression[38,42].

MANAGEMENT

In heart–lung and lung recipients who demonstrate a deterioration in pulmonary function it is extremely important to differentiate bronchiolitis obliterans syndrome from other causes of late allograft dysfunction as the potential for reversibility and the specific therapy indicated may vary greatly. The common causes of late allograft dysfunction are listed in Table 2.

Acute lung rejection, although most common within the first few months after lung transplantation, may occur at any time, and generally responds to augmented immunosuppression. Both infection and post-transplant lymphoproliferative disease would be adversely affected by increased immunosuppression, but are potentially curable with appropriately directed therapy. Anastomotic bronchial stenosis, which may be effectively treated by insertion of a silastic stent, generally occurs within the first 6 months. Recurrence of a variety of primary lung diseases – including sarcoidosis, eosinophilic granulomatosis, and lymphangiolyomyomatosis – has been reported in lung recipients.

Table 2 Causes of pulmonary dysfunction in lung recipients

Obliterative bronchiolitis
Acute rejection
Infection
Post-transplant lymphoproliferative disease
Airway anastomotic stenosis
Recurrence of primary lung disease
Progression of primary lung disease in the contralateral native lung

In single-lung recipients progression of the primary lung disease in the contralateral lung, rather than bronchiolitis obliterans, may account for a significant deterioration in pulmonary function. For example, in patients with chronic obstructive pulmonary disease, hyperinflation of the native emphysematous lung with mediastinal shift and compression of the transplant lung may impair allograft function. In selected patients a pneumectomy 'volume reduction' procedure may result in a significant improvement in both clinical symptoms and pulmonary function, as demonstrated in Figure 5.

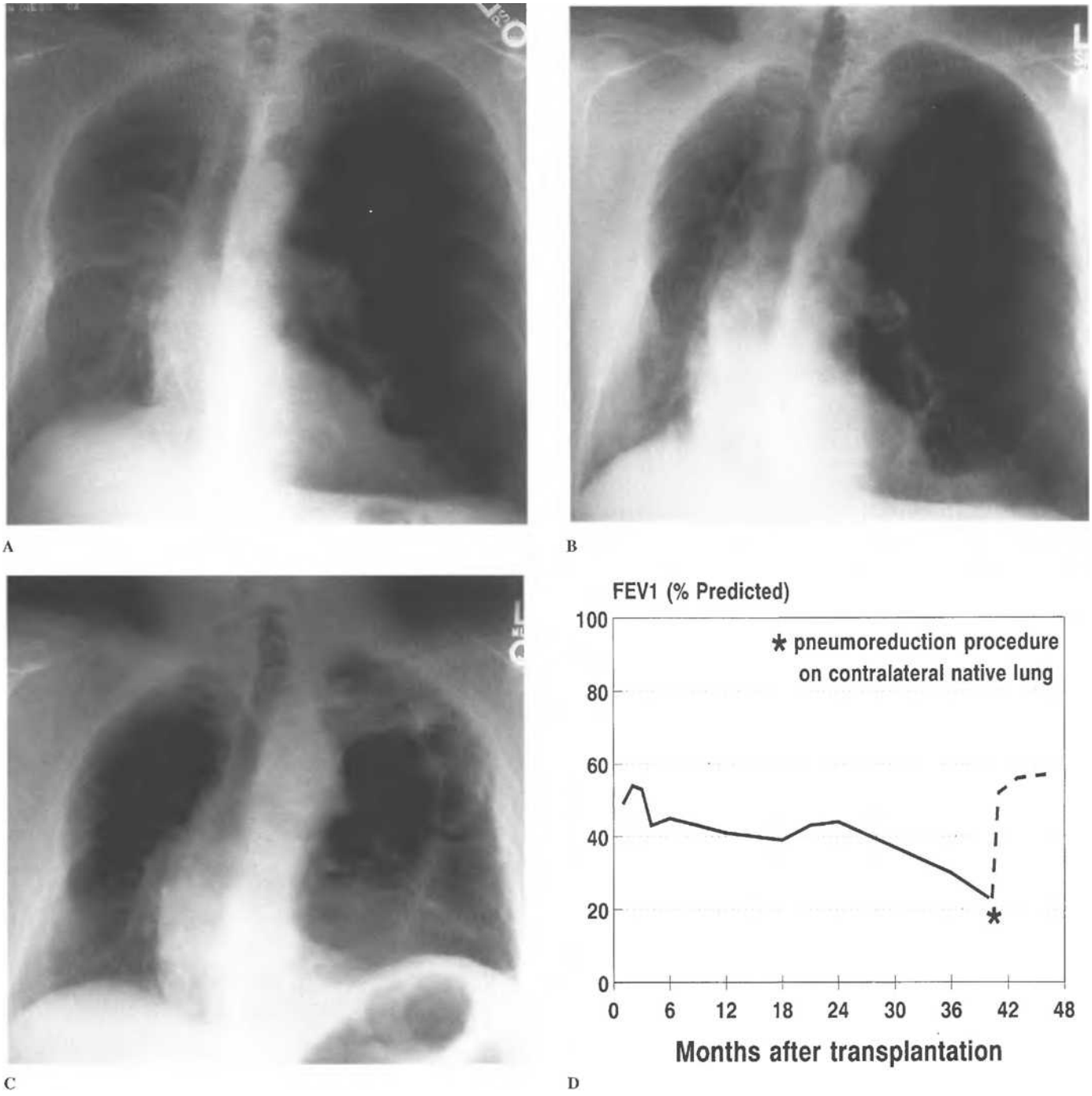

Figure 5 Chest radiographs and pulmonary function data in a single-lung recipient with emphysema. **A**: X-ray 1 month after transplantation. **B**: X-ray 3 years after transplantation showing compression of transplant lung. **C**: X-ray 3 months after unilateral pneumoreduction. **D**: Change in FEV_1 before and after pneumoreduction

Immunosuppression

Augmented immunosuppression has been commonly used in patients with bronchiolitis obliterans syndrome. Although this treatment may be associated with an improvement in both symptomatology and pulmonary function, the effects are generally transient and a significant histologic reversal has not been documented. Close surveillance for infection during this period of increased immunosuppression is essential.

Standard treatment regimens have included one or more courses of high-dose oral prednisone therapy (100 mg/day with gradual taper to maintenance levels) or antilymphocyte antibody preparations. Recently, the addition of inhaled steroid preparations or replacement of cyclosporin with FK506 (tacrolimus) has

been suggested, although clinical experience to date with these regimens has been limited. Although there is no evidence that bronchodilators or aggressive chest physiotherapy reverse the process, these may be beneficial in the treatment of acute infectious exacerbations.

Once a substantial deterioration in lung function has occurred, the likelihood of a response to augmented immunosuppression declines significantly. Therefore, repeated trials of high-dose steroids or antilymphocyte antibody preparations are not warranted, and significantly increase the risk of supervening and potentially fatal infectious complications.

Retransplantation

Due to the variable time-course, the risk of recurrent infections, and the lack of an effective therapy in patients with bronchiolitis obliterans syndrome, early consideration should be given regarding the patient's candidacy for lung retransplantation. Current limitations in donor availability mandate that this option be restricted only to those patients with the highest potential for survival and full rehabilitation after retransplantation.

In heart–lung recipients, because the obliterative airway disease may be associated with accelerated graft atherosclerosis, coronary arteriography is a necessary part of the evaluation prior to retransplantation. The detection of significant accelerated graft atherosclerosis will determine the need for heart–lung replacement rather than lung retransplantation.

Bronchiolitis obliterans has been the most common indication for heart–lung and lung retransplantation. A multicenter series of retransplant procedures included 32 patients in which the indication for retransplantation was bronchiolitis obliterans[59]. Twenty-eight percent of the patients died within 1 month after retransplantation. One-and 2-year actuarial survival rates were 41% and 33%, respectively. The recent experience with single lung retransplantation after heart–lung transplantation suggests a lower early mortality risk than repeat heart–lung transplantation[60].

COMMENT

Bronchiolitis obliterans remains a major impediment to successful long-term outcome after heart–lung and lung transplantation. As treatment options in patients with bronchiolitis obliterans syndrome are extremely limited, and generally unsuccessful, the primary strategy in the management of lung transplant recipients should be directed toward the prevention of bronchiolitis obliterans. At the present time, optimization of immunosuppression to decrease the incidence of early acute rejection episodes, and CMV prophylaxis to decrease in the incidence of CMV pneumonitis, are key areas for potentially minimizing the risk of bronchiolitis obliterans after lung transplantation. The development of techniques for induction of donor-specific graft tolerance holds the greatest promise for the future.

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Pulmonary Retransplantation for Obliterative Bronchiolitis

R.J. NOVICK, H-J. SCHÄFERS, L. STITT, B. ANDRÉASSIAN, W. KLEPETKO, R.L. HARDESTY, A.E. FROST AND G.A. PATTERSON

INTRODUCTION

Despite increasing experience in the postoperative care of lung transplant recipients, obliterative bronchiolitis (OB) develops in up to 40% of patients in the intermediate term postoperatively[1,2]. Although some patients with this condition respond to increased immunosuppression[1,3], in many others chronic allograft dysfunction is resistant to therapy and ultimately causes death from progressive respiratory failure or opportunistic infection. Since 1988 an increasing number of lung transplant recipients with OB have been treated by retransplantation[4-12]. The pulmonary retransplant registry was established in 1991 in order to document the results and determine the predictors of survival after pulmonary retransplantation[5,11]. Recently, after increased patient accrual, the registry data were updated in order to document the determinants of outcome after retransplantation for OB.

PATIENTS AND METHODS

The pulmonary retransplant registry currently contains complete data on retransplant patients from 35 North American and European centers. Patients who have undergone repeat heart–lung transplantation or a heart–lung transplant after a previous pulmonary transplant have been excluded[5,11]. The status of all study patients was updated, with the use of standardized follow-up questionnaires, in late 1994. Only patients retransplanted because of end-stage OB were included in the study cohort.

The 15 parameters listed in Table 1 were analyzed in each patient. The main outcome variables included survival interval after retransplantation, cause of death, functional status and pulmonary function test data in survivors. Study form results were tabulated on the FoxPro database system (Microsoft Corporation, Redmond, Washington, USA) on a 486 DX2/66 MHz computer. Statistical analysis was performed with the SAS Statistical Package, version 6.04 (SAS Institute Inc., Cary, North Carolina, USA). All data were expressed as mean ± standard error of the mean. Actuarial survival was calculated by the Kaplan–Meier method[13] and the statistical difference between survival curves was determined by the Wilcoxon[14] and log-rank tests. Furthermore, Cox proportional hazards methods[15] were used to determine which variables were associated with, and which subset of variables were predictive of, survival after pulmonary retransplantation for OB. The risk ratio of each variable was expressed as a comparison of survival between groups, with a value of 1.00 indicating no survival difference. A p value <0.05 was deemed significant.

Complete pulmonary function test data were obtained in each survivor of retransplantation. Bronchiolitis obliterans syndrome (BOS) stages were assigned according to standardized criteria, based on forced expiratory volume in 1 second (FEV_1) values[1,16]. Furthermore, the changes in absolute FEV_1 values and their percentage decrease at 1 and 2 years after retransplantation were calculated and the statistical difference between values was determined by paired, two-tailed t-tests.

RESULTS

Twenty-six lung transplant centers participating in the pulmonary retransplant registry had performed reoperations for OB as of the closing date of the study. A total of 72 patients have undergone retransplantation for this condition, including 37 patients in 13 North American centers and 35 patients in 13 European centers. The study cohort comprised 43 women and 29 men with a median age of 39 years (range 5–62 years). Prior to their first transplant, 32% had a diagnosis of emphysema, 26% primary pulmonary hypertension or Eisenmenger's syndrome, 17% cystic fibrosis, 17% restrictive lung disease and 8% miscellaneous conditions. The median interval between transplant procedures was 590 days (range 195–2358 days). Twenty-seven patients underwent re-do single lung transplantation for OB, 11 on the ipsilateral side and 16 on the contralateral side. Ten patients underwent re-do double lung transplantation, 14 double lung transplantation after a previous single lung transplant, and 21 single lung transplantation after a previous double lung or heart–lung transplant.

Survival

The actuarial survival of all patients after retransplantation for OB is shown in Figure 1. Of the 72 retransplant recipients, 44 have died and 28 are still living. Despite the high early postoperative attrition a separate actuarial analysis of 90-day postoperative survivors indicated that 63 ± 7% were alive 2 years after retransplantation. The median follow-up in current survivors is 429 days (range 188–1337 days). Twenty-one patients have reached the first anniversary, 12 the second anniversary and five the third anniversary of their retransplants.

The association of the 15 variables that were analyzed in each patient with survival after retransplantation is shown in Table 1. Actuarial survival was not statistically different according to the age, sex or original diagnosis of the recipients, the waiting time or the interval between transplant operations. Life table analysis has confirmed that survival after retransplantation for OB has been improving in recent years ($p = 0.03$ as a continuous variable). In particular, actuarial survival was significantly higher in patients who underwent reoperation from 1990 to 1994, as opposed to 1985 to 1989 ($p = 0.002$). Furthermore, actuarial survival was significantly increased in patients

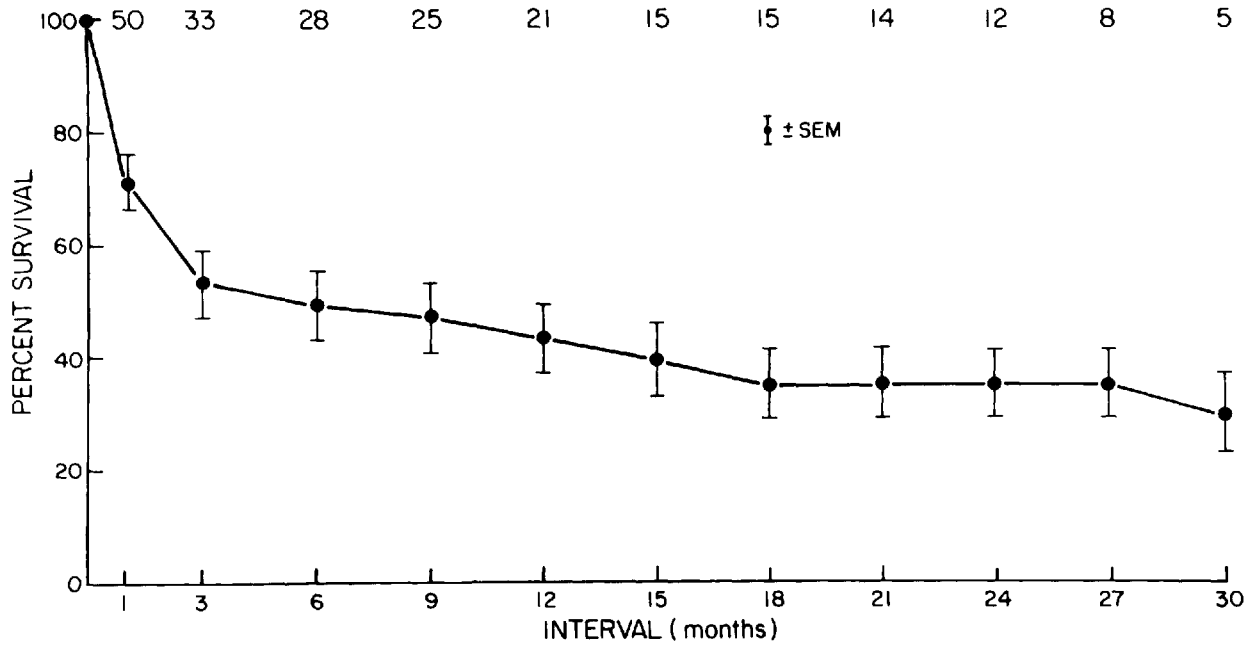

Figure 1 Actuarial survival of 72 patients undergoing pulmonary retransplantation for obliterative bronchiolitis. The number of retransplant recipients alive at each time interval is shown at the top of the graph

Table 1 Association of variables analyzed with survival after pulmonary retransplantation for OB

	Univariate analysis		*Multivariate analysis*	
Variable	*Risk ratio*	p *value*	*Adjusted risk ratio*	p *value*
Age	1.00	0.918	—	n.s.
Sex	0.90	0.726	—	n.s.
Original diagnosis	1.04	0.888	—	n.s.
Waiting time	1.00	0.607	—	n.s.
Interval between operations	1.00	0.255	—	n.s.
Year of reoperation (1990–94 vs 1985–89)	0.25	0.002	0.14	< 0.001
Retransplant center (Europe vs North America)	0.46	0.013	0.43	0.017
Type of reoperation (old graft *in situ* vs not)	1.22	0.517	—	n.s.
ABO blood group identity	0.45	0.018	—	n.s.
Donor CMV positivity	1.37	0.309	—	n.s.
Recipient CMV positivity	0.90	0.754	—	n.s.
Presence of CMV mismatch	2.06	0.089	—	n.s.
Ambulatory before retransplant	0.76	0.385	0.44	0.022
On ventilator before transplant	1.01	0.982	—	n.s.
Institutional experience with > 3 reoperations for OB	0.44	0.008	—	n.s.

OB, obliterative bronchiolitis; CMV, cytomegalovirus; n.s., not significant on multivariate analysis; —, risk ratio not calculated, since variable does not enter into multivariate model.

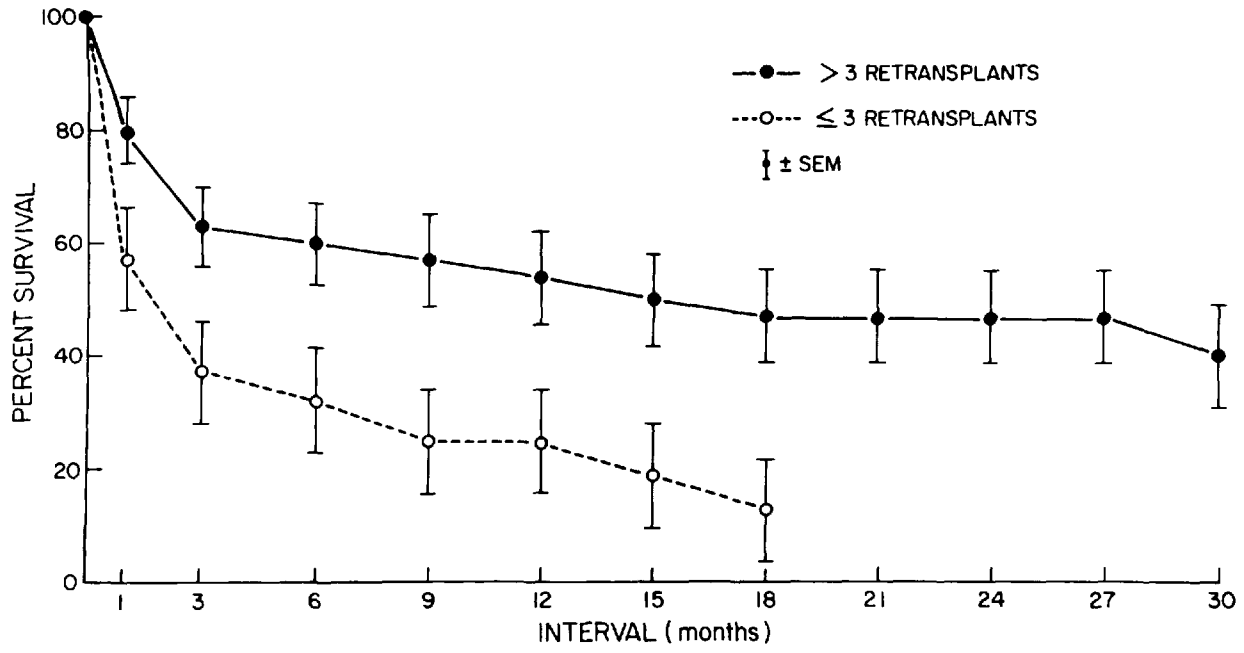

Figure 2 Actuarial survival according to institutional experience with pulmonary retransplantation for obliterative bronchiolitis; $p = 0.008$

undergoing reoperation in centers with experience with four or more pulmonary retransplants for OB (Figure 2), as well as in patients retransplanted in Europe as opposed to North America (Figure 3).

Effect of clinical condition before retransplantation on survival

Before retransplantation only 43% of patients were ambulatory (i.e. able to walk 50 meters with or without assistance). By life

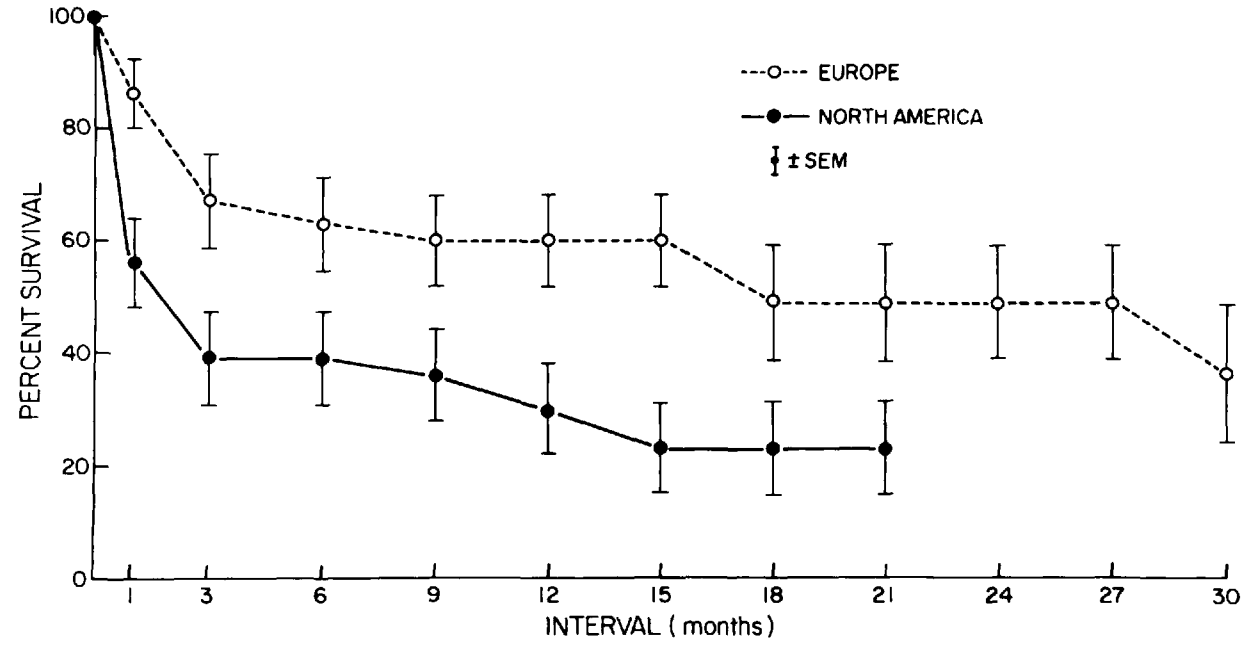

Figure 3 Actuarial survival according to whether retransplantation was performed in a European or a North American centre; $p = 0.013$

table and univariate Cox analysis there were no significant differences in actuarial survival between ambulatory and non-ambulatory patients. Nevertheless, on multivariate analysis, being ambulatory immediately prior to reoperation was predictive of survival (Table 1). Before retransplantation, 25% of patients were ventilator dependent, whereas 75% were not. The survival of ventilated and non-ventilated patients was almost identical (Table 1). No patient with preoperative renal dysfunction requiring dialysis and preoperative hepatic failure survived beyond the third postoperative month after retransplantation.

Impact of the type of retransplant procedure on survival

Actuarial survival did not differ after the five different types of retransplant procedures (Figure 4). In 51% of cases an old, retained contralateral graft remained *in situ* after retransplantation, whereas in 49% of cases all old grafts had been completely explanted. In contradistinction to our last report[11], actuarial survival was not different according to whether or not an old graft remained *in situ* after retransplantation (Figure 5).

Impact of donor–recipient ABO blood group and cytomegalovirus serologic status on survival

Eighty-one percent of patients retransplanted for OB received an ABO-identical graft at reoperation, whereas 19% received a graft that was ABO-compatible, but not identical. Actuarial survival was significantly better in patients transplanted with an ABO-identical graft (Figure 6). In the current series, neither donor nor recipient cytomegalovirus (CMV) serologic status was predictive of survival after retransplantation. Nevertheless, in the nine cases of CMV mismatch (CMV-positive donor, CMV-negative recipient), there was a trend toward decreased survival compared to patients who did not receive a CMV-mismatched graft at reoperation (Figure 7).

Causes of death

The predominant cause of death after retransplantation for OB was infection (29/44 = 66%), followed by acute failure of the second graft (11%), recurrent OB (11%), an airway complication (3%) or other causes (9%). The majority of deaths occurred early postoperatively, and only 11 patients expired beyond 90 days after retransplantation. Infection was the most prominent cause of death at all time intervals after reoperation. Although recurrent OB accounted for a significant percentage of deaths in the intermediate term postoperatively, only five patients in this series died of OB after reoperation for this complication.

Predictors of survival by multivariate analysis

As shown in Table 1, reoperation after 1989, retransplantation in Europe and being ambulatory immediately prior to reoperation were predictive of survival on multivariate analysis. ABO blood group identity and institutional experience with retransplantation for OB, which were significant on univariate analysis, did not enter the multivariate model.

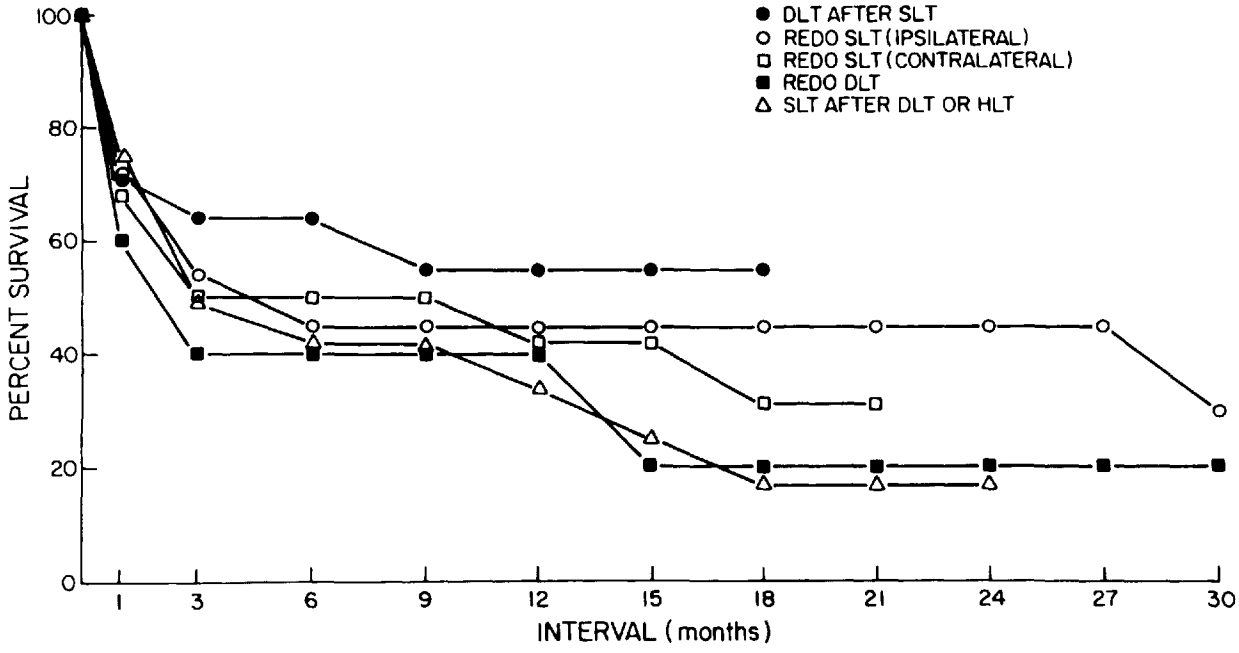

Figure 4 Actuarial survival according to the type of pulmonary retransplant procedure; $p = 0.782$. DLT = double lung transplant, SLT = single lung transplant, HLT = heart–lung transplant

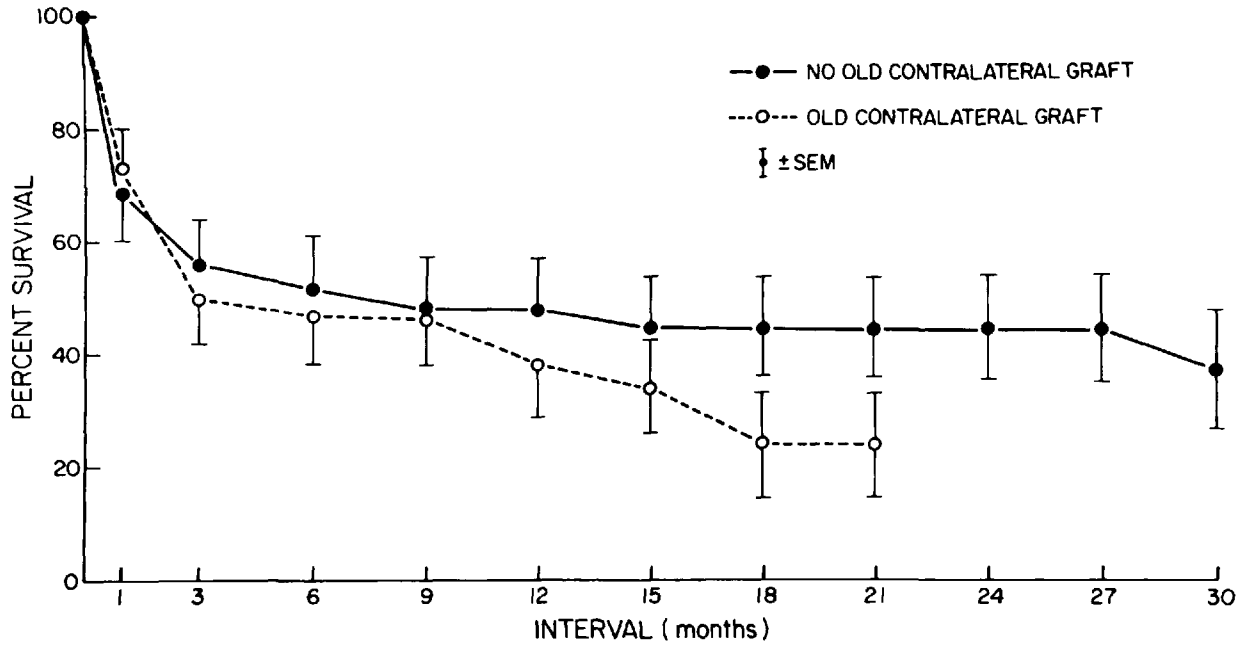

Figure 5 Actuarial survival according to whether an old contralateral graft remained *in situ* after retransplantation; $p = 0.517$

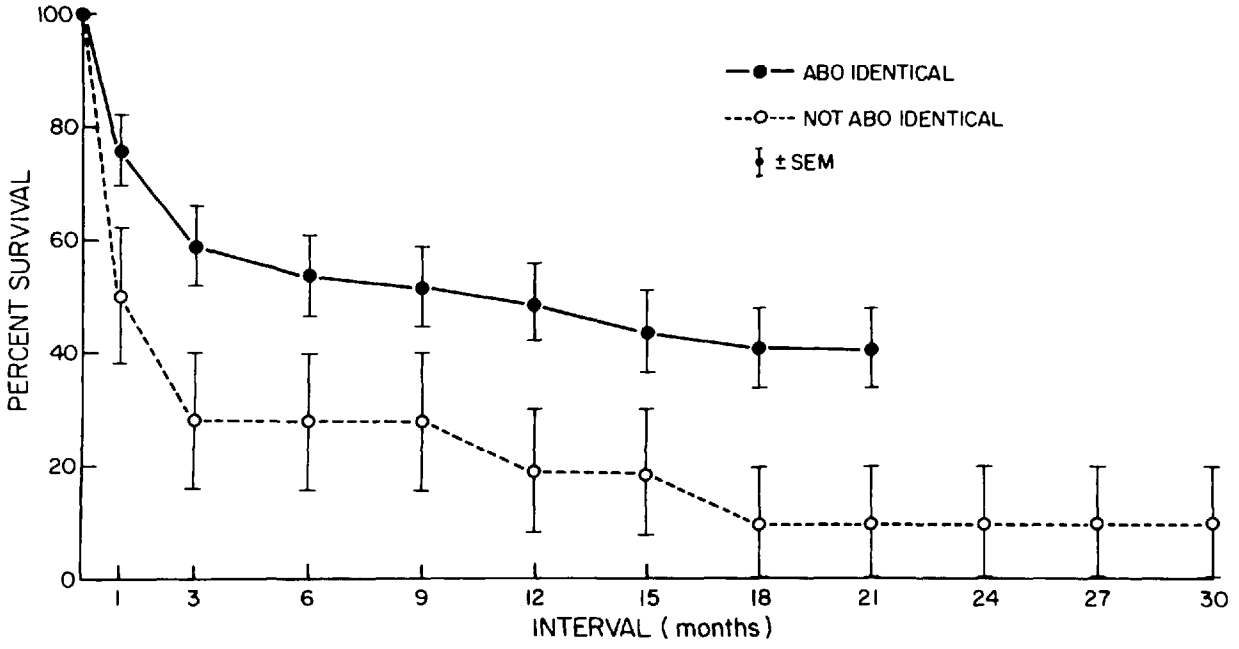

Figure 6 Actuarial survival according to whether an ABO-identical or non-ABO-identical graft was used at retransplantation; $p = 0.018$

Functional status, pulmonary function and recurrence of OB in retransplant survivors

Of the 28 current survivors, 12 (43%) are in functional class I, 11 (39%) in functional class II, three (11%) in functional class III and two (7%) in functional class IV. The BOS stages of retransplant recipients at yearly postoperative intervals are shown in Table 2. Of note is that the prevalence of stage 3 (i.e. severe) BOS was 14% at 1 year, 33% at 2 years and 40% at 3 years after retransplantation. Absolute FEV_1 values decreased by 11 ± 9% at 1 year and 27 ± 10% at 2 years from postoperative baseline values ($p = 0.02$, year 2 versus baseline). There were no

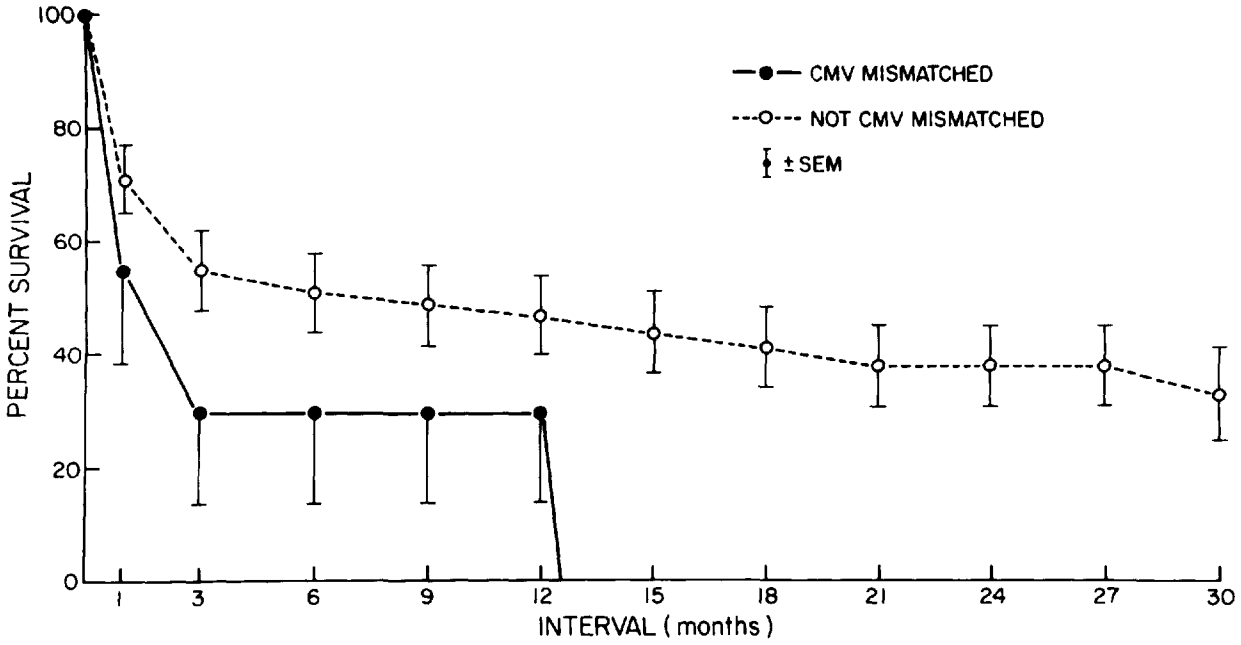

Figure 7 Actuarial survival according to the presence or absence of a cytomegalovirus (CMV) serologic donor–recipient mismatch at reoperation; $p = 0.089$

Table 2 Bronchiolitis obliterans syndrome stages in 1, 2 and 3 year survivors of pulmonary retransplantation for obliterative bronchiolitis*

Postoperative interval	*Number of patients*	*Stage 0*	*Stage 1*	*Stage 2*	*Stage 3*
1 year	21	66%	10%	10%	14%
2 years	12	50%	8%	8%	33%
3 years	5	60%	0%	0%	40%

* Percentages do not add up to 100% due to rounding.

significant differences in BOS stages or the rate of FEV_1 decrease between single- or double-lung retransplant recipients.

COMMENT

Despite recent innovative research on the pathogenesis of OB[17–20], a complete understanding of this condition remains elusive. Although increasing experience in the postoperative care of lung recipients has reduced the prevalence of OB[1,21], the insidious development of OB remains a significant threat to intermediate- and long-term survivors of lung transplantation. Until therapeutic approaches to prevent or mitigate OB are discovered, an increasing number of patients will present with chronic graft dysfunction in the years following transplantation, and the appropriateness of pulmonary retransplantation for OB will continue to be debated.

The pulmonary retransplant registry was established in 1991 to document the outcome and predictors of survival after re-do lung transplantation[5]. There are limitations to this, and previous reports from the registry[5,11] that may make it difficult to draw definitive conclusions from the data presented. These studies have included data collected retrospectively from 26 institutions in North America and Europe, each of which has different preoperative and postoperative protocols and varying experience in primary and re-do lung transplantation. The optimal approach of a prospective, randomized trial of pulmonary retransplantation versus the best alternative therapy in patients with severe OB will probably never be realized, however. Fortunately, the high rate of participation by North American and European transplant centers in the retransplant registry assures that the study cohort and reported outcomes are representative. Furthermore, as the registry has become better known, data are increasingly being collected prospectively. The sizeable number of patients in the registry has increased the statistical power of the outcome analyzes and enabled multivariate analyzes to be used to determine factors that reliably predict survival after pulmonary retransplantation[22]. Despite increasing patient numbers the dynamic nature of the data set increases the probability that the predictors of outcome may change from year to year as experience in the operative and postoperative care of pulmonary retransplant patients continues to increase.

The most important finding of this study is that the results of pulmonary retransplantation for OB are improving. The 1-year actuarial survival for patients retransplanted in recent years has approached 50%. As in primary lung transplantation, infection was a major cause of morbidity and mortality both early and late postoperatively[23]. Of 90-day postoperative survivors, 63 ± 7% were alive 2 years after retransplantation. These data indicate that, with proper patient selection and minimization of early infectious complications, the survival after pulmonary retransplantation for OB can approach that after primary lung transplantation.

In this series, improved results were noted in centers with experience in at least four pulmonary retransplants for OB. The 1-year actuarial survival in patients operated on in these centers was 54 ± 8% compared to 25 ± 10% in patients undergoing retransplantation in centers with less experience. Furthermore, patients retransplanted in Europe appeared to fare better than those reoperated on in North America, with an increased survival in European patients noted in both the life table and univariate Cox analyzes. The improved results in patients reoperated in Europe do not appear to be due solely to increased operative experience, since only three of 13 European centers performed four or more retransplants, as opposed to five of 13 North American centers. Moreover, a detailed comparison of the other covariates listed in Table 1 in European versus North American patients did not readily account for the differing outcome. This fact, and the finding that reoperation in Europe was also found to be a significant independent predictor of survival in the multivariate analysis, indicates that other factors, perhaps involving patient selection, may play a role.

Although donor CMV status at reoperation appeared to play a less important predictive role than in our first paper[5], the presence of a donor–recipient CMV mismatch resulted in a trend toward decreased survival after pulmonary retransplantation ($p = 0.09$). In a large series of primary lung transplantation, donor–recipient CMV matching had no influence on the prevalence of biopsy-proven OB or BOS stage[1]. Furthermore, a recent report from the St Louis International Lung Transplant Registry showed no significant difference in survival for any of the CMV donor–recipient combinations[24]. In our series a significant number of deaths from infection occurred within the first 90 days postoperatively, in which CMV was a major offending organism, in concert with resistant Gram-negative bacteria and fungi. Perhaps the increased doses of immunosuppressive drugs at retransplantation, and a more precarious patient condition prior to retransplantation, accentuated the morbidity from CMV infection in this series.

As noted in previous reports from the retransplant registry[5,11], ventilator dependence prior to retransplantation did not bias postoperative survival. In a recently reported single center experience with pulmonary retransplantation, preoperative ventilator dependence also did not adversely affect survival, but did result in a marked prolongation of the postoperative intensive-care-unit stay[12]. Other reports have confirmed that primary lung transplantation can be performed in patients who have been on the ventilator for less than 3 weeks with a reasonable expectation of success[25]. Other factors, such as preoperative nutritional and ambulatory status, are probably more important than ventilatory status in determining survival after retransplantation. With increased patient accrual to the retransplant registry, being ambulatory immediately preoperatively has become predictive of survival on multivariate analysis. Further follow-up of a larger number of patients is required in order to confirm the importance and predictive value of ambulatory status on survival after retransplantation for OB.

The issue as to whether OB or BOS recur in an accelerated manner after retransplantation for this complication is of major importance. Actuarial data on the prevalence and severity of OB and BOS after primary lung transplantation are sparse. In a single center experience, the freedom from stage 3 (i.e. severe) BOS was identical in primary and secondary lung transplant recipients at 1 year[12]. After 2 years, however, 72% of recipients of primary lung grafts were free of stage 3 BOS, as opposed to only 27% of pulmonary retransplant recipients. In the larger number of OB patients followed in the retransplant registry, 86% were free of stage 3 BOS at 1 year and 67% at 2 years. Furthermore, absolute FEV_1 decreased by only 27 ± 10% at 2 years from postoperative baseline values, similar to the Washington University experience after primary lung transplantation[1]. It is thus evident that although some patients develop rapidly progressive BOS after retransplantation for this condition, the majority have acceptable pulmonary function in the intermediate term postoperatively. There is therefore no evidence at present that BOS occurs in an accelerated manner after a secondary, as opposed to a primary, lung transplant operation.

Until such time as the number of lung grafts available for primary transplantation can be increased, the practice of pulmonary retransplantation will continue to raise ethical dilemmas[11,26]. In recognition of the experimental nature of pulmonary retransplantation it is imperative that only patients most likely to survive be offered the option of reoperation. Current evidence suggests that pulmonary retransplantation should be performed only in highly selected patients with OB who are ambulatory and are operated on in experienced centers. Hopefully, further research will clarify the pathogenesis of OB and lead to therapeutic strategies to prevent chronic graft dysfunction in primary lung transplant recipients, thus reducing the number of patients who require consideration for pulmonary retransplantation.

Acknowledgements

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Lung Transplantation for Cystic Fibrosis

T.M. EGAN

INTRODUCTION

Cystic fibrosis (CF) is the most common lethal genetic disease of Caucasians. Although it is a multisystem disease it is estimated that 95% of patients with CF will succumb from end-stage pulmonary disease[1]. Up to 400 patients with CF die annually in the United States[2]. The past decade has seen remarkable advances in understanding the pathophysiology of CF, including identification of a chloride channel abnormality[3], identification of the gene responsible for the chloride channel[4], and creation of a transgenic mouse with CF[5]. Even before the genetic defect was identified, it was apparent that the chloride channel epithelial abnormality present in CF, evidenced by an altered potential difference[3], was not manifested in the epithelium of heart–lung grafts in transplanted CF patients[6]. This provided assurance that the pulmonary abnormalities observed in CF patients were unlikely to recur following lung transplantation.

Similar remarkable advances have been made in the area of thoracic transplantation. Patients with CF, once considered high-risk candidates for any type of thoracic transplant, are now being transplanted, with mortality rates that are less than those for patients with other forms of end-stage lung disease[7].

HISTORY OF LUNG TRANSPLANTATION IN CF

The first successful heart–lung transplant was performed at Stanford University by Reitz and colleagues[8]. This operation was reportedly first performed in a patient with CF in September 1984 at Harefield Hospital[9]. Meanwhile, at the University of Toronto, techniques were being evolved for isolated lung transplantation. In November 1983 the first successful single lung transplant was performed[10], some 20 years after Hardy's first attempt at the University of Mississippi[11]. To circumvent the necessity of heart transplantation for patients who required bilateral lung replacement, a technique for double lung transplant was developed at the University of Toronto[12]. The first successful double lung transplant using this technique was performed in 1986[13].

This '*en-bloc*' double lung transplant procedure was fraught with a distressing incidence of serious airway complications[14]. Although the *en-bloc* operation was applied in CF patients at the University of Toronto as early as 1987, it became clear that the *en-bloc* double lung transplant had an unacceptable incidence of morbidity and mortality. Noirclerc from Marseilles, France, introduced a technique of bilateral bronchial anastomoses through a sternotomy[15], which substantially reduced the incidence of ischemic airway complications. Safe extraction of lungs through a sternotomy in patients with CF remained an enigma until the so-called 'clamshell' incision was introduced by the thoracic group at Washington University[16]. The transverse incision offered several advantages, including a better exposure of the pleural spaces for safe division of vascular adhesions and the opportunity to sequentially transplant lungs, thus avoiding the need for cardiopulmonary bypass in many cases.

Some controversy still remains regarding the most appropriate transplant procedure for patients with CF. Some centers still adhere to the notion that heart–lung transplantation is a more reasonable alternative because of the low incidence of airway complications. The bilateral sequential technique has addressed many of these issues. By avoiding transplantation of the heart, recipients are not exposed to the risks of cardiac transplantation and the development of graft atherosclerosis[17]. The cardiac denervation that results from heart–lung transplantation delays heart rate response to exercise and may reduce exercise performance[18]. Some surgeons have advocated the so-called 'domino procedure', in which CF recipients of heart–lung grafts donate their native heart to recipients awaiting cardiac transplantation[9]. The increasing popularity of cardiac transplantation is making heart–lung grafts more scarce.

Initial experience with heart–lung transplantation for CF in North America was discouraging. A 1-year actuarial survival of 42% was reported for 33 CF patients undergoing heart–lung transplant at 14 centers in North America between October 1983 and August 1990[19]. Heart–lung transplantation for CF was more popular in European centers. In 1990 Yacoub and colleagues, from Harefield, reported 27 heart–lung transplants in patients with CF over a 4-year period, with only four operative deaths[9]. One- and 2-year actuarial survival was 78% and 72%, respectively. The group from Papworth Hospital, Cambridge, reported a similar

experience with 32 patients undergoing heart–lung transplant for CF, with a 1-year actuarial survival of 73%[20].

Meanwhile in North America, the growth in the number of heart transplant centers and the increasing popularity of heart transplants resulted in a dramatic decline in availability of heart–lung grafts. The bilateral sequential technique of double lung transplant was applied to patients with CF with acceptable morbidity and mortality[7,21,22].

PATIENT SELECTION

In general, lung transplantation is an option for appropriate patients with end-stage lung disease whose risk of death from lung disease exceeds the risks associated with transplantation. Other factors entering into the selection process include issues related to compliance, psychological stability, and an absence of contraindications that would pose an increased risk for the transplant team.

As candidates for lung transplantation, CF patients pose some technical challenges. CF lungs are usually colonized with a variety of multiply resistant organisms that have contributed to the destruction of the organ. The infections result in extensive vascular intrathoracic adhesions and a proliferation of systemic bronchial vasculature, with encasement of hilar structures in large, well-vascularized lymph nodes. In other respects CF patients are attractive transplant candidates. They are frequently young, with life-threatening disease that is confined primarily to one organ system. Their need for ongoing care often results in superior ability to manage a complex medical regimen, and also helps identify patients in whom compliance has been problematic.

Pretransplant evaluation seeks to identify patients whose pulmonary functional status and prognosis justify the risks of transplant, and whose current health problems will not unnecessarily increase the risks of the operation or jeopardize long-term success[23]. Identification of the most appropriate time for transplantation is a difficult aspect of selection. The study by Kerem *et al.* from the Hospital for Sick Children has been helpful in identifying predictors of mortality in CF patients[24]. Over a 12-year period, 673 CF patients were followed at one center. During this interval 190 patients died. Regression analysis was used to determine the relative risk of death within 1 or 2 years from prior measurements of pulmonary function, blood gases, and nutritional status. Among objective measurements, the FEV_1 was the most significant predictor of mortality in this study, but age and sex were also significant for predicting risk. This study found that CF patients under the age of 18 with an FEV_1 <30% predicted had a risk of mortality within 2 years of 50%. Patients older than 18 had a somewhat reduced (40%) risk of death with an FEV_1 <30%. For patients with an FEV_1 <20%, the risk of death within 2 years increased to 70% for young patients and 58% for patients aged 19–44. The risks were higher in females, and weight for height <70% predicted was also particularly worrisome. Patients with room air pO_2 below 55 mmHg or pCO_2 above 50 mmHg also had a 2-year mortality rate above 50%. Pulmonary function tests (PFTs) are not the sole criterion for patient selection. A history of reducing exercise tolerance, increasing requirements for hospitalization or intravenous antibiotic therapy, institution of supplemental oxygen, and difficulty maintaining weight all portend a poor prognosis for CF patients.

In our experience, mortality on the waiting list is associated with a significantly lower FEV_1 than that for listed patients who survive long enough to be transplanted[25]. Of 95 CF patients seen here over a $3\frac{1}{2}$-year interval, 67 patients were listed or transplanted, with 11 patients dying on the waiting list. Only 12% of referrals were judged to be too well at the time of assessment and were followed. Absolute contraindications to lung transplant for CF patients include an inability to tolerate systemic steroids, renal insufficiency (because cyclosporin is so nephrotoxic), a history of previous malignancy, and pan-resistant organisms. Diabetes mellitus is not an absolute contraindication, because diabetes itself does not appear adversely to affect prognosis in CF patients[26]. However, end-organ involvement due to diabetes, such as renal disease or neuropathy, may substantially compromise outcome after transplant, we have not used nutritional parameters to establish suitability for transplant. Nutritional status has been demonstrated to improve after lung transplant, irrespective of the pretransplant diagnosis[27]. The need for preoperative ventilation in CF patients has not been associated with increased perioperative risk in properly selected candidates[28,29].

The profile of organisms recovered from sputum prior to transplant may increase risk, and is an area of controversy in terms of patient selection. Patients with *Pseudomonas cepacia* have an increased risk of an adverse outcome following lung transplantation[30], which has resulted in several programs refusing patients with *P. cepacia*. Our policy has been to offer transplant to those patients with *P. cepacia* who have organisms susceptible to antibiotics *in vitro*. It appears that patients maintain the same strain of *P. cepacia* for prolonged periods, and that some strains may be more biologically aggressive than others[31].

A more difficult problem is the disposition of patients with pan-resistant organisms. Whether these are *P. cepacia* or *P. aeruginosa*, it is difficult to know how to adequately treat these patients perioperatively, and the development of postoperative pneumonia with a pan-resistant organism is usually a lethal complication. Thus, we consider pan-resistant organisms an absolute contraindication to transplantation. An ethical and moral dilemma ensues when a patient is listed with organisms that are sensitive *in vitro* and develops resistance during the waiting period. The critical shortage of suitable lungs for transplant adds to this dilemma, because of the mounting pressure to transplant only those patients who are most likely to survive, for optimal utilization of a scarce resource.

SURGICAL TECHNIQUE

Issues related to donor selection, management and retrieval have been covered elsewhere in this text. Because CF patients tend to be of small stature, prospective donor lungs may be larger, and the population of larger donors is disproportionately high compared with the numbers of CF patients listed. We have performed 'pneumoreduction' procedures to deal with this discrepancy by resection of donor lung tissue after completion of a transplant to match the size of the recipient thorax. In an analysis of 11 patients who underwent some form of pneumoreduction procedure, we demonstrated the safety and practicality of this approach[32].

The recipient procedure begins with intubation and bronchoscopy to attempt to aspirate as much purulent material from

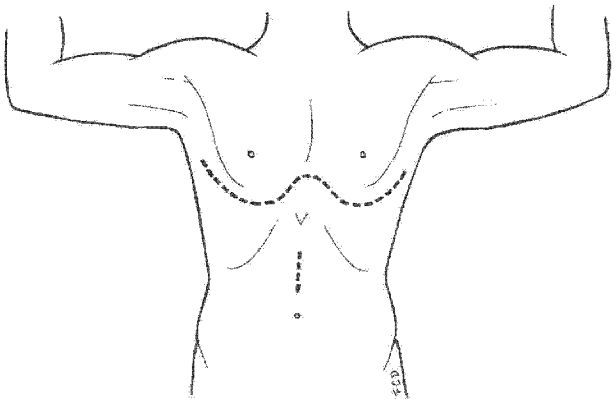

Figure 1 Position of skin incisions for double lung transplant in CF recipients. (Reprinted with permission from ref. 58)

the airways as possible, because the sequential technique requires one-lung ventilation during implantation of the first graft. A left-sided double-lumen endotracheal tube is then positioned in all but the youngest patients, who are managed with bronchus-blocking balloons. The recipient is positioned on the operating table supine, with the arms suspended above. We have developed an attachment to the operating room table to facilitate this positioning. The chest is entered through an anterior transverse bilateral thoracosternotomy in the fifth or fourth interspace. The skin incision is submammary in location (Figure 1). The exposure afforded by this incision is excellent for mobilization of the lungs and division of pleural adhesions. The hila can be readily accessed for control of bleeding from bronchial arteries, and for the performance of the anastomoses (Figure 2).

Manipulation of the lungs often results in augmenting secretion content in the main airways, requiring attention to airway toilet by the anesthesiologist. A preoperative perfusion scan, performed at the time of evaluation, may identify patients who have a discrepant perfusion to one lung. The lung with the least perfusion is usually removed first. If perfusion is relatively equally distributed, then our preference is to perform right pneumonectomy and transplant first.

The pulmonary artery (PA) should be divided distal to its first division, because there is often a considerable size discrepancy between the recipient's PA, which may be larger due to chronic pulmonary hypertension, and the donor's normal-sized PA. The pulmonary veins are divided. We prefer to staple the veins adjacent to the left atrium. The remainder of the inferior pulmonary ligament is divided with electrocautery, the distal bronchial divisions are clamped to minimize egress of purulent secretions, and the bronchus is divided with sharp dissection just proximal to the upper lobe takeoff. Hemorrhage from bronchial arteries is controlled with electrocautery or clips. An atrial cuff is then fashioned by incising the pericardium around the pulmonary venous stumps. On the right side, development of the interatrial groove facilitates securing an appropriate left atrial cuff.

The double lung block is divided into its constituent organs. The donor bronchus is trimmed back to within one or two rings of the upper lobe takeoff. Before placing the organ in the chest, we irrigate the open airway and pleural space with warm Betadine solution to evacuate purulent material in the upper airway. The lung is positioned in the chest surrounded by cold laparotomy sponges. The bronchial anastomosis is performed first. We prefer an end-to-end anastomosis and use polyglycolic acid suture, a running monofilament suture on the membranous portion, and interrupted braided sutures on the cartilaginous portion (Figure 3).

A Satinsky clamp is placed on the left atrium to include the pulmonary venous stumps, which are amputated, and a recipient left atrial cuff is fashioned. This is sutured to the atrial cuff on the donor lung with running 5/0 polypropylene suture. A Debakey vascular clamp is then positioned on the PA stump, the ligatures are excised, and the recipient artery is trimmed back for anastomosis to the donor PA. It is important to trim the PA so that it is of an appropriate length at the completion of the transplant. If the PA is too long, kinking may result. It is also important to align the artery end appropriately so that there is no twisting. Leaving the upper division on the recipient's PA until the time of trimming facilitates this alignment with the upper division of the

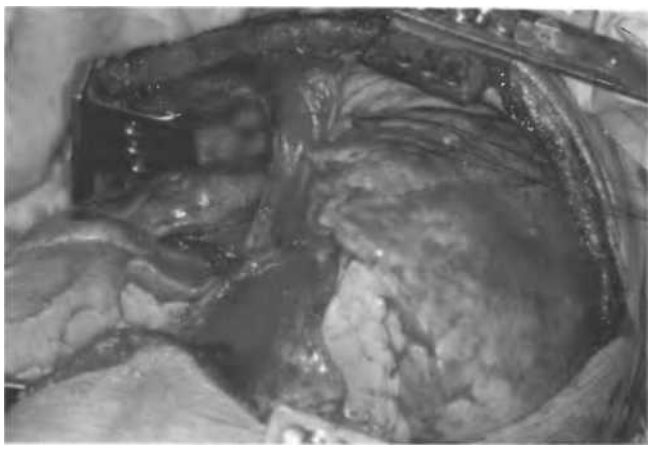

Figure 2 Exposure afforded by the 'clamshell' incision. Both pleural spaces – as well as the great vessels in the mediastinum and pericardium for cannulation – can be accessed easily. (Reprinted with permission from ref. 58)

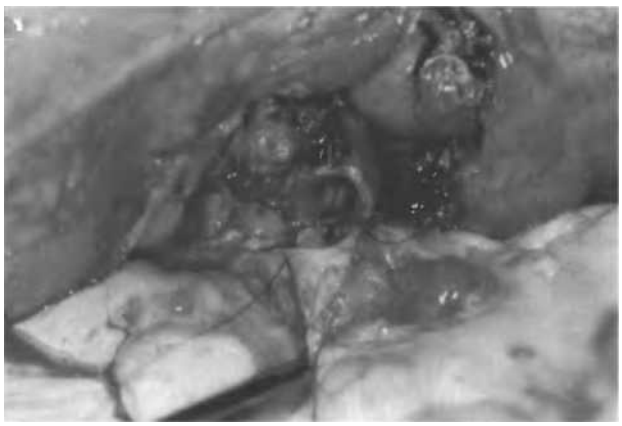

Figure 3 The posterior wall of the bronchial anastomosis has been completed with running Maxon (Davis and Geck) suture. The cartilaginous portion will be approximated with interrupted sutures of Dexon II (Davis and Geck). (Reprinted with permission from ref. 58)

donor lung. The PA anastomosis is interrupted in two or three places, in an attempt to prevent purse-stringing. Prior to removing the vascular clamps, the lung is de-aired. We prefer to do this by back-bleeding from the left atrium, initially allowing egress of air through an open left atrial anastomosis and then anticipating egress of preservation solution and air through the open PA anastomosis. During this maneuver the lung is hand-ventilated. The PA is de-aired by momentarily unclamping the PA while temporarily occluding outflow beyond the anastomosis, forcing air and any debris out through the open suture line before securing it.

Attention is then directed to the opposite side, where pneumonectomy and implantation are carried out in the same manner. Cardiopulmonary bypass are always available, but is generally avoided. Indications to institute bypass are inability to tolerate one-lung anesthesia during pneumonectomy or transplant, or the early development of pulmonary edema in the transplanted lung during contralateral pneumonectomy or transplantation. Should the need for bypass arise, the pericardium is opened in the midline and the ascending aorta and right atrium are cannulated in the routine manner. We have found that the transfusion requirement is substantially higher in patients who undergo the procedure on bypass[7]. The use of aprotinin may reduce transfusion requirements for these patients.

Some controversy exists as to the appropriate method of airway anastomosis. We have continued to use omentopexy in an attempt to improve airway blood flow, and we interpose tissue between the bronchial anastomosis and the PA anastomosis. In a small number of patients we demonstrated that this approach was superior to telescoping anastomosis without omentopexy[33]. However, as more experience has been accrued, it is becoming increasingly clear that omentopexy may not be required for satisfactory airway healing[34–36].

Upon completion of the procedure, two chest tubes are placed in each pleural space through anterior stab wounds. A curved tube over the diaphragm is positioned posteriorly in the gutter, and a straight tube is placed up to the apex on each side. Purse-string sutures are placed around the tubes to secure them and to allow subsequent chest tube removal, because CF lung transplant recipients are so thin that they can leak air through the chest tube tract after removal, resulting in pneumothorax. The sternal edges are re-approximated with wires, and the incision is closed in layers.

POSTOPERATIVE CARE

After transplantation, appropriate analgesic management is imperative to allow for early extubation and ambulation. Preoperative participation in a rehabilitation program of aerobic exercise facilitates early ambulation and early extubation. Epidural anesthesia has been helpful in many patients, but may be precluded because of frequent problems with altered clotting parameters postoperatively. These problems are often caused by mild coexisting hepatic insufficiency related to CF.

Our immunosuppression protocol has consisted of intravenous cyclosporin in an attempt to maintain blood levels at 450 ng/ml (fluorescence polarization monoclonal immunoassay, Abbott Laboratories), azathioprine (2 mg/kg), and antilymphocyte globulin (Atgam, Upjohn, Kalamazoo, MI) at 15 mg/kg daily for 14 days. We avoid introducing systemic steroids until day 14. Episodes of acute rejection diagnosed either histologically or clinically are treated with bolus methylprednisolone (15 mg/kg for 2 days, then 7.5 mg/kg for 1 day). Prophylactic antibiotics are administered for the first week and are tailored to antibiotic sensitivities of preoperative sputum cultures and donor sputum cultures.

Absorption of oral cyclosporin is often problematic in patients with CF. We have used ketoconazole administration to reduce the rate of cyclosporin metabolism[37]. Other programs have used diltiazem for the same purpose[38]. Post-discharge, patients continue to participate in a program of aerobic exercise for 4–8 weeks. Fevers, infiltrates, or graft dysfunction are investigated with bronchoscopy, Bronchoalveolar lavage, and transbronchial biopsy.

RESULTS OF LUNG TRANSPLANTATION FOR CF

Isolated lung transplantation is a relatively new approach to patients with CF. By the end of 1990 the International Registry of the Society for Heart and Lung Transplantation had collected data on only 120 double lung transplant procedures worldwide; 33% of these were for CF[39]. By 1993 over 38% of double lung transplant procedures reported to the Registry were for CF, with over 300 procedures reported in 1993 alone[40]. The number of heart–lung transplant procedures reported to the International Registry has declined steadily since 1989, and CF is a diminishing indication for this procedure[40].

Outcome of CF lung transplant patients has continued to improve. The first report of double lung transplant for CF came from the University of Toronto, where 17 CF patients were transplanted over a 3-year period[21]. There were six deaths in hospital, four of which were caused by bacterial infection with *P. cepacia*. Actuarial survival was 58% at 12 months. Shennib and Noirclerc reported on the combined experience of double lung transplant for CF at the Montreal General Hospital in Montreal, Canada, and the Ste Marguerite Hospital in Marseilles, France. The 25 recipients had an operative survival of 76% and a 1-year actuarial survival of 64%[22]. Most recently, the Registry of the ISHLT reported a 1-year actuarial survival of 70% for all 303 CF recipients of double lung transplants reported over the lifetime of the Registry[41].

Our experience at the University of North Carolina has recently been updated[42]. Between October 1990 and August 1995, 58 CF patients underwent lung transplantation; the sequential bilateral technique was used in 56, and living-related bilateral lower lobe transplants in one recipient. The mean age was 24 years (range 8–45). Hospital stay averaged 29 days (range 14–129). Three patients experienced catastrophic graft failure and were retransplanted urgently; two survived, while one represents the only operative death in the series. Actuarial survival is shown in Figure 4. Cause of death within the first year included pneumonia (four patients; *P. cepacia* in two, viral in two), non-compliance or suicide (two patients), fungal brain infection (one patient), and unexplained increased intracranial pressure (one patient). Lymphoma was responsible for two deaths in the second post-transplant year.

Bronchiolitis obliterans syndrome (BOS)[43] was the predominant cause of death beyond a year. The development of BOS is common, but is well tolerated in many patients. Actuarial

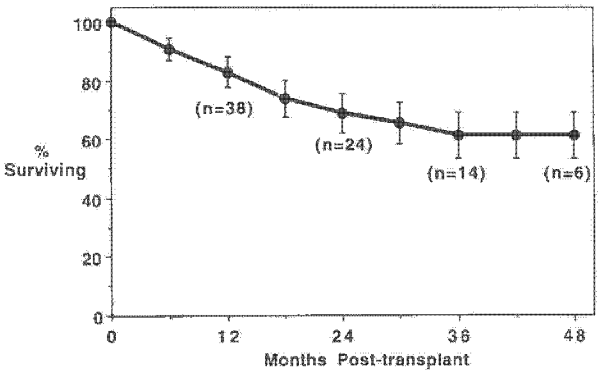

Figure 4 Actuarial survival of 58 lung transplants for CF at the University of North Carolina (± 95% confidence limits)

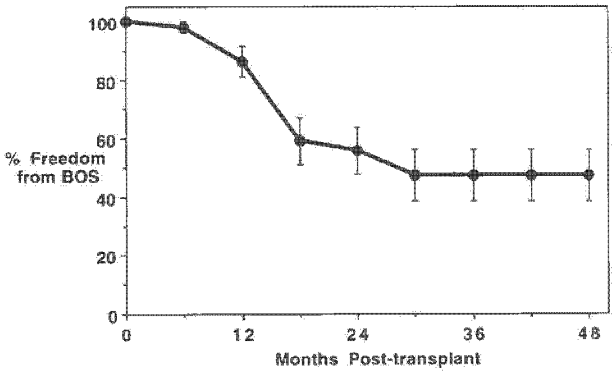

Figure 5 Actuarial freedom from bronchiolitis obliterans syndrome of CF lung transplant recipients at the University of North Carolina (± 95% confidence limits). Deaths from other causes were treated as 'withdrawn' for purposes of this analysis

freedom from development of BOS is depicted in Figure 5. Two CF patients have been retransplanted for severe bronchiolitis obliterans. One succumbed from CMV pneumonia 3 months later, while the second is alive and well 6 months after retransplant.

Postoperative complications have been reviewed in detail elsewhere[7]. Airway complications have been fortuitously infrequent. Three patients developed significant airway dehiscence, which was contained in two patients but was associated with the development of an intrathoracic abscess in our youngest recipient (Figure 6). These all healed without sequelae. No anastomotic strictures have developed, but significant narrowing of the bronchus intermedius has occurred in two patients; this was successfully treated by dilatation and insertion of a Dumon silastic stent[44].

Perioperative pulmonary infections are common, but not more so in CF patients than in non-CF patients at our institution[45]. Sinusitis is a frequent occurrence in long-term survivors. Some programs surgically drain sinuses of CF patients prior to transplant, to reduce bacterial burden[46], but we prefer to delay this procedure in asymptomatic patients until after transplant.

P. cepacia pneumonia was lethal, at $3\frac{1}{2}$ and 5 months postoperatively, in two of eight transplant recipients who grew *P. cepacia* prior to transplant. This organism has been recognized as a cause

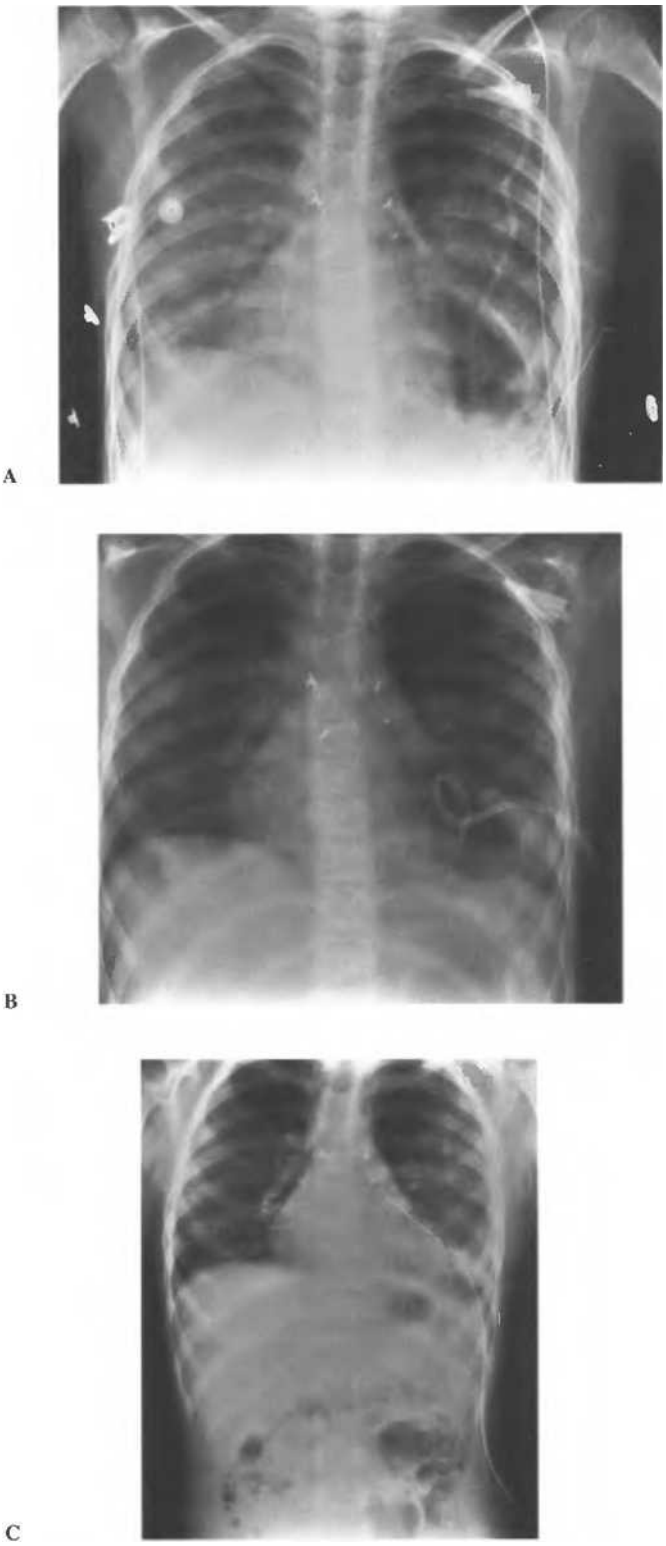

Figure 6 A: Chest X-ray demonstrating loculated air space associated with a leak from a partially dehisced left bronchial anastomosis after double lung transplant. B: A pigtail catheter was positioned into the space, but suction was inadequate to collapse the cavity. C: A no. 24 chest tube was advanced into the space, using the pigtail as a guide, resulting in resolution of the space. The bronchopleural fistula went on to heal without airway stricture

of increased morbidity following lung transplantation for CF at other centers[30], and its presence is considered a contraindication to transplant at some centers. We have continued to offer lung transplant to CF patients who harbour *P. cepacia*, as long as there are demonstrable antibiotic sensitivities *in vitro*.

Despite the incidence of BOS, there has been gratifying improvement in pulmonary function in survivors (Figure 7). Impact on quality of life has been documented by the Papworth group for CF recipients of heart–lung grafts at that institution. There was improvement in all six dimensions of the Nottingham Health Profile among CF transplant recipients[47]. A convincing testament to the utility and value of lung transplant procedures for CF patients comes from the recipients themselves. One of our long-term survivors, who has participated in the Transplant Olympic Games, has written: 'It is hard to take in the magnitude of the miracle and change in lifestyle the transplant has meant to me. I may still die a pulmonary death, someday, but the victory over CF is sweet'[48].

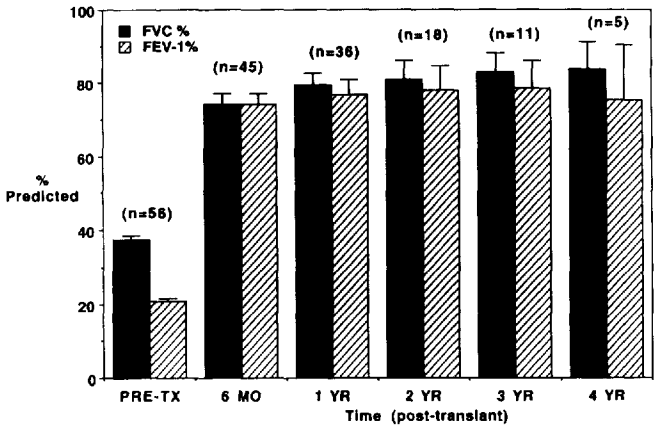

Figure 7 FVC and FEV_1 expressed as percentage predicted, at evaluation for listing (pretransplant) and at annual intervals after transplant (± SEM). The living-related lobe recipient is excluded

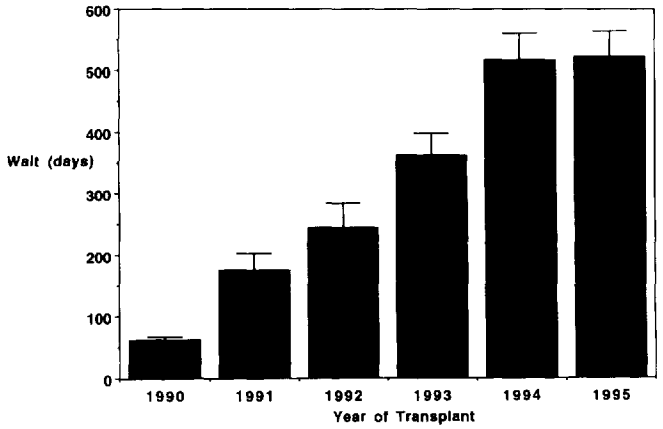

Figure 8 Mean waiting time according to year of transplant for CF recipients at the University of North Carolina (± SEM)

COMMENT

Substantial strides have been made in the field of lung transplantation in the past decade, particularly with regard to patients suffering from lung disease due to CF. There are currently two major impediments to further progress. One is the disturbing incidence and morbidity of bronchiolitis obliterans after successful transplantation. The magnitude of this problem has been outlined above. A better understanding of immune mechanisms of chronic rejection will perhaps allow the evolution of strategies to prevent or curtail the impact of bronchiolitis obliterans. The development of a large-animal model of post-transplant bronchiolitis holds promise for further study[49].

A more pressing problem for potential recipients of lung transplants is the scarcity of suitable lung donors. This has resulted in substantial increases in waiting time for lung transplant recipients. Because of current UNOS organ distribution algorithms, those awaiting bilateral lung transplant in the United States generally wait longer than those awaiting single lung transplant, which increases waiting time for CF patients. The annual increase in mean waiting time (from listing to transplant) for CF patients transplanted at UNC is depicted in Figure 8. This unfortunately translates into increasing numbers of deaths on the waiting list.

This problem has sparked interest in alternatives to conventional organ donors. One approach has been to consider living-related lobe donors[50]. For CF patients this requires identification of two healthy donors with the same or compatible blood types, who are sufficiently large enough (compared to the intended recipient) for a lower lobe to adequately occupy the hemithorax of the recipient. Early results of application of living-related lobe-transplants for patients with CF have been encouraging[51]. However, there are ethical concerns related to obtaining informed consent of donors and subjecting two donors to the risk of lobectomy. Anatomical considerations occasionally place the right middle lobe at risk[52]. For many potential recipients, particularly large males, identification of two appropriate donors is not an easy task.

While xenografts hold promise for eventually alleviating the organ donor shortage, there are substantial immunologic hurdles to overcome[53]. A more practical approach may be to consider lung retrieval after circulatory arrest and death[54]. We have shown a substantial delay in cell death, ultrastructural deterioration and high-energy phosphate depletion after circulatory arrest in ventilated rat lungs[55–57], implying that cadaver lung retrieval may have some merit. This could have a substantial impact on the size of the potential pulmonary donor pool.

Lung transplantation for CF has emerged in the past decade as an exciting therapy that can restore health and quality of life to a group of patients destined to die at a young age. Substantial improvements in actuarial survival following lung transplantation have been reported for CF patients. Bronchiolitis obliterans continues to be the leading cause of death *after* transplant, but failure to live *to* transplant because of scarcity of donors may be a larger problem facing CF transplant candidates.

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62
Lung Transplantation in Infants and Children – Indications, Surgical Techniques, and Special Considerations

J.E. DAVIS AND V.A. STARNES

INTRODUCTION

Lung transplantation (LTx) in infants and children has its roots in the adult LTx experience. The first LTx was performed by Hardy (Chapter 18, Figure 9) *et al.*[1] in 1963 on a patient with squamous cell carcinoma of the lung (Chapter 45). The patient lived for 17 days and died of renal failure. The autopsy results showed intact anastomoses. For the next 17 years there were numerous failures by those who attempted to repeat Hardy's feat until 1981, when Reitz (Chapter 66, Figure 5) *et al.*[1] accomplished the first heart–lung transplant. Then in 1983, Joel Cooper (Chapter 45, Figure 1) *et al.*[3] performed a successful single lung transplant. These advances were mainly due to the introduction of cyclosporin and improved bronchial anastomotic techniques, namely bronchial wrapping with an omental pedicle. These events mark the beginnings of pulmonary transplantation, and the lessons learned were applied to the pediatric heart–lung and lung transplant population in the late 1980s[4,5].

In this chapter we will examine LTx as it pertains to the pediatric patient with particular emphasis on: (a) indications, (b) timing of transplantation, (c) type of pulmonary transplantation, (d) various surgical techniques, and (e) special issues particular to the infant and child.

INDICATIONS

Indications for LTx in the pediatric population typically fall into one of four categories: (a) parenchymal pulmonary disease, (b) primary pulmonary hypertension, (c) secondary pulmonary hypertension, and (d) inadequate peripheral pulmonary vasculature. Decisions of timing and type of transplantation are particular to each diagnosis.

Parenchymal pulmonary disease

Parenchymal disorders of the lung requiring LTx can be divided into two classes – infected and non-infected. Cystic fibrosis and bronchiectasis are commonly accompanied by chronic airway infections. Bronchopulmonary dysplasia, idiopathic fibrosis, bronchiolitis obliterans, pulmonary alveolar proteinosis, congenital diaphragmatic hernia, *Proteus* syndrome, rheumatoid lung, and desquative interstitial pneumonitis are all non-infected diseases that may potentially benefit from LTx. In 1994 the Registry of the International Society for Heart and Lung Transplantation reported that, over the previous 10 years, 61% of lung transplants were carried out for diseases of the parenchyma.

Cystic fibrosis

Cystic fibrosis (CF) represents the most common indication for LTx in children. One in 2000 people carry the recessive gene for CF. When the homozygous state occurs, the individual is afflicted with CF. This is characterized by a defect in mucociliary function, which results in an inability to clear secretions, mucous plugging of the bronchioles, chronic pseudomonal pulmonary infections, chronic sinusitis, malabsorption, and diabetes. Patients typically succumb to their disease in the second to third decade of life.

LTx offers a form of palliation of the chronic destructive pulmonary process that characterizes CF, with results comparable to LTx for other conditions[6]. The evaluation and timing of LTx should be carried out with the natural history of the disease in mind. Signs of deterioration in a patient's clinical course include an increase in the number or severity of pulmonary infections within the past year. Objective data in the form of significantly subnormal pulmonary function tests can also be useful (Table 1).

Table 1 Criteria for lung transplantation in patients with cystic fibrosis*

Clinical signs
Increased number or severity of pulmonary infections
Weight loss
Pulmonary function tests
FEV_1 < 30% predicted post-bronchodilator
P_{O_2} < 55 mmHg
Hypercapnia

* From Trulock EP. Recipient selection. *Chest Surg Clin N Am.* 1993;3:9.

Since CF patients are chronically infected, they should undergo bilateral LTx. Single LTx is contraindicated due to the risk of spillover contamination from a retained chronically infected native lung. Patients with cor pulmonale secondary to long-standing pulmonary disease may need combined heart–lung transplantation.

Contraindications to LTx specific to patients with CF include ongoing active infection with pan-resistant organisms. Some centers consider infection of the sinuses or airways with *Pseudomonas cepacia* or *Aspergillus* to be a contraindication to LTx.

Primary pulmonary hypertension

Primary pulmonary hypertension (PPH) is an idiopathic process that results in vasoconstriction and obliteration of the pulmonary arteries, leading to increased afterload for the right ventricle. The right ventricle generates higher pressures to maintain pulmonary blood flow and this, in turn, leads to more vasoconstriction with vascular endothelial hypertrophy and hyperplasia, which with time become fixed changes[7]. PPH may be present for some time before symptoms occur. Consideration for LTx is usually brought on by symptoms of dyspnea with exertion. Catheterization criteria for PPH are given in Table 2. The timing of transplant in PPH should be based on the projected survival and the usual time lag before a donor is found.

The issue of survival time has been addressed by various groups. Rich and Levy[8] in 1984 examined the characteristics of survivors and non-survivors with PPH. They found that heart rate, right atrial pressure, cardiac index, stroke volume index, systemic vascular resistance, and pulmonary vascular resistance were all discriminators. A prospective study of 194 patients determined the mean survival to be 2.8 years, and that independent predictors of mortality were pulmonary artery pressure, right atrial pressure, and cardiac index[9]. Echocardiographic and Doppler indicators of survival (correlated with cardiac catheterization data) have also been examined[10]. These studies showed that pericardial effusions and Doppler indicators of increased right ventricular impedance correlate with pulmonary artery pressures and mortality. The median period of survival of patients diagnosed with PPH either clinically by hemodynamic criteria, or by echocardiographic/Doppler data, is 2.4 years. Glanville *et al.*[11] reviewed 90 adult patients with PPH and found the mean survival of 27 patients who died while waiting for heart–lung transplantation (HLTx) to be 50 months. The mean time to transplant in the 14 patients who underwent HLTx was 25 months.

Any patient with significant hemodynamic or echocardiographic evidence of PPH should undergo either single or bilateral sequential LTx, and those with severe right and left ventricular failure should have combined HLTx.

Table 2 Criteria for lung transplantation in patients with primary pulmonary hypertension

Resting MPAP > 60 mmHg
PVR > 6 Wood units
PCWP–MPAP > 15 mmHg
RAP > 10 mmHg
Moderate pericardial effusion
Cardiac index ≤ 2.5 l/min per m^2
NYHA III or IV
T-DEC ≤ –300 cm^2/s
AT < 62 ms

MPAP = mean pulmonary artery pressure; PVR = pulmonary vascular resistance; PCWP = pulmonary capillary wedge pressure; RAP = right atrial pressure; NYHA = New York Heart Association functional class; T-DEC = deceleration of Doppler tricuspid early flow velocity; AT = pulmonic Doppler acceleration time.

Secondary pulmonary hypertension

Secondary pulmonary hypertension occurs as a result of congenital heart defects in which significant left-to-right shunts exist. This hemodynamic situation can lead to progressive right ventricular failure, fixed pulmonary hypertension, and the development of Eisenmenger's physiology (ie. right-to-left shunt with cyanosis). The treatment of secondary pulmonary hypertension depends upon the particular cardiac defect as well as the degree of ventricular failure. Significant right and left ventricular failure necessitates HLTx, as do lesions with single ventricle physiology or complex defects in which repair may be suboptimal hemodynamically.

Congenital heart disease with reparable cardiac defects

Pulmonary hypertension in patients with heart defects that are readily repaired can undergo bilateral or single LTx. In the past 10 years, 8.4% of lung transplants have been performed on children with congenital heart disease[12]. Lupinetti *et al.*[13] and Bridges *et al.*[14] have each described series of patients with reparable cardiac lesions who then underwent either single of bilateral LTx. The lesions included atrial septal defect, ventricular septal defect, patent ductus arteriosus, atrioventricular canal, congenital pulmonary vein stenosis, and tetralogy of Fallot with absent pulmonary arteries. More complex lesions such as transposition of the great arteries and truncus arteriosus with secondary pulmonary hypertension could potentially be treated by repair and transplant. In evaluating patients with secondary pulmonary hypertension and congenital heart defects it is important to assess the status of both ventricles. Significant combined right and left ventricular dysfunction should be treated with HLTx.

Congenital heart disease with irreparable heart defects

In some cardiac defects, biventricular repair is not possible, or ventricular dysfunction is irreversible. In these patients with fixed pulmonary hypertension, HLTx is the only option. Unfortunately, there are no studies that examine the critical level of ventricular dysfunction that is irreversible, and the decision is made on a case-by-case basis. This decision becomes important if one considers the relative paucity of heart–lung donor blocks. Only 74 heart–lung transplants in patients less than 18 years old have been performed[15]. In 1994 there were 38 patients on the UNOS Registry waiting list[16]. Pathologies that would be indications for HLTx include complex lesions which have a relatively poor prognosis with primary repair (Table 3).

Congenital heart disease with inadequate pulmonary vasculature

Congenital heart lesions with disorders of the pulmonary arterial and venous systems have been variously treated. In cases in which absent main pulmonary arteries can be reconstructed, bilat-

Table 3 Indications for heart–lung transplantation

Complex lesions
Unbalanced atrioventricular canal
Truncus arteriosus
Congenital mitral stenosis
Aortic valve stenosis
Transposition of the great arteries
Univentricular lesions
Hypoplastic left heart syndrome
Tricuspid atresia with patent ductus arteriosus
Hypoplastic right ventricle
Ebstein's anomaly
Hemodynamic parameters
Left ventricular ejection fraction < 35%
Severe right ventricular failure

eral sequential LTx is an option[14]. Congenital pulmonary vein stenosis can also be corrected at the time of LTx. In those cases where the pulmonary arterial tree cannot be reconstructed, HLTx may be the best option.

PATIENT EVALUATION

Each prospective recipient, regardless of his/her particular diagnosis, should undergo a series of tests (Table 4) (Chapter 5) designed to better characterize the patient's preoperative status as well as identify possible risk factors (e.g. CMV, EBV) that have an impact after transplantation. These tests also assist in determining which patients are not candidates for transplantation due to significant systemic or multisystem disease. Patients with limited life expectancy due to non-pulmonary disease would likely not benefit from transplantation. Significant renal disease (creatinine >1.5 mg/dl and 24-hour creatinine clearance <50 ml/min) would limit the use of cyclosporin due to nephrotoxicity[17]. Hepatic disease, with increased bilirubin level, also places potential recipients at risk for bleeding, infection, poor wound healing, and impaired clearance of cyclosporin.

Table 4 Tests performed during evaluation of pediatric patients for lung transplantation

Blood type and screen
Cytomegalovirus (CMV) antibody
CMV IgM-specific antibody
Epstein Barr virus (EBV) serology
Human immunodeficiency virus (HIV) antibody
Hepatitis A antibody
Hepatitis B surface antigen
Hepatitis B surface antibody
Hepatitis B core antibody
Hepatitis C antibody
Herpes simplex antibody and IgM-specific antibody
Rapid plasma reagin (RPR)
Toxoplasmosis antibody and IgM-specific antibody
Polio neutralization
Varicella–zoster antibody
Measles IgM and IgG
Mumps titer
Rubella titer
Anti-nuclear antibody (ANA) screen
Diptheria pertussis tuberculosis (DPT) titer
Amylase, creatinine, blood urea nitrogen (BUN), electrolytes, chemistry panel, total iron binding capacity (TIBC), serum iron, ferritin, magnesium
Lipid panel
Protime (PT), Partial thromboplastin time (PTT)
Complete blood count (CBC) with differential
Erythrocyte sedimentation rate (ESR)
human leucocyte antigen (HLA) A, B, C, and DR types
Cytotoxic antibody (Panel Reactive Antibodies)
Urinalysis
Blood, urine, stool, and sputum cultures for acid fast bacilli, fungal and bacterial organisms
24-hour creatinine clearance
Properidin (PPD), cocci, *Candida*, tetanus skin tests
Pulmonary function tests
Chest radiography
Electrocardiogram (EKG)
Echocardiography

Patients who have undergone previous thoracic procedures are at an increased risk for significant bleeding, which in some instances can be uncontrollable. Nonetheless, previous operation is not an absolute contraindication to LTx. Patients being supported by ionotropic agents, mechanical ventilation or ECMO are also not automatically excluded from transplantation.

DONOR SELECTION

Potential lung and heart–lung donors should be thoroughly evaluated (Chapters 4 and 48). A complete history and physical examination are essential, and should focus on the mode of brain death and its effects on the donor organs, with specific reference to chest trauma, cardiac and pulmonary contusion, barotrauma, and aspiration. A prior history of malignancy, cardiac ischemia, smoking, drug use, adverse environmental exposure, or recent pulmonary infection should be sought. Donors should have been mechanically ventilated for not more than 4 days. The lung compliance should be normal with peak inspiratory pressures <30 mmHg with a tidal volume of 15 ml/kg. The chest radiograph should be normal. Cultures of sputum, blood and urine should be obtained; organisms found on sputum culture demand fiberoptic bronchoscopic examination with direct bronchial washing cultures. An arterial blood gas should be drawn. Suitable donors have a P_{O_2} of 100 on 40% inspired oxygen and >350 on 100% oxygen. Continual monitoring of the donor until harvest is mandatory, to detect deterioration[18].

Each recipient should have undergone evaluation to determine the acceptable size of the donor organ(s). Patients are listed according to ABO blood group. When the appropriate blood group organ is available, an assessment of size match is made. Lung and heart–lung size matching has previously been made on the basis of chest radiographic measurements – width at the aortic knob, width at the widest point, and length from apex to mid-hemidiaphragm. Recently, matching has been based on the vital capacity of the donor (as predicted by height and sex) compared to the calculated vital capacity of the recipient (Chapter 48).

SURGICAL TECHNIQUES

Excision of donor organs (see also Chapters 48 and 68)

Each thoracic organ retrieval procedure is usually carried out in concert with other procurement teams who will be harvesting various other donor organs, such as kidneys, pancreas, liver and small bowel. Abdominal organ harvests require significantly more

dissection and preparation time before the actual removal of the organs. During this time the integrity of the cardiopulmonary system must be maintained. Constant monitoring of heart rate, blood pressure, and amount of bleeding should be monitored. The chest is opened by a midline sternotomy[19], which also provides increased exposure of the upper abdomen, including the suprahepatic inferior vena cava.

The practice at the Children's Hospital, Los Angeles, is to harvest all donors as either double lung or heart–lung tissue blocks. However, the technique of single lung harvest will also be described for completeness.

Single lung harvest

Single LTx requires consideration of a number of factors including selection of the lung to be transplanted. If a previous thoracotomy has been performed in the recipient, then transplant to the contralateral side would be preferred. The left side is generally preferred due to: (a) the long length of recipient left main stem bronchus, (b) ease of clamping the left atrium, and (c) ability of the left thoracic cavity to accommodate oversized grafts.

The pericardium is resected and the inferior pulmonary ligament divided up to the inferior pulmonary vein. The right and left pulmonary arteries are dissected from the bifurcation to the hilum. Perfusion cannulae are placed in the ascending aorta and main pulmonary artery for instillation of cardioplegia and pulmonoplegia solutions. When the preparation of the abdominal organs is complete, excision of organs can begin. Prostaglandin E_1 is given intravenously. The superior vena cava is ligated and divided, and the inferior vena cava is transected. The tip of the left atrial appendage is amputated, and the aorta is cross-clamped. This maneuver decompresses the systemic and pulmonary venous returns.

At our center, cold Euro-Collins solution (10–20 ml/kg) is initially infused via the pulmonary artery to flush blood out of the lungs, and then University of Wisconsin solution (60–90 ml/kg) is used for preservation. Simultaneously, cold cardioplegia is delivered into the cross-clamped ascending aorta.

The pulmonary veins and a 5 mm cuff of left atrium are then divided from the remainder of the left atrium (Chapter 48, Figure 1). The pulmonary artery is transected at the bifurcation, and the mediastinal tissues are divided with the electrocautery down to the bronchus. The bronchus is then divided between two staple lines close to the carina, and the lung is removed (Figure 1). The graft is triple-bagged and placed in iced saline slush for transport.

Double lung harvest

Both lungs may be harvested *en bloc* for either double[20] (rarely used today) or bilateral sequential[21,22] LTx (illustrated in Chapter 48). In both instances the initial dissection and placement of perfusion catheters are identical to the single lung technique. After infusion of the cardioplegia and pulmonoplegia solutions the heart is excised. (In double lung harvests the posterior wall of the left atrium is retained with the lung block by transecting the left atrium along the interatrial groove.) Bilateral sequential donor lungs should be removed with a 5 mm cuff of atrium around each pair of pulmonary veins. The tissue block is then elevated from inferior to superior with dissection of all soft tissue off the descending aorta and esophagus. The trachea is stapled high, and the block is placed in a bag surrounded with iced saline slush. At the time of implantation each bronchus is divided so that two bronchial rings above the upper lobe bronchus are retained, along with preservation of the soft tissue around the bronchus.

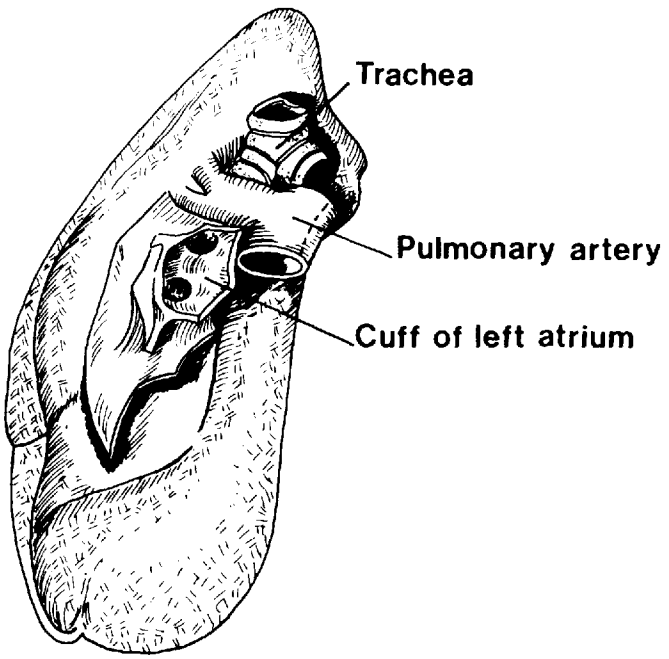

Figure 1 Excised donor right lung. Note the cuff of left atrial tissue around the orifices of the pulmonary veins. In this case a short segment of trachea and left main bronchus (both stapled) remain *in situ*, as does a short segment of pulmonary artery. The right bronchus and pulmonary artery will be trimmed at the time of implantation into the recipient

Heart–lung harvest (see also Chapter 68)

Heart–lung harvest is carried out in the fashion described by Reitz *et al.* in 1982[2] (illustrated in Chapter 68). After median sternotomy, total pericardiectomy, thymectomy, ligation of the azygos vein, and dissection of the aorta, pulmonary artery and venae cavae, a cardioplegia line is placed into the ascending aorta. Prostaglandin E_1 is given[4] at a dose of 125 ng kg^{-1} min^{-1}. The superior vena cava is doubly ligated and divided, and the inferior vena cava is transected. The aorta is cross-clamped and the heart is arrested with cold cardioplegia. The tip of the left atrial appendage is amputated, and the lungs are flushed with University of Wisconsin solution (60–90 ml/kg). Topical cold saline is applied to the organs. The tissue block is resected by dividing the aorta. The posterior attachments of the pericardium and pulmonary ligaments are divided. The heart and lungs are elevated superiorly, and the trachea is dissected high into the mediastinum and is transected proximal to a staple line applied with the lungs inflated. The organs are placed in a sterile plastic bag and immersed in cold iced saline and slush.

Recipient operative procedures

The operative plan for each type of procedure should take into account the possibility of needing cardiopulmonary bypass. Heart–lung, double lung, bilateral sequential lung, and single left lung transplants in patients with femoral vessels too small for cannulation should be performed through a median sternotomy or bilateral thoracotomy with sternal transection (Chapter 50, Figure 1). The latter incision is particularly useful in patients with adhesions, and provides excellent exposure. Single right or left LTx in patients with femoral vessels large enough to accommodate femoral–femoral bypass can be carried out through a thoracotomy incision. Lung translants that require correction of cardiac defects should generally be performed through a median sternotomy, with the possible exception of isolated atrial septal defects.

Single lung transplantation (see also Chapter 50)

Excision of the recipient native lung[20] is performed with cardiopulmonary bypass standby. Through a thoracotomy, the pulmonary veins are circumferentially dissected extrapericardially. The pulmonary artery is dissected as far distal as possible. On the right side the azygos vein may be ligated to mobilize the superior vena cava, and on the left side the ligamentum arteriosus may be divided. The pulmonary artery is temporarily occluded in an effort to determine the need for cardiopulmonary bypass. If the patient remains stable, the pulmonary artery is divided distal to its first branch, the pulmonary veins are divided extrapericardially, and the bronchus is divided just proximal to the upper lobe bronchus. The lung is removed, and the pericardium surrounding the pulmonary veins is dissected circumferentially. An adequate side-biting clamp is placed on the left atrium significantly proximal to the ligated branches. The veins are opened and connected with a longitudinal incision, resulting in a single atrial cuff.

The donor lung is placed in the thorax and the donor venous cuff is anastomosed to the recipient left atrial cuff with a continuous non-absorbable suture (Figure 2). The bronchial stumps are then brought into proximity. The selection of the particular technique of bronchial anastomosis should be based upon the relative sizes of the bronchi. Bronchial stumps of equal size can be anastomosed with a continuous running polypropylene suture without telescoping. Bronchial stumps of unequal size can be anastomosed with a continuous polypropylene suture using a telescoping technique[20]. Alternatively, others employ a continuous non-absorbable suture[23] or interrupted sutures. The pulmonary artery anastomosis is then completed. Back-bleeding from the pulmonary artery effectively flushes air from the pulmonary vasculature, and is a good sign of adequate pulmonary preservation. Omental wrapping of the bronchial anastomosis is not routinely carried out[13,24] (generally, the omentum of the child is not suitable for this purpose). If needed, the bronchus can be covered with pericardium or a pericardial fat pad pedicle[25].

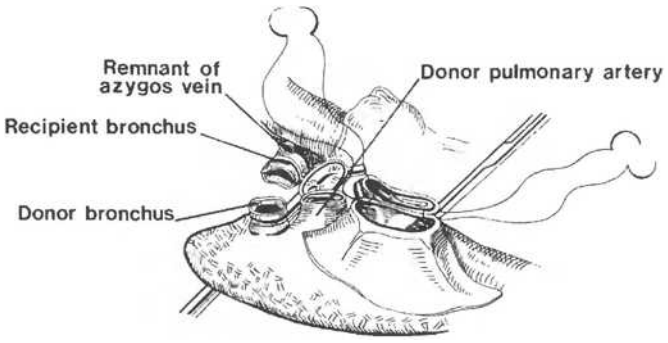

Figure 2 The donor right lung has been placed in the recipient right pleural cavity, and the left atrial anastomosis is in progress, beginning with the posterior wall. This will be followed by anastomoses of the bronchi and, finally, the pulmonary arteries. (The order of anastomosis varies from center to center, with many groups beginning with the bronchial anastomosis)

Bilateral sequential lung transplantation (see also Chapter 50)

The technique of bilateral sequential LTx is identical to the single lung technique. Commonly, in small children and infants, cardiopulmonary bypass is necessary and, if used, can allow irrigation of the native trachea and bronchi in patients with cystic fibrosis (after resection of both native lungs and prior to implantation of the donor lungs[24]). Since cystic fibrosis is a common indication, bilateral thoracotomy with trans-sternal division is usually the approach of choice.

Heart–lung transplantation (see also Chapter 68)

The combined HLTx techniques are those reported in the adult patient[2] (and are illustrated in Chapter 68). An anterior pericardiectomy, with the phrenic nerves preserved on pedicles, is performed. The patient is placed on cardiopulmonary bypass via bicaval and ascending aortic cannulation. The cavae are snared around the cannulae, and the aorta is cross-clamped. The aorta and pulmonary artery are transected. The heart is excised along the atrioventricular groove, and the pulmonary venous orifices are connected on each side. The mainstem bronchi are stapled just proximal to the upper lobe bronchi, and divided. The pulmonary arteries are divided in the hila and the veins are transected. The lungs are removed. The recipient pulmonary artery remnants are resected, with care being taken to preserve the ligamentum and the left recurrent laryngeal nerve. The bronchial stumps are dissected to the trachea, which is transected just above the carina. The donor heart–lung block is then placed in the chest by passing the right lung behind the right phrenic nerve pedicle and the left lung behind the left phrenic nerve pedicle. The tracheal anastomosis is preferred, followed by the right atrial cuff and aortic anastomoses.

SPECIAL CONSIDERATIONS

Immunosuppression

Immunosuppression is based on four drugs – corticosteroids, azathioprine, cyclosporin and tacrolimus (FK506). The current immunosuppression protocol at the Children's Hospital, Los Angeles, is outlined in Table 5.

Table 5 Immunosupression protocol at the Children's Hospital, Los Angeles

Preoperative
Cyclosporine 2 mg/kg i.v. over 2–6 h
Azathioprine 4 mg/kg i.v. over 30 min
Intraoperative
Methylprednisolone 15 mg/kg i.v. when weaning from cardiopulmonary bypass
Postoperative
Cyclosporine 2 mg/kg every 12 h p.o.
Azathioprine 2 mg/kg daily p.o.
Methylprednisolone 5 mg/kg every 8 h p.o. for 24 h, then 0.5 mg/kg every 12 h. Weaned by 0.1–0.2 mg/kg per week until a dose of 0.2 mg/kg per day is reached

Corticosteriods

In the early history of LTx the use of corticosteroids was shown to increase the incidence of bronchial anastomotic complications[26]. This led to the avoidance of steroids in the early postoperative period. Advances in bronchial anastomotic techniques have allowed steroids to be used intraoperatively and postoperatively without an increased incidence of bronchial complications. The issue has now turned to the long-term use of corticosteroids in children. Prolonged use of steroids leads to growth retardation, hypertension, osteoporosis, diabetes, and avascular necrosis[27]. Concern regarding these effects has led some centers to discontinue steroids post-transplantation (usually after 1 year) or to avoid their use altogether. A recent survey[24] of pediatric lung transplant centers indicated that only six of the reported 135 patients were totally managed without steroids, and a further eight had steroids discontinued at some stage during the post-transplant period. Further studies will be needed to assess the safety of this approach.

Cyclosporine versus tacrolimus

The introduction of cyclosporin in the early 1980s allowed organ transplantation to advance significantly. Problems associated with bronchial healing began to dissipate, and cyclosporin was given much of the credit. The introduction of tacrolimus (FK506) has provided an alternative to cyclosporin-based regimens. Tacrolimus, like cyclosporin, exerts its immunosuppressive effect by inhibiting transcription of the interleukin-2 gene[28]. It also has a similar nephrotoxic effect[29]. Unlike cyclosporin, tacrolimus does not cause hypertension, hirsutism, gingival hyperplasia or facial bone growth abnormalities. This is an important difference in the pediatric patient.

Clinical trials using tacrolimus in pediatric heart transplant patients have shown significant therapeutic benefits[30]. Based on these results the transplantation group at the University of Pittsburgh has conducted a trial of tacrolimus in pediatric lung and heart–lung patients[31]. In this small group of patients the incidence of acute rejection has been equivalent to that seen in patients treated with cyclosporin; none required antilymphocyte agents for treatment of rejection. This may be significant, since the development of bronchiolitis obliterans has been associated with the number and severity of acute rejection episodes[32]. The effect of tacrolimus on the occurrence of bronchiolitis obliterans has yet to be determined.

Post-transplantation lymphoproliferative disease (PTLD)

The development of PTLD is related to (a) the degree of immunosuppression and (b) infection with the Epstein–Barr virus (EBV)[33,34]. The pediatric LTx patient is especially prone to the development of PTLD[35]. This unique form of lymphoma usually occurs 12–18 months after transplantation in both nodal and extranodal sites, generally in the abdomen. Up to 40% of patients who develop PTLD undergo remission following a decrease in immunosuppression. EBV serologies are therefore obtained on all recipients and donors. Some centers reduce immunosuppression to either cyclosporin or tacrolimus alone if EBV-negative recipients receive an EBV-positive organ[36], and then monitor aggressively for signs of EBV infection.

Management of secondary pulmonary hypertension and complex cardiac defects

The availability of heart–lung donors does not currently meet the need. In an effort to treat patients with secondary hypertension and congenital heart defects, an increasing number of patients are undergoing combined LTx with cardiac repair. This has the benefit of distributing organs from one donor to a greater number of recipients. In addition, the waiting time for potential LTx recipients is half that of HLTx candidates[37]. The definition of what is a correctable cardiac lesion has not been determined, but certain concepts can help as a guide in the decision as to whether to repair a cardiac lesion or perform HLTx.

The maintenance of a biventricular physiology is mandatory. An attempt to perform a univentricular repair with a systemic–pulmonary shunt and LTx may be technically possible, but conversion to a cavopulmonary connection may not be possible, due to high mean pulmonary artery pressures. Recovery of right ventricular function after LTx does occur, and is therefore not in itself an indication for HLTx[38]. Significant left ventricular dysfunction (LVEF <50%) would also favor HLTx versus LTx alone. Bridges *et al.*[14] have also suggested that significant valvular abnormalities predispose the immunocompromised patient to endocarditis, and therefore HLTx may be indicated. Potential negative factors related to HLTx versus LTx include: (a) the added possibility of cardiac rejection and (b) the tracheal anastomosis, which may possibly be prone to complications[39].

Single versus bilateral sequential lung transplantation for pulmonary hypertension

Primary and secondary pulmonary hypertension affects both lungs, presumably in an equal manner. However, it is not necessary to transplant both lungs in order to alleviate the hypertension. Each type of transplant has its benefits and disadvantages. The use of single LTx distributes lungs to more recipients, and cardiopulmonary bypass can frequently be avoided. Single LTx has been used successfully for a number of indications in patients with pulmonary hypertension.

Fremes *et al.*[40] reported the closure of a patent ductus arteriosus with single LTx for the successful treatment of Eisenmenger's syndrome. Bando *et al.*[41] reported the results of 11 single LTx for

pulmonary hypertension, of whom six died of graft-related disease. The survival of this group, however, was not significantly different from those who underwent bilateral LTx or HLTx for the same indication. Lupinetti *et al.*[13] and Bridges *et al.*[14] each reported series of patients who underwent LTx and cardiac repair. Of the 10 combined patients, seven had died by the time of the reports.

The physiologic concern with single LTx has been the fact that the grafted lung receives nearly all of the blood flow, while ventilation remains equal between the transplanted and native lungs[41]. Given these factors, it would seem prudent to reserve single LTx for patients with primary pulmonary hypertension whose survival is limited[42]. Single LTx combined with cardiac defect repair has a particularly poor outcome and, given the better longevity of patients with secondary pulmonary hypertension[43], bilateral sequential LTx would seem to be the wisest course of action.

Cytomegalovirus and obliterative bronchiolitis

Each recipient and donor undergoes serologic testing for previous cytomegalovirus (CMV) exposure. As decisions regarding donor–recipient matching are not based upon these serologic findings, mismatches in serologic status occur. In addition, all blood products given to transplant patients are CMV-negative. Mismatches (donor CMV+/recipient CMV–, donor–/recipient+) and donor+/recipient+ transplants are treated prophylactically with ganciclovir (Table 6).

Despite prophylactic measures, CMV infection still occurs in pediatric patients with mismatched serologies[45]. This is significant in that the development of obliterative bronchiolitis has been linked to CMV infection in adult LTx recipients[41]. Geist *et al.*[44] have shown that the gene products of CMV infections prevent the inhibitory effects of cyclosporin on interleukin 2, which could allow for normal T cell function and increased rejection. The number and severity of rejection episodes seem to be associated with the development of obliterative bronchiolitis, so efforts to reduce CMV infection and rejection episodes may have a significant impact on patient survival. Current trials examining the effect of tacrolimus versus cyclosporin on the development of obliterative bronchiolitis are ongoing; preliminary results in adults indicate that the number of rejection episodes is decreased in the first 6 months after LTx[45], but the effects on obliterative bronchiolitis are as yet unknown.

Table 6 CMV prophylaxis at the Children's Hospital, Los Angeles

D+/R+ and D+/R–
Days 1–21: intravenous immunoglobulin therapy
Days 14–42: ganciclovir 6 mg/kg per day
Day 42–6 months: acyclovir 100 mg/kg p.o. three times daily
D–/R+
Days 14–42: ganciclovir 6 mg/kg per day
Day 42–6 months: acyclovir 100 mg/kg p.o. three times daily
D–/R–
No prophylaxis

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Airway Complications in Children Following Lung Transplantation

C.B. HUDDLESTON

INTRODUCTION

Historically, disruption of the airway suture line was the major source of morbidity and mortality in the early days of lung transplantation in adults, particularly in the setting of an *en-bloc* double lung transplant[1-3]. Modifications of the technique by wrapping the anastomosis with either omentum or other viable tissues[4] and performing bi-bronchial anastomoses provided reasonable solutions to this problem[5]. Airway anastomotic complications are now a less serious but persistently vexing problem, with an incidence in adult transplants ranging from 10% to 30% of bronchial anastomoses at risk[6-10]. Most of these bronchial complications are due to stenosis. The incidence of airway complications in the pediatric age group, and those factors leading to this set of problems following lung transplantation, are not well defined due to less experience in children. The experience in adults may not be easily translated to children, for a variety of reasons, including the smaller size of the airways being anastomosed and the expected somatic growth that will occur. This is a review of the literature and of our own experience in children, to evaluate the incidence, risk factors and appropriate treatment associated with these complications in the pediatric population subjected to transplantation.

AIRWAY CHARACTERISTICS UNIQUE TO CHILDREN

In general, the bronchus is mature in virtually all aspects shortly after birth. The blood supply is the same as that seen in adults. The bronchial cartilage is somewhat more compliant and soft in infants than in older children, and particularly adults. This may predispose to malacia of the airway when the donor is young. The major issues that could impact on airway complications relate to the smaller size *per se* and the need for growth when transplanting very small children rather than to the biology of the bronchus.

UNIQUENESS OF THE BRONCHIAL ANASTOMOSIS

Bronchial blood supply

Normally there are two or three bronchial arteries arising from the aorta. They enter the lung through the hilar region and divide upon reaching the mainstem bronchus. A plexus is formed in the peribronchial space and small arterioles penetrate the muscular layer of the airway to reach the bronchial mucosa, where they form the submucosal plexus from the mainstem bronchus to the terminal bronchioles. At this level the capillary bed from the pulmonary arterial blood supply communicates with the bronchial capillary system[11,12]. In general, direct bronchial revascularization is not performed in lung transplantation. Thus, blood supply to the bronchus reaches that point by retrograde flow from the pulmonary capillary system into the bronchial capillary system through the submucosal plexus back to the mainstem bronchus to reach the anastomosis. It is this tenuous system of vessels that the proximal donor bronchus depends upon for its nutrient source in the early stages after lung transplantation (Figure 1).

Revascularization of the anastomotic region may be accomplished by either direct bronchial arterial revascularization or 'indirect' revascularization – wrapping the anastomosis with viable tissue which can provide a source for ingrowth of new vessels. Some centers utilize direct bronchial revascularization with a low reported incidence of airway complications. These are all adult patients and relatively small series, however[13,14]. The disadvantages of direct revascularization are that it is cumbersome, adds to an already lengthy procedure, adds ischemic time to the transplanted organs and increases the risks of bleeding.

Indirect revascularization using omentopexy or other viable tissue flaps was at one time the standard[15-17]. There is experimental evidence of ingrowth of new vessels to the area of the bronchial anastomosis as early as 4 days post-transplant[18] and extensive neovascularization of the bronchial circulation via collaterals within 3–4 weeks[19]. However, most have now abandoned this adjunct to lung transplantation, and cover the bronchial anastomosis with viable donor peribronchial tissue; this has provided clinical results similar to that achieved with omentopexy, pericardial flaps and intercostal muscle flaps, and avoids the need for laparotomy or mobilization of other tissues. The donor peribronchial tissue is viable and probably provides for the rapid ingrowth of new vessels to the area of the anastomosis in a fashion similar to omentum, although there is no experimental evidence for this. There is, however, experimental evidence that the bronchus will heal normally with no wrap at all[20], thus taking us

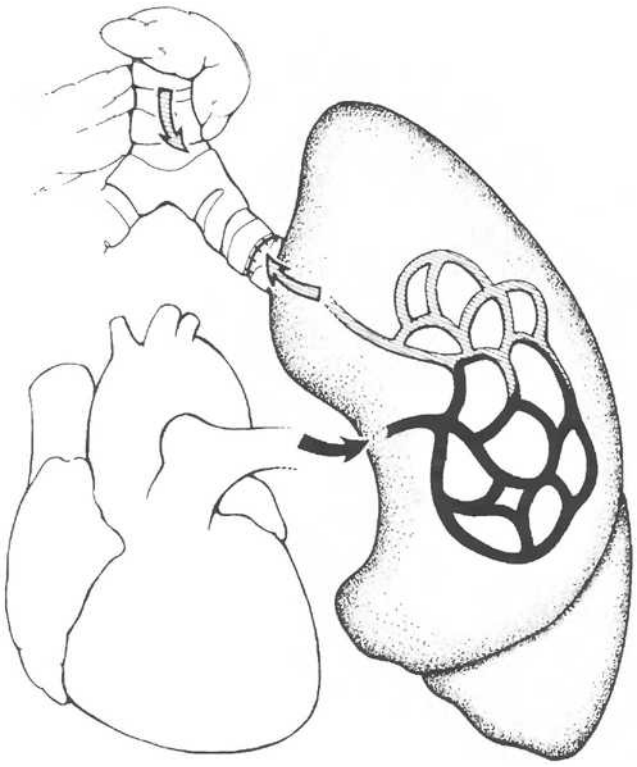

Figure 1 Antegrade flow to the proximal mainstem bronchus stops at the suture line. Nutrient supply to the area of the anastomosis must come retrograde via collaterals between the pulmonary and bronchial circulations

full circle. It is prudent to provide some coverage of the suture line with the peribronchial tissue if for no other reason than to protect the pulmonary artery anastomosis should there be any breakdown at all of the bronchial anastomosis.

It is likely that most airway complications are related to ischemia at the anastomosis. Although omentum and other vascular pedicles may provide indirect revascularization this requires days in which the anastomosis must live off the retrograde perfusion from the pulmonary arterial system. It is relatively common to see bronchoscopic evidence of some ischemia in the proximal donor bronchus – usually a bluish discoloration or, if more severe, a gray appearance that may extend to the level of the orifice of the upper lobe bronchus. This usually resolves over the first week following transplantation. Given that the early blood supply to the anastomotic region is crucial in avoiding airway complications, efforts should be extended to maintain this tenuous collateral microvascular blood flow. Maintenance of excellent pulmonary blood flow, pulmonary preservation, and handling of the proximal donor bronchus are factors which impact upon this.

Pulmonary blood flow

Obviously maintenance of good cardiac output is crucial in the care of any patient postoperatively. In children the luxury of measuring cardiac output by the thermodilution technique is seldom available, except in the teenage group. In general, reliance on clinical assessment of the cardiac output is more frequently necessary – pedal pulses, capillary refill, warmth of extremities, urinary output, etc. In addition, pulmonary vasodilators may have a very positive impact on bronchial healing, as demonstrated by the group from Hannover[21]. They used prostacyclin as a pulmonary vasodilator for 48 hours post-transplant. Heparin was also administered to prevent thrombosis of any small vessels in areas of low flow. They had no significant bronchial complications in 41 anastomoses at risk. We currently use prostaglandin E_1 (PGE_1) in all patients for 48 hours post-transplant in a dose of 25 ng/kg per minute.

Pulmonary preservation

This topic is covered elsewhere in this publication, and has been reviewed in depth by a number of investigators. We use modified Euro-Collins solution (50 ml/kg) after bolus injection of 500 μg of PGE_1. Poor pulmonary preservation and prolonged ischemic times place the microvasculature of the lung at risk; thus the flow via collateral vessels to the bronchial anastomotic area may be diminished. It has been shown experimentally that lungs with better preservation have better healing of the bronchus[22]. In a similar fashion, prolonged ischemic injury to the lung will injure the pulmonary microvasculature and thus diminish the collateral flow to the bronchus. In our series of 79 transplants in 71 patients (153 anastomoses at risk) the ischemic time tended to be longer in those developing complications, but did not reach statistical significance. Interestingly, most bronchial complications were seen on the right (nine versus four). The left lung transplant is always performed first at our center; thus the right lung has on average an additional 40 minutes of ischemic time compared to the left. To truly analyze whether the ischemic time is the causative factor would require randomization of the sequence of the transplants to be certain that other factors are not involved.

Handling of the bronchus

The donor bronchus should be trimmed to within one or two cartilaginous rings of the bifurcation of the mainstem bronchus. This shortens the distance that the collateral blood flow must travel to reach the anastomosis, but still allows sufficient length to perform the anastomosis[23]. When performing a lobar transplant we divide the bronchus just beyond the take-off of the upper lobe. We avoid skeletonizing the donor bronchus and also limit the amount of trauma from forceps grasping. To allow for the potential for growth, monofilament absorbable suture (polyglyconate) is used for the anastomosis. A study in lambs by Friedman *et al.*[24] has demonstrated that this allows for satisfactory growth of the airway anastomosis. Finally, there is the issue of whether the anastomosis should be telescoped. The San Antonio group has provided quite convincing data in adults that telescoping of the anastomosis provides the best results in airway healing[25]. In most cases the donor bronchus is of smaller diameter than the recipient and some telescoping is inevitable. However, in our experience and that of others[26], telescoping with a mattress suture has had a very high incidence of airway stenosis, and we no longer utilize this technique, instead using a simple suture technique.

OTHER RISK FACTORS

Age and size

We reviewed our series to evaluate the impact of the age and size of the patients on their risk for airway complications. Our concern was that small airways would be at greater risk for complications than larger ones. There was no difference between those that suffered bronchial complications in terms of average age (12.1±2.2 years) and those who did not (9.6±2.5 years). Likewise, there was no difference in terms of size between those who developed bronchial complications (28.7±3.0 kg, 132.5±4.7 cm) versus those who did not (24.1±3.7 kg, 118.2±5.7 cm). Looking at this another way, the incidence of bronchial complications in those under 10 kg was 9.1% (2/22); it was 15.8% (12/76) in those greater than 10 kg in weight. Thus, small size and young age did not portend an adverse prognosis in terms of bronchial complications.

Transplant diagnosis

In our series of pediatric lung transplants the pretransplant pulmonary diagnosis was not a risk factor. However, in comparing septic lung disease (basically cystic fibrosis) to non-septic lung disease (pulmonary fibrosis, pulmonary vascular disease, etc.) there was a trend for a higher rate of airway complications in the cystic fibrosis group than in the others lumped together (19.7% vs 9.8%). To evaluate this further, we looked at airway organisms to see if they might play a role in increasing the risk of complications. The presence of *Aspergillus fumigatus* resulted in a higher incidence of complications, but this did not reach statistical significance. Nonetheless, we currently aggressively treat *Aspergillus* both pre- and post-transplant with intravenous and aerosolized amphotericin B when it is cultured from the sputum. The greatest benefit in this is the prevention of a clinically significant *Aspergillus* infection, but it may also prevent airway complications.

Rejection

Rejection is very common following lung transplantation; in our series this occurred to a mild degree in 65–70% at least once in the first 3 months following lung transplantation. Rejection in a more severe form occurs in about half of our patients during the first 3 post-transplant months. Theoretically, rejection could pose a threat to the collateral blood supply to the bronchial anastomosis and there is experimental evidence for this[27,28]. However, it is unusual for there to be significant rejection during the first week post-transplant, and beyond that a new blood supply to the region of the bronchial anastomosis is reasonably well established via the bronchial wrap. Rejection did not prove to be a risk factor for airway complications in our series.

The use of steroids early following lung transplantation was at one time felt to be contraindicated because of its impact on healing in general, and bronchial healing specifically[29]. However, it has subsequently been shown that not only is it not deleterious, but it may be beneficial to have patients on low doses of steroids[30]. It is our practice to use steroids in modest doses early after transplant.

DIAGNOSIS OF BRONCHIAL COMPLICATIONS

The diagnosis of an airway dehiscence following lung transplantation is generally made by bronchoscopy, although chest computerized tomography can delineate these complications quite clearly at times, and provides more precise information in terms of extent of the dehiscence when partial than is generally obtainable from bronchoscopy. The presence of any new mediastinal air beyond 24 hours of the transplant is highly suggestive of this diagnosis (Figure 2). Prior to frank dehiscence, however, there is usually some suggestion of significant airway ischemia by gross inspection of the color of the bronchial mucosa by bronchoscopy. However, we have had one patient who developed complete dehiscence of the bronchus, whose initial bronchoscopy was essentially normal. She cultured *Aspergillus fumigatus* from the cut edge of the bronchus and presumably this was the underlying etiology of this dehiscence.

In our experience the detection of bronchial stenosis has at times been somewhat difficult. In most cases bronchoscopy has been the major diagnostic modality. However, there have been two instances in which the airway appeared reasonably patent in patients still ventilated post-transplant, in whom weaning from the ventilator failed repeatedly. Ultimately the diagnosis was made, stents were placed, and the ventilatory status markedly improved. Pulmonary function tests usually show a fall in FEV_1; this may be due to rejection or distal airway problems. However, the flow–volume loop provides a highly suggestive pattern in which the curves have some concavity[31]. Bronchoscopy is always mandated to confirm the findings and is best done with the patient breathing spontaneously. CT scans are occasionally helpful, but are not very sensitive.

TREATMENT OF BRONCHIAL COMPLICATIONS

Airway complications occur in two forms – dehiscence and stenosis. Complete dehiscence is generally catastrophic. Drainage alone is seldom adequate and an attempt at repair is appropriate as an initial step. It has a high failure rate and will probably result in transplant pneumonectomy. Fortunately this is relatively rare and in fact has occurred in only one of our patients, who interestingly grew *Aspergillus* from the bronchial margin, as mentioned above. Partial dehiscence can be treated expectantly, and generally will heal satisfactorily as long as there is adequate intraluminal drainage. When this involves the cartilaginous portion of the anastomosis there is an increased risk of stenosis in that airway as it heals; when the dehiscence involves only the membranous portion this generally heals without sequelae (Figures 3 and 4).

All patients with significant stenosis of the anastomosis will require some sort of intervention. Dilatation of the anastomosis was performed first in all instances in our patients, using a rigid bronchoscope or angioplasty balloon catheter. Some patients have presented with very tight airway stenoses of less than 2 mm in diameter. It is generally not safe to dilate such stenoses with the rigid bronchoscope, for a number of reasons: to engage the stricture would require a very small-diameter rigid bronchoscope, which usually would not be long enough to reach the stricture in the bronchus; ventilating through the small bronchoscopes will be associated with a significant air leak around the bronchoscope, particularly with an obstructed airway distally; and it is difficult

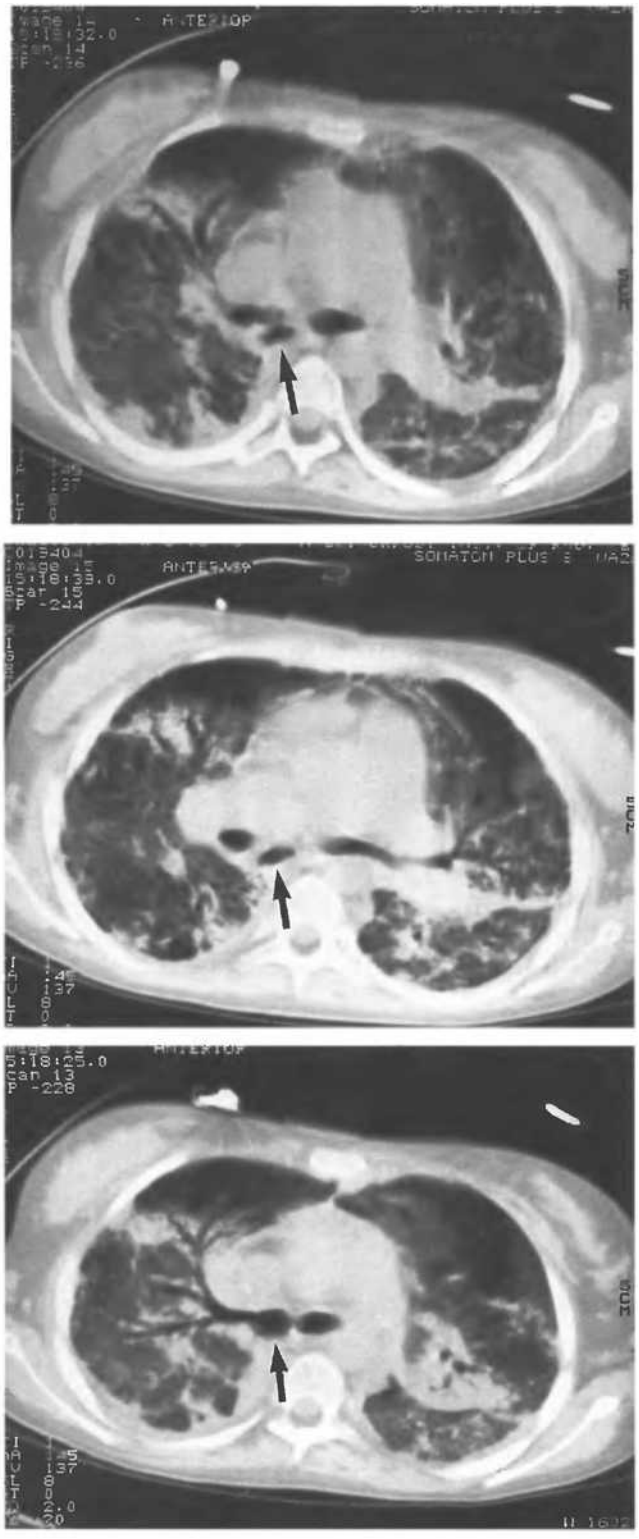

Figure 2 This CT scan of a patient was taken 4 weeks following lung transplantation for cystic fibrosis. **A,B**: Note the presence of air on the right posterior to the bronchus (arrow). There is also significant pulmonary parenchymal disease in this view. **C**: Following the CT scan further inferiorly, it can be easily noted that there is partial disruption at the area of the anastomosis; the two ends are held together somewhat tenuously

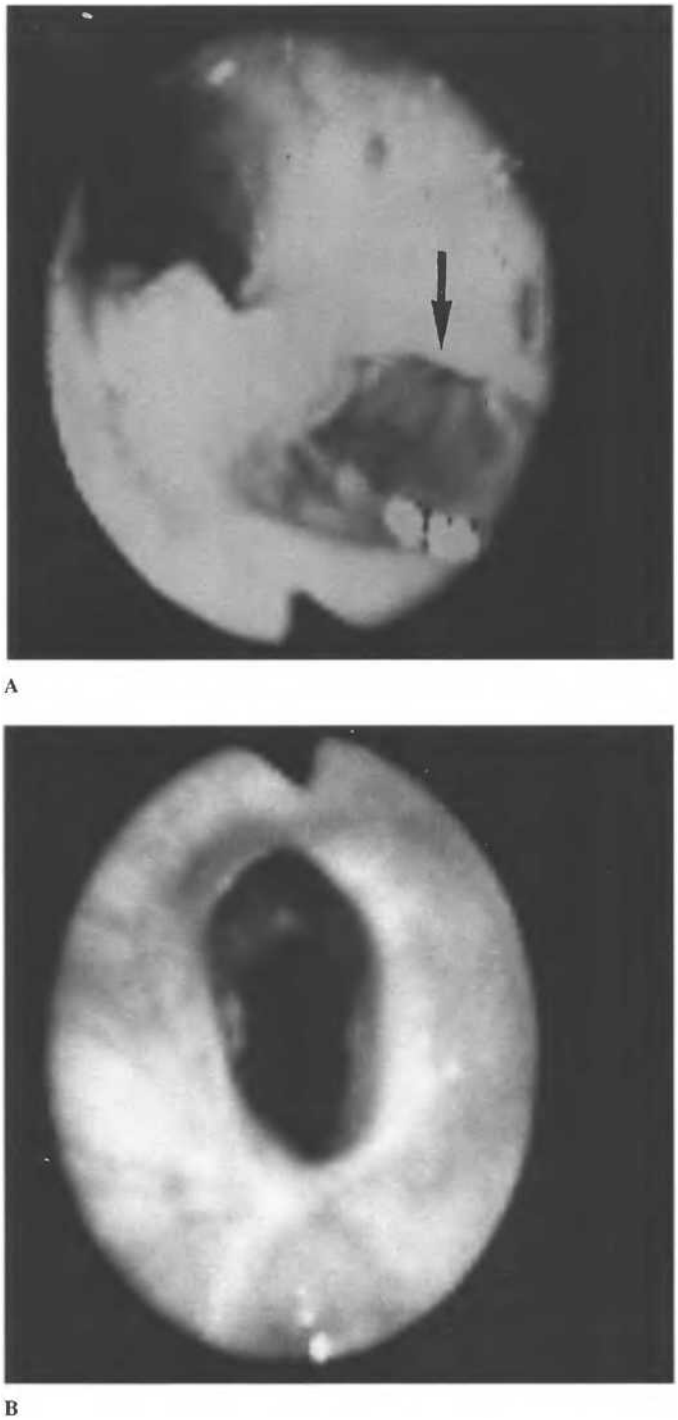

Figure 3 **A**: Bronchoscopic view of the partial dehiscence of the bronchus shown in Figure 2. The arrow points to the disruption and cavity produced. **B**: Bronchoscopic view of the same bronchus 4 months later, showing a nicely open bronchus with minimal or no stenosis

to judge the direction of insertion of the bronchoscope when performing a forceful dilatation, risking disruption of the bronchus. I prefer performing a stretching of the stricture using a rigid optical biopsy forceps or a grasping device through the rigid bronchoscope. Alternatively, an angioplasty balloon may be used over a

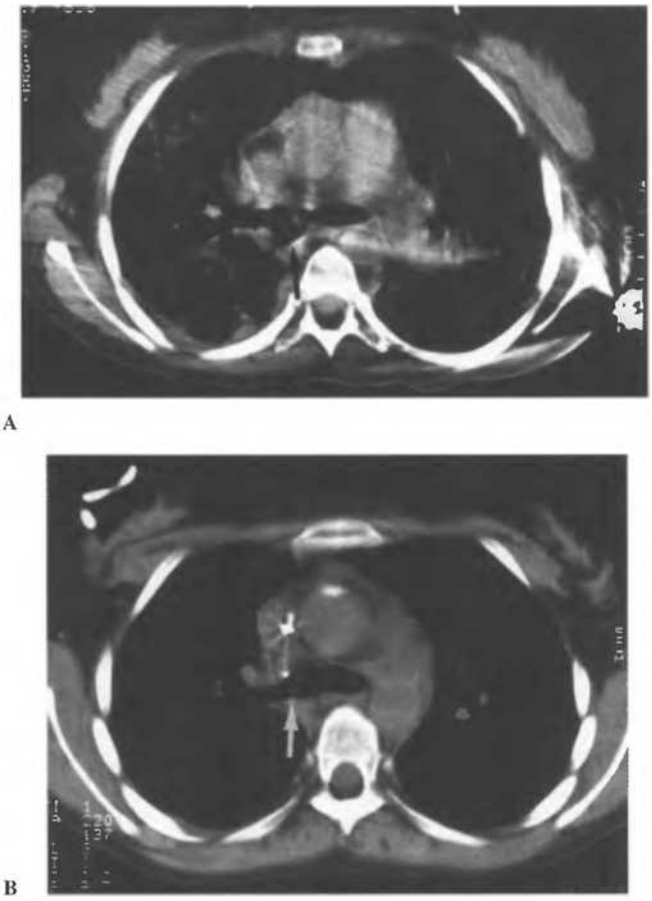

Figure 4 CT scans from the same patient depicted in Figures 2 and 3. **A**: CT scan taken at 4 weeks post-transplant, demonstrating the dehiscence. **B**: CT scan taken 4 months later, showing a healed bronchus and no extrabronchial mediastinal air. Note again that the lumen of the bronchus is wide open with no stenosis

wire if necessary. Fluoroscopic control of placement of this balloon catheter is quite helpful. Once a satisfactory opening in the bronchus is obtained dilatation with progressively larger rigid bronchoscopes is relatively straightforward, although the angioplasty balloon dilatation may be quite adequate on its own. Fogarty balloon catheters cannot be inflated to a sufficiently high pressure to effect a satisfactory dilatation, but may provide an initial opening through which a larger angioplasty balloon may be inserted. We prefer angioplasty catheters with a balloon length of 2–3 cm and a diameter nearly equal to what the expected diameter of the bronchus would be. It is unclear to what pressure these balloons should be inflated, with some going to 4 atm and others to 6 atm[32,33]. We inflate to the maximum advised for that particular balloon (12–14 atm) and have seen no untoward events related to that.

In those patients with recurrent stenosis early following dilatation, silicone stents (E. Benson Hood Laboratories, Pembrook, MA) were inserted over the rigid bronchoscope following dilatation. Although some have favored wire mesh expandable stents[34], we do not, because of their tendency to erode through the wall of the bronchus. Once this has occurred, these stents are virtually impossible to remove, and allow granulation tissue to grow through them, creating a recurrent stenosis that is extremely difficult to manage.[35]

There are four important factors associated with placing these stents:

(1) The external diameter and length must be carefully assessed. Generally speaking, the smallest external diameter stent is 6 mm. This provides an internal diameter of 4 mm. For obvious reasons placing a larger stent will result in a better long-term result. We have used the 6 mm stents for children less than 18 months, 8 mm stents for ages 18 months to 10 years, and 10 mm stents for those greater than 10 years of age. The length of the stent should be the shortest that will do the job, and usually is 10–13 mm. It is common for a right-sided stent to project partially into the trachea a short distance. These stents come in standard sizes, but custom-made stents are available and the difference of 1 mm in length or diameter may be quite crucial in a small infant or child.
(2) The bronchus must be dilated first to the same diameter as the stent. It is virtually impossible to insert a stent of larger external diameter than the lumen of the bronchus.
(3) Granulation tissue should be removed as much as possible. Generally speaking, granulation tissue begins to form only after there has been much manipulation of the airway, such as repeated dilatation. Therefore, I believe stents should be placed after the first recurrence of stenosis, particularly if it occurs early. Once present, this tissue tends to grow over either end of the stent, and once over the distal end the stent must be removed (Figure 5). This leaves very few options in treatment, and may lead to sleeve resection of that segment of bronchus, upper lobectomy, or re-transplantation.
(4) The distal end of the stent must not obstruct the upper lobe bronchus (Figure 6). The suture line for the anastomosis is one or two cartilaginous rings away from the upper lobe orifice. This translates to no more than 3–4 mm distance in children. I make every effort to ensure that the upper lobe bronchus is open, principally using a thin flexible bronchoscope (2.2 mm diameter) to go through the stent once placed, and into the upper lobe orifice. Frequently fluoroscopy is performed to confirm the location (Figure 7).

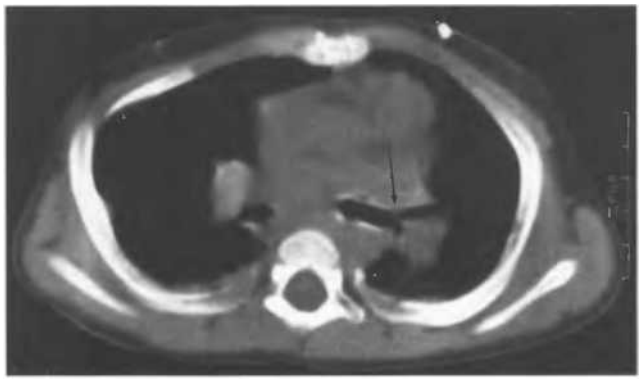

Figure 5 CT scan of a patient with a stent in place. There is granulation tissue growing over the distal end of the stent (arrow)

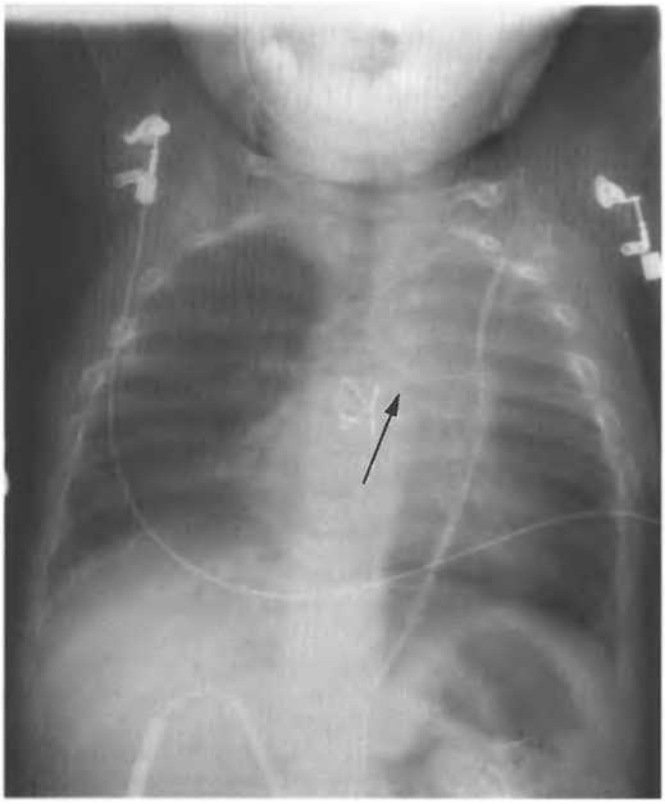

Figure 6 This is a plain portable chest radiograph of a patient immediately following placement of a left bronchial stent. The left upper lobe is atelectatic due to the stent occluding the left upper lobe bronchus. The stent may be faintly seen positioned in the left mainstem bronchus (arrow)

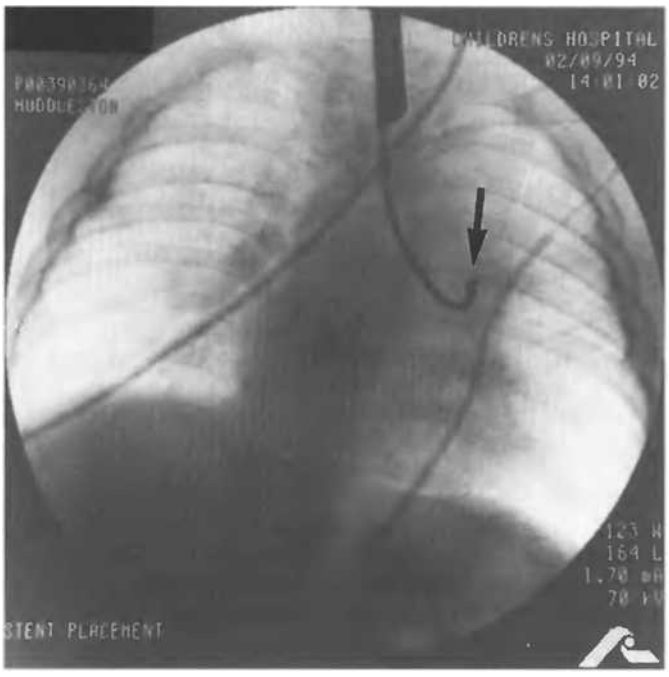

Figure 7 This chest radiograph was taken during fluoroscopy following completion of stent placement in the left mainstem bronchus of a child. To confirm that the upper lobe orifice is patent distal to the stent a small flexible bronchoscope is inserted into the orifice (arrow)

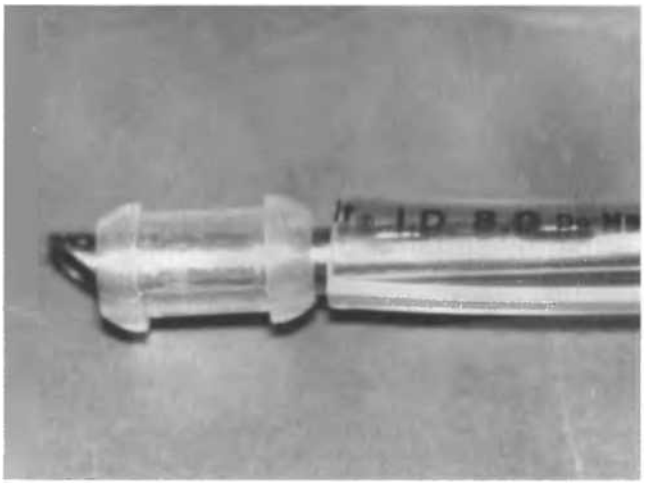

Figure 8 This is the assembly we use for placement of a silicone stent. An appropriately sized bronchoscope is selected and an endotracheal tube of the appropriate internal diameter is cut to the proper length so that the tube and the stent fit on the entire length of the bronchoscope

We have placed stents in six of our patients. Our technique is derived from that described elsewhere[36]. The stent is loaded onto an appropriately sized rigid bronchoscope with an endotracheal tube cut to the proper length loaded on behind that (Figure 8). The bronchoscope should be adequately lubricated for ease of removal of the stent. The entire assembly is then passed into the trachea, advanced down to the level of the stenosis and positioned carefully while viewing through the bronchoscope, usually with the telescope. All this must be done somewhat expeditiously because ventilation is significantly compromised while the assembly is passed down into the mainstem bronchus, since the side holes of the bronchoscope are occluded by the endotracheal tube. The stent is then held in position as the rigid bronchoscope is removed by maintaining the position of the endotracheal tube loaded onto the rigid bronchoscope behind the stent. Once the rigid bronchoscope is removed, ventilation can be resumed through the endotracheal tube still in the airway. Although the stents are not radiopaque, one can vaguely see these on X-rays (Figure 6). In small children placement can be very time-consuming, but it is imperative that precise placement is achieved to assure that the stricture is appropriately treated and the upper lobe bronchus is patent.

These stents are left in place for a minimum of 6 months, and preferably 12 months. Following removal of the stent the airway caliber has generally remained stable (Figure 9). We have not noted growth in the airway by gross inspection in these patients; however, the follow-up has been relatively short. Granulation tissue may occur at either end of the stent, and should be removed either with laser or by resection. Should it become particularly bothersome distal to the stent, the stent may have to be removed and the airway treated with periodic dilatation. I have found balloon dilatation to be generally less traumatic than dilatation with the rigid bronchoscope, and thus perhaps less likely to stimulate growth of granulation tissue.

The group in Hannover has performed sleeve resection of the stenotic segment with good results in adults[37]. Although this has

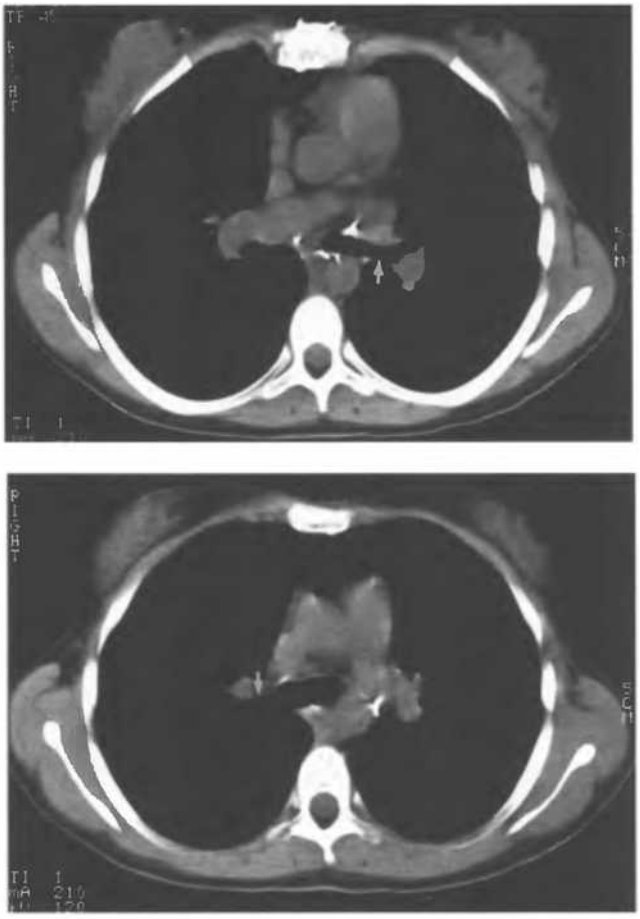

Figure 9 CT scans taken on a patient who had previously required bilateral stent placement for bronchial stenosis. The stents had been out for 1.5 years at the time these CT scans were taken. The bronchi are both widely patent

some appeal I think it would be a very difficult procedure in children, and I would not recommend it. When the stenosis extends down into the upper or lower lobe bronchi, lobectomy, or potentially retransplant, may be the only solution. Obviously these are somewhat extreme cases. Retransplantation carries with it greater immunologic uncertainty, unpredictable waiting time, the consequences of reoperation, and overall results which are not as good as first-time transplants[38]. However, when the stenosis extends down into the lobar orifices there may not be a plausible alternative treatment option.

COMMENT

The incidence of airway complications in children undergoing lung transplantation is similar to that reported elsewhere in adults. Dehiscence is now fortunately a fairly rare occurrence, with stricture formation becoming the primary complication. Factors important in prevention include strict adherence to meticulous surgical and organ preservation techniques and simple end-to-end anastomosis rather than telescoped anastomosis in children. Endoscopic management of bronchial complications following lung transplantation is a challenging task. We have preferred initial dilatation with subsequent silicone stent placement in recurrent or severe strictures. This has provided satisfactory results and no deaths secondary to the airway problem itself in our series.

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Living Donor Lobar Lung Transplantation

J.E. DAVIS AND V.A. STARNES

INTRODUCTION

Utilization of lung transplantation (LTx) is limited by donor availability. In the USA approximately 10% of patients listed for LTx will die in any given year. As LTx has become more accepted the pool of possible recipients has grown from 149 in 1988 to 2000 in 1995. During this same period the number of potential donors has remained almost constant. Living donor lobar LTx techniques offer a viable alternative form of treatment to patients with end-stage lung disease who might otherwise die while waiting for cadaveric LTx. While the first living donor LTx was reported in 1990[1], living donor lobar LTx (LDLLTx) has become an accepted form of treatment for adults and children with end-stage lung disease at the University of Southern California (USC) School of Medicine[2].

INDICATIONS

The indications for LDLLTx are the same as for cadaveric LTx. LDLLTx is specifically indicated when patients who have been listed for cadaveric LTx begin deteriorating. Potential donors are usually evaluated prior to the recipient's deterioration. At USC, 31 of 34 patients who have undergone LDLLTx have had cystic fibrosis (CF). Patients with pulmonary hypertension, pulmonary fibrosis, and obliterative bronchiolitis have also been transplanted. In all cases the severity of the pulmonary compromise has been significant. The mean pCO_2 and pO_2 have been 65 ± 5 and 70 ± 5 mmHg, respectively. In the patients with CF the FEV_1, FVC and FEF_{25-75} were all <50% of predicted.

Exclusion criteria are the same as for whole LTx.

DONOR EVALUATION

Donors for LDLLTx are usually selected from a pool of individuals related to the recipient, although one donor was the spouse of a relative. Donors undergo a battery of tests, including ABO blood group determination, room air arterial blood gas measurement, spirometry, echocardiography, ventilation/perfusion lung scanning, chest radiography, and computerized tomography. These tests serve to evaluate the ABO and anatomic compatibility of the donor lobe and the potential risk to the donor. Viral serologies and lymphocytotoxic crossmatch are also performed. The psychological and social impact upon the potential donor is assessed.

The following criteria have been utilized for donor acceptance[3]:

(1) Age ≤55 years.
(2) No significant past medical history.
(3) No recent viral infection.
(4) Normal echocardiogram.
(5) Normal electrocardiogram.
(6) Normal chest radiograph.
(7) Oxygen tension >80 mmHg on room air.
(8) FEV_1 and FVC >85% predicted.
(9) No significant pulmonary pathology on CT scan (completely normal on donor side).
(10) No previous thoracic operation on donor side.

SURGICAL TECHNIQUES

Donor procedures

Two donors are utilized for each recipient, with each donating a right or left lower lobe. The larger donor is the preferred right lower lobe donor due to the smaller size of the right versus the left lower lobe. Harvesting of the lobes is done simultaneously by two separate surgical teams.

Epidural anesthesia and a double-lumen endotracheal tube are used. Fiberoptic bronchoscopy is performed prior to the procedure to exclude infection or inflammation, and to confirm bronchial anatomy. Patients are placed in the decubitus position and posterolateral thoracotomy is performed. The technique of lobectomy is modified to maximize the length of donor pulmonary artery and vein as well as bronchus. Dissection is generally carried out on the side of the remaining lobe, to minimize air leaks in the recipient.

Donor right lower lobectomy

The right lower lobectomy is begun by division of the inferior pulmonary ligament up to the inferior pulmonary vein using electrocautery. The mediastinal pleura is dissected anterior to the superior pulmonary vein and posterior to the inferior aspect of the take-off of the right upper lobe bronchus. The pulmonary artery is then located within the fissure (Figure 1), and the anatomy of the branches is carefully defined, especially to the middle lobe. The distance between the superior segmental artery to the lower lobe and the middle lobe artery is variable and determines the length of the pulmonary artery cuff that can be obtained.

The venous drainage of the middle lobe is determined to ensure that it does not arise from the inferior pulmonary vein. The pericardium surrounding the inferior pulmonary vein is then incised. This will allow placement of vascular clamp on the left atrium (Figure 2).

The fissures are then divided using a 75 mm GIA stapler (US Surgical Corp., Norwalk, CT, USA) and any raw areas of parenchyma are cauterized.

The lung is then reinflated and 10 000 units of heparin and 500 mg of methylprednisolone are given intravenously. After 5–10 min of ventilation the lung is again collapsed and a vascular clamp is placed on the pulmonary artery between the superior segmental artery of the lower lobe and the middle lobe artery in such a fashion as to maximize the cuff of artery distal to the clamp (Figure 1). A vascular clamp is placed on the left atrium (Figure 2). The pulmonary artery and inferior pulmonary vein (with left atrial cuff) are divided.

The right lower lobe bronchus is exposed as little as possible in order to preserve the blood supply. The bronchus is transected along a line from (a) above the segmental bronchus to the superior segment inferiorly to (b) just below the take-off of the middle lobe bronchus (Figure 3). The lobe is removed, wrapped in a cold moist sponge and taken to the back table for preservation.

The transected pulmonary artery in the donor is repaired with a running 6/0 polypropylene suture, and the left atrium is closed with a running 4/0 polypropylene suture. The divided bronchus in the donor is then closed with interrupted simple sutures of 5/0 polypropylene.

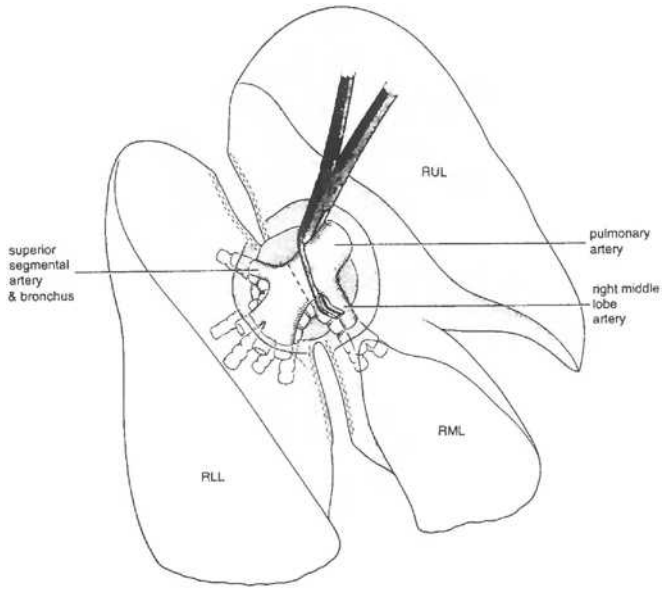

Figure 1 Dissection and division of the pulmonary artery for donor right lower lobectomy (from ref. 3)

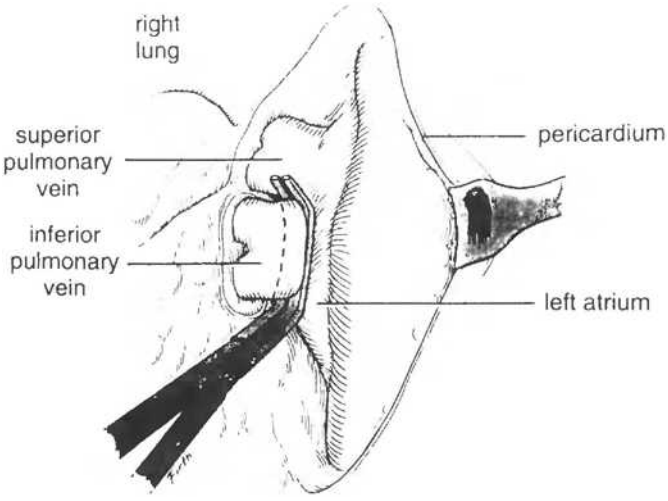

Figure 2 Dissection of the right inferior pulmonary vein so that a vascular clamp can be placed on the intrapericardial left atrium (from ref. 3)

Donor left lower lobectomy

Harvesting of the left lower lobe is also begun by dividing the inferior pulmonary ligament up to the inferior pulmonary vein. The pulmonary artery is identified within the major fissure and

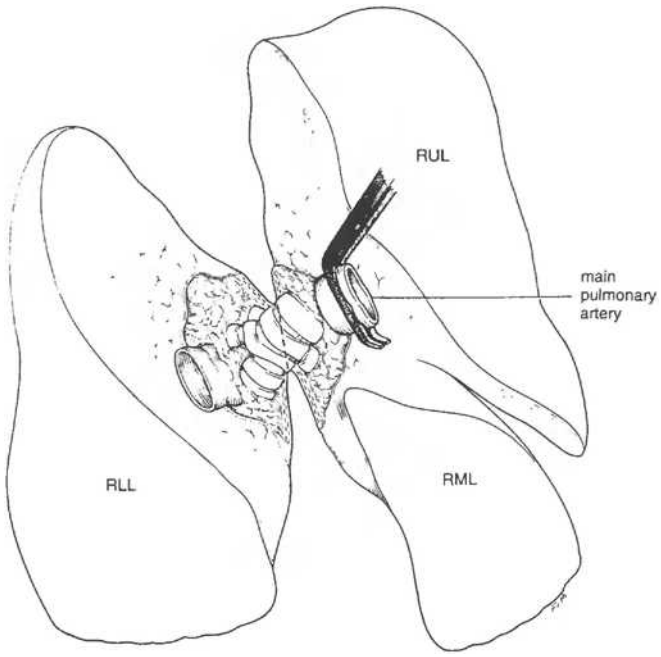

Figure 3 Dissection and division of the bronchus to the right lower lobe (from ref. 3)

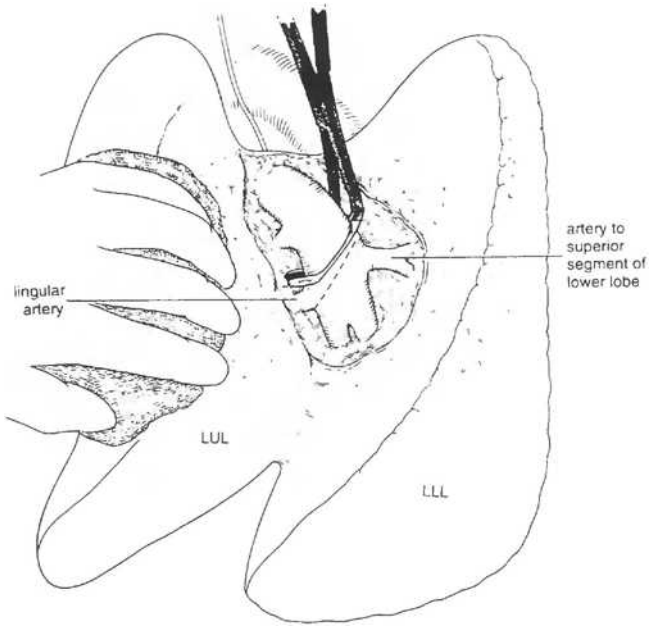

Figure 4 Dissection and division of the pulmonary artery for donor left lower lobectomy (from ref. 3)

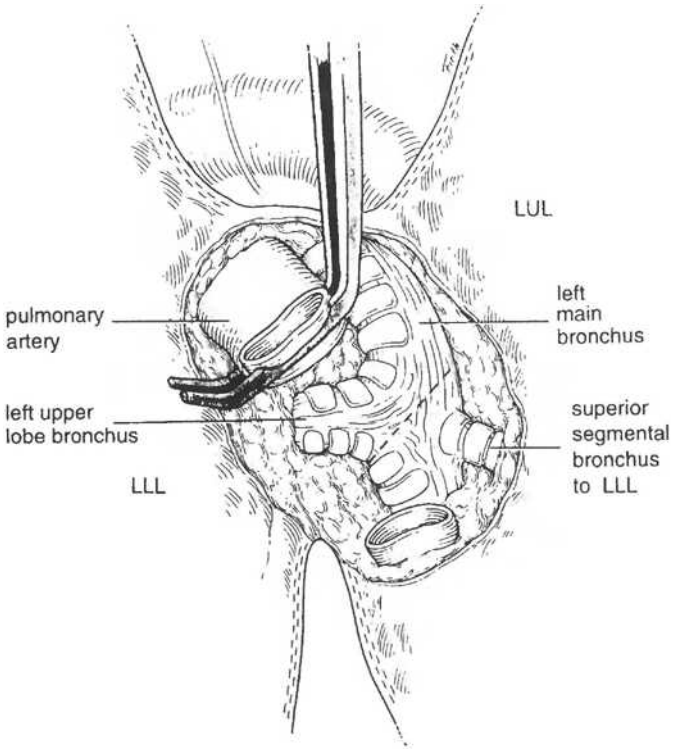

Figure 5 Dissection and division of the bronchus to the left lower lobe (from ref. 3)

exposed (Figure 4). The branches to the superior segment of the lower lobe and the lingula are identified and mobilized. A lingular artery located too far distally can be ligated if small. The pericardium is then opened anterior to the hilum and the inferior pulmonary vein is identified and dissected circumferentially. Division of the fissure is then completed using an automatic stapling device.

The lung is reinflated and heparin and methylprednisolone are administered. After ventilation, the lung is again deflated and the pulmonary artery is clamped as proximal as possible to the superior segmental artery of the lower lobe (Figure 4). The inferior pulmonary vein is isolated with a cuff of left atrium using a vascular clamp. The artery and vein are transected and the bronchus is exposed (Figure 5).

The main bronchus is traced proximally until the lingular bronchus is identified. The bronchus is then divided tangentially from (a) the junction of the lower lobe bronchus and the lingular bronchus to (b) a point approximately 3 mm superior to the superior segmental bronchus of the lower lobe (Figure 5). The lobe is removed to the back table for preparation. The artery, atrium and bronchus in the donor are closed as described for the right side.

Pulmonary preservation

Prior to removing the lobe from the donor, prostaglandin is given to dilate the pulmonary vasculature. Once at the back table, the bronchus of each lobe is intubated with a small endotracheal tube and the lobe is ventilated with 100% oxygen. The preservation fluid is administered in a retrograde fashion to ensure complete perfusion of the lobe. The pulmonary vein is cannulated, and the lobe is flushed with at least 1 liter of modified Euro-Collins solution or until the arterial effluent becomes clear and the parenchyma is white. The lobe is then transported to the recipient operating room. During preservation, preservation fluid is prevented from flooding into the bronchus.

Recipient procedure

After the induction of general anesthesia, the patient is placed in a supine position with each arm abducted 90° and each elbow flexed 90°. The forearms are gently wrapped to the anesthesia screen frame. The chest is prepared and draped for bilateral thoracotomy with transverse sternotomy (Clamshell incision). The incision is opened widely and adhesions are lysed. The hilum is dissected on both sides. Heparin is given and cardiopulmonary bypass is instituted via the ascending aorta and the right atrium. Recipient pneumonectomies are performed (Figure 6). Care is taken to preserve lengthy cuffs of recipient artery, vein and bronchus.

The right donor lobe is placed into the right chest and the bronchial anastomosis is performed first (Figure 7). The stapled end of the recipient bronchus is excised and an end-to-end anastomosis is performed using a running 4/0 polypropylene suture. Telescoping is utilized to compensate for differences in donor–recipient bronchus diameters.

The venous anastomosis is completed by anastomosing the superior pulmonary vein to the lobar vein using a running 4/0 polypropylene suture (Figure 8). The venous clamp is then removed to allow back-bleeding. Finally, the arterial anastomosis is completed with a running 5/0 polypropylene suture (Figure 9).

The right lobe is perfused while the left lobe is implanted in a like fashion. The patient is weaned from cardiopulmonary bypass and the wound is closed.

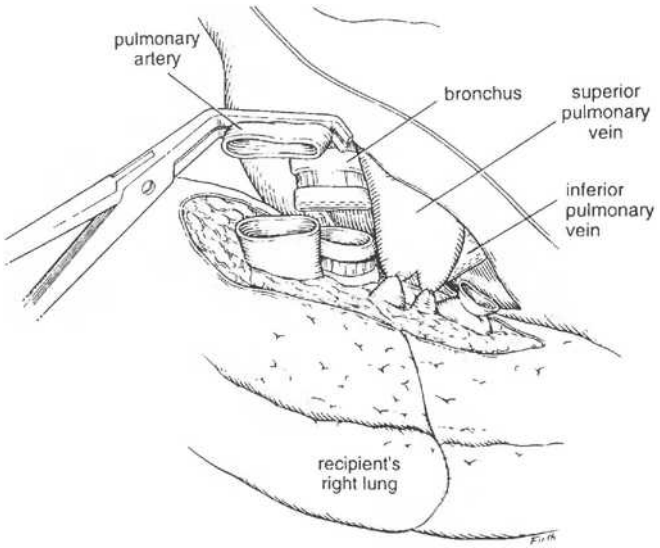

Figure 6 Recipient right pneumonectomy (from ref. 2)

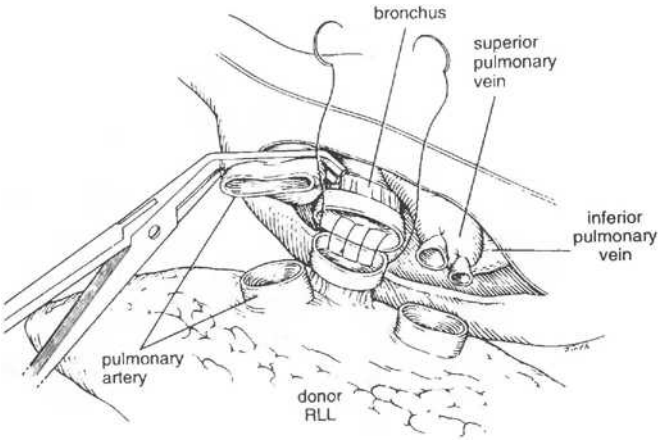

Figure 7 Right lower lobe (RLL) implantation – bronchial anastomosis (from ref. 2)

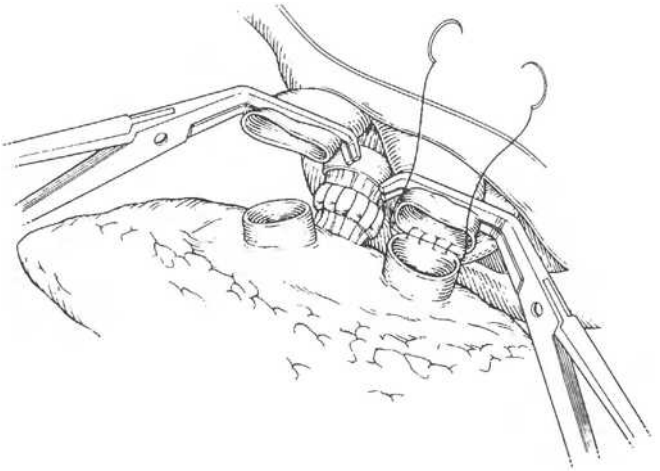

Figure 8 Right lower lobe implantation – pulmonary venous anastomosis (from ref. 2)

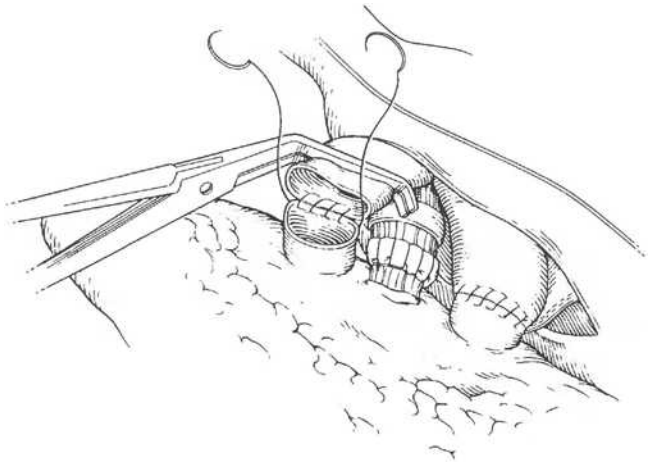

Figure 9 Right lower lobe implantation – pulmonary arterial anastomosis (from ref. 2)

Immunosuppression is administered as in conventional cadaveric LTx.

RESULTS

Thirty-four patients have undergone LDLLTx. Twenty-three of these required supplemental oxygen or mechanical ventilation prior to transplant. Twenty-five procedures were done as an emergency procedure, or urgently due to clinical deterioration. The mean survival time is 20 months, with a 1 year survival of 66%. There have been seven in-hospital deaths and four late deaths. Rejection has occurred in 16 patients (0.07 rejection episodes/patient month). Postoperative infections have occurred in 23 patients, most being pseudomonal pneumonias. Fungal or cytomegalovirus infections occurred in 14 patients.

FEV_1 and FVC increased from 23% to 68% and 37% to 67% of predicted, respectively. The most dramatic improvement occurred in the FEF_{25-75} measurements (9% to 68% of predicted). Clinically, the patients improved from NYHA Class 3.1 to 1.1 ($p<0.001$).

COMMENT

A number of unique donor-specific and recipient-specific issues arise when LDLLTx is being considered.

On an individual basis the surgeon must decide whether the potential recipient will benefit more from a lobar transplant using live donors or from a conventional cadaveric LTx. LDLLTx has the advantages of: (a) immediate availability of donor organs, (b) assurance of the health of the donor lobes due to extensive preoperative evaluation, and (c) control of the timing of the operation. The disadvantages are: (a) a slight but real risk of morbidity and potential mortality to the donor and (b) the increased technical difficulty of lobar transplantation.

The fate of mature lobes transplanted into immature individuals has been studied without conclusive results[4,5]. Kern *et al.*[6] have shown in animals that the mature transplanted lobe undergoes hypertrophy, but the number of functioning alveolar units does not

increase. The possible development of emphysematous changes in the transplanted mature lobe is a concern which will require further study.

In summary, LDLLTx offers an alternative to cadaveric LTx that results in equivalent survival and morbidity. In addition, patients whose life expectancy is less than the usual waiting period for LTx can be effectively palliated with this procedure.

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Results of Lung Transplantation and Factors Influencing Survival Based on the St Louis Lung Transplant Registry

M. POHL AND J.D. COOPER

NTRODUCTION

The St Louis International Lung Transplant Registry was begun n 1988 and contains information from 1983 when the first successful single lung transplantation was performed for a patient with pulmonary fibrosis. As a voluntary registry it helps to document the indications, complications, and results of lung transplantation. Since that first transplant in 1983, transplantation has become a viable alternative for patients suffering with end-stage ung disease. The following information is based on data reported o the Registry from 121 centers, as of 1 January 1995.

REGISTRY DATA

Of these centers there are 45 active centers in the USA (those having reported at least one lung transplantation since 1 January 994) and 32 active centers outside the USA (Figure 1). As of anuary 1995, over 3836 lung transplants were registered – 2346 ingle lung transplants (SLTx) (1160 right, 1181 left, five unspecified as to side), and 1490 bilateral transplants (230 en bloc louble (EBD), 1252 bilateral sequential transplants (BLTx), and ight unspecified) with an equal distribution of men (1950) and vomen (1884) transplanted. There is an increasing number of BLTx (Figure 2) being performed per year compared to the number of SLTx. A small number of EBD lung transplants continue to be performed.

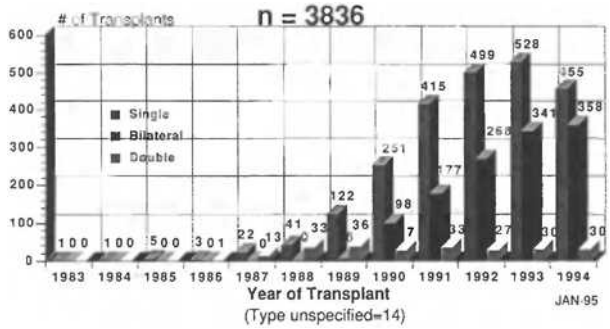

Figure 2 Number of lung transplants per year by type ($n = 3836$)

SURVIVAL FOLLOWING LUNG TRANSPLANTATION

Survival of lung transplant recipients continues to improve each year (Figure 3). The 1-year actuarial survival for all lung transplant recipients was 71%, the 2-year survival 64%, and the 6-year survival 43% (Figure 3). Most of the reported transplants have

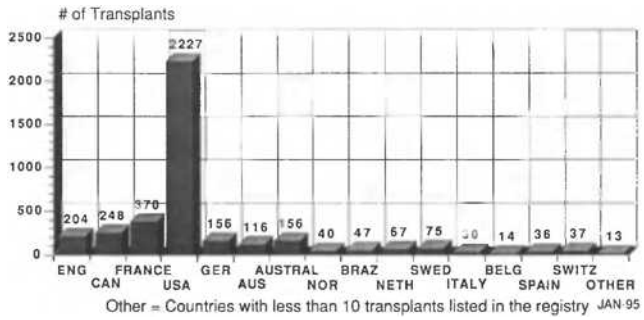

Figure 1 Number of lung transplants by country

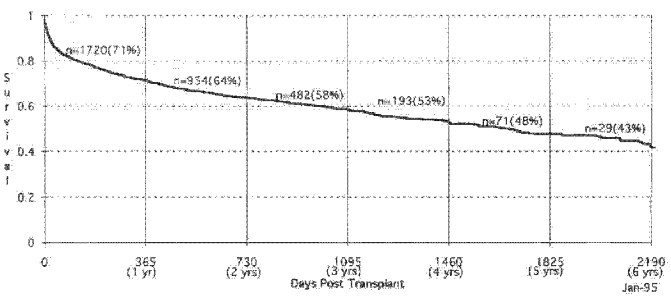

Figure 3 Six-year actuarial survival of patients with lung transplants ($n = 3739$)

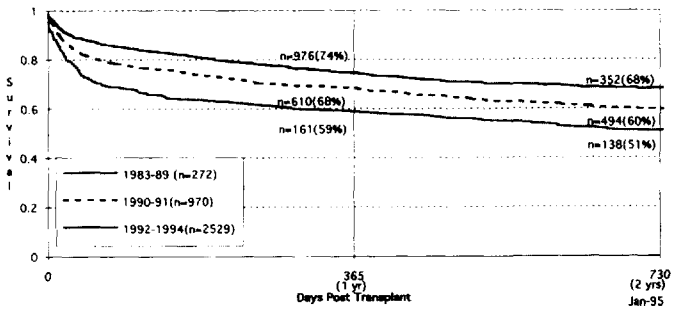

Figure 4 Two-year actuarial survival of patients with lung transplants by transplant era

been performed during the last 4 years. Figure 4 depicts the fact that patients who undergo a lung transplant today have a better actuarial survival than those patients who underwent transplantation before 1989. Because of this increasing survival of recent transplants, an upward trend in actuarial survival should occur in the years to come.

Influence of type of transplant (SLTx vs BLTx)

Of note is the difference between patients undergoing BLTx and SLTx. Although a much smaller group of patients has undergone BLTx, these patients have a statistically significant better actuarial survival than those who have undergone SLTx and a much higher actuarial survival than those who have undergone EBD transplant (Figure 5). More lung transplant centers are beginning to employ BLTx rather than SLTx, possibly due to these actuarial statistics and to the greater reserve of pulmonary function conferred on someone who subsequently develops bronchiolitis obliterans or rejection. With the severe shortage of lung donors, institutions are faced not only with the medical decision of who gets transplanted, but also with the dilemma of using SLTx to provide a lung for a greater number of recipients versus the possibility of improved long-term results following bilateral replacement.

Table 1 shows a breakdown for SLTx and BLTx for each major diagnosis. Bilateral transplants have been employed for patients with cystic fibrosis (with rare exceptions) because of the presence of chronic infection in both lungs. For other conditions, treated by either single or bilateral transplantation, the type of transplant

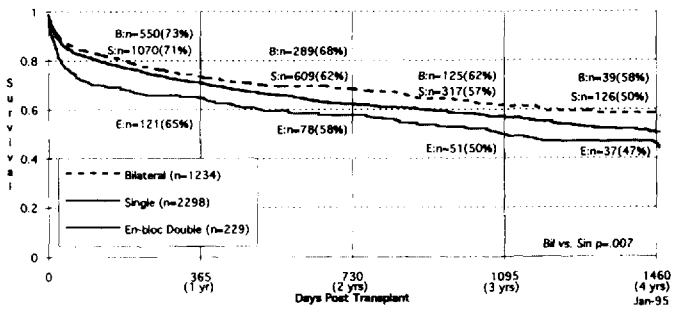

Figure 5 Four-year actuarial survival of patients with lung transplants by transplant type

Table 1 Indications for lung transplantation (excludes en bloc double lung transplants)

Indication	*n*	*Single lung (n = 2346)*	*Bilateral lung (n = 1252)*
COPD	1109	912	197
Interstitial pulmonary fibrosis	618	561	57
Cystic fibrosis	505	2	503
α_1-Emphysema	430	299	131
PPH/Eisenmenger's	364	231	133
Other	557	341	231

COPD = chronic obstructive pulmonary disease; PPH = primary pulmonary hypertension

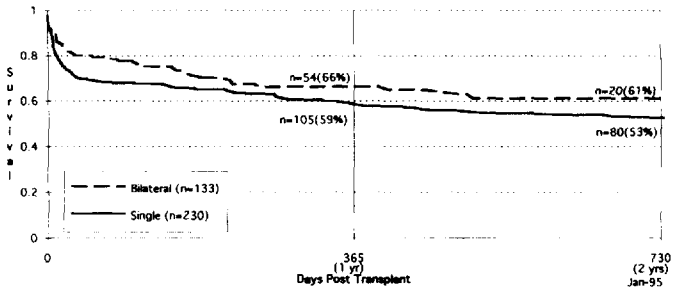

Figure 6 Two-year actuarial survival of patients with lung transplants for primary pulmonary hypertension or Eisenmenger's syndrome by transplant type

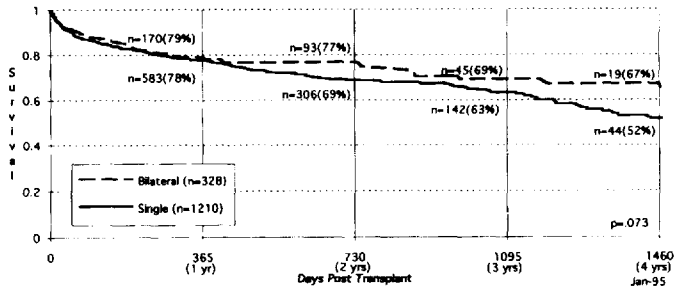

Figure 7 Four-year actuarial survival of patients with lung transplants for α_1-antitrypsin emphysema and chronic obstructive pulmonary disease by transplant type

employed appears to have no significant influence on survival rate. In Figures 6 and 7 the survival curves for SLTx and BLTx recipients are demonstrated for patients with primary pulmonary hypertension (PPH)/Eisenmenger's syndrome and for those with chronic obstructive pulmonary disease (COPD)/α_1-antitrypsin deficiency, respectively. One might expect the additional pulmonary reserve conferred by a bilateral transplant to favorably influence survival, but recipient diagnosis appears to exert a more significant influence on post-transplantation survival (Figure 8).

Influence of underlying pulmonary disease

The number of conditions for which lung transplantation is being successfully employed continues to expand. The most common indication (Figure 9) continues to be COPD, followed by

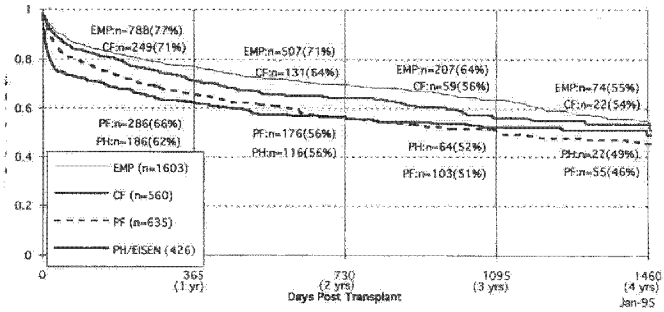

Figure 8 Four-year actuarial survival of patients with lung transplants by diagnosis

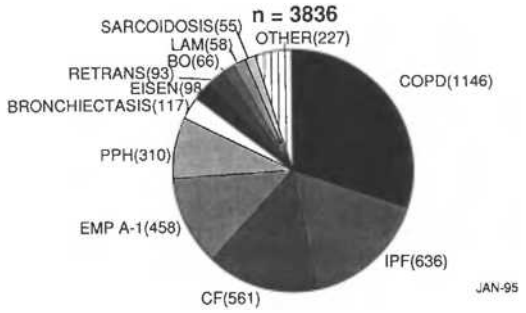

Figure 9 Number of lung transplant performed for various diagnoses (n = 3836)

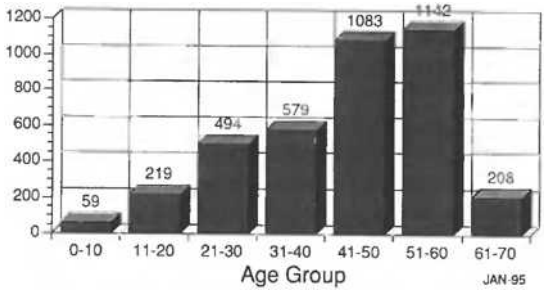

Figure 10 Number of lung transplant by age group

Interstitial pulmonary fibrosis (IPF), and cystic fibrosis (CF). Lung transplantation has become a viable treatment for patients with PPH, who at one time would have received a combined heart–lung transplant. Although SLTx have been performed most often for this condition (230 patients), the use of BLTx (133 patients) is increasing very significantly.

Influence of age of recipient

The largest number of transplant recipients (2225) continues to be between 41 and 60 years of age (Figure 10). Since the age cut-off for lung transplantation at many institutions is 60, it is of continued interest to evaluate the actuarial survival for those patients who are over the age of 60 at the time of transplantation. Figure 11 shows that this group of patients had a 61% actuarial survival at 1 year, compared to the total experience of 71%, and had a 50% survival at 2 years, compared to the total experience of 64%. One must note, however, that there is a small number of patients in this population group, making the statistical comparison relatively uneven.

Pediatric lung transplantation has continued to increase slowly over the years with 151 pediatric transplants (patients less than 16 years of age) being registered (Table 2). At 1 year this group had an actuarial survival of 66%, decreasing to 57% at 2 years (Figure 12). Most pediatric transplants continue to be performed for cystic fibrosis, with the next largest group being those with PPH (Table 2). Most pediatric transplants received BLTx, with the largest recipient age group being the 11–16-year-olds.

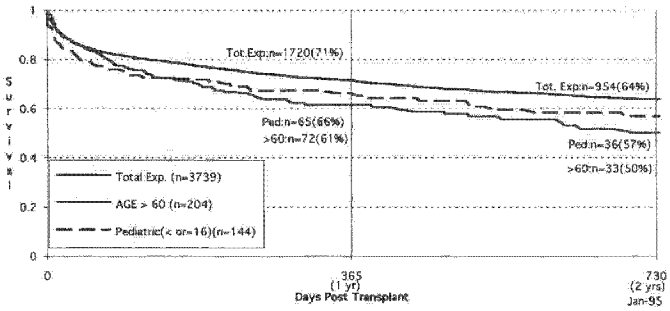

Figure 11 Two-year actuarial survival of patients with lung transplants by age group

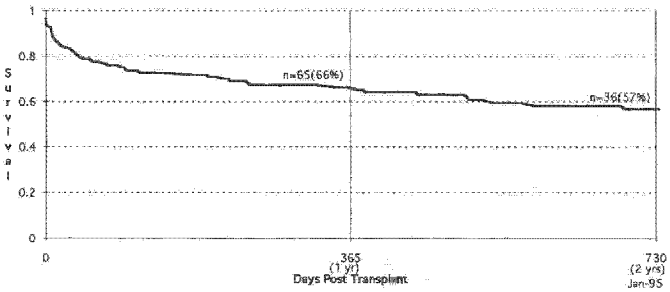

Figure 12 Two-year actuarial survival of pediatric patients with lung transplants

Table 2 Indications for lung transplantation in pediatric age groups (0–16 years)

Indication	*0–5 (n = 27)*	*6–10 (n = 32)*	*11–16 (n = 92)*	*Total (n = 151)*
Cystic fibrosis	0	16	54	70
Interstitial pulmonary fibrosis	2	1	9	12
PPH	8	2	12	22
Eisenmenger's	1	1	3	5
Retransplant (bronchiolitis obliterans)	0	5	3	8
Retransplant (other)	0	2	5	7
Other	16	5	6	27

Influence of cytomegalovirus (CMV)

CMV infection remains the major source of morbidity following all types of organ transplantation. The incidence and severity of CMV infection can be reduced by matching CMV-negative recipients with CMV-negative donors. However, the constant shortage of donor lungs makes it increasingly difficult to reject a potential donor solely because of CMV status. Currently, with improved methods of prophylaxis and treatment for CMV, a CMV mismatch does not influence overall survival (p=n.s.) (Figure 13). There is no significant difference in actuarial survival between CMV-negative recipients who received a CMV-negative donor and those who received a CMV-positive donor.

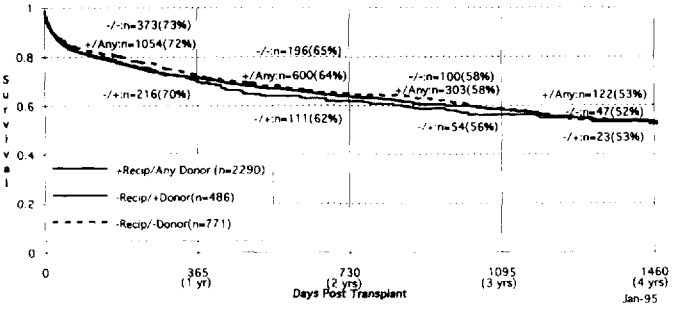

Figure 13 Four-year actuarial survival of patients with lung transplants based on donor and recipient CMV status

Table 3 Causes of death in patients with lung transplants occurring ≤ 90 days post-transplant (n = 675)

Cause of death	*n*	*Percentage of deaths < 90 days*	*Percentage of total transplants*
Infection (other than CMV)	178	26	5
Primary lung failure	86	13	2
Heart failure	54	8	1
Airway dehiscence	43	6	1
Hemorrhage	41	6	1
Multi-organ failure	38	6	< 1
Rejection	37	5	< 1
CMV infection	37	5	< 1
Other	161	24	4

Table 4 Causes of death in patients with lung transplants occurring > 90 days post-transplant (n = 601)

Cause of death	*n*	*Percentage of deaths > 90 days*	*Percentage of total transplants*
Acute rejection/bronchiolitis obliterans	159	26	4
Infection (other than CMV)	157	26	4
Respiratory failure	34	6	< 1
CMV infection	34	6	< 1
Malignancy	32	5	< 1
Hemorrhage	21	4	< 1
Multi-organ failure	20	3	< 1
Heart failure	12	2	< 1
Other	132	22	3

Causes of death

The leading causes of death following lung transplantation are listed in Tables 3 and 4. Twenty-six percent of the 675 recipients who died within 90 days of transplantation died from infection (other than CMV), making it the most common cause of death for these recipients (Table 3). Primary organ failure was the second most common cause of death at 13%. The major causes of death for the 601 patients who died more than 90 days post-transplantation were rejection (both acute and bronchiolitis obliterans) (26%) and infection (other than CMV) (26%) (Table 4). Most late deaths associated with infection occurred as a result of augmented immunosuppression for chronic rejection.

COMMENT

Lung transplantation continues to be a viable alternative for patients with end-stage lung disease. The actuarial survival of patients undergoing lung transplantation has steadily improved over recent years. Two major obstacles are: (a) the increasing number of recipients requiring a lung transplant compared to the number of suitable lung donors who become available and (b) the continued problems of acute rejection and bronchiolitis obliterans once these patients are transplanted. It will only be with an increase in organ donation, and the development of more effective immunosuppressant agents and protocols, that we will see an increase in the number of lung transplants performed and an improvement in patient survival.

66
Transplantation of the Heart and Both Lungs – Experimental Background and Early Clinical Experience

E. BECERRA, J. KAPLAN AND D.K.C COOPER

INTRODUCTION

Transplantation of the heart and both lungs only became a clinical reality in the 1980s, largely due to the introduction of cyclosporin, which enabled the patient to be immunosuppressed adequately during the first 2–3 weeks without the need for a corticosteroid. The ability to immunosuppress the patient sufficiently without a corticosteroid allowed time for healing of the tracheal suture line, which previously was a major source of early complication following this operation.

Research workers have been interested in heart–lung transplantation, however, for many years[1], and three clinical attempts at the procedure were carried out in the 1960s and 1970s.

EXPERIMENTAL BACKGROUND

Initial studies

The earliest attempt to transplant the heart and both lungs was by Carrel (see Chapter 18, Figure 1) at the beginning of this century, though this involved only transplantation into the neck of a recipient cat[2]; lung edema occurred with distension of the right side of the heart.

In 1946, Demikhov (Figure 1) transplanted the heart and lungs of a dog, and the recipient survived for 2 hours without its own organs, but it was not until 1949 that more prolonged survival was obtained[3]. The technique used was ingenious (Figure 2) as it enabled the blood supply to the brain to be maintained continuously throughout the operation, with the exception of 2–3 minutes at one critical stage. Demikhov took care to dissect out the phrenic and vagus nerves of the recipient with the intention of preserving the innervation of those structures, particularly the diaphragm, below the region of the heart and lungs. At this stage the right lung was removed, to facilitate later parts of the operation.

After preliminary mobilization, the donor heart–lung preparation was removed from the animal by clamping and dividing the thoracic aorta, the inferior vena cava, the brachiocephalic and subclavian arteries, and the superior vena cava. During transfer

Figure 1 Vladimir Demikhov, who, working in relative isolation in the USSR, carried out extensive experimental work in the field of heart and heart–lung transplantation in the 1940s and 1950s

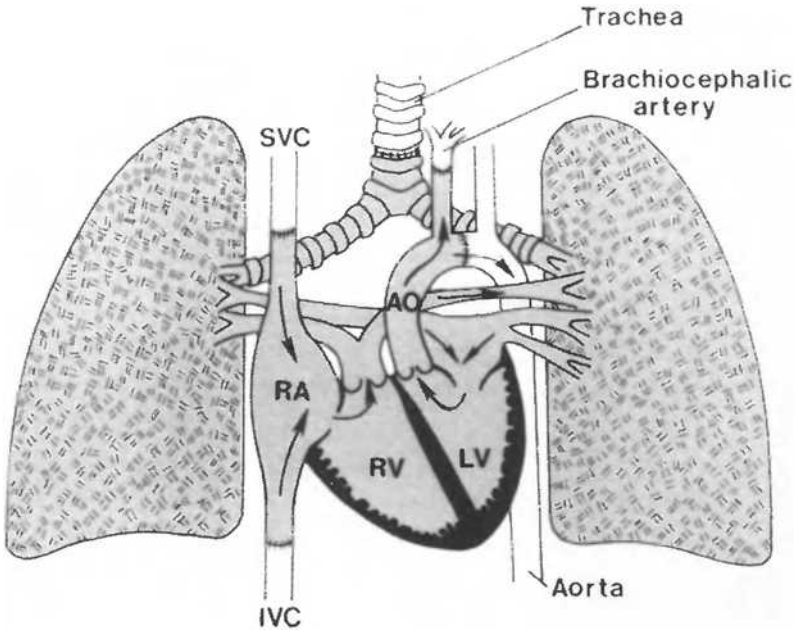

Figure 2 The completed operation of orthotopic heart–lung transplantation, using the technique described by Demikhov[3]

the donor heart–lung was kept viable by its own closed-circuit circulation, blood from the left ventricle being pumped into the arch of the aorta, from whence it passed through the coronary vessels supplying the myocardium and into the right atrium, the right ventricle, and the lungs; oxygenated blood was returned to the left atrium. This form of heart–lung preparation was subsequently the basis of a means of transporting and temporarily preserving the heart and lungs[4–8].

The various vascular anastomoses were made either by suturing or by 'quick connects' over prosthetic tubes. During the inferior vena caval anastomosis the blood supply to the lower half of the body was temporarily interrupted for 15–20 minutes. The tracheas of the transplant and recipient were then connected, either by means of a special tube or by silk sutures, using a technique which avoided interference with respiration.

Of 67 attempts at this procedure, only six dogs survived for more than 48 hours, with only two surviving for more than 4 days. Early deaths were from technical problems and thromboses at the various anastomoses, particularly of the brachiocephalic artery. Those dogs which did recover from the immediate effects of the operation appear to have been quite well for the few days until their demise. Respiration was generally slow, in the region of 12 per minute, and the pulse rate variable, though frequently fast. Certain dogs appeared to recover remarkably well, walking about their kennels, drinking water, eating meat, and reacting briskly to their surroundings. (One of them was even sent by train from Ryazan to Moscow on the fourth postoperative day and on arrival at its destination 'ran up the stairs by itself').

Several important observations and conclusions have resulted from Demikhov's pioneering studies. Most significant is the fact that following total replacement of the heart and both lungs many of these dogs did breathe spontaneously, and apparently adequately, until death, which did not appear to be the result of respiratory insufficiency unless caused by bronchopneumonia. This is a particularly important finding, but one that was not confirmed by all subsequent workers. Secondly, the respiratory rate was variable. On the day following operation one dog had a respiratory rate of 18 per minute. On the second postoperative day it was noted to have a pleural effusion; attempts to aspirate this led to vomiting for 5 minutes, after which the dog was dyspnoeic 'and the respiratory rate rose to 135 per minute'. Four and a half hours later the rate returned to 12 per minute. Thirdly, the transplanted heart was able to maintain an adequate circulation for 6 days, and, despite the fact that it was totally denervated and neither atrium had been left *in situ*, it also showed considerable variation in rate.

During the period of Demikhov's studies, other workers, notably Marcus, Wong, and Luisada[9,10], were also studying heart–lung transplantation. Marcus *et al.* developed a technique for transplanting the heart and both lungs into the abdomen, thus giving the recipient two sets of heart and lungs (Figure 3). The purpose of this latter experiment was to determine whether the donor heart and lungs could be used as a pump–oxygenator unit to deliver oxygenated blood to a limited part of the host's body. Among their conclusions these authors suggested the possibility of using a heterologous heart–lung preparation as an extracorporeal pump during intracardiac procedures. They also commented that the transplanted heart might act as an accessory pump to decrease the workload of the native heart, even if only temporarily.

Matejicek in 1956 briefly reported a study of the transplantation of the heart and right upper lobe of the lung into the chest, but no results were reported[11].

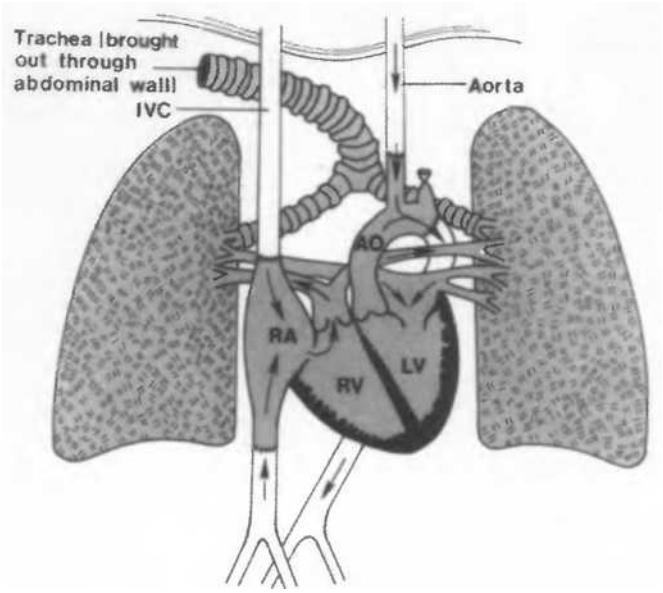

Figure 3 The donor heart–lung preparation transplanted into the recipient aorta–vena caval circulation in the abdomen, using the technique described by Marcus *et al.*[9,10]. The transplanted organs were able to function in accessory support of the host animal

Advent of supportive techniques

With the advent of supportive techniques, total heart and lung excision and replacement became more feasible. In 1953 Neptune *et al.* reported the use of hypothermia to sustain life in the recipient while transplantation was proceeding[12]. The surgical technique, which became the basis for most of the subsequent experimental studies during the next 15 years, involved anastomosis or 'coupling' of the trachea, SVC, IVC and aorta. The longest surviving animal recovered spontaneous respiration but died after 6 hours.

In 1957 Webb and his colleagues introduced cardiopulmonary bypass for the same purposes[13–15]. After a variety of experiments in dogs, Webb and his co-workers came to the conclusion that simultaneous bilateral pulmonary denervation of the heart and lungs, or even bilateral hilar stripping, resulted in respiratory dysfunction or even paralysis which made autotransplantation or allotransplantation of the heart and lungs impracticable.

They did suggest that, while transplantation of the heart with one lung was technically more difficult, the technique might be feasible for use in patients with pulmonary hypertension. Such transplantation circumvented respiratory paralysis by leaving one innervated lung in the recipient. Continuing respiration appeared to be dependent on 'feedback' afferents from the respiratory system; they believed their studies indicated that respiratory paralysis was not due to phrenic nerve damage, excessive vagal or sympathetic dissection, or periods of shock accompanying the extensive dissection and trauma of the actual transplant. Accordingly, they concluded that transplantation of the heart combined with both lungs was probably a physiological impossibility.

Other investigators, however, reported the resumption of spontaneous respiration in dogs surviving after cardiopulmonary transplantation[16–19], and it was observed that oxygenation remained adequate despite the fact that the respiratory pattern was greatly altered; the tidal volume was increased and the respiratory rate diminished[17].

Lower (see Chapter 18, Figure 6) and his colleagues[17] carried out heart–lung transplantation in six dogs, two of which survived until the fifth postoperative day. They were ambulatory, active, and eating until they became lethargic on the fourth day, at which time they began to die from respiratory insufficiency. These authors felt that their studies confirmed earlier work that the bronchial arterial supply to the lungs could be sacrificed without resulting necrosis, but the question of the possibility of prolonged survival after pulmonary denervation remained unanswered. It was evident that the sacrifice of peripheral innervation, which necessarily accompanies pulmonary transplantation, resulted, in the cases reported, not in respiratory paralysis but in an altered respiratory pattern which resembled that observed after bilateral cervical vagotomy. The operation appeared technically feasible, and spontaneous respirations with an altered pattern appeared to be sufficient to sustain life until allograft rejection supervened.

Both of the 5-day-surviving dogs died from microscopic changes suggesting rejection of the lungs. The changes of acute rejection in the myocardium were less extensive than these authors had observed previously with cardiac allografts of longer duration. This observation has been confirmed by many subsequent workers both in the experimental animal[20,21] and in patients undergoing heart–lung transplantation[21,22]; the lung is generally more rapidly rejected than the heart and, in fact, the heart may be protected in some way by the lungs.

De Bono[19], using Neptune's technique, obtained six surviving dogs in which spontaneous respiration of an apparently normal pattern occurred for periods of 2–10 hours, at which times the dogs were sacrificed. He pointed out that pulmonary edema, caused by a number of factors, diminished the ventilatory capacity and compliance of the lungs[19]. Similar observations of a changed respiratory pattern, which was often inadequate, were found by Longmore and his colleagues in 1969[6] and by Grinnan *et al.* in 1970[23]. Longmore's group simplified the operation by anastomosing the two right atria rather than both the SVC and the IVC. Only three anastomoses were now required – tracheae, right atria, and aortae (Figure 4). This technique forms the basis of that used currently in clinical practice (Chapter 68).

Further light was thrown on the effect of denervation of the lungs on subsequent respiratory function by studies on unilateral and bilateral lung transplantation. Bilateral lung denervation or bilateral lung transplantation resulted in a similar change in respiratory pattern. These studies are discussed in Chapter 45, and clinical observations are summarized in Chapter 54.

Heart–lung transplantation in primates

In 1967, Nakae and his colleagues[24] carried out extensive pulmonary denervation in the dog, cat and monkey, and recognized the ability of primates to withstand total lung denervation. A normal pattern of spontaneous breathing was found in primates after lung denervation. These authors predicted that long-term

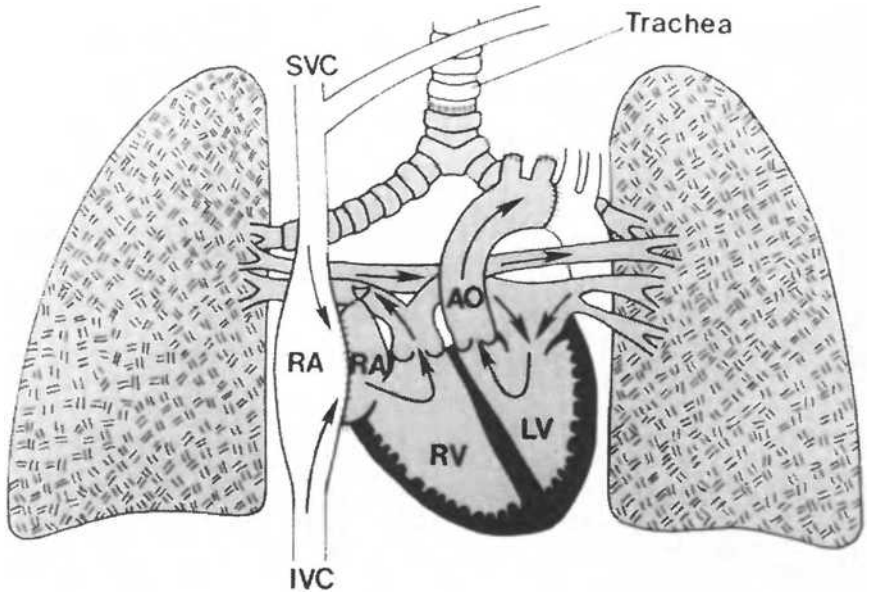

Figure 4 The completed operation of orthotopic heart–lung transplantation, using the technique described by Longmore *et al.*[6]

survival could be achieved in this species. It was evident that primates tolerated total denervation of the lung, and did not require the Hering–Breuer reflex as did the dog, since spontaneous respiration, controlled by the mid-brain, was preserved. This observation was confirmed in 1972 by Castaneda and his colleagues[25], who reported long-term survival of 6–24 months after heart–lung autotransplantation in the baboon.

Both of these studies lent some degree of confirmation to the earlier work by Haglin and his colleagues in 1963[26], who showed that total denervation of both lungs did not prevent a return of adequate spontaneous respiration in primates, though it did in dogs.

Introduction of cyclosporin

Reitz and his colleagues[27], working with rhesus and cynomolgus monkeys, made significant contributions to the development of cardiopulmonary transplantation in the late 1970s and early 1980s, obtaining long-term survival through the introduction of immunosuppression with cyclosporin. Several contributions were made by this group; they (a) demonstrated that cardiopulmonary bypass was preferable to hypothermic circulatory arrest to support the recipient during heart–lung transplantation; (b) established that median sternotomy provided the best approach to the chest cavity for this operation; (c) established that immunosuppression with cyclosporin and azathioprine, without a corticosteroid during the first 14 days to avoid its deleterious effect on tracheal healing, could be successful in preventing acute rejection; and (d) described the successful management of post-transplant lung edema by fluid restriction and the administration of furosemide.

With regard to the technical aspects of their work, they utilized a low tracheal anastomosis, as used previously by many other workers, and a single right atrial anastomosis as proposed originally by Longmore *et al.*[6]. The relatively heavy immunosuppressive regimen used resulted in the development of histiocytic lymphoma in some of their experimental animals.

As a result of their studies they proposed that endomyocardial biopsy could be used to diagnose both cardiac and pulmonary allograft rejection[27,28]. In retrospect this proved to be an unreliable method of diagnosing lung rejection, as rejection rarely occurs simultaneously in both organs, pulmonary rejection being more frequent than cardiac rejection[20–22,29].

EARLY CLINICAL EXPERIENCE

The operation was first performed clinically by Cooley (Chapter 77, Figure 1) on 31 August 1968[30]. The patient was a 2-month-old infant with a complete atrioventricular canal defect, pulmonary hypertension and pneumonia. The patient required reopening for bleeding, and died 14 hours after the initial transplant operation. In December 1969 Lillehei performed the second such operation on a 43-year-old patient with emphysema and pulmonary hypertension[31]; the patient survived 8 days, dying from pneumonia. The third operation was performed in Cape Town by Barnard (see Chapter 18, Figure 10) in July 1971[32,33]. The bronchi, rather than the trachea, were chosen as the site of anastomosis of the air passages, as it was believed at that time that this would preserve both the blood supply to the recipient carina and the cough reflex in the carinal area more satisfactorily. The patient did well initially, but died on the 23rd day following the development of a right-sided bronchopleural fistula, which necessitated right pneumonectomy, and which was followed by septicemia.

All three of these early patients were immunosuppressed with only azathioprine and corticosteroids, as cyclosporin was not then available.

It was not until another 10 years had elapsed that a fourth transplant of the heart and both lungs was reported, on this occasion (9 March 1981) by Reitz (Figure 5) and his colleagues at Stanford University[28,34,35]. The availability of an improved immunosuppressive regimen, including cyclosporin, and a better understanding of both the reimplantation syndrome and the blood supply of the trachea and bronchi, resulted in the first long-term survival of such a patient. This first patient was a 45-year-old woman with primary pulmonary hypertension, who underwent heart and lung transplantation using the surgical technique and immunosuppression developed in this group's experimental program. She suffered two acute rejection episodes, both of which were reversed successfully and, 10 months later, showed normal exercise tolerance.

Two other patients underwent the same operation during the following 4 months[36]. One of them was a 29-year-old woman with a complex transposition of the great vessels who had undergone previous cardiac surgery. Dense adhesions led to technical problems and a coagulopathy associated with the long period of cardiopulmonary bypass. Renal, hepatic and pulmonary complications followed, the patient dying on the fourth postoperative day. Similar experiences at several centers resulted in a reluctance of surgeons to attempt this procedure in patients who had undergone previous surgery of the chest[37]. Despite the hazards presented by adhesions from previous surgery, retransplantation of the heart and lungs was successfully accomplished[37].

Although the results of transplantation of the heart and both lungs are still inferior to those which can be expected after heart transplantation alone, the improved immunosuppressive regimen made available by the introduction of cyclosporin and increasing experience in the management of patients undergoing this procedure offer the possibility of long-term survival in well-selected patients[38].

Figure 5 Bruce Reitz, who, working in Shumway's group at Stanford University in the USA, performed important experimental work on heart–lung transplantation. In 1981 he led a team that carried out heart–lung transplantation in a patient who became the first to survive long-term

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Transplantation of the Heart and Both Lungs – Indications, Selection, and Evaluation

V.R. KSHETTRY AND R.M. BOLMAN III

INTRODUCTION

The successful clinical heart–lung transplantation by Reitz *et al.*[1], is a landmark in the annals of thoracic organ transplantation. Since 1981 over 1500 heart–lung transplants have been performed worldwide[2]. The aim is to increase the life expectancy of severely ill patients with cardiopulmonary disease and to enhance their quality of life. To meet this aim, expert knowledge of the course of their disease and of the prospects for a successful transplant is necessary. Advances in surgical technique, organ preservation, and post-transplant care have improved survival and long-term graft function.

The success of heart–lung transplantation depends, in large part, on the selection of appropriate candidates. With an ever-increasing pool of potential recipients, and a limited number of donors, it is imperative to select patients who are most likely to benefit.

INDICATIONS

Indications for heart–lung transplantation have evolved with the introduction and current success of lung transplantation[3–5]. Single and bilateral lung transplants are increasingly being done for diseases formerly thought appropriate for combined heart–lung transplants. The increased demand for heart transplants has reduced the number of available heart–lung blocks. Currently, the need for heart–lung transplantation is determined by the degree of right and left heart dysfunction, the presence or absence of severe coronary artery disease, and the complexity of congenital heart disease, in conjunction with end-stage disease of the lungs (Table 1).

Primary pulmonary hypertension

Primary pulmonary hypertension (PPH) is a progressive, irreversible, and usually fatal disease; over two-thirds of patients die within 2 years after diagnosis[6]. Although medical interventions have prolonged life for specific subsets of patients[7,8], the long-term prognosis without a heart–lung or lung transplant remains poor.

Table 1 Indications for heart–lung transplantation

Primary pulmonary hypertension
Systemic or two-thirds systemic pulmonary artery pressures
Severe right heart failure with high-dose diuretic therapy
3 to 4+ tricuspid regurgitation
Right ventricle ejection fraction < 20%
Eisenmenger's syndrome
Irreparable intracardiac defect
Severe right heart failure
Pulmonary parenchymal disease
Progressive pulmonary disease with (1) cor pulmonale or (2) severe left ventricular dysfunction due to advanced coronary artery disease, valvular disease, and cardiomyopathy

Primary pulmonary hypertension patients may require a combined heart–lung transplant if they have: (a) systemic or two-thirds systemic pulmonary artery pressure; (b) severe right ventricular dysfunction (as demonstrated by severe tricuspid valve regurgitation); (c) a right ventricle ejection fraction less than 20%; or (d) a significant diuretic requirement due to persistent right heart failure. Any concomitant cardiac condition (coronary artery disease, cardiomyopathy, etc.) is a definite indication for heart–lung transplantation in a patient with PPH.

Eisenmenger's syndrome

Survival is difficult to predict for patients with Eisenmenger's syndrome. The rate of symptomatic deterioration in this group (compared with PPH patients) is slower, and may occur in a stepwise fashion. There is always a risk of sudden death, paradoxical embolism, or cerebral abscess. Right ventricular failure that is unresponsive to diuretic therapy has a poor prognosis. An exercise-induced decrease in oxygen saturation below 60% suggests serious disability and a poor prognosis[9]. Patients with cardiac defects more complex than a ventricular or atrial septal defect or a patent ductus arteriosus require a heart–lung transplant.

Pulmonary disease

Some patients with progressive pulmonary disease – such as interstitial pulmonary fibrosis, cystic fibrosis, and obstructive lung disease – develop cor pulmonale with irreversible right heart decompensation. Other patients may have severe left ventricular dysfunction due to advanced coronary artery disease. Such patients are a poor risk for a lung-only transplant; instead, they require a combined heart–lung transplant.

SELECTION CRITERIA

All potential heart–lung recipients must have end-stage pulmonary vascular or parenchymal disease. Their functional capacity is so limited that current activity levels are intolerable or inadequate for a satisfactory quality of life. Recipients are deemed to be experiencing the last 2–3 years of their natural lives.

If possible, a transplant should be discussed with potential recipients early in the course of their progressive decline in health. In this way the patient comes to view the transplant as one of several treatment modalities, rather than a last-ditch effort. The waiting time for heart–lung transplants varies in different geographic regions; therefore, patients should be evaluated well ahead of time, if they are to survive in a stable condition until transplanted. Because of the limited availability of donor organs the following criteria are a guide for recipient selection. The criteria are designed to help determine the severity and nature of the underlying illness, the prognosis, and any contraindications (Table 2).

Age

Recipient age under 50 years has been an arbitrarily selected criterion. However, an upper age limit is not supported by any clear data. The only valid concern regarding age as a criterion is the ethical dilemma of allocating scarce donor organs.

Disease stage

Heart–lung transplant recipients must be in New York Heart Association (NYHA) class III or IV, defined as severe functional limitation in performing activities of daily living. However, it is important to evaluate patients before they reach class IV. Many such patients may not be optimal transplant candidates. It is imperative to critically evaluate recurrent right heart failure, whether treated or not. Patients with an advanced degree of right heart failure with ascites and liver failure are not optimal candidates for any thoracic organ transplant. Patients in NYHA class IV who have disabling symptoms at rest, or who cannot walk 600 feet in 6 minutes, may already be too severely ill for a successful heart–lung transplant.

Table 2 Heart–lung recipient selection criteria

Age
< 50 years
Disease stage
New York Heart Association stage III or IV
End-stage pulmonary vascular or parenchymal disease associated with severe right ventricular dysfunction or severe tricuspid regurgitation
Body weight
Within 20% of ideal range
Psychological profile
Stable personality
Good compliance
Family support
Corticosteroid use
None to < 20 mg prednisone or equivalent per day

Body weight

Weight above or below the predicted range is associated with increased morbidity. Obese patients have an increased incidence of postoperative atelectasis, pneumonia, and difficulty in physical rehabilitation[10]. Similarly, malnourished patients are at greater risk for such postoperative complications as infection, wound healing, and physical deconditioning[11], although a preoperative trial of hyperalimentation may improve their nutritional status. Patients whose body weight is more than 140% or less than 75% of their ideal body weight should not be on a transplant list. The best candidates are within 20% of their ideal body weight as adjusted for their height, sex, and ethnic origin.

Psychosocial profile

Recipients must be psychologically and socially stable for the transplant to succeed, given its extraordinary demands on their lifestyle. The burden of a severe illness, a stressful operation, changed finances and relationships, and possible relocation, can be overwhelming, even under the best circumstances. A stable personality profile, as well as solid support from family or friends, are paramount.

Corticosteroid use

In the early years of lung transplantation the consensus was that corticosteroid use impaired tracheobronchial healing[12]. However, as experience in this field has grown, successful airway healing has been achieved in patients on low-dose corticosteroids[13]. Tracheal anastomosis after heart–lung transplantation has a low incidence of airway complications. Patients taking up to 20 mg per day of prednisone or the equivalent can be safely transplanted. However, any large-dose steroid use should be appropriately tapered pretransplant.

Prior thoracic surgery

Postoperative hemorrhage after heart–lung transplantation has been a major cause of early mortality. Prior thoracic surgery was long considered a contraindication. However, with increased experience and refined surgical techniques, patients are now evaluated on an individual basis, and prior thoracotomy or sternotomy are now relative contraindications only. Nonetheless, patients with extensive pleural scarring are still excluded from heart–lung transplantation.

CONTRAINDICATIONS

To improve the results of heart–lung transplantation, certain absolute and relative contraindications have been defined (Tables 3 and 4). In general the operation is not offered to patients who have systemic diseases with multisystem involvement. Multiorgan involvement limits full recovery post-transplant. Similarly, patients with active malignancy are excluded.

Table 3 Absolute contraindications for heart–lung transplantation

Systemic or multisystem disease
Active malignancy
Active extrapulmonary infections, including hepatitis B and C, and immunodeficiency virus
Active pulmonary fungal infections
Irreversible renal dysfunction (serum creatinine > 2 mg/dl, creatinine clearance < 50 ml/min)
Irreversible hepatic dysfunction (total bilirubin > 2.5 mg/dl)
Mechanical ventilation
Chronic high-dose steroid therapy (> 20 mg prednisone or equivalent/day)
Current drug, alcohol, or tobacco abuse
Unstable psychological profile

Table 4 Relative contraindications for heart–lung transplantation

Body weight outside 20% of ideal range
Peptic ulcer disease
Steroid use (10–20 mg prednisone or equivalent per day)
Free of malignant disease > 5 years
Prior thoracotomy or sternotomy

Patients with active extrapulmonary infections are at high risk of developing sepsis in the face of immunosuppression. Active fungal pulmonary infections require aggressive treatment and close evaluation.

Most recipients develop some degree of renal insufficiency due to the transplant surgery and the nephrotoxic effects of cyclosporin. All require sufficient renal reserve to withstand this period. Those with pre-existing renal failure whose serum creatinine level is greater than 2 mg/dl, and whose creatinine clearance is less than 50 ml/min, are at high risk for developing irreversible renal failure post-transplant[14].

Many recipients develop hepatic dysfunction secondary to right heart failure. If this persists despite diuretic therapy, and the total bilirubin level remains greater than 2.5 mg/dl, severe liver failure can develop post-transplant[15].

Mechanical ventilation is also a contraindication to heart–lung transplantation. Often such patients require prolonged ventilation and tracheostomy, and become ventilator-dependent[16].

Patients with ongoing psychiatric illness or continuing alcohol or drug abuse are excluded from transplantation, because experience has shown that they may be unable to comply with the rigorous post-transplant regimen. Similarly, patients who currently use tobacco are excluded; former smokers must have abstained for at least 6 months. Compliance with smoking cessation is strictly enforced with random testing of urine for nicotine metabolites.

EVALUATION

Potential heart–lung recipients undergo a careful history and physical examination. After initial screening a thorough evaluation is carried out to determine suitability for a transplant. Blood tests should be done to determine the complete blood count; platelet, electrolyte, and creatinine levels; liver function; blood group and HLA typing; antileukocyte antibody screen; HIV status; and viral antibody titers for cytomegalovirus, Epstein–Barr virus, herpes simplex virus, and varicella–zoster. Also needed are pulmonary function tests, cardiac catheterization, and MUGA scan for right and left ventricular ejection fraction. Psychological, social, dental, and gynecologic evaluations should also be performed. Other tests may be indicated by the history and physical examination. Our evaluation protocol is summarized in Table 5.

Once the patient is selected and listed for a heart–lung transplant, a considerable wait usually ensues. During this period the patient's medical and physical condition must be optimized, with regular outpatient evaluation by the transplant physicians. Any deterioration in the patient's status that might complicate the transplant is aggressively treated. However, some patients may need to be temporarily or permanently taken off the transplant waiting list. Close communication with the patient, family members, the transplant team, and the referring physician is essential to expedite prompt, appropriate, and compassionate care.

COMMENT

Indications, selection criteria, and evaluation for heart–lung transplantation are complex. The process requires teamwork involving several medical disciplines. Potential recipients must be carefully chosen to ensure that scarce organ resources are used wisely. Most important, patients and family members must be treated with skill, dignity, and respect.

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Table 5 Heart–lung recipient evaluation

Hematology
Complete blood count with differential
Coagulation studies
PT, PTT, platelet count

Chemistry
Glucose and electrolyte panel, Po_4, amylase, AST, total bilirubin, alkaline phosphatase, thyroid function tests, TSH, total protein, albumin, carboxyhemoglobin
24-hour urine creatinine clearance

Immunology
ABO typing and screen
HLA A, B, C, and DR typing and panel reactive antibody (PRA)
Quantitative immunoglobulins with G subclasses I, II, III, IV

Virology/serology
Titers for CMV, EBV, VZV, HSV, HIV, toxoplasmosis
Hepatitis profile (A, B, C)

Microbiology
Sputum for routine culture and fungus
Urine analysis and culture

Tests
Chest X-ray (PA and lateral, and AP supine at 40 inches height)
Stool guaiac × 3
12-lead EKG
MUGA scan (first pass right and left ventricular ejection fractions)
Lung scan with quantitative perfusion imaging
6-minute walk test
Echocardiogram (with estimate of RV pressures) with bubble study
Cardiac catheterization. Must include pulmonary artery pressures and pulmonary vascular resistance. Left ventriculography and coronary arteriography if > 40 years old
Pulmonary function tests
CT of chest without contrast (including high-resolution cuts)
Bilateral mammogram for female patients ≥ 35 years
PPD (five test units), mumps, and *Candida* (adults only) skin tests for patients with no history of positive PPD or verified TB
Skeletal X-rays: spine (thoracic and lumbar); hip, bilateral
Complete dental examination by local dentist

Vaccination
Pneumovax (pneumococcal vaccine) 0.5 ml intramuscularly (only if patient has not received it before)

Consults
Transplant surgeon, cardiologist, and pulmonologist
Social services
Neurologist
Psychologist
Transplant coordinator
Gynecologist for female patients (PAP smear and pelvic exam)
Chaplain

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Transplantation of the Heart and Both Lungs – Organ Procurement and Recipient Surgical Techniques

V.R. KSHETTRY AND R.M. BOLMAN III

INTRODUCTION

Since its introduction in 1981[1], clinical heart–lung transplantation has developed into a viable treatment for patients with end-stage cardiopulmonary diseases. Original surgical techniques have been well described[2]. As the worldwide experience has increased, several refinements in the selection and care of donors, and in surgical technique for recipients, have contributed to improved outcome. These issues are discussed in this chapter.

HEART–LUNG PROCUREMENT

A shortage of donors continues to restrict the number of heart–lung transplants. Of all organ donors only 15% are suitable for heart–lung donation[3]. Prompt and efficient care of donors by a dedicated team of health-care professionals is a prerequisite to success.

In the early experience of clinical heart–lung transplantation, lack of a suitable lung perfusate necessitated moving the donor to the recipient hospital. Research was directed at developing preservation methods that would allow distant procurement. Initially, autoperfusion was used, but the cumbersome technical requirements of this setup precluded broad application[4,5]. Next, profound systemic cooling of the donor on cardiopulmonary bypass (before procurement) was introduced, with good clinical outcome[6]. However, the need for cardiopulmonary bypass equipment at the donor hospital limited the use of this modality.

Finally, Euro-Collins solution for pulmonary artery flush and preservation was successfully used in a canine lung model[7]. This simple method of lung preservation, combined with topical cooling, is now used worldwide for distant organ procurement in humans. This combined method allows ischemic times beyond 4 hours, and achieves excellent graft function[8,9].

Prospective donors under age 50 are further evaluated for normal cardiac function and gas exchange, arterial oxygen greater than 100 mmHg on inspired oxygen of 0.4, and peak airway pressure of less than 30 mmHg on normal tidal volume. The chest radiograph should be normal, and pulmonary secretions minimal. The presence of fungus in any amount, or of Gram-negative bacteria in large numbers, contraindicates donation: the risk of post-transplant infection increases morbidity and mortality. Also excluded are donors with a history of penetrating or blunt chest trauma with lung contusions or hemothorax. Criteria for suitable heart–lung donors are listed in Table 1.

The donor and recipient should be matched according to ABO blood group, and the lymphocytotoxic cross-match should be negative (Table 1). ABO identity between the donor and recipient is recommended to prevent graft-versus-host disease in the form of hemolytic anemia.

The size match between the donor and recipient is important; the donor lungs must not be too large. The height and weight of

Table 1 Heart–lung donor selection criteria

Age
< 50 years
History
< 20 pack-year smoking
No significant chest trauma
No tracheobronchial aspiration
No prior cardiopulmonary operation
Immunology
ABO identify
Lymphocytotoxic crossmatch for sensitized patients (i.e. those with panel-reactive antibody > 10%)
Pulmonary function
Clear chest radiograph
Pa_{O_2} 100 mmHg or greater on F_iO_2 of 0.4
Lung compliance normal (peak airway pressure < 30 mmHg on normal tidal volume)
Hemodynamics
Minimal inotropic support (dopamine hydrochloride < 10 $\mu g\ kg^{-1}\ min^{-1}$)
Good right and left heart function on echocardiogram
Microbiology
No obvious pulmonary sepsis
No purulent pulmonary secretions
No fungal organisms or large numbers of Gram-negative organisms
Size match
Lung volume same as or less than the recipient's

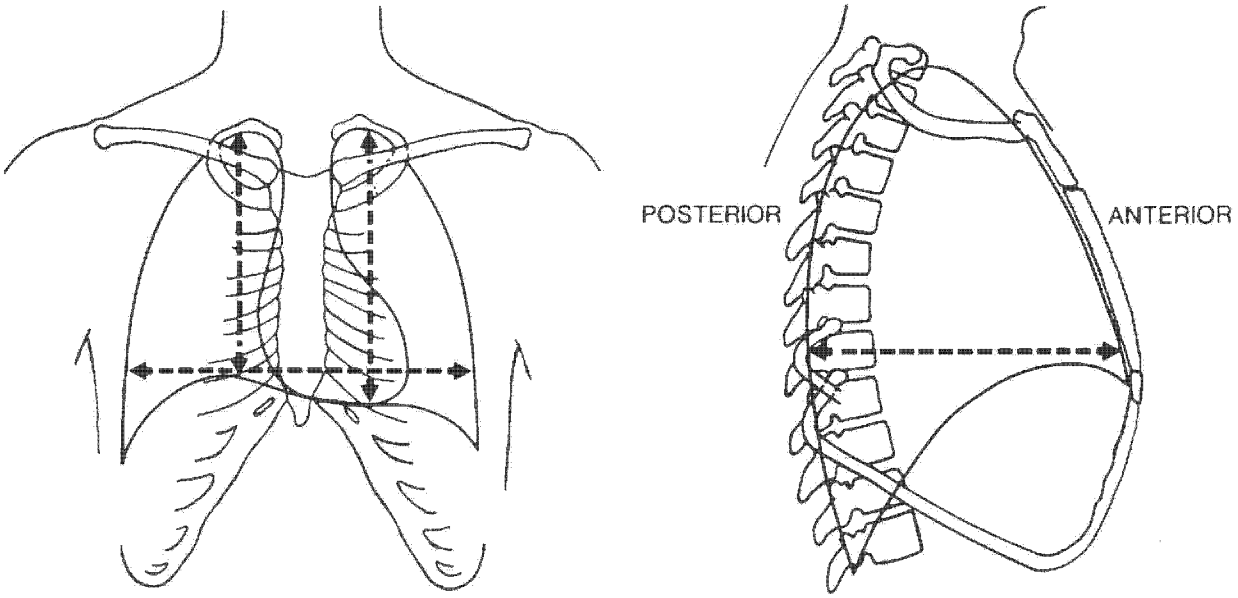

Figure 1 Measurements of anteroposterior and lateral chest radiographs found helpful in assessing relative sizes of donor organs and recipient thoracic cavity. All measurements are made on chest radiographs taken in the supine position with the camera at a set distance from the radiographic plate. Measurements include (1) vertical distance from the apex of the pleural cavity to the diaghragm on both right and left sides; (2) the transverse diameter at the widest point of the chest (this is usually near at the costophrenic angle); (3) the anteroposterior diameters measured on the lateral chest radiograph from anterior surface of the vertebral column to the posterior surface of the sternum, and from the posterior curvature of the ribs to the back of the sternum, both of these measurements being made at the mid-sternal and diaphragmatic levels

the donor and the recipient should be about the same. Height, in particular, is a better indicator of relative lung size than weight[10]. A chest roentgenogram, taken in full inspiration, may also be a useful guide for size match. Especially crucial are the vertical measurements from the apex of the lung to the dome of the diaphragm, and the transverse measurements at the level of the arch of the aorta and the dome of the diaphragm (Figure 1).

Careful assessment and management of the donor's fluid and electrolyte status, before and during procurement, are critical. Fluid overload must be avoided. The donor must be maintained as dry as possible, consistent with stable hemodynamic function and perfusion of any other organs being procured.

Surgical technique

Heart and lung procurement occurs routinely as part of a multiple organ retrieval operation. The chest is opened through a midline sternotomy. Both pleural cavities are entered. The lungs are inspected for evidence of contusion or laceration. The pericardium is opened and the heart inspected. Any evidence of myocardial contusion or other injury is noted. The overall contractility of the left and right ventricles is also noted. The coronary arteries are palpated for coronary artery disease.

The pericardium is attached to the edges of the sternotomy with sutures. The superior and inferior vena cava and ascending aorta are all encircled in preparation for organ removal. Purse-string sutures are placed in the ascending aorta and main pulmonary artery for insertion of cardioplegia and pulmonary flush cannulae. The trachea is then exposed through the posterior pericardium between the aorta and superior vena cava, at a level 2–3 cm cephalad to the carina. This dissection can be facilitated by ligation and division of the innominate vessels. Unnecessary dissection of the trachea should be avoided, to limit damage to the peritracheal tissue which contains a blood supply from coronary collaterals (Figure 2).

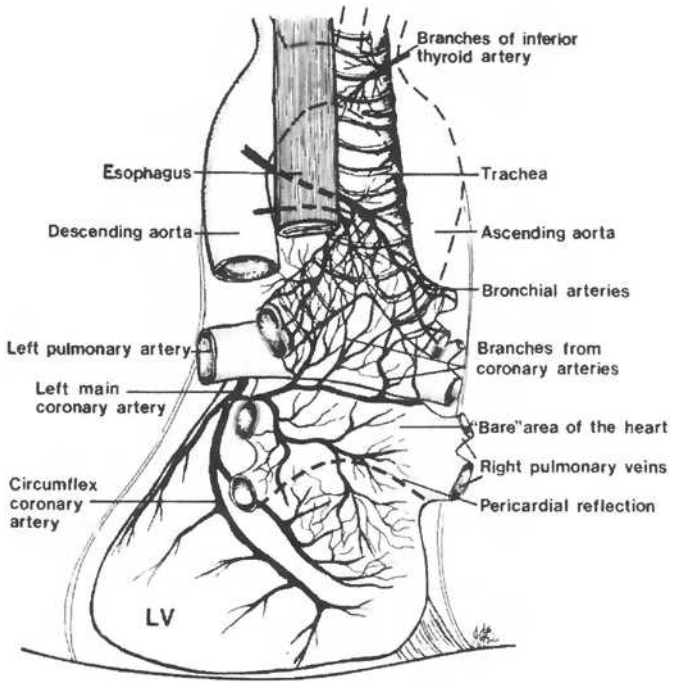

Figure 2 Posterior view of heart and trachea, showing blood supply to the trachea, carina and bronchi. (LV = left ventricle)

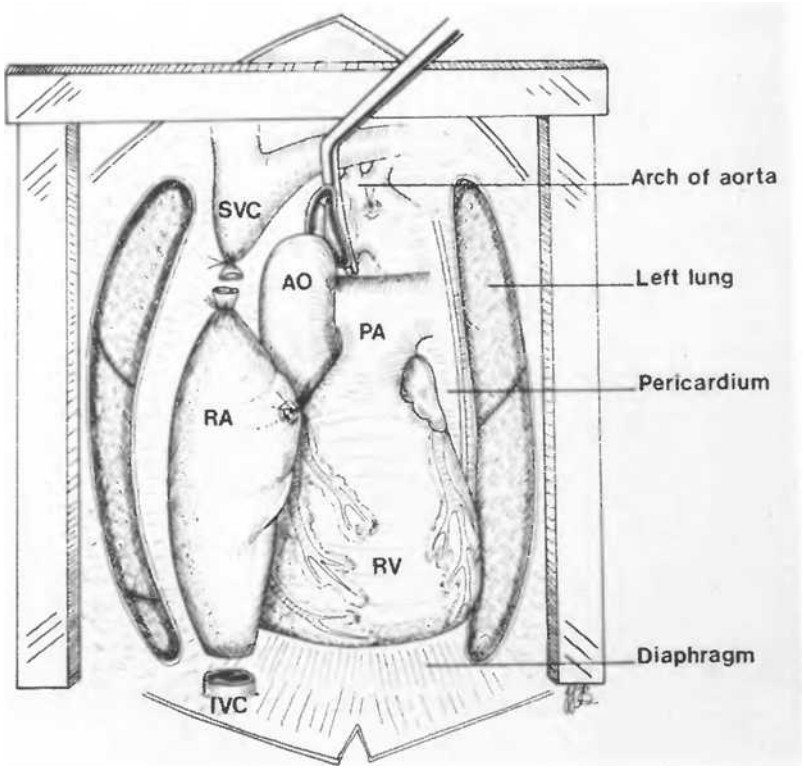

Figure 3 Excision of donor organs. A median sternotomy has been performed, and pericardiectomy carried out. Both pleural cavities have been opened to allow inspection of the lungs. Cooling of the heart and lungs can be carried out by (1) a pump-oxygenator (bringing about total body cooling) and the infusion of a cardioplegic agent, or by (2) simultaneous infusion of a cardioplegic agent into the ascending aorta and a 'pulmoplegic' agent into the main pulmonary artery. In this figure cooling has been by pump-oxygenator. The aortic and right atrial cannulae have already been removed, but the sites of cannulation in the arch of the aorta and right atrial appendage are indicated. The cardioplegic infusion cannula is not shown. The superior vena cava has been doubly ligated and divided. The inferior cava has been divided. The ascending aorta has been cross-clamped as high as possible and divided. (Abbreviations used in this chapter: SVC = superior vena cava; RA = right atrium; IVC = inferior vena cava; RV = right ventricle; PA = pulmonary artery; PV = pulmonary vein; AO = aorta)

When the abdominal viscera are mobilized and ready to be removed, retrieval of the heart and lung block can proceed. Intravenous heparin at a dose of 400 units/kg of body weight is given. All central venous lines are removed. Removal of the organs begins with ligation and division of the superior vena cava and azygos vein. The inferior vena cava is divided flush with the right atrium, which allows the heart to empty. The aorta is cross-clamped at the base of the innominate artery. Cardioplegic solution is infused into the aorta and cold modified Euro-Collins solution into the main pulmonary artery. The tip of the left atrial appendage is amputated to allow the pulmonary preservation solution to drain out and prevent distension of the left heart.

Topical cooling with normal saline at 4°C helps preserve the organs. During infusion of the preservation solution the lungs are gently ventilated with room air. When about 1 liter of cardioplegia solution and 3–4 liters of modified Euro-Collins solution have been infused, the organs can be removed. The aorta is transected just proximal to the crossclamp (Figure 3). Both inferior pulmonary ligaments are divided. The endotracheal tube is withdrawn. The trachea is stapled as high as possible with a stapling device with 4.8 mm staples, and divided (Figure 4). The lungs remain partially inflated, to prevent atelectasis during storage.

The heart–lung block is then detached from the posterior mediastinal attachments, with the surgeon working cephalad to caudad using electrocautery (Figures 5 and 6). The area of the posterior trachea must be approached with special care. It is extremely important not to enter the trachea inadvertently during this dissection (Figure 7). It is also important to leave all adventitial tissue surrounding the trachea intact, to avoid devascularization of this vital structure. The heart–lung block is then removed, placed in cold saline, and packaged for return to the recipient hospital (Figures 8 and 9).

RECIPIENT OPERATION

Heart and lung transplantation routinely requires cardiopulmonary bypass. Given the enormous surgical field in this operation, coupled with the need for total anticoagulation, the potential for life-threatening hemorrhage is great. Much attention must be directed to achieving hemostasis at all stages of the operation.

Initiation of cardiopulmonary bypass

The chest is opened through a midline sternotomy. Both pleural spaces are entered. Any adhesions are divided and made hemostatic before heparin is administered. The pericardium is opened longitudinally in the midline; stay sutures are used to retract it. The

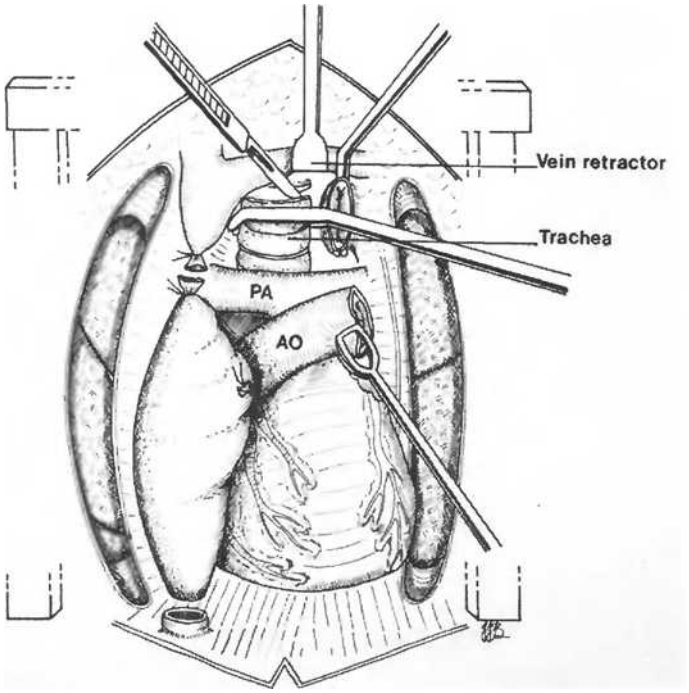

Figure 4 The ascending aorta has been retracted downwards and to the left, exposing the trachea, which has been clamped and divided as high as possible (after withdrawal of the endotracheal tube)

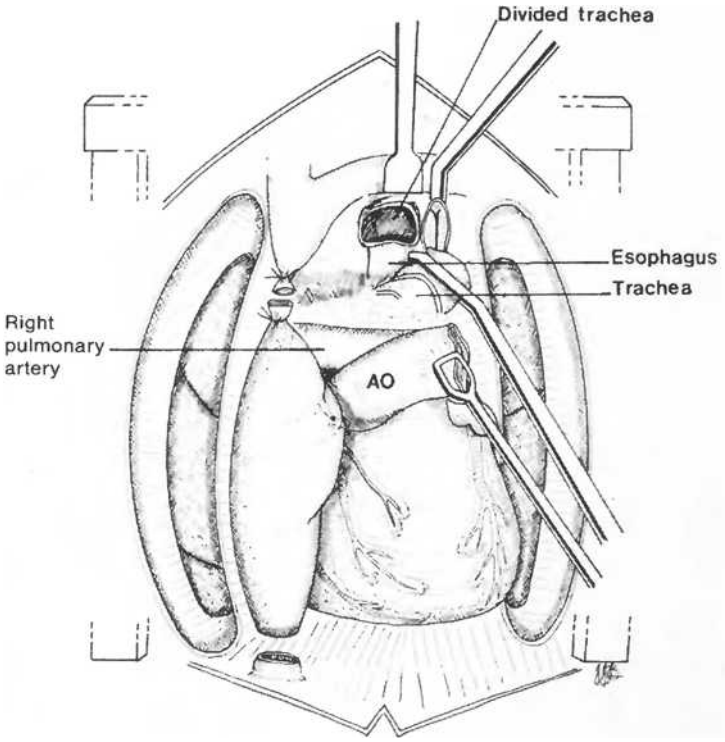

Figure 5 Mobilization of the heart away from the posterior mediastinal tissues is begun in a craniocaudal direction by retracting the distal trachea anteriorly and downwards, exposing the esophagus and descending aorta

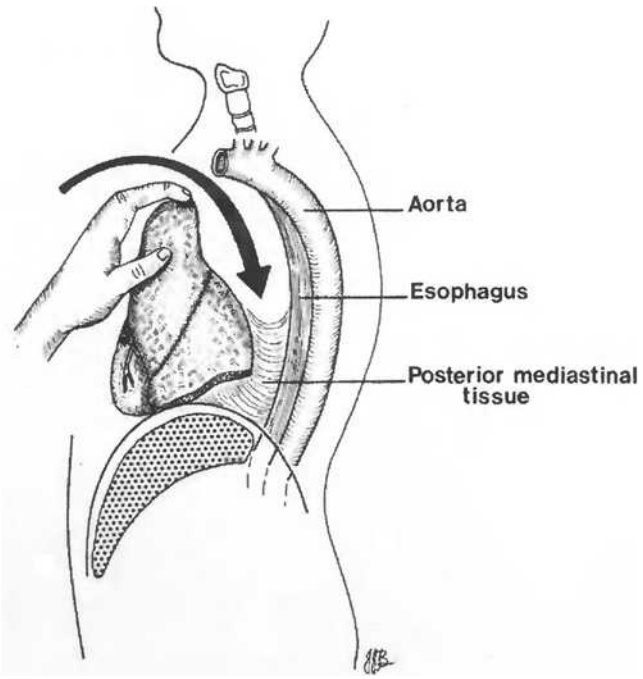

Figure 6 Indicates the plane of the dissection between heart (and lungs) and posterior mediastinal structures (esophagus and descending aorta)

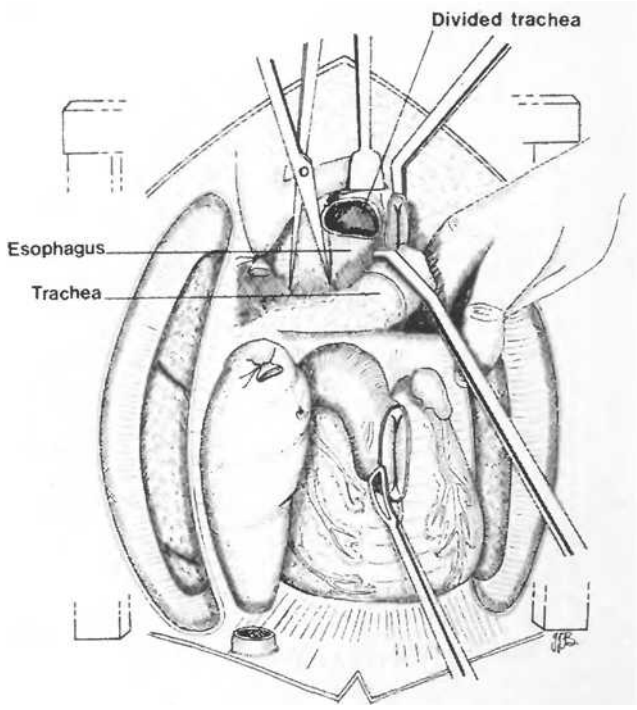

Figure 7 To facilitate the dissection, the surgeon's fingers are inserted posterior to the heart to retract this organ forwards and downwards

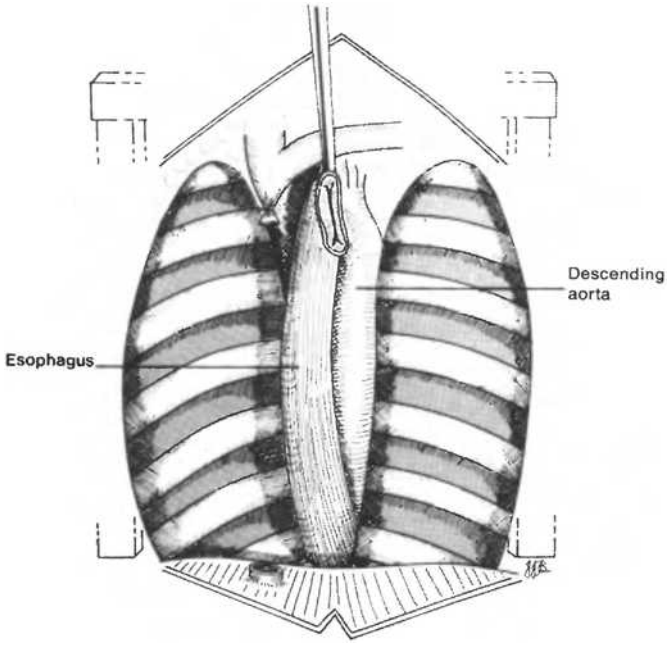

Figure 8 Major structures remaining after removal of the heart and lungs from the thoracic cavity

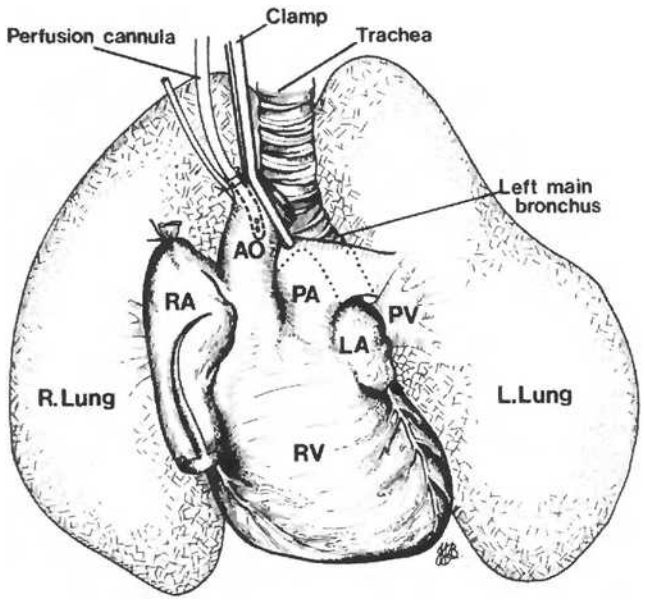

Figure 9 The excised organs. The cardioplegic perfusion catheter remains *in situ*. The right atrium has been incised in preparation for insertion into the recipient

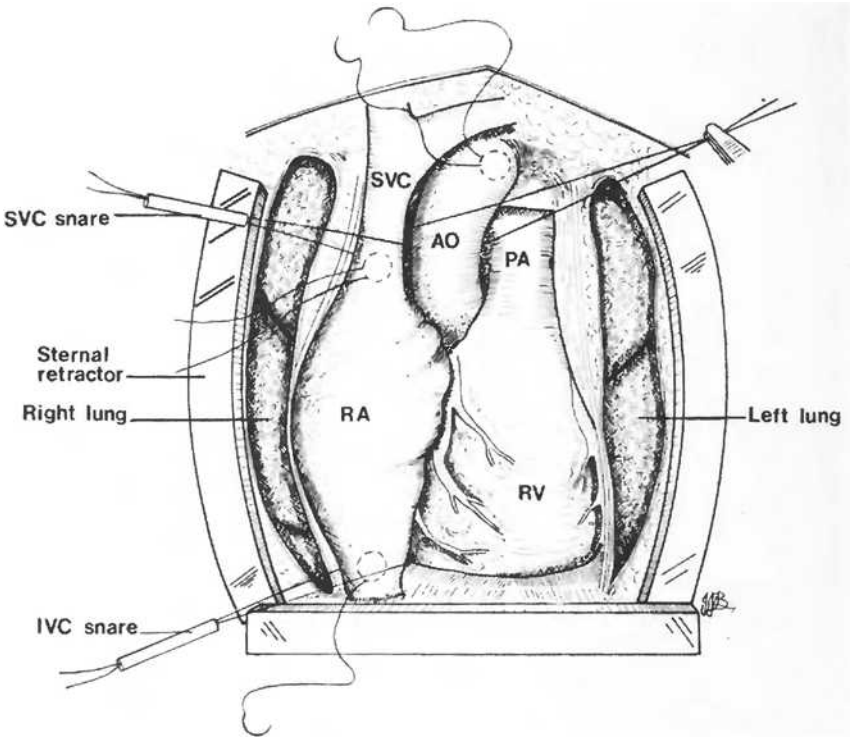

Figure 10 A median sternotomy has been performed. The ascending aorta has been mobilized from the pulmonary artery. Tapes have been passed around the ascending aorta, superior (SVC) and inferior (IVC) venae cavae. Purse-string sutures have been placed in the aorta, SVC and IVC. The pleura has been opened on each side anterior to the phenic nerves, exposing the lungs. (Abbreviations used in this chapter. SVC = superior vena cava; RA = right atrium; IVC = inferior vena cava; RV = right ventricle; PA = pulmonary artery; LA = left atrium; LV = left ventricle; AO = aorta)

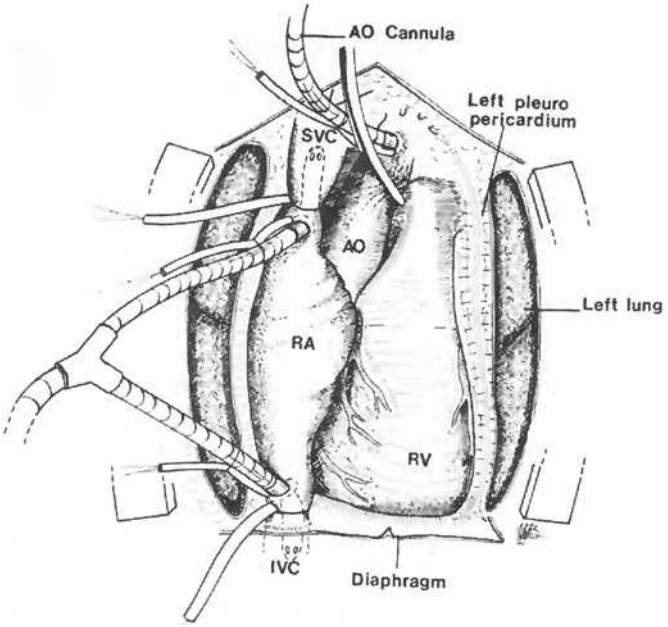

Figure 11 The aorta, SVC and IVC have been cannulated, and the ascending aorta cross-clamped

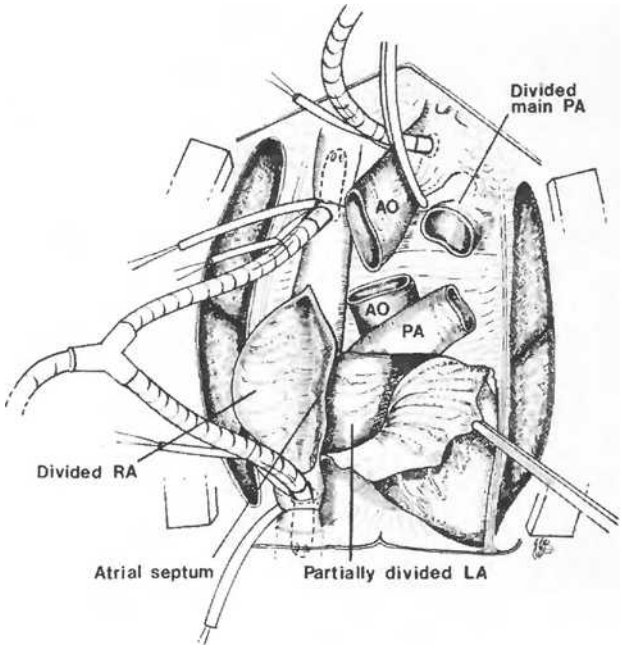

Figure 12 The aorta and pulmonary artery have been divided distal to their respective valves. The right atrium has been divided, and the left atrium is in the process of division

ascending aorta is mobilized from the pulmonary artery. Tapes are placed around the ascending aorta and the superior and inferior venae cavae. Heparin is administered and preparations are made for cannulation (Figure 10). The aorta is cannulated at the base of the innominate artery. The superior and inferior venae cavae are cannulated via the right atrium (Figure 11). If the superior vena cava is enlarged, a right-angled venous cannula should be used. This facilitates exposure and performance of the right atrial, or separate superior and inferior caval, anastomoses. Cardiopulmonary bypass is then instituted. Both venae cavae are snared over the cannulae. The body is systemically cooled to 28°C.

Excision of recipient heart and lungs

First, preparations are made for cardiectomy. The aorta is cross-clamped just proximal to the aortic cannula. The aorta is transected at the level of the commissures of the aortic valve. The right atrium is transected, beginning midway between the tip of the right atrial appendage and the junction of the superior vena cava and right atrium. This incision is carried inferiorly, leaving an adequate cuff of right atrium anterior to the venous cannulae. Care is taken to avoid injuring the Swan–Ganz catheter, if present. Superiorly, the incision is carried to the root of the aorta. The left atrium is incised just posterior to the aorta with a no. 11 knife blade. With both atria open, the right atrial septum is divided well anteriorly. The incisions in the septum and the lateral atrial wall join at the ostium of the coronary sinus. The pulmonary artery is divided at its midpoint. The recipient cardiectomy is completed by an incision along the atrioventricular groove on the left side (Figure 12).

Attention is then directed to the removal of the lungs. Electrocautery is used on low setting. A pedicle of pericardium and phrenic nerve is created bilaterally. An incision is made in the pericardium at the level of the main pulmonary artery, with great care taken to avoid injury to the phrenic nerve. The phrenic nerve must be repeatedly visualized, both medially and laterally, on the pericardial pedicle during this dissection. The incision is carried caudally from the pulmonary artery to the diaphragm, again with great care taken to avoid injury to the phrenic nerve. An opening large enough to permit passage of the donor lung into the pleural space is created. It is not necessary to carry this incision cephalad to the main pulmonary artery; in fact, especially on the left side, the recurrent laryngeal nerve may be injured if this incision is carried above the pulmonary artery. The phrenic nerve lies very close to the pulmonary hilum on the right side and proceeds posteriorly in its course towards the diaphragm. Thus, it is very easy to injure the phrenic nerve on the right side, unless this structure is repeatedly visualized during the dissection of the phrenic pedicle.

The recipient pneumonectomy follows. Under direct vision the inferior pulmonary ligaments are divided with electrocautery and surgical clips if necessary. A TA-90 stapler is passed extrapericardially around the left pulmonary hilum, with care taken to avoid injury to the phrenic nerve. The hilum is stapled, and the lung is removed (Figure 13). Similarly, the right pneumonectomy is performed. The staple lines are bilaterally made hemostatic, with electrocautery, clips, and sutures if necessary.

With the diseased heart and lungs removed, the posterior mediastinum and trachea must be prepared for implantation of the donor heart–lung block. The posterior left atrium is removed, leaving a small cuff attached to the right atrium. A passage is created posterior to the right atrium through the posterior pericardium, to allow the right lung to enter the right pleural space (Figure 14). All structures in this plane – from the diaphragm to the trachea – must be divided, to prevent any tension on the tracheal suture line.

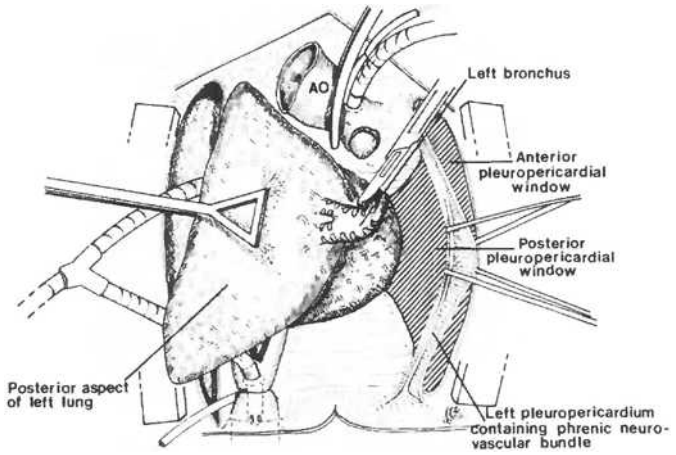

Figure 13 The left pleuropericardium has been incised posterior to the phrenic nerve. The left lung has been withdrawn from the left cavity into the pericardial cavity by passing it posterior to the phrenic neurovascular pedicle. The left bronchus has been dissected out and is about to be stapled and divided

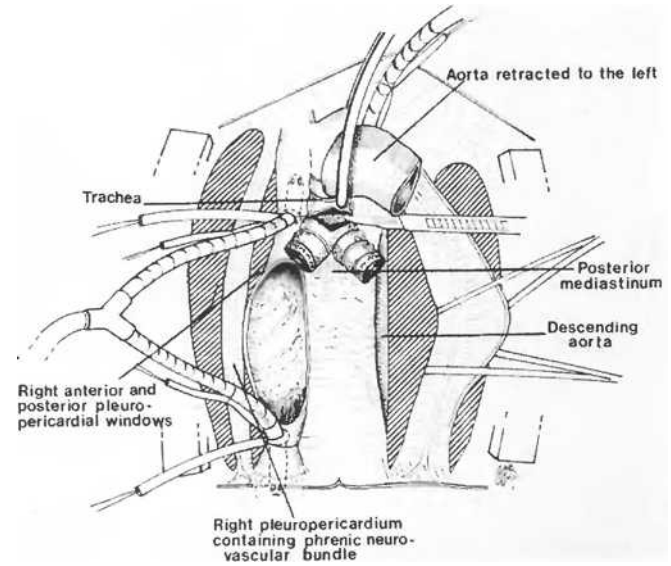

Figure 14 An incision has been made in the right pleuropericardium posterior to the right phrenic neurovascular bundle. The right lung has been withdrawn into the pericardial cavity, and has been excised (as was the left lung). The remnants of the two bronchi and lower trachea have been mobilized, and are being excised

In transecting the left atrium, it is important to avoid buttonholing the posterior right atrium. The atrial septum effectively becomes the outside wall of the right atrium after heart and lung transplantation. Therefore any small septal defects, a patent foramen ovale, or other openings must be secured; this area of the heart is completely inaccessible after the donor organs are implanted. We have found it useful to approximate any remaining left atrial tissue posterior to the atrial septum to improve hemostasis.

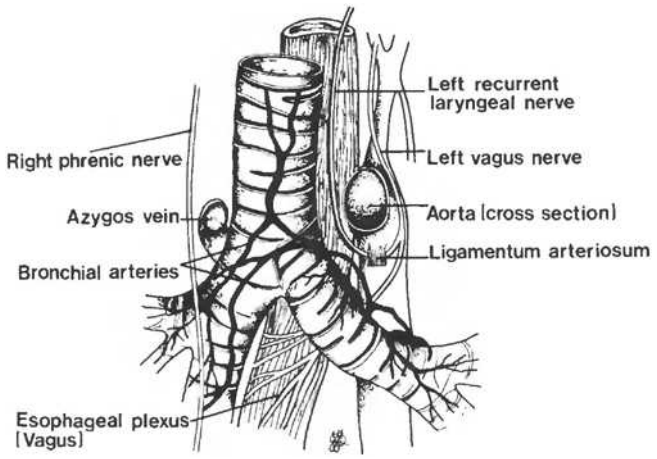

Figure 15 Drawing to illustrate the proximity of major thoracic nerves to the trachea, bronchi, and aorta. The potential sites of damage of these nerves during the operation of transplantation of the heart and lungs are obvious

The recipient trachea is then mobilized via the posterior pericardium between the aorta and superior vena cava. To gain access to the trachea the entire right pulmonary artery must be removed. The remnant of left pulmonary artery should be left in place, to avoid injury to the recurrent laryngeal nerve. The trachea is circumferentially mobilized at the level of the carina and divided. At this point, vessels accompanying the trachea must be carefully controlled with surgical clips. Extensive dissection in this area should be avoided, to prevent damage to neurovascular structures which lie in proximity to this anatomic area (Figure 15).

The recipient thoracic cavity is now prepared for implantation of the donor organs. Hemostasis must be immaculately secured in the posterior mediastinum, surrounding both pulmonary hila, in the inferior pulmonary ligaments, surrounding the remnant of left pulmonary artery, surrounding the trachea, and at any points where pleural adhesions were divided. Access to any of these points after the donor heart and lungs are implanted is at best difficult, and at worst impossible. Surgical clips or suture ligatures are preferred to obtain hemostasis.

Implantation of donor organs

The donor and recipient tracheae, aortae, and right atria must be anastomosed. The donor heart–lung block is brought to the operative field. The donor trachea is transected one tracheal ring above the carina; all of the adventitia must be left in place surrounding the carina and main stem bronchi. Aggressive skeletonization of these structures risks devascularization and subsequent anastomotic dehiscence.

Cultures are taken from the donor trachea, to help identify donor-transmitted bacterial pneumonia. The donor superior vena cava is reinforced, and an incision is made from the inferior vena caval orifice toward the right atrial appendage, staying well away from the area of the sinoatrial node. The donor atrial septum is inspected for any atrial septal defects, which are closed with a running suture. The heart–lung block is then placed in the recipient chest. The heart is positioned in the pericardial cavity. The right

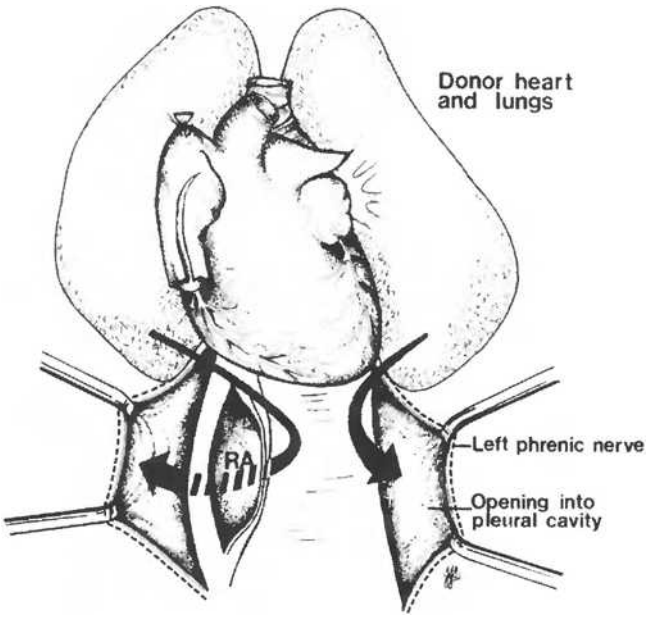

Figure 16 The donor heart and lungs will be inserted into the recipient chest by passing the donor right lung posterior to the recipient right atrium and right pherenic neurovascular bundle. The donor left lung will be passed posterior to the left phrenic neurovascular bundle. An incision has been made in the wall of the donor right atrium from the orifice of the IVC into the atrial appendage

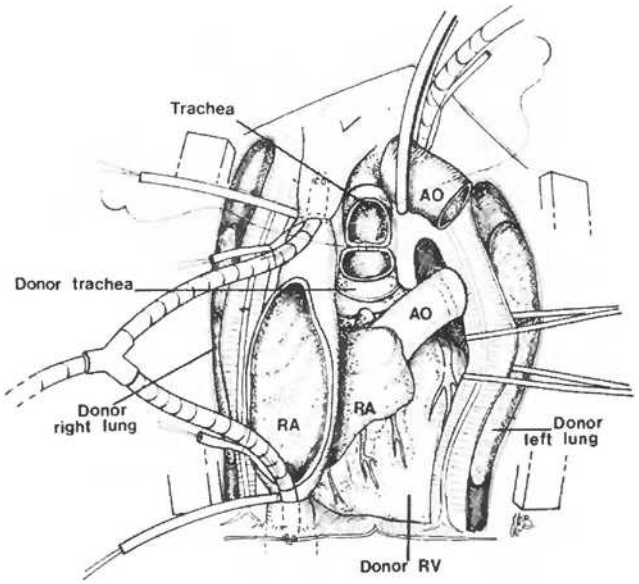

Figure 17 The donor heart and lungs are now in position in the recipient's chest. The tracheal anastomosis is being carried out. (The stay sutures at the junction of the membranous–cartilaginous tracheas are not shown)

lung passes behind the recipient right atrium and right phrenic nerve; the left lung passes behind the left phrenic nerve (Figure 16).

Implantation begins with anastomosis of the donor and recipient tracheas, which are joined end-to-end with a running 3/0 polypropylene suture (Figure 17). If desired, the tracheal anastomosis can be wrapped with donor pericardium. The donor right atrium is positioned anterior to the recipient right atrium (Figure 18). The oblique incision in the donor right atrial wall can be extended, if required, to match the orifice of the recipient right atrium. The right atrial anastomosis is performed with a continuous 3/0 polypropylene suture. The donor and recipient aortas are then shortened and anastomosed end-to-end with running two-layer 4/0 Prolene sutures; an inner horizontal mattress layer and an outer running layer are used (Figure 19). When the anastomosis is completed, a vent site is placed in the ascending aorta. The crossclamp is removed to restore perfusion to the graft.

A Swan–Ganz catheter, which had been removed from the heart before the cardiectomy, is repositioned into the pulmonary artery. The caval tapes are removed. After at least 30 minutes of reperfusion and multiple maneuvers to remove air from the transplanted heart, the patient can be weaned from cardiopulmonary bypass. The amputated left atrial appendage and the pulmonary preservation cannulation site must be closed. Where visible, the suture lines are checked carefully for hemostasis and are reinforced if necessary. If hemostasis appears adequate, protamine is given to counteract the heparin, and decannulation is accomplished (Figure 20). Each pleural space is drained by a large-bore right-angled catheter over the diaphragm and a large-bore straight chest catheter to the apex. The chest cavity is copiously irrigated with antibiotics. The chest is closed in a routine fashion.

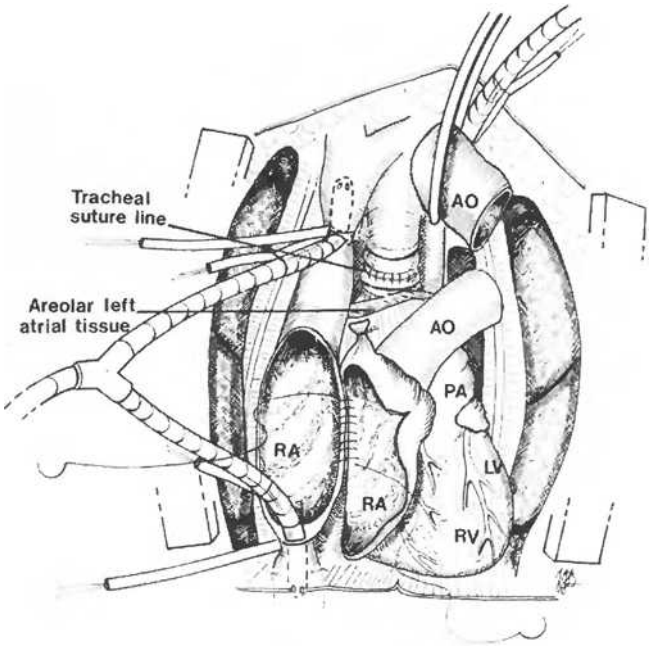

Figure 18 The tracheal anastomosis has been completed. The areolar tissue around the donor left atrium which is used to cover the site of the tracheal suture line is indicated, but in this drawing has not been sutured over the anastomosis. The two right atria are in process of being anastomosed

SPECIAL TECHNICAL CONSIDERATIONS

Postoperative bleeding

Bleeding has been a major cause of early postoperative morbidity and mortality after heart–lung transplants[11–13]. Life-threatening

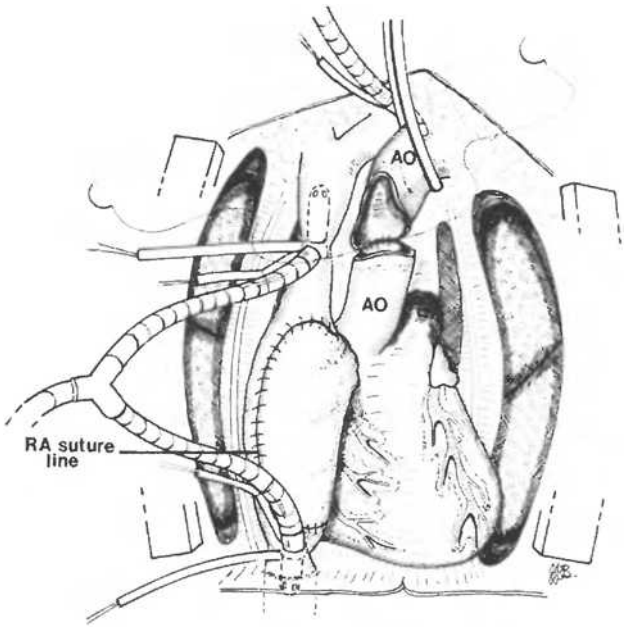

Figure 19 The right atrial anastomosis has been completed. The aortic anastomosis is in progress

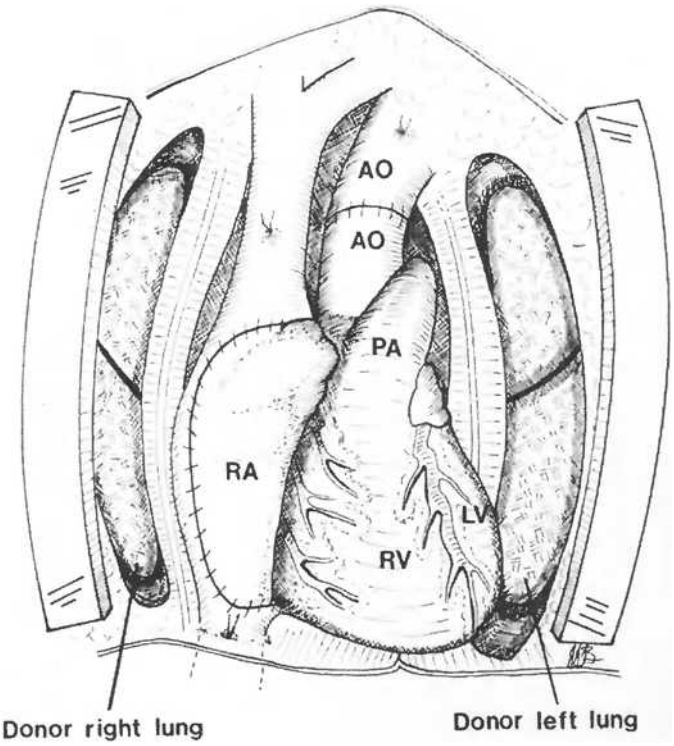

Figure 20 The aortic anastomosis has been completed, and all cannulae have been removed

bleeding can develop in patients with intrathoracic adhesions due to previous surgery, or with abundant enlarged bronchial arteries and pleural collaterals due to underlying disease. However, as experience in heart–lung transplantation has grown, several strategies have emerged that help reduce postoperative hemorrhage[14,15].

Posterior mediastinal attachments of the donor graft can bleed after implantation. Careful inspection and ligation of bleeding tissue will improve hemostasis. Similarly, after the reversal of heparin, the phrenic nerve neurovascular pedicles should be inspected for bleeding sites, which are secured with suture ligation.

Meticulous hemostasis of the recipient posterior mediastinum, before the heart–lung block is implanted, is paramount. Most of the posterior pericardium should be left intact without undue dissection or excision. The mediastinal parietal pleura should be sutured to the lateral edge of the pericardium with running 4/0 polypropylene sutures. Hemostasis of the peribronchial tissue should be carefully maintained during dissection of the carina; clips or suture ligatures are preferred to electrocautery. Areas around inferior pulmonary ligaments should be observed and bleeding sites controlled. Pulmonary adhesions should be divided before giving heparin; electrocautery and argon beam coagulation can help reduce bleeding.

Coagulopathy may be induced by either preoperative medications or hepatic failure. Its effect can be compounded by the use of cardiopulmonary bypass during the transplant. Aprotinin, a serine protease inhibitor derived from bovine lung tissue, has been shown to decrease postoperative bleeding and blood product transfusion requirements in patients undergoing open-heart surgery[16–18]. Aprotinin augments postoperative hemostasis after heart–lung transplantation[19]. Decreased bleeding and blood product transfusions benefit the right heart and pulmonary function postoperatively. However, there is no substitute for meticulous surgical technique and attention to detail.

Patent ductus arteriosus

Some patients with Eisenmenger's complex and elevated pulmonary artery pressures may have a patent ductus arteriosus that is not visualized on preoperative testing. In this group of patients, controlling the PDA during surgery can be difficult. The ductus tissue is very friable; ligation should not be attempted before preparations are completed for cardiopulmonary bypass. During the recipient cardiectomy, a previously undiagnosed PDA may become apparent if unexplained bleeding occurs when the aorta is transected after crossclamping. Systemic perfusion may be greatly reduced as the blood is shunted into the lungs. Division and control of a PDA may require circulatory arrest techniques to provide good visualization. Secure closure is accomplished with Teflon-supported sutures, after which the transplant can proceed.

Torsion of the grafted lungs

During passage of the right and left lungs into the recipient chest, great care should be taken to avoid torsion of either pulmonary hilum. Each lobe of the lung should be identified and the position of the lungs checked, to ensure that rotation of lung or lobe is not present at the hilum. Such defects are impossible to correct after the anastomosis is completed.

Cardiac bicaval anastomosis

In orthotopic heart transplantation, anastomosis of the donor atrium to the recipient atrium alters the anatomic size and geometric shape

of the atria, as seen on echocardiography[20,21].Consequently, mitral and tricuspid regurgitation are frequent. To circumvent these problems, alternative anastomosis techniques have been described[22]. Heart–lung transplantation involves only right atrial anastomosis; to reduce tricuspid regurgitation, bicaval anastomoses can be performed – a technique favored by our group recently. To use this technique, some simple modifications are required during procurement. To obtain maximum length the superior vena cava should be transected above the azygos vein. The inferior vena cava should be divided at the diaphragmatic reflection. The recipient superior and inferior venae cavae should be cannulated with right-angled cannulae, which facilitate the bicaval end-to-end anastomoses.

POSTOPERATIVE CARE

Intensive-care unit

Patients are nursed in the intensive-care unit until extubated. Strict attention to fluid management is maintained. Fluid overload and renal impairment are common, due to the effects of cardiopulmonary bypass and cyclosporin therapy. Diuresis is achieved with diuretics and low-dose dopamine (2–3 μg/kg per minute) by intravenous infusion. Patients also receive intravenous isoproterenol for 2–3 days, which has beneficial chronotropic effects and reduces pulmonary vascular resistance. Cardiovascular performance is optimized to improve oxygen delivery.

Early weaning from the ventilator is encouraged, based on physiologic respiratory parameters. The inspired oxygen (F_iO_2) on the ventilator is kept at the lowest possible level, to keep the arterial oxygen (Pa_{O_2}) around 80 mmHg or to maintain an arterial oxygen saturation of 90% or greater. Positive end-expiratory pressure (PEEP) is used, as needed, to maintain adequate oxygenation. PEEP and peak inspiratory pressure (PIP) are kept below 10 cmH_2O and 30 mmHg, respectively. For the first 3 days chest roentgenograms are obtained twice a day. Diffuse pulmonary opacities may be seen, due to preservation injury, which usually resolves after conservative treatment with diuretics and pulmonary toilet. Fiberoptic bronchoscopy is carried out within the first 24 hours to assess the tracheal anastomosis, and thereafter as indicated by the patient's clinical condition, chest radiograph, or arterial blood gases.

Immunosuppression

All of our recipients receive triple-therapy immunosuppression, a protocol introduced at the University of Minnesota in 1983 for heart transplants[23] (Table 2). Cyclosporine (CsA) is started preoperatively at 4–6 mg/kg, depending on renal function. Postoperatively, CsA is administered at 1–2 mg/h as a continuous intravenous infusion. In addition, CsA is given orally or via nasogastric tube at 4–6 mg/kg per day in two divided doses 12 h apart. Whole blood CsA levels are checked every day for the first 10 post-transplant days and every other day thereafter. Oral CsA doses are adjusted to maintain a level of around 300 μg/l in the first month post-transplant (as determined by high-performance liquid chromatography). The correlation between blood CsA concentration and effect is weak, but concentrations less than 100 μg/l in the immediate post-transplant period are frequently associated with rejection. Similarly, the correlation between blood CsA concentration and toxicity is relatively poor but, in general, risk of toxicity increases significantly with levels greater than 350 μg/l. As the time post-transplant increases, the need for frequent CsA blood level monitoring decreases. After 3 months post-transplant, monthly monitoring is sufficient for stable patients.

Table 2 Immunosuppression for heart–lung recipients

Preoperative
CsA 4–6 mg/kg orally depending on renal function
AZA 2–3 mg/kg orally

Intraoperative
MP 500 mg intravenously at the time of reperfusion

Postoperative
CsA
  Oral (nasogastric) 4–6 mg/kg per day in two divided doses, 12 h apart
  Intravenous 1–2 mg/h by continuous infusion
  Dosage adjusted to achieve whole blood CsA level of 300 μg/l
AZA
  Oral or by nasogastric tube 2–3 mg/kg per day
  Dosage decreased if WBC count < 5000 mm^3
MP
  Intravenous 125 mg every 8 h for three doses
Prednisone
  Oral 0.5 mg/kg per day in two divided doses beginning on day 1

Maintenance
CsA 5–6 mg/kg per day in two divided doses (adjusted to maintain blood CsA level of 200–300 μg/l)
AZA 1.5–2.5 mg/kg per day (decreased if WBC count < 5000 mm^3)
Prednisone tapered to 0.1 mg/kg per day by 3–6 months (depending on clinical course)

CSA = cyclosporine; AZA = azathioprine; MP = methylprednisolone; WBC = white blood cell

Azathioprine (AZA) is administered preoperatively at 2–3 mg/kg. Postoperatively, AZA dosage is targeted to maintain a white blood cell count of 4000–5000 cells/mm^3.

Methylprednisolone (MP) is administered intraoperatively at the time of reperfusion at 500 mg intravenously. Postoperatively, MP is given intravenously at 125 mg every 8 h, for a total of three doses. Low-dose (0.5 mg/kg per day) oral prednisone is begun on postoperative day 1. In our early experience with heart–lung transplants, oral prednisone was withheld for the first 2 weeks, to promote healing of the airway anastomosis. However, most patients received pulse MP therapy 1–3 weeks post-transplant to treat pulmonary rejection – and their airway anastomosis still healed satisfactorily. Our current practice, therefore, is to maintain low-dose prednisone from postoperative day 1. By 3–6 months post-transplant, prednisone has been tapered to 0.1 mg/kg per day.

Infection prophylaxis

Infections are the leading cause of morbidity and mortality after a heart–lung transplantation[24,25]. Surveillance cultures of sputum, urine, and blood, and viral antibody titers should be routinely monitored. In our institution all patients receive perioperative vancomycin for 24 h and cefamandole until all drainage catheters

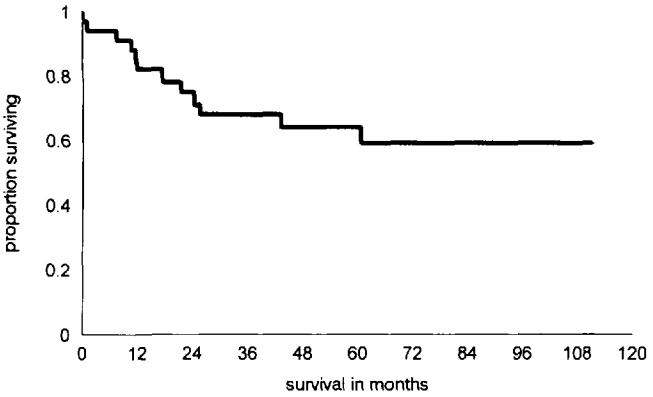

Figure 21

and monitoring lines are removed. Further antibiotic therapy depends on the results of donor bronchial secretion cultures. Every attempt should be made to treat identified infections only, and to avoid indiscriminate use of antibiotics, lest fungal or resistant bacterial overgrowth develops.

Heart–lung recipients are especially prone to pneumonias, particularly those caused by opportunistic organisms such as cytomegalovirus (CMV) and *Pneumocystis carinii*. Recipients who are CMV-seronegative pretransplant receive CMV-negative blood and blood products. In addition, donor or recipient CMV-seropositive status requires treatment with intravenous ganciclovir at 5 mg/kg twice a day for 14 days, then 5 mg/kg per day for 8 weeks. The dose of ganciclovir is adjusted according to renal function. Mycostatin is given by mouth for 3 months post-transplant. Trimethoprim-sulfamethoxazole is given indefinitely to prevent infections caused by *Pneumocystis* and *Nocardia* organisms.

RESULTS

Improved patient selection, surgical techniques, and postoperative care have reduced 30-day mortality after combined heart–lung transplantation to around 20%. However, the outcome for long-term survival has not changed markedly. The international Society for Heart and Lung Transplantation report 1-, 5- and 10-year survival rates of 59%, 42%, and 20%, respectively, among 1567 heart–lung transplant recipients worldwide[26]. In our institution the heart–lung transplant program (begun in 1986) has survival rates of 82% at 1 year and 64% at 5 years (Figure 21).

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Lung and Heart–Lung Transplantation: A Review of Progress and Current Status based on the Registry of the International Society for Heart and Lung Transplantation

G.B. HAASLER AND J.D. HOSENPUD

INTRODUCTION

The past decade has witnessed the rise of lung transplantation (LTx) as a viable clinical entity, and has seen substantial revisions in the concepts underlying the techniques and indications for LTx and heart–lung transplantation (HLTx). From rather inauspicious beginnings in the late 1960s and 1970s, LTx has emerged as a modality useful for treating both end-stage primary lung disease in a variety of settings[1–17] and the pulmonary sequelae of congenital heart disease[4,7,15,18–21]. Over 500 single lung, 300 double lung, and 100 heart–lung transplants were carried out in 1994 (Figures 1–3). Single (SLTx) and double (or, more correctly, bilateral single) (DLTx) lung transplantation have resulted in substantial reversibility of right heart dysfunction secondary to pulmonary vascular disease in both primary and secondary pulmonary hypertension, allowing preservation of the patient's own heart where formerly HLTx was considered a necessity[19,22]. This has resulted in a significant reduction in the number of heart–lung transplants yearly (Figure 3). In addition, the routine use of DLTx

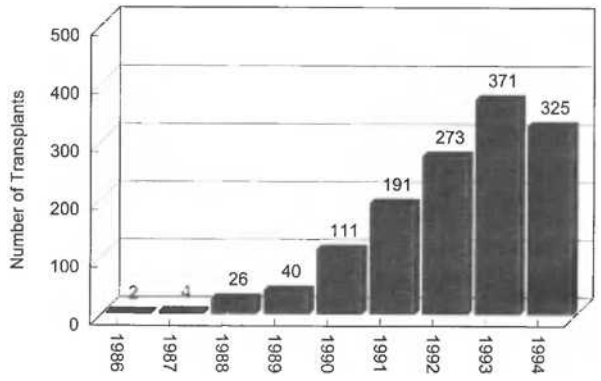

Figure 2 Number of bilateral and double lung transplants performed 1986–1994. This includes both the *en-bloc* DLTx (done earlier) and the more recent bilateral sequential SLTx, which has become the major way in which two lungs are transplanted currently. The volume may be stabilizing due to non-increase of the donor pool. However, 1994 data may still reflect some incomplete acquisition. (Source: ref. 10)

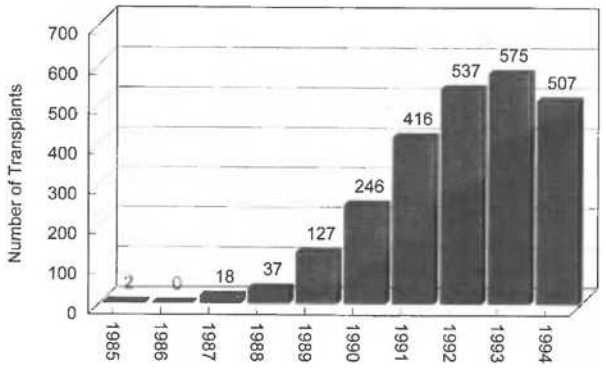

Figure 1 Number of SLTx performed 1985–1994. There was a progressive increase in the number of SLTx carried out annually until 1993. Data for 1994 may reflect some incomplete acquisition. However, the number of transplants performed may be stabilizing due to restriction of the donor pool. (Source: ref. 10)

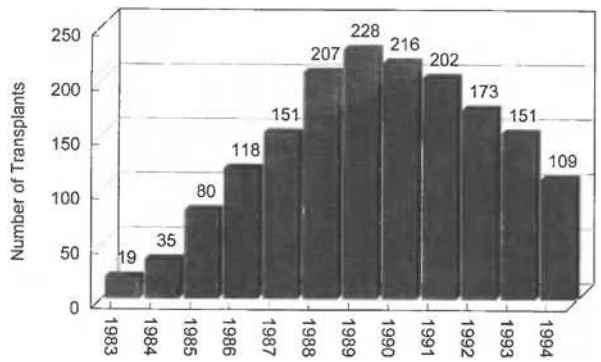

Figure 3 Number of HLTx performed 1983–1994. The number of HLTx has clearly decreased since 1989 with the realization that SLTx and bilateral pulmonary replacement could often allow recovery of a failing heart. In addition, increasing success in LTx for cystic fibrosis has resulted in fewer HLTx being carried out for the indication. (Source: ref. 10)

in septic conditions, such as cystic fibrosis and bronchiectasis, has demonstrated that transplantation and immunosuppression can be carried out in a unique setting of infection where significant residual bacterial colonization still exists in other body reservoirs[2,23,24].

The techniques and principles that have led to the resurgence and modern development of LTx have proven almost as pliant as the organs themselves. Principles such as the avoidance of routine corticosteroids and omental wrapping of bronchial anastomoses have not been proven as essential as once thought[25–28]. Other developments such as an increased awareness of the lungs' metabolic activities have resulted in specific pulmoprotective techniques that are unique to LTx and not just borrowed from other organ transplant technology[3,29–34]. Constant improvements in techniques and in perioperative care are allowing patients with a variety of conditions and attendant medical problems to safely undergo pulmonary replacement[4,7,35]. As selection criteria progressively change and liberalize, it remains to be seen what effect these policies will have on organ availability and mortality statistics.

This chapter will examine successes, trends and problems in LTx and HLTx based on current literature and data from the International Society for Heart and Lung Transplantation (ISHLT) Registry.

HISTORICAL PERSPECTIVE

James Hardy (Chapter 18, Figure 9) of Mississippi carried out the first clinical human LTx in 1963 (Chapter 45). At that time, with there being very limited knowledge and means, the patient survived for 18 days, expiring of renal failure[4]. Over the next 15 years approximately 40 lung transplants were carried out worldwide, without a single long-term survivor. The reasons for failure in these patients were chiefly technical, with bronchial dehiscence the major source of mortality[2]. Recipient selection involved extremely ill patients, with perhaps predictably poor outcomes. Following this period, clinical LTx was thought to be unfeasible. While solid-organ transplantation continued to develop in the renal and cardiac arenas, LTx remained confined to a few animal laboratories.

Pioneering work by Morgan and others[2,4,36], suggested that improved vascularization of the ischemic donor bronchus would be possible using the omentum as a wrap. Using three other clinical and scientific premises – (a) the rise of cyclosporin A as a new immunosuppressive agent, (b) the desire to avoid large doses of steroids which inhibit healing, and (c) the selection of patients who were not yet so ill as to be preterminal, and therefore could be improved clinically prior to transplantation – Joel Cooper (Chapter 45, Figure 1) and others at the Toronto General Hospital Program carried out several successful SLTx for idiopathic pulmonary fibrosis[8,16,17,37]. It was initially thought that pulmonary fibrosis, without clinical sepsis and with a shrunken contralateral lung, would be the ideal setting for future LTx, although the number of patients with pulmonary fibrosis was small compared to other pulmonary disorders.

Heart–lung transplantation had been carried out for primary pulmonary hypertension and secondary pulmonary hypertension due to congenital cardiac disorders. It was rapidly recognized that SLTx could offer a superb low-resistance circuit for blood flow that would allow recovery of right-sided cardiac function alone. The transplanted lung could function not only as an organ of oxygenation but as the ideal 'runoff' for an overloaded right ventricle. The implications of this concept have been enormous[19,20,22,38], resulting in a progressive decline in the performance of HLTx (Figure 3) when it was discovered that even a severely compromised right ventricle could recover substantial function if left ventricular function was still intact. (A parallel experience in pulmonary endarterectomy for chronic pulmonary emboli demonstrated reversibility of right ventricular ejection fractions as low as 10%.)

A variety of combinations of heart and lung transplantation were subsequently carried out, including SLTx, DLTx, SLTx with heart transplantation[39], and SLTx or DLTx with concomitant intracardiac repair of a congenital defect[15,19–21,38]. Current indications for SLTx, HLTx, and DLTx are outlined in Figures 4–6 and Tables 1 and 2. Emphysema (acquired and congenitally predisposed) constitutes the single largest group of patients obtaining SLTx. Nearly equal numbers of patients with emphysema and cystic fibrosis receive DLTx. The original indication for SLTx, idiopathic pulmonary fibrosis, currently forms a fairly small group at 16.7%, reflecting the large increase in LTx for emphysema.

Technical advances had improved in clinical HLTx also[35,40]. Confidence over the ability to sustain a transplanted heart–lung

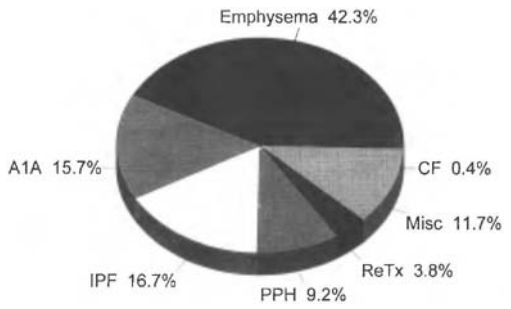

Figure 4 Indications for adult SLTx. Acquired and congenital (A1A) emphysema constitute the majority of SLTx. The original primary indication for SLTx, idiopathic pulmonary fibrosis (IPF), currently constitutes a relatively small percentage of cases. Patients with primary pulmonary hypertension constitute a fairly small group (in both SLTx and bilateral DLTx). A certain number of patients with congenital heart disease and secondary pulmonary hypertension are included in the miscellaneous category. (Source: ref. 10)

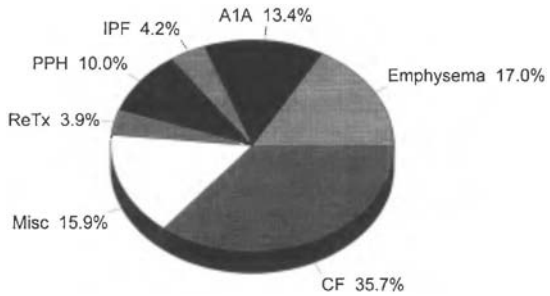

Figure 5 Indications for adult bilateral/DLTx. Emphysema and septic lung disease due to cystic fibrosis constitute the major indications. (Source: ref. 10)

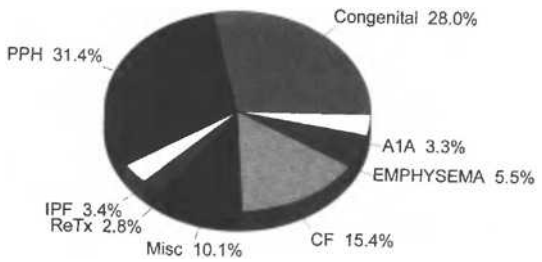

Figure 6 Indications for adult HLTx. Primary pulmonary hypertension and congenital heart disease with secondary pulmonary hypertension constitute the major indications. Patients with emphysema who undergo HLTx are primarily those with additional left ventricular dysfunction. (Source: ref. 10)

Table 1 End-stage lung diseases suitable for single or bilateral lung transplantation (see also Figures 4 and 5)

Idiopathic interstitial pulmonary fibrosis
Pulmonary fibrosis of occupational etiology
Sarcoidosis
Toxin-induced irreversible pulmonary disease (non-narcotic)
Eosinophilic granuloma
Primary pulmonary hypertension
Acquired or congenital emphysema (not volume reduction or bullectomy candidates)
Chronic pulmonary embolic pulmonary hypertension (not amenable to thrombo-endarterectomy)
Scleroderma
Cystic fibrosis
Bronchiectasis
Bronchiolitis obliterans
Lymphangioleiomyomatosis
Multiple arteriovenous malformations (not amenable to other measures)
Other less common interstitial or obstructive lung diseases as determined by individual consideration

Table 2 Indications for heart–lung transplantation (see also Figure 6)

Congenital heart disease (with anatomic abnormalities *not* reparable) with pulmonary hypertension
End-stage lung disease with left ventricular dysfunction with or without clinical right heart dysfunction
Primary pulmonary hypertension (with LV dysfunction)
Cystic fibrosis (usually bilateral lung transplant now)

block led surgeons to consider HLTx for cystic fibrosis[1,4,23,24,41] and to domino procedures, wherein a patient with primary pulmonary disease and secondary right ventricular hypertrophy would receive a heart–lung block from a donor, while the recipient's own heart ('conditioned' with secondary right ventricular hypertrophy after functioning for many months in the presence of pulmonary vascular disease) would be transplanted into a person requiring chiefly a heart transplant, but with moderate elevations of pulmonary vascular resistance[42–45]. While ingenious and successful by short-term consideration, ethical reservations were voiced on several fronts regarding this modality, given the uncertainties of future cardiac graft vasculopathy (chronic rejection). The overall utilization of heart–lung blocks has decreased over the past few years (Figure 3) with a corresponding increase in the number of SLTx.

Bilateral LTx proved necessary for a variety of conditions, including bronchiectasis and cystic fibrosis, where a SLTx might be soiled by a remaining septic lung (Table 1). Isolated reports of SLTx with contralateral pneumonectomy for cystic fibrosis were published[46,47], but generally the DLTx has been preferred. Post-pneumonectomy space problems can be avoided in this way, and lung function appears to be better after DLTx in young people.

Initially, it was thought that DLTx was also routinely required for emphysema because of the fear that a SLTx would be overwhelmed by a more compliant hyperinflated remaining lung[4,5,9,12,48]. While this appeared to occur in some instances, further experience suggested that lung reduction of the remaining lung could be carried out, resulting in satisfactory decompression of the transplanted lung. Increasing awareness that unilateral transplanted organ compression appeared more likely due to graft dysfunction than to compression led to a better understanding of the pathophysiology in these transplants. The advent of SLTx and DLTx for emphysema[12,13,49,50] led to a large cohort of patients becoming immediately available for transplantation (Figures 4 and 5). In addition, the emphysema patients, who were generally more stable than other patients preoperatively, proved to be more stable postoperatively with consequently better survival[7,10] (Figure 7).

Techniques for DLTx initially followed that of HLTx[51], with a single tracheal anastomosis, a single pulmonary arterial anastomosis, and a single left atrial anastomosis, constructed with the patient's heart arrested on cardiopulmonary bypass. This procedure had significant complications in the form of tracheal anastomotic problems, low cardiac output, and neurologic sequelae of cardiopulmonary bypass[4,52]. The recognition that DLTx could be carried out much more easily, and with greater safety, often avoiding cardiopulmonary bypass altogether, using a sequential bilateral SLTx technique, further reduced the risks and problems associated with DLTx[52–54]. This significantly affected the early mortality after DLTx. Concomitant improvements in critical care, peri-transplant infection control, and earlier recognition and treatment of rejection have also been the subject of significant study, and have contributed to improved clinical outcomes[4,16,17,55–58].

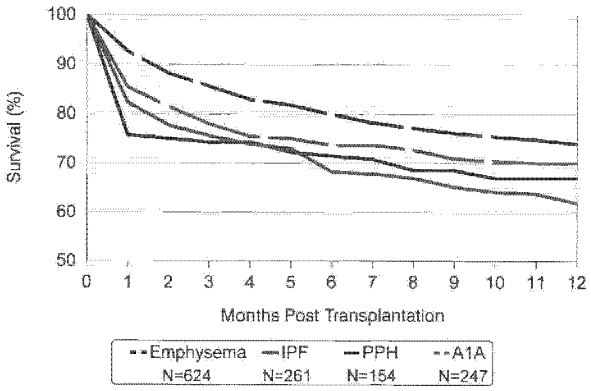

Figure 7 Actuarial patient survival following SLTx by indications. Patients with acquired emphysema constitute the largest group and have the best long-term survival. A significant component to survival is the initial postoperative course, with pulmonary hypertension patients faring the worst. After the first month the courses are approximately parallel, with late complications of LTx representing the main reasons for eventual death. (Source: ref. 10)

IMPROVEMENTS IN DONOR LUNG SELECTION AND DONOR MANAGEMENT

Early efforts in LTx involved the use of a donor situated in an adjacent operating room to that of the recipient, or required transport of the donor to the recipient hospital[2,37]. The management of donors has undergone significant improvement since lungs were first considered for transplantation[4,59–63]. Since kidney viability and transplantation were early priorities of most organ procurement agencies, some of the policies which would favor the kidneys, such as vigorous hydration of the donor, would in fact hurt the lungs due to the development of pulmonary edema. In addition, the importance of optimal pulmonary management early on in the post-injury period was not clearly appreciated as it related to organ donation. Aggressive pulmonary care early on after a neurologic event was thought to cause wide swings in intracerebral pressure, and was therefore considered inadvisable. Subsequently, the understanding that aggressive suctioning of pulmonary secretions, careful identification and control of aspiration pneumonias, and judicious fluid management were required to keep the lungs in a condition that might favor donation became better appreciated.

The lung is in a unique position as a transplantable solid organ in that it is constantly in communication with the outside environment, being subjected not only to inhaled material, but also to substances pushed through by positive pressure ventilation and intravenous routes (such as air and embolic material). The realization that the metabolic functions of the lung could also be compromised by shock led to improved understanding of apparent lung dysfunction after injury.

The basic criteria for lung donation have not changed over the years in that evidence of adequate oxygenation (P_{O_2} greater than 300 or 350 on 100% O_2 and 5 cm of PEEP) is required by most groups. Certainly, unilateral pulmonary dysfunction in the donor, which has been explained on a non-septic etiology, has been increasingly recognizable in patients suffering from post-traumatic pulmonary contusion, and may allow use of the contralateral lung. Whereas initially it was thought that lungs without an absolutely clear chest radiograph could not be used, this has been shown to be an erroneous assumption. Aggressive bronchoscopy may reveal airway plugging that can be safely cleared out, improving donor oxygenation. While 'marginal donors' have occasionally been used successfully[64,65], the basic requirement for fairly adequate oxygenation persists, and situations must be carefully individualized by considering donor history and recipient status.

Lung procurement and preservation techniques have similarly evolved with the rest of the science. Initially, lungs were rapidly harvested from a donor in an adjacent operating room and preserved in an atelectatic state by cold immersion[4,8,37]. Subsequently, the need to obtain organs from more distant locations – and the recognition that explanted, cold lungs continue to exhibit aerobic metabolism – led to the development of other pulmoprotective techniques. The first major change involved perfusion and flushing of the donor organs with a balanced electrolyte solution. Euro-Collins solution was accepted as a superb physiologic solution, and was modified with glucose and magnesium to allow metabolism to occur in the excised lung. It was recognized that the lung should be kept inflated in order to avoid atelectasis, loss of surfactant, and continued air substrate for the lungs' metabolic activities. With this, preservation of the lung improved substantially, allowing long-distance transport with a subsequent decrease in the incidence of primary graft failure[28,31,32,34,59]. In addition, the recognition that better cooling and delivery of washing substances could occur by administering prostaglandin E1 as a pulmonary vasodilator appeared to result in better lung preservation.

While much interest focuses currently on issues of lung preservation, no solution or perfusion technique has been demonstrated to be clearly superior over another for preservation of the lung. For harvesting of the heart–lung block, where heart utilization, either separately or as part of a HLTx, is planned, the use of University of Wisconsin (UW) solution has been demonstrated in cardiac transplantation to result in earlier return of function and fewer rhythm problems for the cardiac portion. For lung function, UW solution has been shown to extend ischemic time in laboratory animals[33]. Active research is taking place with other solution additives to reduce lipid peroxidation (allopurinol and lazaroid compounds)[29,66].

RECIPIENT SELECTION

Significant interest has also focused on the optimum timing of LTx and on the physical condition of the transplant recipient in order to favorably influence outcome. Initial patients selected for early attempts at LTx in the mid-1970s were frequently ventilator-dependent, extremely ill patients in poor physical condition who proved unable to withstand the rigors of transplantation. Much work has subsequently focused not only on determining the optimum point in a person's disease where LTx should occur, but also on the condition in which the patient is able to be brought for transplantation[9,35]. The realization that patients could not be transplanted with high expectations of success if they were nearly dead led to extensive interest in the natural history of various pulmonary disorders and indices of eventual failure[9,35,40,67]. In each of the major disease entities (emphysema, cystic fibrosis[68], pulmonary hypertension[67], pulmonary fibrosis[28]), indices of deterioration were identified and assessed in the context of the natural history of that disease.

In emphysema, the onset of oxygen use, more frequent hospitalizations, and increasing pulmonary exercise disability were felt to represent increased risk for death over the next 2 years compared to the outcome that LTx could offer[28]. As the post-transplant results for emphysema have improved, LTx has appeared even more attractive. These functional characteristics appeared to be more reliable as indicators than any absolute value of spirometric function, although most early patients undergoing LTx for emphysema have had FEV_1 determinations <30% of predicted. The non-transplanted emphysema patient who deteriorates to the point where he or she begins to require night-time oxygen has a predicted 2-year survival of 60%, down from 80% expected when not requiring oxygen[28].

With pulmonary hypertension, similar criteria were developed in the form of progressive disability, mean pulmonary arterial pressure $\geq$50 mmHg, decreased cardiac index $\leq$2.5 l/m^2, mean right atrial pressure $\geq$10 mmHg, syncopal episodes, and the development of hemoptysis. Because pulmonary hypertension, particularly of the idiopathic variety, is associated with an often

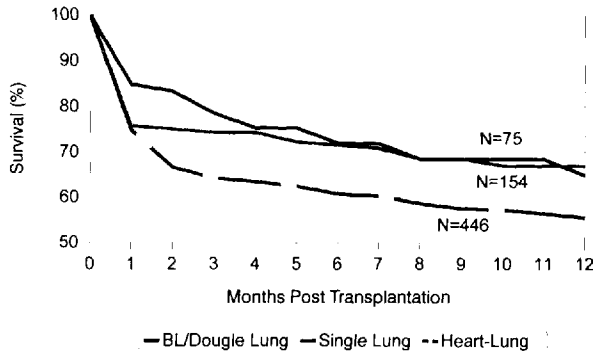

Figure 8 Actuarial patient survival for PPH following LTx by procedure. Patients undergoing bilateral LTx, as opposed to SLTx, have an initial better in-hospital course. This may reflect that initial adjustments in cardiac output and residual pulmonary hypertension may play a major role in the early post-operative course after transplantation. It is rather striking, however, that these curves converge at 4 months and remain parallel thereafter. This may reflect the total dependence on transplanted tissues that the patients with DLTx face. As in other categories where HLTx is carried out, survival is significantly poorer than after SLTx or DLTx. (Source: ref. 10)

unpredictable progression, it is the trend towards increasing events or more frequent hospitalizations that identifies the need for transplantation[22,28]. Whether patients with pulmonary hypertension should be considered for SLTx or DLTx is still a controversial topic. Lower pulmonary pressures and improved heart function are seen with DLTx or HLTx[22]. However, organ availability and the critical condition of some of these patients may restrict this choice. The SLTx is comparatively flooded with blood from the time of transplant, making the postoperative period potentially more difficult. The influence of the choice of transplant procedure (single or double) on long-term survival therefore remains a concern. ISHLT Registry data (Figure 8) suggest decreased survival of SLTx versus DLTx recipients within the first 6 months, although later survival appears superimposable. The early results of SLTx for primary pulmonary hypertension are certainly worse than those for the other conditions requiring SLTx (Figure 7).

The recognition of the complex milieu of cystic fibrosis led to identification of several predictive factors for mortality in this condition. These were the increasing need for hospitalization, the development of weight loss, a rising P_{CO_2}, and an FEV_1 <30% of predicted[1,3,23,68]. In cystic fibrosis patients the assessment of the referring pulmonologist in identifying early signs of inevitable deterioration was considered a key component of assessing the patient for LTx. The importance of proper selection of patients became more acute in cystic fibrosis for other reasons also, given that these patients frequently had highly aggressive resistant organisms present in the tracheobronchial tree that would most likely remain after transplantation[3]. Most cystic fibrosis patients have reservoirs of *Pseudomonas aeruginosa* and *Pseudomonas cepacia*, as well as *Aspergillus*, in their respiratory tree, particularly in their sinuses[3]. The recognition that sinus disease could prove a major problem in the postoperative period led to prophylactic sinus drainage procedures. While these have not stood the test of time as being essential (and have sometimes proven meddlesome), the attention which was focused on all sources of persistent bacteria, as well as the need to control any infection, has led to a general improvement in the perioperative management of transplanted cystic fibrosis patients.

The need for nutritional supplementation has been noted in all areas of LTx, most notably in cystic fibrosis and chronic lung disease patients. Frequently, both groups represent a nutritionally depleted patient population and nutritional supplementation, coupled with an appropriate exercise regimen, can help these patients enormously[70]. Calcium supplementation may also be indicated, as osteoporosis is frequently seen in this patient population.

Initially, good-risk patients were selected, while patients on mechanical ventilators or in older age groups (>60 years) were automatically excluded. Subsequently, as LTx has become more successful, many programs have chosen to offer LTx to older individuals, as well as to those who appear more ill, and often to cystic fibrosis patients who require short-term mechanical ventilation immediately prior to the transplant procedure[28,59]. In addition, whereas mechanically ventilated patients were initially excluded, the realization that stable, non-infected mechanically ventilated patients may benefit from LTx has led to a reassessment of this requirement[28]. It remains to be seen whether the patient selection criteria, as they are liberalized, will result in a subsequent decrease in overall survival rate.

The criteria for selection that have been developed in patients with emphysema are now facing a new consideration – the rise of lung reduction surgery for certain patients with this condition (Chapter 89). The concept of this surgery involves physiologically reducing the size of the lungs that can be demonstrated to be overdistended and that have focal areas of worse disease[71]. In general, poorly perfused and poorly ventilated areas are preferentially excised, in contrast to lung parenchyma which is obviously functional. Ongoing changes in approach for patients with different types of emphysema and multiple medical problems, or in whom different anatomic technical considerations exist (such as previous surgery, which might preclude lung reduction but not LTx), may alter the potential recipient profile or post-LTx survival for this disease entity in unpredictable ways.

DEVELOPMENT OF CHANGES IN IMPLANTATION TECHNIQUES AND PERIOPERATIVE MANAGEMENT

The bronchial anastomosis is generally avascular. Healing relies on the presence of collateral circulation from the pulmonary artery and lung parenchyma flowing retrograde into the bronchial circulation, in order to nourish the bronchial anastomotic region. Depending on the initial condition of the donor, the status of the donor bronchus can be quite variable. For the most part, donors with diffuse tracheobronchitis or aspiration pneumonitis are avoided, although those with mild degrees of tracheobronchitis in an otherwise clear lung field, and no obvious history of major aspiration, can be utilized safely.

Techniques for carrying out the bronchial anastomosis have changed substantially over the past 10 years. When initially conceived it was felt that bronchial anastomoses, because of their inherently avascular nature, would require wrapping in omentum in order to be properly sustained[4,37]. Some impressive early studies demonstrated revascularization of ischemic bronchial stumps by the use of an omental wrap[36]. A variety of techniques had been employed prior to this, including the telescoped anastomosis that

has currently become more popular, but other factors with respect to wound healing and organ rejection resulted in these anastomotic techniques being unsuccessful in the past. It has become apparent that rejection can alter donor bronchial blood flow, and perhaps increase the effect of ischemic injury. This would partly explain why distal bronchial complications rarely accompany standard bronchial sleeve lobectomies.

As the omentum was used frequently for wrapping of bronchial anastomoses, the complications inherent in its use (ileus, additional pain, as well as prolonged operating time) became less desirable, and surgeons began to seek alternatives. The work of Calhoon *et al.* at the University of Texas[26] demonstrated that safe donor-to-recipient anastomoses could be carried out without an omental wrap, using sutures placed in a figure-of-eight pattern and a telescoping anastomotic technique. The importance of bronchial stump length and length of the donor bronchus became increasingly obvious. If the donor bronchus was kept fairly short (one or two rings) proximal to the lobar bifurcation, bronchial anastomotic healing was improved.

It had been previously thought that corticosteriods exerted such a negative impact on bronchial anastomotic healing that these were to be entirely avoided. However, it became clear that, despite the original desires to limit steroid use, steroids were frequently being employed in the management of these patients for the treatment of presumed rejection episodes. Furthermore, fairly large steroid doses were being given at these times. As shown by Calhoon *et al.*[26], small doses of steroids in the preoperative patient or significant routine doses of methylprednisolone in the immediate postoperative period, did not seem to impair bronchial anastomosis healing with the telescoping technique. This new 'tolerance' of steroids occurred in a complex milieu of increasing knowledge regarding lung preservation and bronchial anastomotic techniques, and more familiarity with post-transplant care. Increased awareness regarding ischemic times and the importance of pulmonary perfusion, and the more careful use of immunosuppressive agents all contributed to a better understanding of the transplant process, and probably improved survival of the bronchial anastomosis and pulmonary graft.

The telescoped bronchial anastomosis[26] has become increasingly utilized, being used in bilateral LTx as well as in HLTx. The safety of this anastomosis compared to other techniques has also contributed significantly to early post-transplant survival. In addition, abdominal complications of omental harvest are avoided. In pediatric situations the small size of the telescoped anastomoses was associated with strictures, and therefore end-to-end techniques have proven more useful[15].

At the same time as techniques for constructing the bronchial anastomoses were being advanced, those anastomoses that failed were also being handled with increasing expertise and better results[72,73]. The judicious use of lasers, transbronchial debridement, and occasionally endobronchial stents, resulted in salvage of many patients who might otherwise have lost their grafts and their lives. While avoidance of bronchial anastomotic problems is clearly the goal, it appears that these problems will continue to find occasional incidence and require expert management. Techniques of direct bronchial arterial revascularization appear to be effective, but add significant complexity to donor organ procurement and the recipient operation. These approaches have not been accepted as standard in the major centers.

Vascular anastomotic techniques for the pulmonary great vessels have also changed. The technique of constructing an atrial anastomosis for the pulmonary vein confluence, instead of individual pulmonary venous anastomoses, was first suggested by Metras in 1949[4,74]. A venous anastomosis at the atrial level has proven to be a much more feasible and forgiving anastomosis than anastomosis of each pulmonary vein. Experimental studies have shown that individual venous anastomoses are certainly possible, but the atrial suture line offers significantly larger and more substantial tissues for suturing.

Initially, the concept of an atrial anastomosis was extended to the double lung *en-bloc* procedure wherein all four pulmonary veins were anastomosed to the left atrium as a single donor block. This large single anastomosis, carried out on the back wall of the heart, required cardiopulmonary bypass (CPB), cardiac arrest, and elevation of the heart with opening of the chambers and significant subsequent risk of air embolism. The realization that two lungs could be sutured separately and sequentially into a patient without CPB[52,53], or at least without cardiac arrest, led to a significant reduction in potential morbidity and mortality. Publication of the first major series of bilateral sequential LTx[52] demonstrated an 89% in-hospital survival rate, a previously unachieved success rate. The pulmonary arteries, rather than being anastomosed at the level of the main pulmonary artery, were anastomosed separately at the more peripheral levels of the right and left pulmonary arteries. The bilateral LTx experience also led to the realization that current pulmonary protective means were fairly satisfactory since acceptable function, with excellent immediate Po_2 levels, were achieved up to 10 h after ischemia for the second lung (of the bilateral sequential pair).

The use of adjunctive CPB during lung implantation has been a subject of some debate. There are surgeons on each side of the issue, some recommending bypass during bilateral LTx procedures and others not recommending it[4,15,26,75]. There are situations in which CPB must be used, such as in HLTx and in SLTx or DLTx for pulmonary hypertension (where one lung is generally not sufficient to sustain the circulation in the presence of the severe hypertension and subsequent right heart failure). On the other hand, for diseases such as cystic fibrosis or other bilateral LTx procedures, the question arises whether CPB has advantages that balance or override the potential disadvantages of coagulation disturbances[15,26], need for additional surgery and, in some cases, the need for an additional incision (in the groin).

Most patients undergoing CPB for DLTx can be easily placed on CPB through the chest, as can the occasional SLTx patient with a large chest cavity due to emphysema. For SLTx through a lateral thoracotomy for pulmonary hypertension, a groin incision is usually required. Advocates of CPB say it makes preliminary dissection easier and protects the first lung after it is implanted if the second lung is to be placed sequentially. In addition, the employment of CPB allows for simultaneous lung implantation, which is impossible without CPB. This technique has been used frequently in children[15]. The use of adjunctive drugs, such as aprotinin given to reduce bleeding, and leukocyte-depleting filters to minimize reperfusion injury, has allowed some additional gains secondary to the use of CPB.

Direct revascularization of the bronchial artery has remained of interest to surgeons. A variety of anastomotic techniques to restore blood flow directly to the bronchial artery have been at-

tempted. These have included: (a) saphenous vein grafts between the aorta and the bronchial artery, (b) internal mammary artery pedicles, as well as (c) buttons of aorta attached to bronchial arteries that could be implanted directly. None of these has proven essential so far, although the techniques clearly result in excellent bronchial anastomotic revascularization.

Anesthetic considerations have also certainly evolved over the years[76,77] (Chapter 49). A major component of LTx mortality has always been related to the operative procedure itself. Anesthetic techniques allowing greater stability of donors and recipients have clearly influenced current outcome. Careful hand ventilation in emphysematous patients, and transesophageal echocardiography for continuous monitoring of right ventricular function, are among the specific anesthetic techniques that have contributed to improved intraoperative results, although these adjuncts are not required in every case.

ADVANCES IN POSTOPERATIVE CARE

The postoperative management of LTx patients has become increasingly more sophisticated. While basic premises have changed little, and the diagnosis of rejection is still sometimes more art than science, management has steadily improved until we now have substantial familiarity with the normal pattern of events seen after LTx[4,25,73,78–83]. By itself, this has allowed earlier recognition and treatment of potentially life-threatening problems. For diseases such as cystic fibrosis[3], the early aggressive treatment of anticipated bacterial problems has become standard practise. Recognition of sinusitis as a potential major septic problem after operation has allowed preoperative examination of CT scans, and preemptive treatment as needed. Where cystic fibrosis patients tend to have extremely erratic cyclosporin levels due to variations in absorption, management of early cyclosporin dosing with augmented doses of pancreatic enzymes and/or the use of i.v. dosing, or more soluble oral cyclosporin preparations, has allowed for more stable levels in the early postoperative period.

For all patients the simultaneous progress occurring in aggressive pain management techniques (e.g. epidural catheters and intrathecal injections) has also influenced post-transplant recovery, allowing early extubation and reducing the risk of pneumonia. The use of thoracic epidural catheters has proven useful, not only for posterolateral thoracotomies but also for sternotomy incisions and bilateral submammary incisions.

The recognition of rejection as a nearly inevitable, yet highly treatable, event in the early postoperative period has led to earlier suspicion and treatment[84]. The diagnosis of rejection is made primarily by clinical criteria, with the development of sudden fever, a decrease in oxygenation, leukocytosis, and a diffuse infiltrate on chest radiograph, often perihilar without discrete localization to one side or the other (as might be seen with infection)[4,85]. Much attention has been paid to standardizing reporting of lung biopsy rejection grades, and to determining bronchoscopic protocols for the early and consistent diagnosis of rejection[54,86–90]. Between six and nine areas of lung are biopsied using transbronchial biopsy techniques. This seems to provide adequate tissue for identification of rejection. In addition, recognition of Leu-7 lymphocytes[91], as well as other compounds such as interleukin-2R[92] and hyaluronic acid[93] in bronchoalveolar lavage (BAL) fluid during rejection, may offer cytologic as well as chemical markers for rejection[91].

Growing experience with immunosuppressive therapy[94,95], including the emergence of tacrolimus (FK506) (to help induce graft 'tolerance') and OKT3[96] (to help reverse steroid-resistant rejection[97]) as alternatives to cyclosporin for difficult cases of rejection, or for long-term therapy, has suggested that better initial graft 'tolerance' may be achieved and that refractory rejection may be reversed. Different immunosuppressive regimens are associated with specific patterns of early rejection[3,4,26]. In antilymphocyte-globulin-treated patients, there is an almost inevitable occurrence of a rejection episode within the first 9 or 10 days, which generally responds readily to steroid bolus therapy. For subsequent episodes, additional steroids may be given as needed, and immunosuppressive regimens augmented or diminished depending on effectiveness or the development of intolerable side-effects.

Despite bronchoscopic techniques and experience, the suspicion and recognition of rejection remains as much an art as it is a science. A high level of clinical suspicion is required, and at times the willingness to try a dose of steroids and observe the response is needed. If treatment of rejection does not rapidly return the lung to a pre-rejection baseline, then open-lung biopsy may be required to further differentiate rejection from infection.

Advances in the treatment and recognition of cytomegalovirus (CMV) disease have also been among the major contributions of the past 10 years[25,56,98–102] (Chapter 57). Early mortality was significant when CMV matching was not considered or tested for[103]. The development of rapid spin shell vial assays for CMV has allowed rapid diagnosis of CMV infection with minimally invasive techniques. The recognition of CMV as a major pathogen for early mortality and morbidity[5,10,98,99,104,105], as well as a later mediator of obliterative bronchiolitis (chronic rejection)[10,105,106], has been key to understanding the natural history of chronic transplant failure.

Whereas patients who are CMV-negative and receive a CMV-positive organ are at the greatest risk, it has also become clear that patients who have already tested positive for CMV are still at substantial risk from reactivation of their CMV. Although the need for aggressive prophylaxis of CMV disease is very clear in the mismatched transplant (donor (D)+/recipient (R)–), it has been realized that patients who have been previously exposed and are CMV-positive are still at fairly high risk for CMV disease after transplantation. The best prophylactic regimen for these two groups is still hotly debated. A number of empiric protocols for treatment of CMV in high-risk populations have been advanced and have resulted in a substantial reduction in CMV infection[57,100]. The length of prophylactic therapy required to prevent symptomatic CMV infection is not at all clear, and the commonly accepted treatment of patients with D+/R– status for 90–100 days is largely empiric. The period of prophylaxis for patients who are R+/D– (or +) is even less clear-cut, with various periods of drug therapy being employed. Full-blown CMV pneumonitis can still carry up to a 50% mortality, so early recognition and treatment are important. Ganciclovir, which is now available in both oral and i.v. forms, has clearly revolutionized the treatment of CMV pneumonitis. However, it appears that the major benefit of the drug as a prophylactic agent may be to defer the initial seroconversion of patients to a time when they are more stable, and able to tolerate a minor CMV infection.

Table 3 Risk factors for 1-year single lung transplant mortality (n = 1034; US data 1987–1994)

Variable	*Odds ratio*	*p-Value*	*95% Confidence interval*
Ventilator	4.09	0.002	1.69–9.88
Congenital	3.57	0.001	1.67–7.62
Previous Tx	3.59	0.003	1.56–8.29
Recipient with PPH	2.45	< 0.001	1.52–3.94
Recipient with IPF	1.57	0.016	1.09–2.27
Recipient 40–59 years	1.63	0.015	1.10–2.42
Recipient ≥ 60 years	2.67	< 0.001	1.55–4.58
CMV:Donor+/Recipient–	1.58	0.036	1.03–2.41

CMV mismatching (i.e. the insertion of a CMV+ organ into a CMV– recipient) carries with it a persistently increased risk of mortality at 1 year (Table 3). This may be due to overt infection, or to a less well-defined detrimental CMV effect on the body.

While the donor shortage has not permitted the use of only CMV-matched organs, according to the St Louis Lung Transplantation Registry the mortality at 1 and 2 years post-transplant is not different as a result of CMV mismatching (Chapter 65). In contrast, the ISHLT Registry 1995 Report demonstrates a 1.58 odds ratio mortality risk at 1 year for CMV– recipients receiving a CMV+ donor organ (Table 3).

RECOGNITION AND MANAGEMENT OF OBLITERATIVE BRONCHIOLITIS

It has long been suspected from the experience in HLTx that obliterative bronchiolitis (OB) might represent lung rejection. That this process was not unique to HLTx was suspected early during the SLTx and DLTx experience. It has subsequently become clear that OB is the major manifestation of chronic lung rejection. Currently, most center experiences suggest that 40% of patients who undergo LTx will develop OB within a 5–10-year period. In some the process will advance to severe pulmonary insufficiency and retransplantation will have to be considered. While there remains no good management for OB, some patients may respond to increased steroids, and the bronchiolitis process may stabilize if augmented immunosuppression is instituted at the time of recognition. The predisposing factors to OB that have been identified include CMV-mismatch and repeated CMV infections and multiple episodes of rejection in the early postoperative period. This knowledge has led to CMV donor–recipient matching (where possible) and to careful management of CMV infection postoperatively.

The diagnosis of OB may be exceedingly difficult[107]. The finding of pulmonary interstitial infiltrate in the absence of infectious symptoms is suggestive, but not diagnostic. Several CT scan studies have suggested patchy pulmonary vascular infiltrates and vascular pruning associated with some bronchiectatic changes as suggestive of OB. Pulmonary function studies will demonstrate decreased FVC and FEV_1, as well as a decrease in flow mechanics, particularly the FEF 25–75%. The most definitive test remains lung biopsy, by either a transbronchial or an open route. Both of these diagnostic techniques are fraught by the fact that OB may be a patchy process, and therefore difficult to identify due to sampling errors. Multiple bilateral transbronchial biopsies are generally required, and these must be taken from at least the terminal bronchiole level. The yield with open lung biopsy also depends on location of the process. The use of CT scans to identify suspicious areas has helped in biopsy planning.

Earlier recognition of rejection episodes, more complete treatment of such episodes, increased surveillance (possibly requiring repeat biopsies), and increased recognition of the impact of CMV disease on the development of OB may be contributing in a major way to improved survival of LTx and HLTx. Since the major late morbidity after HLTx appears to be a deterioration in lung function due to the development of OB, rather than the development of cardiac graft atherosclerosis, it makes sense to continue to make major efforts in this regard. While improvement in technical aspects of HLTx may be a major factor causing improved survival in this arena, the importance of long-term surveillance for OB cannot be overestimated. OB will continue to be the major problem faced by most LTx recipients who survive beyond the initial 6 months. Effective strategies for the prevention or management of OB, once established, may well constitute the next major leap forward in LTx.

HEART–LUNG TRANSPLANTATION

The overall patient survival after HLTx has improved by about 10% over the past 8 years[10]. Despite this, the long-term survival of patients undergoing HLTx remains lower than that of patients undergoing either heart or lung transplantation alone. Current 1-year survival (according to the 1995 ISHLT Report) is 56%, with 10-year survival being less than 20% (Figure 9).

In the current era the indications for HLTx have changed substantially. Fewer patients are receiving HLTx for cystic fibrosis, because these patients usually undergo bilateral LTx[22]. In addition, a number of patients with pulmonary hypertension with or without congenital heart disease are being given lung, as opposed to heart–lung, transplants (often with correction of the underlying heart defect). The change from requiring HLTx to being able to survive with LTx alone will by itself improve survival in patients with these conditions, although this will be hard to measure. The increased risk associated with HLTx is primarily related to the magnitude of the surgical procedure and to the possibility of technical and perioperative problems. The availability of aprotinin for better control of perioperative bleeding, and greater familiarity with the surgical techniques, will undoubtedly continue to improve results of HLTx. In the meantime, fewer HLTx procedures are being carried out for end-stage lung disease, except where it is associated with left ventricular dysfunction or intractable ventricular arrhythmias.

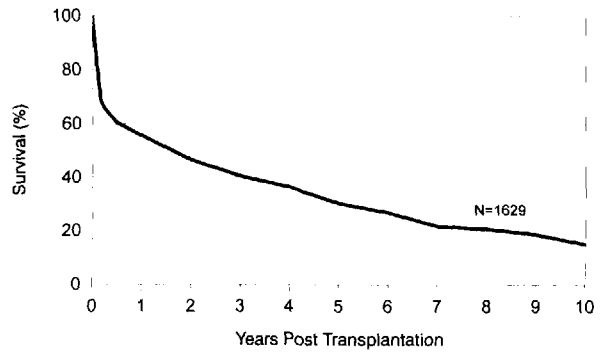

Figure 9 Actuarial patient survival following HLTx up to 10 years after transplantation. These are the longest available follow-up data. No comparable SLTx or DLTx data are as yet available. Short-term results have certainly demonstrated SLTx or DLTx to be superior to HLTx in certain diagnostic categories. However, since diagnostic categories are different, and indications for transplantation are changing, long-term data may be less meaningful than initially thought. (Source: ref. 10)

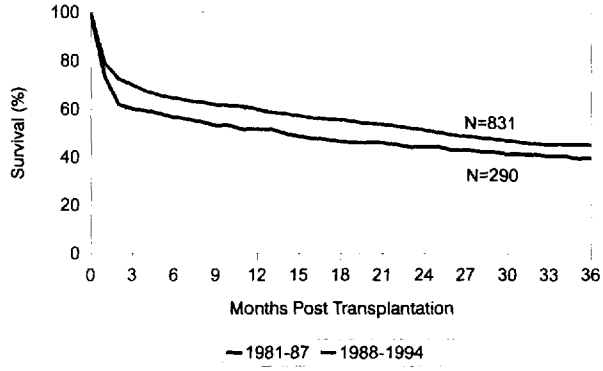

Figure 10 Actuarial patient survival following HLTx by era. Selection criteria and initial perioperative management of HLTx patients have clearly improved in the current era (1988–1994). Long-term morbidity and mortality may be chiefly due to other factors that have not changed substantially. (Source: ref. 10)

Initial experiences with HLTx for cystic fibrosis resulted in a fairly acceptable 5-year survival (36%)[1,23,24,41], but one must remember that, at that time, the techniques of SLTx and DLTx had not yet developed to a point that allowed definite comparison. Once LTx had evolved to a more sophisticated level, results in early survival after bilateral sequential LTx appeared to be superior to those obtained with HLTx[3,52]. Because of concerns relating to the development of graft vascular disease (jeopardizing long-term cardiac allograft survival) HLTx is now employed less frequently.

In the 12th Official Report of the Registry of the ISHLT[10] the major incremental improvement seen is in survival during the first 8 months after HLTx (Figure 10). Subsequently, the survival curves are essentially parallel between the two eras of HLTx (prior to and subsequent to 1988). This suggests that, although perioperative management has improved, no major improvement in long-term survival has been achieved. In cystic fibrosis patients undergoing HLTx, studies have shown no recurrence of cystic fibrosis, the major morbidity being the development of OB.

RISK FACTORS FOR MORTALITY AFTER LUNG TRANSPLANTATION

In the light of the foregoing discussion, the most recent Registry information from the ISHLT has identified several risk factors for mortality after SLTx, DLTx, and HLTx.

After SLTx, several factors leading to several-fold incremental risk of mortality were identified (Table 3). These included: (a) being on a ventilator at the time of LTx (four-fold risk), (b) having a non-emphysema indication for LTx such as primary pulmonary hypertension, congenital heart disease, or idiopathic pulmonary fibrosis, (c) being >60 years of age (2.67-fold risk), and (d) being CMV mismatched. Both CMV mismatching and (e) recipient age between 40 and 59 conferred approximately a 1.6-fold increased 1-year mortality. The highest risks were associated with (f) having had a previous transplant, (g) having congenital heart disease, or (h) being on a ventilator. The fairly high risk of age >60 years should cause serious pause when considering patients in this age group, especially in light of the donor shortage. Patients who are on a ventilator are clearly comparable to the original cohort of LTx patients of the late 1970s, in whom LTx was simply not successful, partly due to the patients being in an advanced state of debility. The high risk of death in patients with congenital heart disease is most likely related both to the presence of pulmonary hypertension and to the additional need for intracardiac surgery.

Table 4 identifies risk factors for mortality 1 year after a bilateral sequential LTx or double LTx. The recipient's status again proved to be of key importance, particularly if the patient was on a ventilator at the time of transplant. Earlier transplantation (before 1990) and *en-bloc* double lung transplants (frequently from the same era) conferred significantly increased mortality. In addition, the older donor, whose lung function was probably not as good as a younger donor's, conferred a 2-fold increase in the mortality risk after DLTx.

In HLTx, once again, patient status at the time of the procedure was a key determining factor (Table 5). Patients sick enough to require mechanical ventilation at the time of transplantation had almost a 7-fold increase in mortality at 1 year. The combination of end-stage cardiac and pulmonary failure, which is frequently the indication for HLTx, suggests a profoundly ill patient. With

Table 4 Risk factors for 1-year bilateral/double lung transplant mortality (n = 484; US data 1987–1994)

Variable	*Odds ratio*	p-*Value*	*95% Confidence interval*
Ventilator	4.07	< 0.001	1.90–8.68
En-bloc double	1.69	0.05	1.00–2.84
Year of Tx < 1990	2.7	0.006	1.33–5.45
Donor ≥ 45 years	2.09	0.018	1.13–1.35

Table 5 Risk factors for 1-year heart–lung transplant mortality (n = 318; US data 1981–1994)

Variable	*Odds ratio*	p-*Value*	*95% Confidence interval*
Ventilator	6.77	0.004	1.85–24.8
Recipient male	2.06	0.003	1.29–3.29
Donor ≥ 40 years	2.04	0.045	1.02–4.08
Year of Tx < 1990	1.6	0.5	1.00–2.55

the overall results for HLTx being far worse than for SLTx or DLTx, it is questionable whether patients requiring HLTx who deteriorate to the point of mechanical ventilation should be transplanted at all. The heart–lung block being implanted could, alternatively, be used for two or three other patients, hopefully with a much greater degree of success.

Figures 11 and 12, demonstrating the actuarial LTx survival for patients with emphysema by procedure, certainly established bilateral LTx as the procedure of choice for emphysema. Survival appears somewhat better at 12 months post-transplantation (79% versus 75%), and clearly establishes that patients with a DLTx do not survive as well as those undergoing bilateral LTx. To some extent these groups are not completely contemporary in that DLTx was for the most part discarded once successful bilateral LTx was demonstrated.

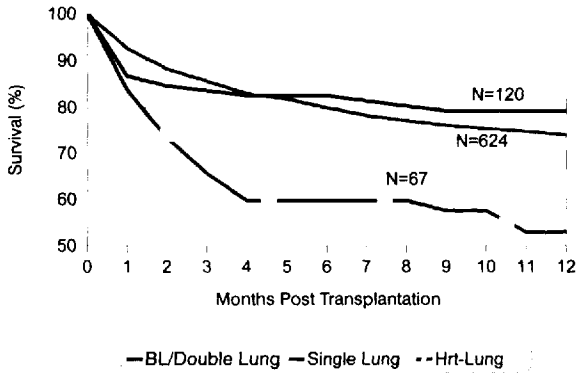

Figure 11 Actuarial patient survival following LTx for emphysema by procedure. Patients clearly do better after LTx without heart transplantation. This may well represent a statistical model for comparing patients with other diseases where choice of transplant is an option. The crossover of survival in SLTx versus DLTx between early and late phases is of interest, and requires observation in future studies. (Source: ref. 10)

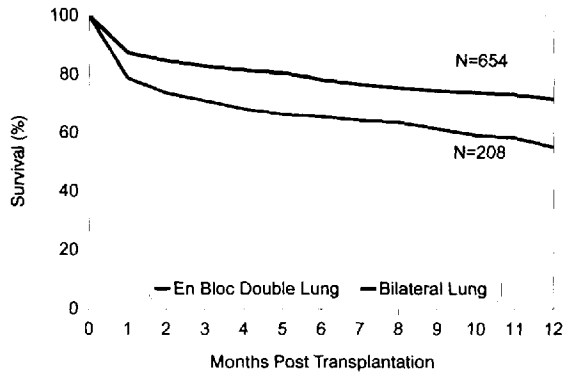

Figure 12 Actuarial patient survival following bilateral LTx by procedure. Patients who have undergone DLTx by the *en-bloc* technique have clearly not fared as well as those undergoing the newer bilateral LTx. While there is a significant initial difference in survival, which is probably accounted for by technical problems, the later separation of the curves may also reflect the fact that bilateral LTx has been carried out in more recent years than the *en-bloc* DLTx. (Source: ref. 10)

Patients with primary pulmonary hypertension appear to do better with a bilateral procedure initially[22] (Figure 8). However, within 1 year the results of SLTx lung versus DLTx are comparable. Once again, patients undergoing HLTx for primary pulmonary hypertension do substantially worse than patients undergoing LTx without cardiac replacement. This gives additional credence to the fact that HLTx confers greater mortality risk, questioning the validity of the 'domino' operation.

The results of SLTx demonstrate that patients with idiopathic pulmonary fibrosis (IPF) do somewhat worse than the group as a whole. This is not only true acutely (Figure 7), but this diagnosis appears to influence late survival, even in patients who survive 1 year (Figure 13). The mechanisms responsible for the increased risk of IPF patients even late post-transplantation is unclear.

Patients undergoing bilateral LTx clearly do better if they are younger. Figure 14 demonstrates bilateral LTx survival by age. There is an initial difference in mortality in the perioperative period of approximately 5%. With time this is maintained, suggesting that the major increased risk in older patients is confined to the first 2 months. After this there are parallel survival curves at 1 year. Following the initial postoperative period there is an approximate 10% incremental mortality for every year after bilateral LTx (Figure 15). Therefore, at 3 years post-transplant, an approximate 50% survival may be expected. Figure 16 shows the

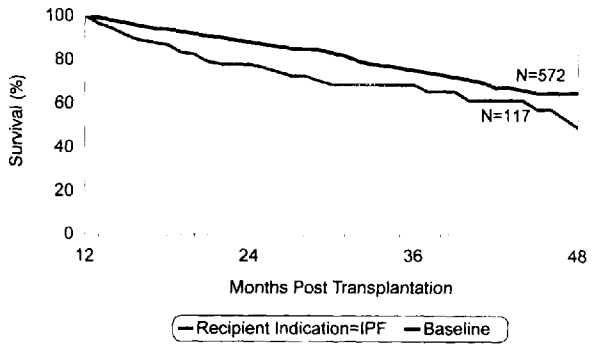

Figure 13 Long-term risk factors following SLTx in the USA (1988–1994). Patients receiving SLTx for idiopathic pulmonary fibrosis appear to have an incremental worsened risk of death compared to other groups. Additional comparisons can be seen in Figure 7. (Source: ref. 10)

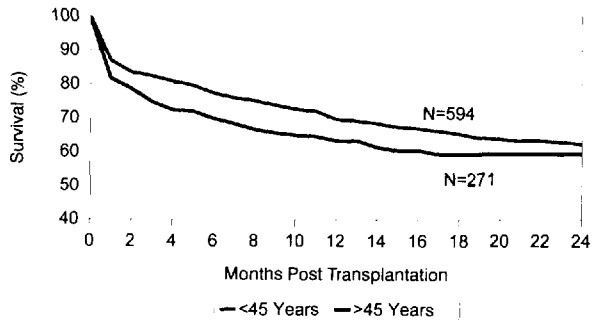

Figure 14 Actuarial patient survival following bilateral LTx by age. Patients >45 years of age at the time of transplantation do somewhat worse than patients <45 years. (Source: ref. 10)

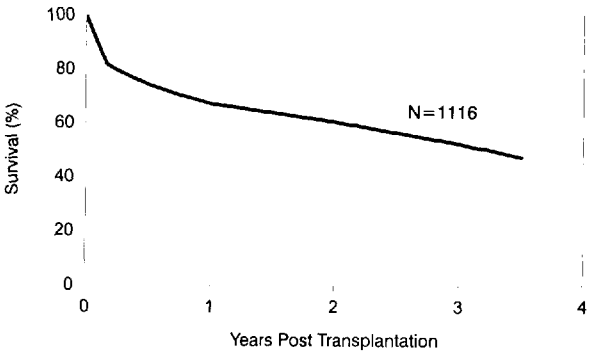

Figure 15 Actuarial patient survival following bilateral/DLTx. One-year survival is 67%, falling to 47% at 3 $\frac{1}{2}$ years. Risk factors contributing to mortality at 1 year are outlined in Table 4. (Source: ref. 10)

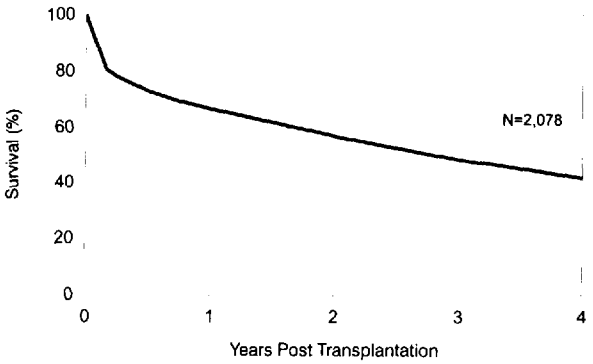

Figure 16 Actuarial patient survival following SLTx. Survival is 67% at 1 year, 57% at 2 years and 42% at 4 years. The risk factors contributing to mortality at 1 year are outlined in Table 3. (Source: ref. 10)

long-term survival for all patients receiving SLTx, and this is only minimally worse than the bilateral LTx survival statistic.

PEDIATRIC LUNG TRANSPLANTATION

The development of pediatric LTx[15] has occurred in two separate clinical patient groups where significantly different problems are faced. The first is that of the neonates, infants and young children. The second is that of older children in their teens.

Major indications for LTx or HLTx in neonates and very young children include: (a) congenital heart disease with complex cyanotic problems, including anomalous pulmonary venous drainage (often after an initial attempt at correction); and (b) other irreparable cardiac disorders with associated anomalies of the pulmonary circulation or intractable pulmonary hypertension. In the older age group, pulmonary hypertension may be seen in the presence of better-tolerated congenital defects, and may be corrected by a SLTx with concomitant repair of the congenital cardiac defect. In addition, the older age group contains patients with cystic fibrosis who become increasingly symptomatic as they enter their teens and young adult life. Both surgical techniques and mortality vary widely in this group of patients. Cardiopulmonary bypass is employed for all HLTx and frequently for bilateral LTx (for cystic fibrosis or pulmonary hypertension) and SLTx (for pulmonary hypertension). As one might expect, the additional difficulties inherent in managing small children with complex conditions, extensive medication needs, and difficult postsurgical courses, result in a higher mortality, which is highest during the first 3 months. After pediatric HLTx a nearly 20% difference is seen in survival at the end of the first 3 months, with the younger children at significantly greater risk[10] (Figure 17). Thereafter the survival courses are essentially parallel.

In pediatric LTx certain technical modifications have also proven necessary due to the small size of the structures involved. According to Spray and Huddleston[15], telescoping anastomoses in children (as advocated by Calhoon *et al.* for adults[26]) lead to bronchial stenosis with progressive granulation tissue or tracheomalacia. End-to-end bronchial anastomoses have therefore been preferred more recently over the telescoping technique. Absorbable suture material is desirable to maximize growth potential in vascular anastomoses. Omental wraps are also not generally utilized, because of the small, tenuous nature of the omentum in most children; pericardial fat pads have been utilized instead.

The double-lumen tube employed for split-lung ventilation in adults is not generally feasible in smaller children. For this reason cardiopulmonary bypass has proven more necessary in children than in adults. In addition, the management of thick secretions in cystic fibrosis is more difficult through smaller airways; use of a

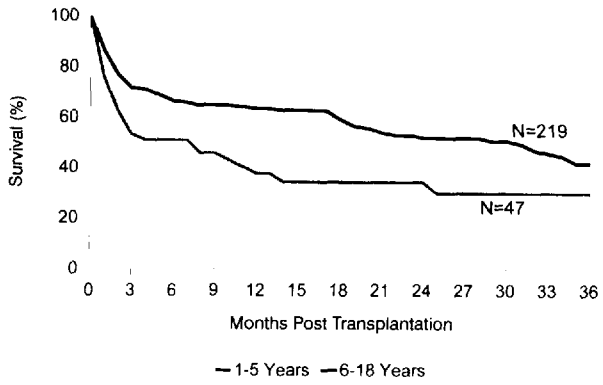

Figure 17 Actuarial patient survival following HLTx in the pediatric group by age. (Source: ref. 10)

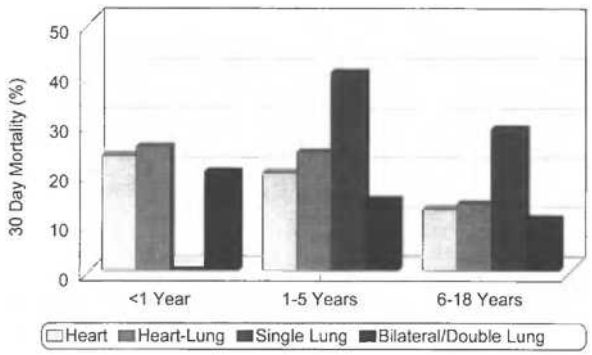

Figure 18 Thirty-day mortality for all thoracic organ transplants in the pediatric group by age

standard single-lumen endotracheal tube has made intraoperative secretion management much easier.

Thirty-day mortality for all thoracic transplants in children is shown in Figure 18. The results are only slightly worse than in adults.

COMMENT

Results of both LTx and HLTx continue to improve annually. Significant improvement has been seen over the past several years due to progress in several contributory basic scientific and clinical arenas. Technical achievements, and an increased familiarity with the potential technical problems associated with LTx and their management, have resulted in improved perioperative survival rates. New techniques for surgical anastomoses, and increased knowledge of various support procedures and pharmacologic management, have reduced mortality from technical failures, early rejection, and infection.

The major problems facing patients with lung and heart–lung transplants now and for the next several years will be chronic rejection and unusual infection problems. The role of viral infections continues to change as new viral pathogens are identified and evolve genetically. Post-transplant Epstein–Barr viral-related malignancies[46,108] are a constant reminder of the delicate equilibrium mankind maintains with surrounding pathogens by way of his finely tuned immune system. CMV has remained a major long-term risk factor for organ loss, general debility, and prolonged morbidity. Major problems still occur from the side-effects of our most effective immunosuppressive agents, including renal failure (secondary to cyclosporin), and osteoporosis[109] and stress fractures (due to steroids) – to mention only two of the most important. Progress in these areas will undoubtedly allow improved survival and quality of life for LTx and HLTx patients.

Whether or not the primary condition may recur after LTx (e.g. cystic fibrosis, sarcoidosis, or interstitial pneumonitis)[6,11,110] and the significance of minor degrees of recurrence still remain to be clarified. So far, cystic fibrosis has not recurred in transplanted lungs, although sarcoidosis and giant-cell interstitial pneumonitis have.

As progress continues in the management of thoracic transplant patients, we have seen organs being offered to patients with a variety of additional diseases and in older age groups. Some of these trends may impact on survival statistics negatively. The persistent overall shortage of donors for both LTx and HLTx continues to be a major problem that prevents the more widespread use of these technologies. The realization that acceptable results may be achieved using less-than-ideal donors may allow for some increase in the donor pool. Whether this will impact significantly on waiting-list mortality remains to be seen. Clearly, management of donors or critically ill patients who may become donors needs to be optimized as much as possible. General improvements in critical care may result in healthier lungs being presented for donation[111]. The financial environment currently faced by transplant programs, recipients, and potential recipients alike will continue to exert a strong restraining influence on transplant volume. Future financial considerations may further restrict the numbers of centers carrying out transplants. This may also impact significantly on both the number of transplanted patients and the results reported by transplant programs. The need to maintain a statistically acceptable survival rate may significantly influence choice of both recipients and donors.

From a programmatic perspective the best results will be achieved by utilizing only the best donors and best recipients. Because LTx continues to represent a high risk for patients (a one-way road from which there is no turning back) and enormous financial expenditure to patients and their insurance companies, the need for quality outcomes must continue to take precedence over the number of transplants performed. As patients who are in better physical condition will sometimes be given preference over patients who are in worse condition, the natural history of these diseases (that require transplantation) must be carefully reviewed at intervals to see whether or not transplantation is clearly of benefit with regard to survival of the recipient. Patients with emphysema show slow natural deterioration, and survival may not be significantly different in those who undergo LTx from those who do not. Certainly, LTx or HLTx can result in a spectacular early improvement of lifestyle and unquestionable prolongation of survival. However, this comes at significant cost in terms of additional hospitalizations, medication, and the risk of eventual graft failure. The results of retransplantation remain poor (Figure 19), and undoubtedly reflect a number of factors (Chapter 60).

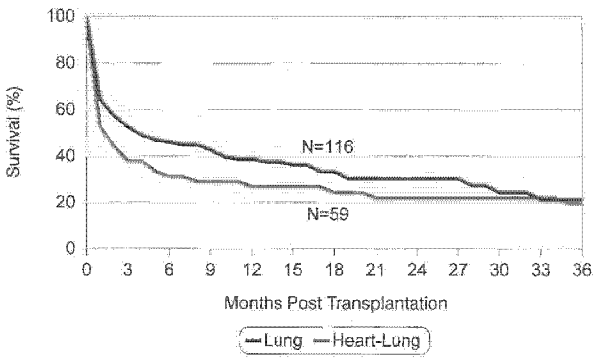

Figure 19 Actuarial patient survival following lung and heart–lung *re*transplantation. There is a sharp initial perioperative mortality for patients undergoing either lung or heart–lung retransplantation. The 1-, 2-, and 3-year actuarial survival rates for lung retransplantation are 39%, 30% and 21%, respectively. (Source: ref. 10)

The poor results must make us pause to reflect on the current utilization of scarce donor organs in settings where long-term success is so unlikely[112].

Education of the public regarding organ donation, and the need for discussing this issue amidst family and religious contexts, must remain a major effort for the medical establishment. Many usable organs are continuing to be wasted, and lawmakers must look seriously at options such as implied consent. Even if lung donation remains possible in only a small proportion of organ donors, an overall increase in the number of lungs available would clearly result from an increase in the overall number of donors becoming available. As successes in transplantation continue to be brought to the public's attention through the media, organ donation may become more acceptable and better understood by an increasing percentage of the population. It is only in this way that we can take maximum advantage of natural organs which, after thousands of years of evolution, remain the most durable and functionally complete substitutes for failing hearts and lungs.

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New Pharmacologic Immunosuppressive Agents

S. TREHAN, D.O. TAYLOR AND D.G. RENLUND

The wizardry necessary for the management of existing non-selective drug regimens recalls the dances, chants and songs practiced by the Babylonians in accord with their concepts of numerology, astrology and fetishism[1].

INTRODUCTION

The last quarter-century has seen the evolution of cardiac transplantation from a rare experiment to an accepted therapy for end-stage heart failure. The success of this endeavor has been primarily credited to an improved understanding of the immunologic mechanisms associated with allograft rejection and our ability to modify these with the available armamentarium of immunosuppressive agents.

Clinical immunosuppression originated from the observation of profound immunodepressive effects of X-ray irradiation[2]. However, the narrow window between insufficient and excessive effects of X-ray irradiation limited its role in the transplant enterprise. Chemical immunosuppression, initiated with the weak, non-selective antiproliferative agent azathioprine and glucocorticoids, entered its adolescence with the discovery of cyclosporin, which displayed an action relatively selective for T cells.

Contemporary immunosuppressive strategies in clinical transplantation administer relatively large doses of potent agents with the consequent penalty of considerable toxicity. The toxicity profiles of the aforementioned agents have precluded further enhancement of allograft protection from rejection without a significant risk of infection or malignancy. Further, to date no therapy prevents allograft coronary vasculopathy, which is a major determinant of long-term survival following cardiac transplantation.

Current practice tends towards the purgative approach to immunosuppression without due attention to a fine immunologic balance between suppression and surveillance. Recently, a large number of newer immunosuppressive agents have graduated from the laboratory to extensive clinical trials of their safety and efficacy. Optimal use of these agents demands an understanding of their mechanisms of action and of basic transplant immunobiology. The following section will present a brief overview of the immunologic responses and the effector mechanisms associated with transplant rejection.

IMMUNOLOGY OF REJECTION

The understanding of immunology of acute and chronic rejection is helpful in management of patients, and is essential for making any real progress in designing new and more specific methods of immunosuppression. The primary immune response is allorecognition, a process by which the graft is recognized as foreign. The major histocompatibility complex (MHC) is a genetic region which codes for specific products devoted to providing extracellular representation of foreign antigens. The MHC-encoded class I and class II molecules provide peptide-binding sites which evoke the effector responses upon recognition of the foreign peptide by the antigen-specific receptors of the T lymphocyte[3].

The majority of T cells have T cell receptors (TcR) with α and β chains, and identify the antigen present in the form of the peptide in the groove of MHC molecules. CD4 and CD8 proteins present on the reciprocal peripheral T lymphocytes react with class II and class I MHC molecules respectively[4,5]. The relative importance of these subsets of T lymphocytes has come to light only in the recent past, as it has been observed in animal experiments that rodents without any T cells do not reject transplanted organs. However, when repopulated with CD4 cells alone the same animals are able to reject allografts. The CD8 cells usually cannot do this; hence, CD4 cells are both necessary and sufficient to cause rejection, although they may recruit other cells (i.e. macrophages, cytotoxic T cells, NK/LAK cells, B cells) into the process rather than cause the damage themselves[6,7].

Transplantation immunity can be induced in either of two ways: direct activation of T lymphocytes by the so-called 'passenger donor lymphocyte' (PDL), a bone marrow-derived cell present within the allograft, or alternatively an indirect pathway where the peptides derived from allogeneic proteins are taken up and processed by specialized host antigen-presenting cells (APC) such as interstitial dendritic cells (DC). The recognition of

allogeneic 'foreign' allopeptides is then self HLA restricted, which may restrict the ability of the activated effector cells to find target structures expressed on the allograft[8].

The importance of PDL for induction of immune responses has been highlighted in several reviews[9,10] and clearly suggests that the activation of T cells relies not only on the recognition of these cells as allogeneic but also on the immunostimulatory capacity of these cells. They have to present donor MHC-class II antigens with bound allopeptides to peripheral T cells[11] and provide reciprocal accessory bindings such as those between leukocyte function antigen, LFA-1 (CD11a/CD18), and intercellular adhesion molecule, ICAM-1(CD54), and between CD28 and CTLA-4 with CD80 (B7) and B70/B7-2[12,13]. If the graft-derived passenger cells lack the immunostimulatory capacity they have to be degraded and presented to the T cells, following processing of the allogeneic peptides by the host immunostimulatory cells, i.e. antigen-presenting cells[8]. Murine experiments have shown that T cell recognition of foreign peptides on cells which lack immunostimulatory capacity does not result in activation[14].

Hence, T cell activation requires both an antigenic stimulation and a co-stimulatory signal. The TcR in association with the CD3 complex, consisting of four or five non-polymorphic polypeptide chains, is clearly involved in antigen-specific recognition and triggering of transmembrane signals leading to cellular activation and signal transduction[13]. There appear to be two early signal transduction pathways, the inositol-phospholipid pathway and the tyrosine kinase pathway (Figure 1). The former pathway results in the cleavage of phosphatidyl-inositol biphosphate (PIP2) into inositol triphosphate (ITP) and diacylglycerol (DAG), which act as second messengers and result in increased intracellular calcium. The calcium influx activates calcineurin, a calcium-sensitive phosphatase, which dephosphorylates the nuclear factor of activated T cells (NFAT-1). The transcription of IL-2 mRNA is under the regulatory control of NFAT-1, which activates the promoter region of the gene resulting in production of IL-2[13,15].

The TcR coupling to the tyrosine kinase pathway remains a mystery, but it is well recognized that one of the substrates of tyrosine phosphorylation, a 70 kDa tyrosine phosphoprotein ZAP-70, associates with the CD3-ζ (zeta) subunit[16], and possibly has a role in augmentation of IL-2 production via the CD28 co-stimulation pathway, which stabilizes the AP-1 transactivating factor. The loss of function of this co-stimulatory signal results in functional anergy and consequent inadequacy of IL-2[17]. The co-stimulation pathway is induced by surface proteins expressed on APC, namely CD80 (B7/BB1), which have a reciprocal binding ligand on T cells in the form of CD28 and CTLA-4, and this appears to be independent of increase in cytosolic calcium or activation of protein kinase C[17–19].

Once the process of allorecognition and subsequent signal transduction resulting in transcription of specific genes is complete, the next phase of the immune response to the allograft begins with clonal proliferation of cytotoxic lymphocytes, which occurs primarily due to the growth factor effects of IL-2. Interleukin-2 also induces higher levels of synthesis of other cytokines such as IFN-γ, TNF-β and B cell growth factors (IL-4, IL-5, and IL-6) (Figure 2). The cytokines facilitate the activation of macrophages and other inflammatory cells, and the production of allospecific anti-graft antibodies by B cells which can recruit complement and cause damage to vascular endothelium[20,21]. An additional function of cytokines is to increase the expression of class I and class II MHC antigens, and expression of adhesion molecules such as ICAM-1, granule membrane protein GMP 140, and VCAM-1 in response to IFN-γ, TNF-β and IL-1[22,23]. Furthermore, it has been noted that rejecting cardiac allografts have increased expression of ICAM-1, VCAM-1 and MHC class II on the capillary endothelia[24].

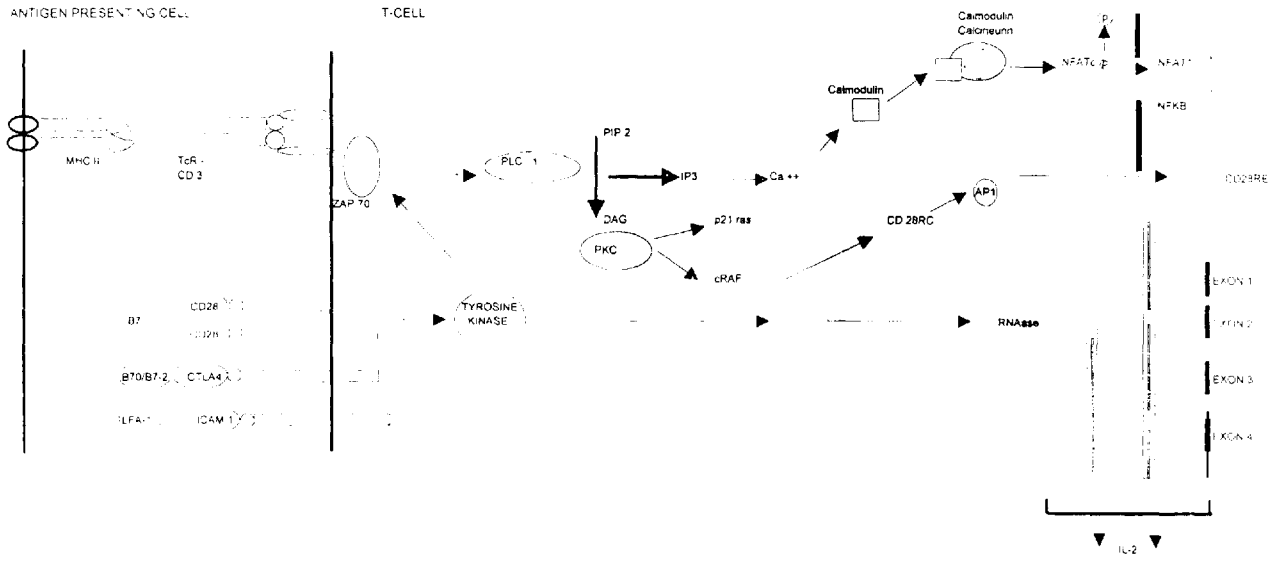

Figure 1 Molecular basis of early signal transduction pathways and co-stimulation for IL-2 production. APC, antigen-presenting cell; IL-2, interleukin 2; PLCγ1, phospholipase C-γ1; PIP_2, phosphatidylinositol biphosphate; IP_3, inositol triphosphate; DAG, diacylglycerol; PKC, protein kinase C; NFATc, nuclear factor of activated cells cytoplasmic component. Modified with permission from ref. 17.

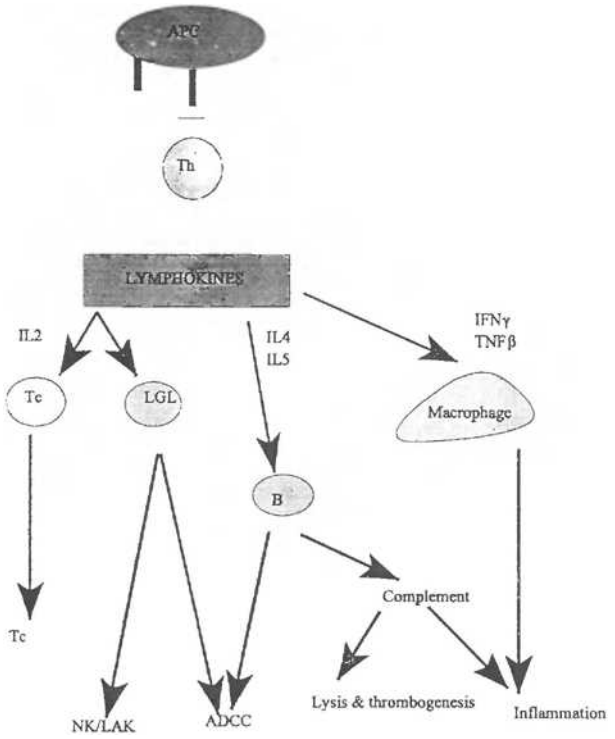

Figure 2 The rejection cascade. The initial activation of T helper cells requires exposure to the MHC class II–peptide complex on the antigen-presenting cell leading to production of a variety of lymphokines once the T helper cells are activated. The principal lymphokines involved with the rejection process are IL-2, IFN-γ and TNF-β, while the IL-4, IL-5 and IL-6 are involved as B cell growth factors. IL-2 is involved in activation of cytotoxic T cells, natural killer (NK) and lymphokine-activated killer (LAK) cells, and also stimulates large granular lymphocytes (LGL) to take up IgG antibodies on their surface receptors and participate in antibody-dependent cell mediated cytotoxicity. Modified with permission from Rose ML, Yacoub M. Immunology of heart and lung transplantation. Edward Arnold; 1993:4

The combination of clonal expansion of allospecific cytotoxic T lymphocytes and the production of cytokines leads to the eventual cascade of rejection culminating in graft death. We have now expanded our horizons of knowledge of the immunologic response to the allograft substantially, and this enables us to develop specific therapeutic approaches which should be more effective and safer than those in contemporary use. The newer immunosuppressive drugs and modalities are listed in Table 1.

Table 1 Classification of newer immunosuppressive agents

Early T cell activation inhibitors	Cyclosporin G, FK-506
Late T cell activation inhibitors	Rapamycin, leflunomide
Antimetabolites	Mycophenolate mofetil, mizoribine, brequinar
Receptor antagonists	Deoxyspergualin, monoclonal/ polyclonal antibodies
Suppressor/regulator inducers	SKF 105685, photopheresis
Others	Castanospermine, discodermolide, LF 08-0299, bryostatin, enisoprost, SC 45662 (5-LO)

NORVALINE CYCLOSPORIN G (SDZ OG 37-325)

The search for an immunosuppressive cyclosporin derivative that would surpass the performance of cyclosporin A (CsA) in clinical practice has been extremely difficult. Over 700 natural, synthetic or semisynthetic analogs of CsA have been produced[25]. Norvaline-cyclosporin, formerly known as cyclosporin G, is a naturally occurring cyclosporin, isolated, like CsA, from the fungus *Tolypocladium inflatum* Gams, which differs from CsA at the amino acid residue in position 2 of the peptide ring in that the α-aminobutyric acid is replaced by L-nor valine (Figure 3)[26].

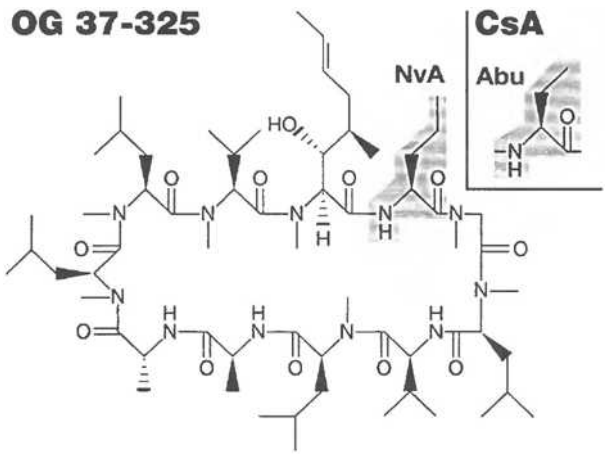

Figure 3 Molecular structure of Norvaline-cyclosporine G. Reproduced with permission from Hiestand PC, Traber R, Borel JF. Pharmacological studies with Norvaline cyclosporin in comparison with cyclosporin A – a summary. Transplant Proc. 1994;26:2999

Mechanism of action

The bulk of experimental data suggests that the mechanism of action of Norvaline-cyclosporin G is similar to CsA, if not identical[27,28]. Norvaline-cyclosporin G uses the same pathway as CsA to inhibit lymphokine gene activation following signal transduction after the TcR–CD3 ligation. While the final pathway leading to lymphokine gene transcription remains to be fully elucidated, a number of required intermediary steps are known. At least two pathways, one leading to activation of *ras* and one involving a calcium-dependent event leading to the activation of the serine–threonine phosphatase calcineurin, appear to cooperate for lymphokine gene transcription[29–32]. Cyclosporine binds to a family of specific intracellular receptors termed cyclophilins, and it is the cyclosporin–cyclophilin complex which inhibits calcineurin and hence the T cell lymphokine gene transcription process[33] and the production of IL-2, IL-3, IL-4, TNF-α, IFN-γ, and GM-CSF, among others (Figure 4)[34,35].

Pharmacokinetics

Norvaline-cyclosporin G is rapidly absorbed following single oral dosage, although the extent of absorption appears to decrease with increasing dosage, e.g. 90% to 62% with an increase of dose

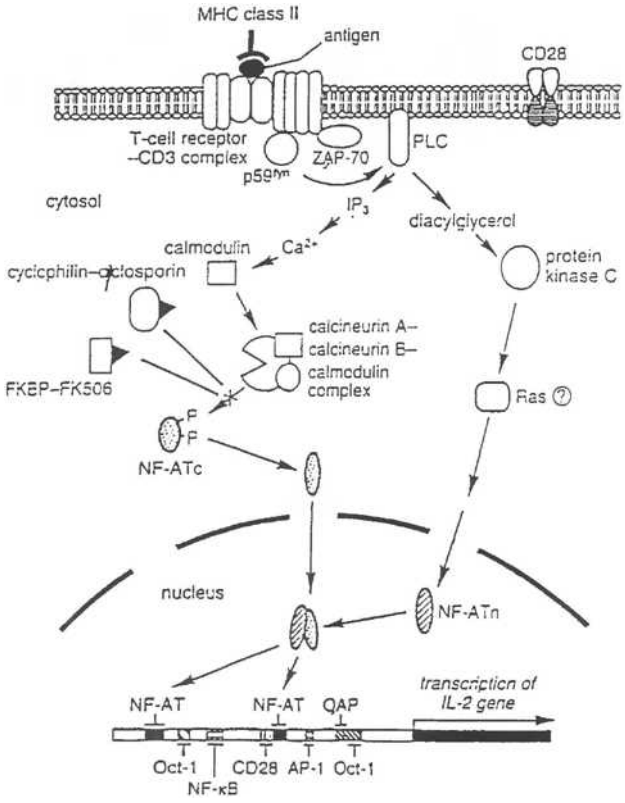

Figure 4 Mechanism of action of cyclosporins and tacrolimus: inhibition of the T cell receptor-mediated signal transduction pathway. Reproduced with permission from Liu J. FK506 and cyclosporin, molecular probes for studying intracellular transduction. Immunol Today. 1993;14:292

from 150 mg to 600 mg. Peak serum concentrations occur 2–3 hours after an oral dose[36]. Absorbed Norvaline-cyclosporin G is slowly but extensively metabolized prior to excretion, and to date seven major metabolites have been identified. The immunosuppressive activities of the metabolites are less than one-tenth those of the parent compound[37].

Norvaline-cyclosporin G, much like CsA, has a non-linear distribution in blood with a plasma:blood ratio of 0.8, and its metabolites tend to associate preferentially with erythrocytes in a manner that appears to be related to relative polarity. In the plasma, most of the Norvaline-cyclosporin G is bound to high-density lipoproteins. The elimination from the blood is biphasic, with a terminal half-life of 9–11 hours[38]. The overall disposition of Norvaline-cyclosporin G is independent of dose, with predominantly fecal excretion. Only 3% of the oral dose is renally excreted as compared to 6% for CsA[36].

Experimental pharmacology

The experimental *in vitro* and *in vivo* studies from the Sandoz laboratories comparing Norvaline-cyclosporin G to CsA suggest that the former has essentially the same pharmacologic profile and similar immunosuppressive potency[39–41]. *In vitro* Norvaline-cyclosporin G is as potent as CsA in the suppression of mitogen-induced cell proliferation, mixed lymphocyte reaction, cell-mediated lympholysis, and immune interferon production[25,41]. *In vivo* Norvaline-cyclosporin G is equipotent with CsA in preventing delayed-type hypersensitivity reaction to oxazolone or tuberculin, and localized graft-versus-host (GvH) reaction in rats, and in prolonging heterotopic neonatal cardiac allograft survival in the mouse ear. Some of these results have been reproduced by independent investigators in experimental allografting of kidney, heart, heart and lung in rats[42–47], kidney and liver in dogs[45–47], and heart in primates[48].

Norvaline-cyclosporin G has also been successfully tested in several autoimmune models such as Freund's adjuvant arthritis, in which it inhibits both the developing and established disease[49], and collagen arthritis in mice[50], and in experimental autoimmune uveoretinitis in rat[51,52]. While all these studies suggest equipotent potential of Norvaline-cyclosporin G, decreased efficacy in rat lung and heart, and in cynomolgus monkey heart allograft models has been reported[48,53].

Clinical trials

The clinical transplantation studies were designed to demonstrate that Norvaline-cyclosporin G is as effective as CsA in maintaining graft and patient survival, and that Norvaline-cyclosporin G has a better safety profile, particularly with less renal dysfunction.

An open-label, multicenter phase II study in primary cadaveric renal transplant recipients was conducted at seven US transplantation centers[54]. Preliminary results from 153 patients, after 4 months of follow-up, suggest similar rejection rates in the high-dose Norvaline-cyclosporin G and CsA groups with a higher number of rejection episodes in the low-dose Norvaline-cyclosporin G group. Four allografts were lost in the CsA and low-dose Norvaline-cyclosporin G groups each with a 100% graft survival in the high-dose Norvaline-cyclosporin G group. An initial trend for lower serum creatinine was noted in the high-dose Norvaline-cyclosporin G group, an advantage which was maintained for 16 weeks; however, following that the difference was no longer evident.

In a randomized, double-blind, phase II trial at Ohio State University Medical Center, 44 primary cadaveric renal transplant recipients were randomized to receive either Norvaline-cyclosporin G at a dose of 6.25 mg/kg per day or CsA at a dose of 10 mg/kg per day. Dosage of the study drug was titrated to clinical response and serum creatinine, and not to blood level. The number of patients experiencing at least one rejection episode was similar in both groups (11 in Norvaline-cyclosporin G and 10 in CsA), two grafts were lost in the Norvaline-cyclosporin G group with none lost in the CsA group. The patients who experienced rejection in the Norvaline-cyclosporin G group had lower whole blood trough levels compared to those who did not reject within the same group, suggesting that the starting dose of 6.25 mg/kg may be inadequate.

Toxicity

The available toxicology studies on Norvaline-cyclosporin G demonstrate that prolonged administration of the drug to rats at a high dose of 45 mg/kg/day resulted in slight nephrotoxicity and hepatotoxicity after 26 weeks. In contrast CsA showed a

higher toxic potential in this study, with 40% of the rats dying at the end of this study with undoubtedly more distinct renal morphological changes than those produced by Norvaline-cyclosporin G[41].

The gingival hyperplasia and hematologic abnormalities seen in dogs were of minor degree, and comparable to those seen in cyclosporin-treated animals. No teratogenic effects were seen in either rats or rabbits, and no mutagenic potential was observed in bacteria. Embryotoxicity was observed only in rats given 60 mg/kg per day, a dose that also caused maternal toxicity[41].

The data from the two phase II clinical trials in the US revealed that patients treated with Norvaline-cyclosporin G had improved renal function, lower serum creatinine and significantly higher GFR as measured by inulin clearance at 3 and 6 months when compared with CsA (46±5 vs 35±3 ml/min at 3 months, 44±9 vs 31±5 ml/min at 6 months respectively)[55,56]. Elevation of liver enzymes was noticed in 30–40% of patients receiving Norvaline-cyclosporin G and appeared to be transient and dose-dependent.

Future potential

The clinical data suggest that Norvaline-cyclosporin G in adequate starting dosages may be equally effective as CsA and possibly less nephrotoxic. If future trials confirm this advantage with no further risks, Norvaline-cyclosporin G could replace CsA.

TACROLIMUS-FK506 (PROGRAF)

Tacrolimus was discovered in 1984 by Fujisawa scientists in Japan. It is a macrolide antibiotic produced by the fungus *Streptomyces tsukubaensis*[57]. The drug demonstrated several biological activities *in vitro* but its immunosuppressive properties were most impressive. The molecular structure of tacrolimus (Figure 5) is unrelated to cyclosporin, and the two drugs have different cytosolic binding sites; however, their mechanism of action is quite similar.

Mechanism of action

Tacrolimus interferes with a Ca^{2+}-sensitive T cell signal transduction pathway, thereby preventing the activation of specific transcription factors (such as NFAT-1 and NF-IL-2A) involved in lymphokine gene expression. Four intracellular receptors for tacrolimus (FK binding proteins, FKBP) have been characterized by molecular weight, localization and activity[58–65]. The individual functions of each of these receptors are detailed in Table 2.

Stimulation of the TcR/CD3 complex results in mobilization of intracellular calcium and activation of calcium-calmodulin-dependent serine/threonine phosphatase calcineurin. Calcineurin dephosphorylates nuclear factor of activated T cells (NFAT-1) which acts as a transcription factor that binds to IL-2 promoter and up-regulates IL-2 gene transcription. Tacrolimus–FKBP12 complex binds to calcineurin and inhibits the modulation of NFAT-1, effectively blocking the subsequent steps necessary for IL-2 gene transcription and the activation cascade (Figure 4)[66–70].

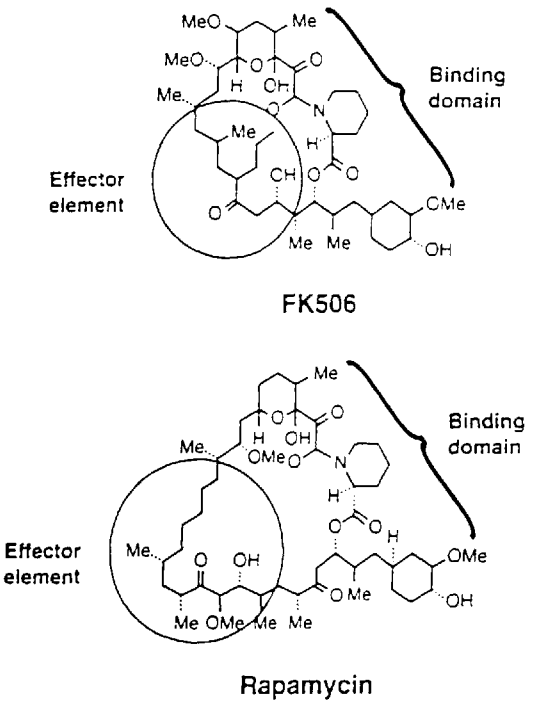

Figure 5 Molecular structure of tacrolimus (FK 506) and sirolimus (rapamycin). Modified with permission from Morris R. Modes of action of FK 506, cyclosporin A, rapamycin. Transplant Proc. 1994;22:3272

Table 2 FK binding proteins

FKBP 59	Heatshock protein p59, a hetero-oligomeric glucocorticoid receptor in the cytosol
FKBP 25	Nuclear receptor combines with casein kinase II, a serine protein kinase involved in cell and ribosome synthesis
FKBP 13	Major rapamycin-binding protein found in ER which may regulate degranulation
FKBP 12	12 kDA cytosolic protein with peptidyl-prolyl *cis–trans* isomeric activity in T cell activation which mediates immunosuppressive effects

Pharmacokinetics

The absorption of tacrolimus from the gastrointestinal tract after oral administration is variable. Peak blood and plasma levels are obtained at 1–3.5 hours after an oral dose, bioavailability averages 12–27%. In liver transplant recipients the drug is 73% protein-bound in plasma and highly bound to erythrocytes. The disposition of tacrolimus from whole blood is biphasic with a terminal elimination half-life of 11.7±3.9 hours in liver transplant recipients and 21.2±8.5 hours in healthy volunteers[71].

Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 enzyme system. In humans less than 1% of the drug is excreted unchanged in the urine. Of the 10 known metabolites, two may have immunosuppressive activity. Excretion is predominantly through the biliary tract, and even mild hepatic dysfunction can alter metabolism and clearance. Tacrolimus is not dialyzable[72–75].

Experimental pharmacology

Tacrolimus affects the earliest steps of T cell activation. The T cell proliferative responses are highly sensitive to inhibition by tacrolimus, including proliferation in response to alloantigen, phytohemagglutinin and concanavalin A, anti-CD2 and anti-CD3, and it further inhibits long-term IL-2-stimulated survival, and primary and secondary generation of cytotoxic cells.

Tacrolimus does not affect T cell proliferation once activation has occurred, and does not inhibit the effector function of NK cells. The suppression of B cell proliferation and immunoglobulin production is limited to the T cell-dependent antigens and stimulation of receptors which utilize the Ca^{2+}-dependent signal transduction pathway. Further, it does not reduce the production of immunosuppressive cytokine IL-10 by T-helper 2 cells[76,77].

Tacrolimus has been demonstrated to prevent rejection of solid-organ allografts in various animal models. In rat skin[78], lung[79], heart[78,80–83], liver[80,83], limb[84] and even multivisceral models[80] allograft survival is prolonged, and in many situations the drug is able to reverse ongoing rejection[85,86]. In dogs, renal[87], hepatic[88], and pancreatico-duodenal[89] allografts have prolonged survival. In non-human primates, cardiac[90,91] and renal[87] allografts survive longer with tacrolimus. The drug has also prevented or delayed onset of hereditary or induced autoimmune reactions in animals, such as collagen-induced arthritis[92], experimental allergic uveitis[93], experimental glomerulonephritis[94] and diabetes in BB rats[95].

The xenogeneic transplant model has provided a more rigorous test of the efficacy of tacrolimus, as in this setting both cell-mediated and humoral mechanisms of rejection operate. Tacrolimus alone is found to be inadequate in the above circumstance; however, in combination with other antiproliferative agents such as brequinar, mycophenolate mofetil or 15-deoxyspergualin it prolongs survival of cardiac, hepatic and pancreatic islet xenografts[96,97].

Clinical trials

The first clinical use of tacrolimus was in the treatment of chronic refractory allograft rejection in liver transplant recipients who were not responding to conventional immunosuppression. About 50–70% of these patients had both clinical and histopathological improvement upon conversion to tacrolimus-based regimens, and 75% of the patients were still alive 3 years following conversion, with 65% of the liver allografts still functioning[98].

Since the initial experience three large trials have been performed comparing tacrolimus to CsA in primary liver transplantation. The results and conclusions from these trials have been summarized in Table 3[99–101]. The common finding among all the studies was that tacrolimus is associated with fewer episodes of acute or steroid-resistant or refractory rejection. Patient and graft survival was better than, or the same as with cyclosporin in all studies; however, the US Multicenter group reported increased incidence of side-effects with tacrolimus[99].

The use of tacrolimus in renal transplantation was initially as rescue therapy for refractory rejection. In a series of 35 patients, those with ongoing acute cellular rejection, 71% were able to be successfully rescued[98]. The results of a phase II trial of tacrolimus and prednisone, when compared with a historical control group receiving a cyclosporin-based regimen, show no significant difference in patient and graft survival at 1 year, rate of rejection or steroid-resistant rejection. However, the incidence of steroid dependence and hypertension was lower in the tacrolimus group[102].

The reported clinical experience with tacrolimus in cardiac transplantation has been limited to the University of Pittsburgh. Armitage *et al.* have reported on 72 patients who were prospectively entered into a primary prophylaxis study of tacrolimus and corticosteroids for immunosuppression in cardiac transplantation. The overall 1-year survival was 92% with an actuarial freedom from rejection at 90 days of 41% and an average of 0.95 episodes of rejection per patient. When compared with a historical control group treated with cyclosporin-based regimen, the rate of recurrent rejection (28% vs 48%) was lower in the tacrolimus-treated group[103]. A phase II randomized, comparative, open-label, prospective, multicenter study is now under way and the results of this study should be available in the next 2 years.

Favorable results have also been reported with lung transplant[104], bone marrow transplant[105,106] and autoimmune disorders such as uveitis[107], psoriasis[108] and autoimmune hepatitis[109]. Additional long-term randomized studies are needed to determine the role for tacrolimus in these conditions.

Table 3 FK-506 in liver transplantation

Site and number of patients	*One-year patient survival*	*One-year graft survival*	*Freedom from rejection*	*Other salient features*
University of Pittsburgh, n = 129 (101)				
FK-506	92%	88%	63%	(1) FK-506 had the ability to treat refractory rejection in CsA group. (2) Freedom from steroid use and lower incidence of hypertension.
CsA	85%	79%	42%	
European FK-506 study, n = 545 (100)				
FK-506	84%	81%	61%	(1) Enhanced patient and graft survival
CsA	79%	77%	50%	
US multicenter FK-506 Liver Study Group, n = 529 (99)				
FK-506	88%	82%	32%	(1) FK-506 better in preventing acute steroid-resistant and refractory rejection (2) Greater incidence of adverse effects requiring withdrawal from study (14.1% vs 4.9%)
CsA	88%	79%	24%	

Toxicity

The wealth of clinical experience with tacrolimus demonstrates that it is tolerated as well as cyclosporin and does not cause hirsutism, gingival hyperplasia or facial dysmorphism, as CsA does. Insomnia, tremors and headache were the most frequently reported side-effects in patients taking oral tacrolimus, while those being administered intravenous drug reported headache, nausea, vomiting, and hyperesthesia.

The spectrum of renal toxicity is similar to that of cyclosporin. The neurotoxicity of tacrolimus includes seizures, akinetic mutism, coma, aphasia, focal deficits, psychosis, and encephalopathy. Hyperglycemia in liver transplant recipients is reported in 47% and 29% in the US and European randomized trials, respectively, and may require treatment.

The incidence of EBV-related lymphoproliferative disorders with tacrolimus is reported to be 1.6%, which is comparable to that with cyclosporin[110].

Drug interactions with tacrolimus have not been extensively investigated but, due to potential for additive renal toxicity, it must be carefully co-administered with drugs that may be associated with renal dysfunction such as amphotericin B, aminoglycosides and cisplatin. Co-administration of cyclosporin with tacrolimus results in additive nephrotoxicity. Reciprocal antagonism is seen between tacrolimus and rapamycin. Drugs which are metabolized by the cytochrome P-450 IIIA system, or drugs which induce this enzyme system, may alter blood levels of tacrolimus.

Future potential

While the early optimism for tacrolimus seems to have ebbed, it still remains at the forefront of the immunosuppressive armamentarium likely to be inducted into clinical practice in a similar role as cyclosporin. If the data from cardiac transplant studies suggest that it is more effective, with decreased dependence on concomitant immunosuppression and lower incidence of hypertension, then it will be an attractive alternative to cyclosporin.

RAPAMYCIN (SIROLIMUS)

Rapamycin (sirolimus) is a new immunosuppressive agent, structurally similar to tacrolimus. Sirolimus was originally isolated in a discovery program for novel antifungal agents. It is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, an actinomycete which was isolated from a soil sample collected from the Vai Atore region of Easter Island[111,112]. Sirolimus is a potent antifungal agent and several yeasts, including *Candida albicans*, and filamentous fungi are sensitive[113,114]. Sirolimus is also active in several murine tumor models. The effects of sirolimus on the immune system were briefly examined in experiments on autoimmune diseases in rodents[115] and then not actively pursued until the discovery of tacrolimus with which sirolimus shares structural resemblance (Figure 5)[116].

Mechanism of action

Sirolimus belongs to the class of macrocyclic immunosuppressants that block the T cell proliferation between G1 and S phases of the cell cycle. However, its effects are distinct from those of CsA or tacrolimus, the other macrocyclic immunosuppressants in this class. In order to mediate their effects, CsA, tacrolimus and sirolimus must each bind to a cytosolic target protein generically known as immunophilins. The first immunophilin to be identified was cyclophilin, which binds to CsA, and subsequently the family of FK binding proteins (FKBP) were described which bind to tacrolimus and sirolimus as a result of their identical binding domains[117]. The cyclophilin–CsA and the tacrolimus–FKBP complexes interfere with the calcium-dependent signal transduction pathways for IL-2 gene expression; however, the FKBP–sirolimus complex seems to affect calcium-independent pathways which are still not completely elucidated. The molecular target for sirolimus akin to calcineurin remains elusive to intensive research in this area, but the following is known about its biochemical target.

(1) The sirolimus–FKBP complex is necessary for inhibition, since molar excess of tacrolimus can block rapamycin's effect[117].
(2) Sirolimus at immunosuppressive concentrations inhibits IL-2-stimulated p70 S6 kinase and its subsequent activation[118,119].
(3) The sirolimus–FKBP complex has no inhibitory effect on the p70 S6 kinase activation in a cell-free system, suggesting that it possibly forms a ternary complex mediating inhibition of the kinases[120].
(4) The p70 S6 kinase activation is not directly involved in S phase entry for cell proliferation; however, it has been demonstrated that IL-2-stimulated expression of serine threonine kinase p34 cdc2, that is known to be required for G1 to S transition, is a target of rapamycin, TOR[121].
(5) Sirolimus blocks cell cycle progression at a point where many early-to-mid-G1 cell cycle regulatory proteins have accumulated, i.e. cyclins D2, D3 and E, yet are unable to execute their function, possibly due to lack of a triggering event[121].
(6) The activation of the cyclin E/cdk2 kinase complex, which is inhibited by sirolimus, may be the potential triggering event required for progression into S phase (Figure 6)[121].
(7) Sirolimus prevents the down-regulation of inhibitory peptide I kappa B alpha by CD28 stimulation, as the continued down-regulation of I $\kappa\beta\alpha$ leads to enhanced nuclear translocation of C-rel (CD28 response element binding factor), which in turn results in increased transcription of IL-2[122].
(8) Sirolimus and CsA act synergistically *in vitro*, whereas tacrolimus and sirolimus are selective reciprocal antagonists for all parameters tested[123].

Pharmacokinetics

Phase I pharmacokinetic studies of rapamycin have been published[124]. Following a single intravenous dose the pharmacokinetics are non-linear, and the drug appears to distribute out of the blood compartment. Its long terminal half-life, more than 13 hours, indicates slow clearance. The distribution of sirolimus, when studied with human blood incubated with radiolabeled drug, reveals that 94% of the drug is contained in red blood cells and less than 3% is distributed to other cells; only 3% is in the plasma

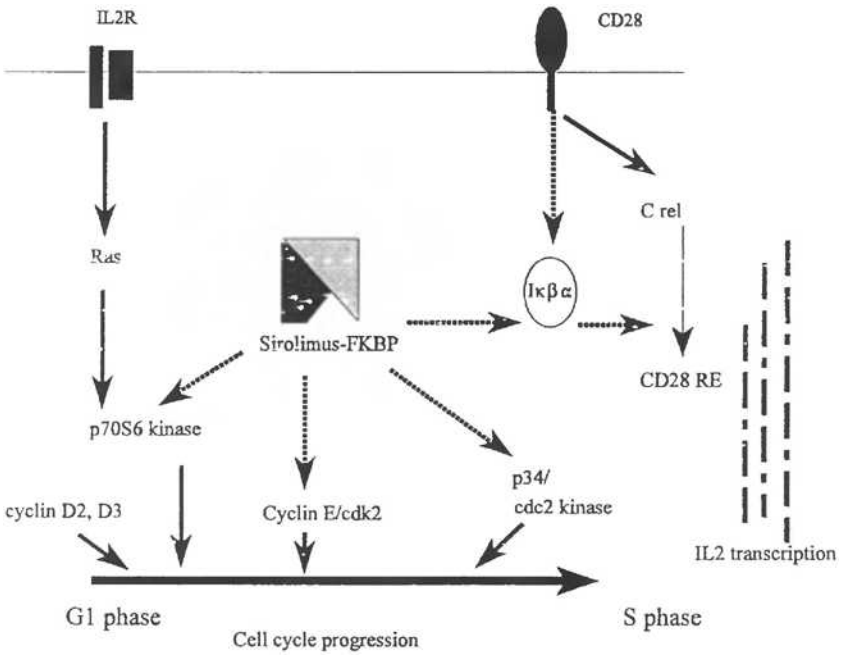

Figure 6 Model for the mechanism of action of sirolimus. The sirolimus–FKBP complex results in dephosphorylation and inactivation of p70 S6 kinase which, though not directly responsible for progression of cell cycle, participates in the process with other cycle-dependent kinases (cdk) which are also inhibited. Sirolimus also inhibits CD28-mediated down-regulation of $I\kappa\beta\alpha$, which is a regulatory peptide for CD28 response element and prevents IL-2 transcription. Broken lines indicate inhibitory effects; solid lines indicate stimulatory effects. Modified with permission from Samelson LE, editor. Lymphocyte activation. Chem Immunol. 1994;59:144

fraction and 97.5% of this fraction is bound to proteins, leaving only 0.175% of the total drug free in blood[125]. A reverse-phase high-performance liquid chromatography assay is now available for quantitating levels of sirolimus in whole blood, with a lower limit sensitivity of 1.0 $\mu g/l$[126].

Sirolimus is lipid soluble with poor oral absorption, the bioavailability being reported to be as low as 1.6%[127].

Experimental pharmacology

Sirolimus inhibits murine, porcine, and human T lymphocyte proliferation induced by mitogenic lectins, antigens, crosslinking of cellular receptors with monoclonal antibodies, i.e. CD3 or CD28, alloantigens, phorbol esters, calcium inophores and lymphokines (IL-2, IL-4, IL-6)[128,129]. Sirolimus inhibits the proliferation of activated T cells even when it is added up to 12 hours after stimulation, whereas CsA and tacrolimus lose their effect when added 2 hours after stimulation[130]. Further IL-2-dependent T cell proliferation is inhibited by sirolimus, unlike CsA or tacrolimus which are unable to suppress lymphokine-mediated proliferation[130].

Sirolimus inhibits calcium-dependent proliferation of B cells with a potency equal to that of tacrolimus, but about 70-fold higher than that of CsA. Sirolimus also inhibits lipopolysaccharide-induced B cell proliferation, which is a calcium-independent pathway resistant to inhibition by tacrolimus or CsA[130,131]. Sirolimus also inhibits spontaneous and pokeweed mitogen induced, IL-2 plus *Staphylococcus aureus* Cowan I-stimulated production of immunoglobulins from human B cells, and IL-4-stimulated IgE production by human peripheral blood mononuclear cells[132].

The immunosuppressive activity of sirolimus has been investigated in several animal models of organ transplantation. Sirolimus is known to prolong survival of heterotopically transplanted hearts in mice[133], rats[134], and cynomolgus monkeys[135], and orthotopic renal transplants in rats[136], dogs[137], pigs[137] and baboons[138]. Sirolimus was effective in the engraftment of islet cells in streptozocin-induced diabetic mice and prolonged graft survival to 56±11 days compared to 22 ± 4.7 days in untreated animals, at a dose of 0.1 mg/kg. However, 10–50-fold higher doses are not as effective in maintaining normoglycemia, presumably due to sirolimus's toxic effect on islet cells[139].

Sirolimus is also able to reverse ongoing rejection in rat cardiac allografts in a dose-dependent manner, and when used in combination with CsA they were able to prolong survival of allografts 10-fold as compared to use of CsA alone (10±2 vs >100 days)[140]. The synergism of sirolimus and CsA for prophylaxis of rejection has also been reported by other investigators[134], and the ability of Sirolimus to reverse ongoing rejection in non-heart, experimental kidney and small bowel allografts has also been demonstrated[136].

Sirolimus has also been shown to inhibit arterial intimal thickening in response to alloimmune and mechanical injury, and may provide a novel strategy in the prevention of allograft proliferative arteriopathy[141]. Sirolimus is also able to induce alloantigen-specific immunologic tolerance in mouse ear–heart models, and treatment with sirolimus combined with donor bone marrow sensitization is superior to that of bone marrow plus thymectomy[134,142].

Sirolimus has also been studied in several models of autoimmune diseases and its efficacy in prevention of experimental allergic uveoretinitis in a dose-dependent manner is well estab-

lished[143]. Sirolimus has been shown to be effective in other models such as mice collagen-induced arthritis, NOD diabetes, and rat adjuvant arthritis[144].

Clinical trials

Several phase I studies to assess the safety and pharmacokinetic profile of the drug are presently ongoing, and the results should be available soon.

Toxicity

A complete toxicity profile of sirolimus is not currently available. The animal toxicology data suggest minimal adverse effects. Sirolimus causes insignificant changes in renal function with no histologic changes, and appears to be less nephrotoxic than CsA[145]. In rats, mild focal myocardial necrosis, glucose intolerance and thymic involution have been noted[146].

Future prospects

Sirolimus' unique immunosuppressive profile and remarkable efficacy in a variety of organ transplantation models suggest significant clinical potential. Its ability to control acute, ongoing, chronic allograft rejection, as well as effects on allograft vasculopathy and possible induction of immunologic tolerance, make it an attractive armament in the immunosuppressive arsenal; however, its ultimate role will depend on the phase II and III trials, and its therapeutic and safety profile relative to other immunosuppressants.

LEFLUNOMIDE (HWA 486)

Leflunomide (LFM), formerly known as HWA 486, was derived from a series of compounds synthesized as agricultural herbicides by the scientists at Hoeschst AG. It is a small-molecular-weight isoxazole derivative which has shown remarkable potential as an immunosuppressant in experimental autoimmune disorders and organ transplantation. LFM is a prodrug and is rapidly metabolized in most animal species and humans to the immunologically active component A771726 (Figure 7)[147]. LFM has no structural or chemical resemblance to any other immunosuppressants.

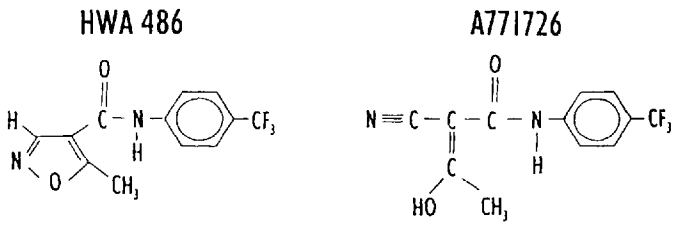

Figure 7 Molecular structure of leflunomide. Reproduced with permission from Chong AS, Xiao F, Xu X, *et al. In vivo* and *in vitro* immunosuppression with leflunomide. In: ? Recent developments in transplantation medicine, vol. 1: Newer immunosuppressive drugs. Glenview, IL: Physician and Scientists Publishing Co.; 1994:165

Mechanism of action

The precise mechanism of action of LFM is not completely understood at present, but it is believed to mediate its immunosuppressive effects by inhibition of the enzyme tyrosine kinase which results in inhibition of tyrosine phosphorylation, an important event in the signal transduction pathway after the engagement of the TCR–CD3 complex and the IL-2 receptor[148,149]. In addition to activation of the TCR–CD3 complex there are a unique set of co-stimulatory molecules such as CD28 and CTLA4, which play an important role in augmenting IL-2 production and preventing the induction of anergy, perhaps by prevention of degradation of IL-2 mRNA by a complex array of biochemical events (Figure 1)[17,150].

Though LFM blocks T cell proliferation stimulated by allogenic cells, anti-CD3, IL-2, or anti-CD28 plus PMA, it does not alter the expression of IL-2R and only partially reduces IL-2 production by activated T cells, suggesting that it acts in the T cell activation sequence later than cyclosporin, and resembles the activity of rapamycin[151]. It is also likely that LFM acts later in the signal transduction pathway, by inhibiting phosphorylation involving other kinases and substrates, which may account for its antiproliferative effects[148,152], or by its effects on the co-stimulatory CD28-mediated pathways.

Pharmacokinetics

LFM is rapidly metabolized to its active component A771726, which is water-soluble, is stable and represents 90% of the metabolites of LFM found in serum of humans and animals. The half-life of the active metabolite varies in the different species from 10 to 30 hours. The data from clinical trials in rheumatoid arthritis suggest that the drug has an excellent safety profile with no toxic effects[153].

Experimental pharmacology

LFM is insoluble in water, and the *in-vitro* experiments are usually performed using the soluble metabolite A771726, which inhibits proliferation of immune mediator cells and other cell lines including epidermal cells, carcinoma cells, fibroblasts, macrophages, bone marrow cells and T and B lymphocytes in ascending order of sensitivity; B lymphocytes are the most susceptible[153]. A771726 may also possess anti-inflammatory properties.

A771726 inhibits T cell proliferation stimulated by allogenic challenge in a one-way mixed lymphocyte reaction, anti-CD3 monoclonal antibody plus PMA, and anti-CD28 monoclonal antibody plus PMA, whereas cyclosporin can only inhibit the proliferation stimulated by anti-CD3 plus PMA[154]. A771726 also inhibits the *in-vitro* generation of murine B cell plaque-forming colonies in response to T-dependent antigen such as sheep RBC, even when added to a 5-day assay, and the murine B cell proliferation stimulated by a T-independent pathway such as anti-IgM or LPS (lipopolysaccharide). The proliferative responses of other cells such as mast cells and promyelomonocytic progenitors induced by IL-3, cellular responses to TNF-α, G-CSF, etc., are also inhibited by A771726[6–8].

LFM has demonstrated remarkable efficacy in prevention of rejection of skin[155], kidney[155] and heart[156] allografts in rats. Its per-

formance in kidney and skin transplantation is comparable to cyclosporin, and is superior to azathioprine and corticosteroids. Further, LFM is able to induce permanent allograft tolerance[155], a phenomenon which was further enhanced by donor-specific blood transfusion. The rat cardiac allograft model also established the comparable proficiency of LFM in prevention of acute rejection, but superiority was noted in the ability to treat established rejection when compared to cyclosporin[156]. LFM has been particularly impressive in its ability to inhibit the development of allospecific antibodies, halt the increase in IgM antibodies when LFM administration is delayed up to 4 days post-transplantation, and inhibit the class switch and development of allospecific IgG antibodies[152,157].

LFM has also had encouraging results in cynomolgus monkey cardiac allograft experiments, with successful prolongation of graft survival at varying doses and in combination with cyclosporin[158]. LFM has also been found effective in delaying acute rejection and prolonging survival in concordant hamster-to-rat cardiac xenografts, while used as a solitary agent and in combination with cyclosporin it provides indefinite survival and control over xenoreactivity[159].

LFM has been shown to inhibit smooth muscle proliferation stimulated by mitogens and intimal thickening in response to balloon catheter injury[158].

Clinical trials

The clinical trials with LFM have until now been restricted to patients with rheumatoid arthritis, and no data are available on its use in clinical transplantation. The rheumatoid arthritis experience suggests that LFM is well tolerated, with no major toxicities and marked clinical and laboratory evidence of resolution of disease[153].

Toxicities

No major toxicities are reported in humans either in the American Rheumatism Association trial in patients with severe rheumatoid arthritis or in the European experience. Animal experiments have suggested anemia and loss of appetite as possible dose-related toxicities[158]. Further studies are needed to develop its toxicological profile.

Future prospects

LFM is certainly an attractive agent, with its ability to suppress both the cellular and the humoral mechanisms in rejection, and possibly prevent intimal proliferative response to injury, but probably the greatest attraction remains its suppression of xenoreactivity. It warrants further animal and human trials to reach its potential in the realm of clinical organ transplantation.

MYCOPHENOLATE MOFETIL (RS 61443, CELL-CEPT)

Mycophenolate mofetil (MM) (formerly known as RS 61443; Syntex, Palo Alto, CA) is a morpholinoethyl ester of mycophenolic acid (Figure 8). Mycophenolic acid was first isolated in 1898 by Gosio[160], from a *Penicillium* culture, while searching for the toxin in maize believed at that time to cause pellagra. The first half of this century led to the analysis of its chemical structure and elucidation of its antibacterial, antifungal, antiviral, antitumor and then immunosuppressive properties[161–167]. Following initial disappointing results the drug was relegated to virtual orphany, and its only use was limited to the treatment of refractory psoriasis[168,169].

Mechanism of action

Renewed interest in the potential of mycophenolic acid as an immunosuppressant arose with the elucidation of purine synthesis pathways in lymphocytes by Allison *et al.*[170–172]. The two major pathways of purine synthesis are schematically entailed in Figure 9. The *de-novo* pathway relies upon formation of inosinate (inosine monophosphate) from amino acids and other precursors, and its conversion to xanthylate (xanthine monophosphate) by the enzyme inosinate dehydrogenase, IMPDH. Xanthylate is then converted to guanylate, which undergoes phosphorylation to form guanosine triphosphate (GTP) to be used for RNA, DNA, protein and glycoprotein synthesis. Inosinate is converted to adenylate by a different set of enzymes. Guanylate and adenylate can also be formed in most cells directly from guanine and adenine via the salvage pathway utilizing hypoxanthine–guanine phosphoribosyl transferase (HGRPTase) and adenine phosphoribosyl transferase (ARPTase). The proliferating lymphocytes lack the salvage pathway for synthesis of purines[170].

The inhibition of inosinate dehydrogenase results in depletion of guanylate within the cells, resulting in antiproliferative effects which seem to be more pronounced on lymphocytes selectively due to the absence of the salvage pathway. Mycophenolic acid is a reversible, non-competitive inhibitor of eukaryotic inosinate dehydrogenase[171,172]. Contrary to earlier reports, mycophenolic acid probably does not inhibit guanylate synthetase, the enzyme which catalyzes the conversion of xanthylate to guanylate[172]. Lymphocytes depleted of guanine nucleotides become fixed in the S phase of the cell cycle and cannot proliferate[173].

Mycophenolic acid has been proven to have preferential effects on lymphocytes for another reason. The type II isoform of IMPDH, which predominates in proliferating B and T lymphocytes, is about four times as sensitive to inhibition by mycophenolic acid than is the type I isoform, expressed in most cell types[172].

Mycophenolic acid has its predicted cytostatic effect on lymphocytes; however, its oral bioavailability is poor and hence the synthesis of a morpholinoethyl ester of mycophenolic acid, mycophenolate mofetil (MM), which has twice the bioavailability in primates when compared to the parent compound, was an exciting new development and further studies have been done using this derivative[174–176].

Pharmacokinetics

MM is rapidly absorbed, chemically intact, across the gastrointestinal tract and is rapidly converted to free mycophenolic acid by ester hydrolysis, primarily in the liver. MPA is then conjugated in the liver with glucuronic acid to form mycophenolic acid glucuronide (MPAG), an inactive metabolite[177]. MPAG is largely

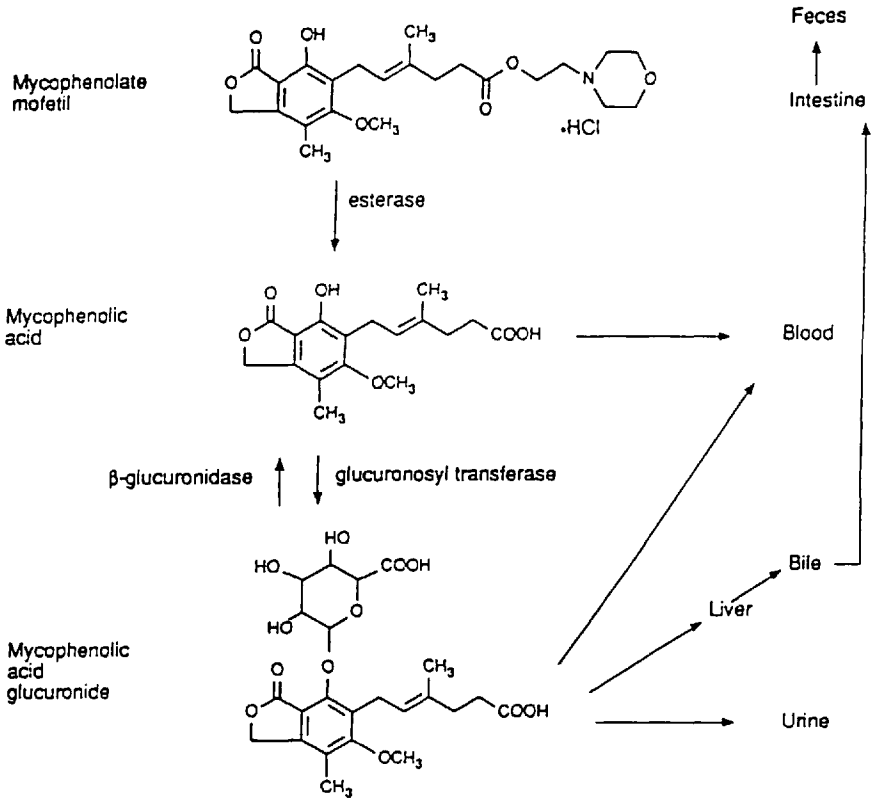

Figure 8 Molecular structure of the morpholinoethyl ester of mycophenolic acid (mycophenolate mofetil) and its glucuronide, and sites of their interconversion and excretion. Reproduced with permission from Allison AC, Kowalski WJ, Muller CD *et al.* Mechanisms of action of mycophenolic acid. Ann NY Acad Sci. 1993:696;67

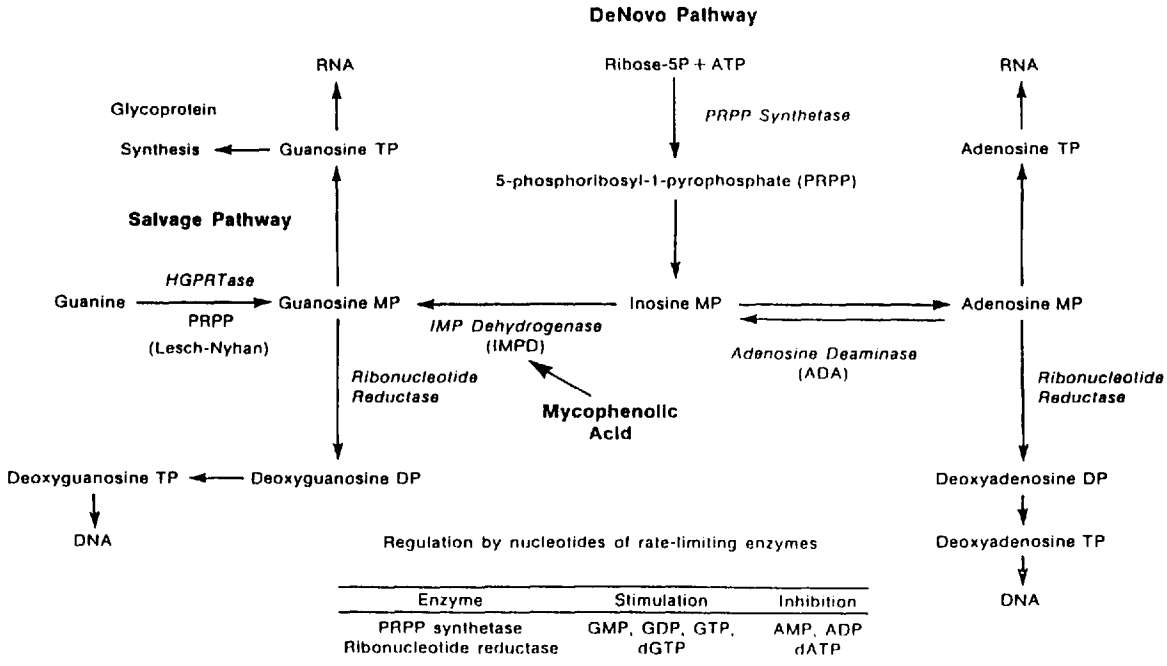

Figure 9 Pathways of purine biosynthesis, showing the central position of inosine monophosphate (IMP). Mycophenolic acid inhibits IMP dehydrogenase thereby depleting GMP, GTP, and dGTP. Reproduced with permission from Allison AC, Kowalski WJ, Muller CD *et al.* Mechanisms of action of mycophenolic acid. Ann NY Acad Sci. 1993;696;64

excreted into the bile where extensive enterohepatic circulation occurs. MPA and MPAG are found in equal amounts in the plasma of MPA-treated rodents. MPAG can be hydrolyzed back to MPA *in situ* by β-glucuronidase, an enzyme found in a variety of cells[178].

Plasma levels of MPA peak within an hour of a single oral dose, with a secondary peak occurring 6–8 hours later, caused by extensive enterohepatic circulation. MPA is metabolically stable and over 90% of the oral dose is recovered intact (as MPAG) from the urine and feces of the test animals[177].

Experimental pharmacology

MPA inhibits mitogen-induced proliferation of rat and mouse spleen cells[179,180] and is a potent inhibitor of proliferative responses of human lymphocytes *in vitro*[181–183]. It inhibits human T lymphocyte proliferative responses to both calcium-dependent and calcium-independent mitogens. It inhibits human B lymphocyte proliferative responses to both T cell-dependent and T cell-independent mitogens[179,184]. MPA and MM inhibit proliferative responses to alloantigenic stimulation (mixed lymphocyte reaction) in human lymphocytes[179,184,185] and inhibit secondary proliferation of alloreactive T cells in human cell culture.

MPA inhibits polyclonal antibody production by B cells stimulated with pokeweed mitogen (T cell dependent) and *Staphylococcus aureus* Cowan I mitogen (T cell-independent)[179]. In addition, MPA inhibits the anti-tetanus-toxoid immunoglobulin G response in human lymphocytes[185,186], an antigen-specific antibody response of memory B cells. MPA inhibits the proliferative response of fibroblasts and endothelial cells only at high doses[179].

MPA primarily affects the late events in lymphocyte response because: (a) MPA does not inhibit IL-2 production, IL-2 receptor expression, or IL-2 mRNA production in human mitogen, alloantigen or anti-CD3 antibody-stimulated lymphocytes[180,187]; (b) MPA does not prevent IL-1 production by blood monocytes[179]; and (c) MPA and MM will inhibit MLR responses when added as late as 3 days after stimulation[179,185].

MPA inhibits proliferating lymphoblastic B cell lines transformed by Epstein–Barr viruses and also induces their differentiation into mature cells[179,188,189]. Clinically attainable concentrations of MPA inhibit proliferation of human smooth muscle cells, which is relevant to effects on proliferative arteriopathy[190]. MPA-mediated depletion of GTP inhibits the transfer of fucose and mannose to glycoproteins, some of which are adhesion molecules facilitating attachment of leukocytes to endothelial cells and to target cells[191]. Further immunoprecipitation studies have shown that one of the lymphocyte glycoproteins affected is VLA-4, the ligand for VCAM-1 on activated endothelial cells[192,193].

MM is an effective immunosuppressant in a variety of animal transplant models. MM used as a single agent prolongs cardiac allograft survival in mice[194,195], rats[195–198] and monkeys[197]; hepatic and renal allografts in dogs[199,200]; pancreatic islet allograft in mice[201,202]; and cardiac allografts in donor-sensitized rats[203]. Survival of rat cardiac allografts is dramatically prolonged when MM is used in combination with cyclosporin[193,203], and brequinar sodium (DUP785)[198]. Canine renal allografts also demonstrate improved survival with the combination of MM and cyclosporin[200], and MM with tacrolimus[204]. MM can arrest progression and reverse established cardiac allograft rejection in rats[195,198] and renal allograft rejection in dogs[200]. MM is also effective in one of the most immunologically challenging models of hamster-to-rat xenografts. MM prolonged survival of hamster-to-rat cardiac and hepatic xenografts[205,206] and its efficacy was further enhanced in combination therapy with tacrolimus[205], with deoxyspergualin and splenectomy[50], and with cyclosporin and brequinar sodium[207].

MM decreases the degree of allograft arteriopathy in the rat cardiac allograft model[197]. MM decreases the intimal proliferation in the rat aortic allograft model[208,209] and in the mechanically injured rat carotid artery model[210].

Clinical trials

The first clinical experience with MM was reported by Sollinger *et al.*[211] in a dose-ranging, safety and pharmacokinetic study in primary cadaveric renal transplant recipients. Forty-eight patients were enrolled into the study, which was stratified to eight groups with dose range of MM from 100 mg/day to 3500 mg/day. All patients received quadruple induction (anti-lymphocyte globulin, cyclosporin A, prednisone and MM) followed by triple maintenance therapy (cyclosporin, prednisone and MM). Follow-up ranged from 2 to 9 months. The drug was well tolerated and was discontinued only in three patients (one non-compliance with protocol, one hemorrhagic gastritis, one acute tubular necrosis). No evidence of organ toxicity or bone marrow suppression was noted. Though not designed as an efficacy study the retrospective analysis suggested significant correlation between the dose and incidence of rejection, with the fewest episodes seen in patients receiving an MM dose of at least 2000 mg/day.

The above study was followed by a multicenter pilot rescue study for refractory renal allograft rejection[212]. Seventy-five patients with biopsy-proven acute rejection, refractory to one course of OKT3/ATG were enrolled, with MM substituted for azathioprine at a dose of 2000–3000 mg/day. Fifty-two of 75 patients (69%) were successfully rescued, which was an impressive result considering the advanced state of rejection in this cohort. In 19 of 75 patients (25%) the drug was discontinued after treatment failure, and in all these patients the allograft was eventually lost. Only four patients had the drug discontinued due to side-effects, believed to have been drug-related. All the side-effects were related to the gastrointestinal tract, colitis, hemorrhagic gastritis and pancreatitis. The overall infection rate was 40% with the spectrum of infections quite characteristic of immunosuppressed patients. In one center the patients with successful rescue were placed on maintenance therapy with MM, and when compared with historical controls there was significantly improved rejection-free graft survival and creatinine clearance[213]. MM was recently approved by the Food and Drug Administration for use in renal transplantation, based on three large multicenter, prospectively randomized, placebo-controlled pivotal trials. Though not yet published these studies suggest that MM is superior to azathioprine in renal transplantation.

Klintmalm *et al.*[214] reported on a multicenter trial for refractory liver allograft rejection with the same definition and entry criteria as the kidney trial. Among 23 patients there were 21 responses, 14 complete and seven improved. Sixteen of 21 patients

continued with the therapy. One patient died from overwhelming sepsis and two underwent retransplantation. Two patients discontinued the drug due to presumed drug-induced cholestasis. Diarrhea, nausea, leukopenia and cholestasis were the most frequently reported side-effects. Liver allografts in three humans have been retained in good functional status using MM with low-dose prednisone but no cyclosporin[215].

MM was first used in human heart transplant recipients in an 8-week, uncontrolled, non-randomized dose–response, pharmacokinetic, and safety study involving 30 patients with Utah grade 3 rejection (includes ISHLT grades 1B, 2, 3A)[216]. Dose range varied from 500 mg/day to 3000 mg/day. Two of six patients (33%) receiving 500 mg/day progressed to moderate rejection (Utah grade 4, ISHLT grade 3A or 3B) versus only 2/24 (8%) receiving 1000 mg/day or a higher dose. Eight of 30 patients had the drug discontinued per protocol, four for progressing rejection and four for persisting rejection. Only one patient had the drug discontinued due to gastrointestinal toxicity. The above results have been confirmed in subsequent studies in patients with mild or moderate rejection with favorable response[217,218].

In a study on chronic maintenance therapy with MM in heart transplant recipients, Taylor *et al.*[219] reported on 33 patients with mean follow-up of 23.7±2.2 months. Only four patients were withdrawn from the study due to side-effects, which included leukopenia, persistent nausea and gastrointestinal hemorrhage. Twenty-eight episodes of mild rejection (ISHLT 1B or 2) (0.5 patient-year) and nine episodes of moderate rejection (ISHLT 3A or 3B) (0.2 patient-year) occurred during therapy with MM. The low rate of recurrent rejection in this group is certainly encouraging. A large randomized, double-blind, multicenter comparative study of MM versus azathioprine in combination with cyclosporin and corticosteroids is currently in progress, and preliminary results of 6-month follow-up should be available in 1996.

Toxicity

Gastrointestinal toxicities have been consistently reported with MPA and MM in both animal and human trials. Nausea, abdominal cramps, diarrhea, soft stools and vomiting are the most commonly reported symptoms, occurring in almost a third of the patients[220]. Urinary tract problems including dysuria, urgency and frequency of urination were reported in 13%. Epinette *et al.* reported on long-term follow-up (up to 13 years) of 76 patients enrolled in a compassionate use protocol of MPA for patients with psoriasis[220a]. Seventy-two percent reported gastrointestinal problems similar to those described earlier; however, the problems decreased significantly after the second year, with diarrhea and nausea being the most common, but only in 5–15% of patients.

Bone marrow suppression was rarely reported in early studies, but from clinical transplant studies an incidence of 11–15% has been reported. Since transplant recipients are on several drugs which may have propensity to cause myelosuppression, assessing the contribution of MM to leukopenia is difficult.

Malignancy is a well-known risk of chronic immunosuppression, but in the long-term follow-up study of 13 years involving patients with psoriasis the risk of malignancy was no greater than that for the general population. MM has been demonstrated to have teratogenic effects in rats and rabbits, but there are no data available in humans.

Future prospects

The unique mechanism of action, high degree of activity and clinical tolerability probably benefit going beyond mere treatment of acute rejection, with a possible role in preventing proliferative arteriopathy and lowering the risk of post-transplant lymphoproliferative disorder. All these features give MM strong credentials to replace azathioprine in standard maintenance immunotherapy.

MIZORIBINE (BREDININ)

Mizoribine (MZB), or bredinin, is a novel imidazole nucleoside which was isolated from the soil fungus *Eupenicillum brefaldianum* in 1974, and was noted to have immunosuppressive activity[221]. It mirrors the mechanism of action of mycophenolate mofetil and is a potent reversible inhibitor of eukaryotic inosine monophosphate dehydrogenase (IMPDH), which results in its antiproliferative effects on lymphocytes which are completely dependent on the *de-novo* pathway for synthesis of guanine nucleotides[222]. The details of purine synthesis pathway and the role of IMPDH inhibitors in selectively inhibiting proliferating lymphocytes have been cited earlier in the chapter.

Pharmacokinetics

MZB is a water-soluble, weakly acidic compound[221]. The pharmacokinetic data from renal transplant recipients suggest considerable variation in the ability to absorb MZB, and correlation was found between the oral dose and serum trough levels[223]. Animal experiments have shown that 57% of the drug is excreted in the urine in 4 hours, with 85% excreted unchanged in the urine in 24 hours[3]. Further, the elimination of drug and creatinine clearance correlate so that the drug accumulates, with impaired renal function[224].

Experimental pharmacology

MZB causes a dose-dependent, reversible inhibition of DNA synthesis in pure cultures of alloantigen or mitogen-stimulated human T lymphocytes. The antiproliferative effects can be reversed by addition of guanosine, and direct measurements of GTP pools in T lymphocytes in the presence of MZB show significant depletion[222]. MZB suppresses the delayed-type hypersensitivity response to PPD and abrogates the hemagglutinin production in response to sheep RBC, suggesting that its effects include suppression of both cell-mediated and humoral immunity[225]. It has also shown efficacy as an immunosuppressant in animal models of autoimmune diseases.

MZB has been extensively studied in canine models of renal, cardiac and pancreatic allograft transplantation[226,227]. It significantly prolonged survival of renal allografts when used in combination with cyclosporin with no additive hepatotoxicity or myelosuppression. Reports of its synergy with cyclosporin have been confirmed in other experimental transplantation models, such as rat heterotopic heart and rat partial lung transplants[228].

Clinical trials

Most of the evidence for the efficacy of MZB in clinical transplantation comes from Japanese studies with kidney transplant re-

cipients. MZB in combination with cyclosporin and prednisone appears to be very promising, with improved graft survival and less toxicity when compared to standard triple therapy with cyclosporin, azathioprine and steroids[229-231]. In 61 patients with haploidentical living-related kidney transplants the group receiving MZB had similar survival and serum creatinine when compared to the group receiving azathioprine, but the MZB regimen had significantly less myelosuppression and incidence of systemic infection[229], which has been corroborated by other investigators[230]. In an open randomized phase II study to assess tolerance and efficacy of MZB as an alternative to azathioprine in cadaveric renal transplants, the investigators came to the following conclusions after a 12-month follow-up: MZB is a safe, well-tolerated and effective alternative to azathioprine. It significantly reduced the incidence of acute rejection and leukopenia[231].

MZB has also been successfully used as a topical solution in preventing corneal transplant rejection[232].

Toxicity

Canine renal allograft experiments have demonstrated that dogs develop severe hemorrhagic enteritis when treated with high doses of MZB, especially in the presence of impaired renal function[226]. Morphologically there is angionecrosis of intestinal submucosal arteries with resultant mucosal necrosis. Similar enterotoxicity has also been described in humans[86]. The incidence of leukopenia is less than 10% and the drug appears to be less hepatotoxic and myelosuppressive than azathioprine[229].

Future prospects

The use of MZB, a selective inhibitor of purine biosynthesis, offers several advantages over azathioprine, including: equivalent or improved graft survival, improved toxicity profile with decreased incidence of leukopenia and hepatotoxicity, favorable effects on prophylaxis of vascular (humoral) rejection, and possibly decreased incidence of post-transplant lymphoproliferative disorders.

BREQUINAR SODIUM (NSC 368390, DUP 785)

Brequinar sodium (BQR), also known as NSC 368390 or DUP 785, is a synthetic quinoline carboxylic acid analog (Figure 10)[233] which acts as an antimetabolite and exhibits immunosuppressive and antitumor characteristics. The drug was initially developed and studied as an anti-cancer agent; however, its success in clinical oncology has been relatively modest. Thus it is now primarily being investigated as an immunosuppressive agent.

Mechanism of action

BQR achieves its antiproliferative effect by non-competitively and reversibly inhibiting the *de-novo* pathway of pyrimidine synthesis[233,234]. BQR inhibits the activity of dihydroorotate dehydrogenase (DHODH), which catalyzes the conversion of dihydroorotate to orotate, and this disruption of the *de-novo* pathway of pyrimidine synthesis results in the depletion of the intracellular nucleotide precursors pool (UTP and CTP), which are essential for formation of DNA and RNA. The lymphocytes lack an alternate salvage pathway for pyrimidine synthesis and are completely dependent upon the *de-novo* pathway which renders them uniquely susceptible to the activity of BQR during the active, proliferative phase of the immune response[235]. The inhibitory effect of BQR can be reversed by addition of exogenous uridine but not cytidine, suggesting that BQR may inhibit not only DHODH, but also the enzyme cytidine deaminase which converts cytidine to uridine (Figure 10)[236].

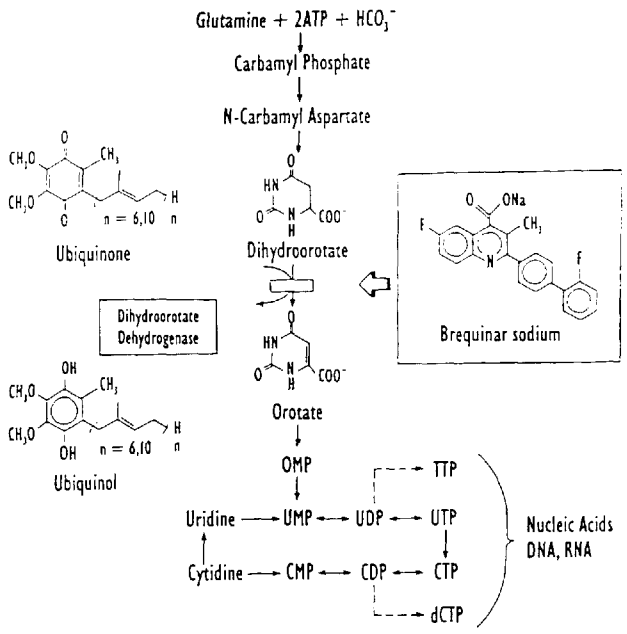

Figure 10 Mechanism of action of brequinar sodium and pathways of pyrimidine synthesis. Reproduced with permission from Cramer DV, Makowka L. Brequinar sodium. In: Recent developments in transplantation medicine, vol. 4: New immunosuppressive drugs. Glenview, IL: Physicians and Scientists publishing Co.; 1994:112

Pharmacokinetics

The initial pharmacokinetic studies were performed in cancer patients and the available data suggest excellent bioavailability in excess of 90%[237]. The compound is water-soluble and can be administered easily in an oral or intravenous form. Peak levels occur 2–4 hours after an oral dose. The steady-state volume of distribution of BQR is in proximity of 8 l/m^2 with biexponential clearance following an intravenous dose, and a mean terminal $T_{\frac{1}{2}}$ of 8–15 hours[238,239]. A variety of dosing schedules have been investigated, and it appears that the most effective and least toxic was when BQR was administered on an alternate-day schedule[240].

The metabolism of BQR has not been clearly delineated, but the primary site of metabolism is believed to be the P-450 cytochrome oxidase system in the liver with excretion primarily in the feces (66%) and urine (23%). No secondary metabolites appear to be active. Co-administration of cyclosporin increases the plasma BQR concentrations significantly without a concomitant change in cyclosporin levels[241].

The activity of BQR can be monitored either by monitoring the drug levels which can be measured using a sensitive reverse-phase HPLC assay[242] or by evaluating its effects on lymphocytes by means of a whole blood mitogen stimulation assay (MSA)[243]. The MSA consists of incubation of heparinized whole blood from patients treated with BQR in a culture medium with phytohemagglutinin. Clinical studies in patients with psoriasis, and in renal and liver allograft recipients, have shown close correlation between plasma drug levels and lymphocyte responsiveness to mitogen stimulation.

Experimental pharmacology

BQR has a broad spectrum of immunosuppressive activity, as evident from the *in-vitro* models of lymphocyte function. BQR eminently inhibits a wide variety of cellular immune responses including alloantigen or mitogen-induced proliferative responses and *in-vivo* suppression of graft-versus-host and delayed-type hypersensitivity responses[244]. The concentration of BQR required for inhibition of B cell responses is relatively higher but still attainable in clinical use. It abrogates the antibody response to sheep RBC or pneumococcal polysaccharide antigenic stimulation[243]. It is the effect on the humoral arm of the immune response which makes BQR an attractive agent, since current cyclosporin-based regimens do not provide efficient protection against B-lymphocyte-mediated immune response.

BQR is reported to prolong allograft survival in rat, pig, and primate models, and also xenograft survival in the rodent model. Rat models have provided the most comprehensive information on the ability of BQR to prevent rejection and, not unlike other immunosuppressive agents, the efficacy of the drug differs in different organ systems, as does the ability to induce permanent graft tolerance[240,243]. Graft survival is prolonged for the period of treatment and, upon discontinuation of the drug, graft rejection occurs within a few days to weeks, reflecting the reversible nature of pyrimidine synthesis inhibition[245].

BQR is also effective in treatment of active ongoing rejection, the rescue being most effective when treatment is started 6–8 days post-transplant of liver allografts in rats[246]. Its ability to prevent graft rejection as a single agent in larger species has been limited by species-specific toxicities. Dogs and pigs are extremely sensitive to its gastrointestinal and hematological side-effects even at relatively small doses, whereas subhuman primates are less sensitive and show significant benefit in cardiac allograft survival either with BQR alone or in combination with cyclosporin[247].

Since humoral immune responses play a significant role in rejection of xenogeneic transplants and in presensitized recipients, the use of BQR in these situations has been investigated and found to prolong graft survival and prevent accelerated rejection[248,249]. The combination of cyclosporin with BQR exhibits synergistic interaction in both allograft and xenograft models[250]. Further, the combination of BQR, cyclosporin and rapamycin is effective in prolonging heart allograft survival[251], and the combination of two antimetabolites BQR and mycophenolate mofetil (RS61443) also induces prolonged survival of rat cardiac allografts with no increase in toxicity[207].

Clinical trials

The initial clinical experience with BQR was in patients with solid malignancies, which generated an extensive database for subsequent studies in organ transplantation. Arteaga *et al.* reported on the effects of a 5-day course of intravenous BQR in 45 patients with refractory solid tumors. The toxic side-effects included nausea, vomiting, malaise, anorexia, diarrhea, mucositis, hepatotoxicity, dermatitis, anemia, leukopenia and thrombocytopenia. Dose-limiting toxicities included thrombocytopenia and desquamative maculopapular dermatitis usually associated with mucositis[239]. In a similar group of 43 patients reported by Schwartsmann *et al.*, who were treated with a single dose every 3 weeks, the toxicity profile was almost identical to that seen with daily dose administration and the incidence increased with the dose[252]. The phase II trials in 53 lung cancer patients[253] and 19 patients with squamous cell carcinoma of head and neck[254] revealed no objective benefit from treatment, and characterized poor response of the tumors to treatment with BQR. The side-effects reported in these trials were similar to those seen in phase I trials, including myelosuppression, gastrointestinal disturbances, mucositis and skin rashes.

The phase I studies in organ transplant recipients to investigate the effects of BQR in single-dose and multiple ascending doses have been reported[237]. Eighteen clinically stable recipients of orthotopic liver transplants who had normal liver function tests were given a single dose of BQR ranging from 0.5 to 4.0 mg/kg. There were minor complaints of headache and diarrhea, which resolved without treatment, and no serious toxicities reported. Ten other patients received 15 oral doses on alternate days, 1 week following the single intravenous dose of 0.5–2.0 mg/kg. The side-effect profile was similar to the previous group, with headache and diarrhea being major complaints. There were also five minor infections reported in seven patients who completed the protocol. Despite widely varied blood levels there was significant immunosuppressive activity in all patients when assessed by whole blood mitogen stimulation assays (MSA). Further studies are currently in progress to develop a better understanding of dosing drug levels MSA and clinical outcome.

Toxicity

BQR is an antimetabolite and toxicity is manifested in tissues with rapid cell turnover such as bone marrow, gastrointestinal tract, lymphoid system and skin[255,256]. There are significant differences in the sensitivity of different species to the drug, but the profile of side-effects is the same in experimental animals and among patients in phase I clinical trials. The primary dose-limiting toxicities are thrombocytopenia, dermatitis and mucositis[239]. The non-competitive and reversible nature of enzyme inhibition allows for rapid reversal of side-effects by discontinuation of the drug and replacement of pyrimidine nucleotide. uridine.

Future prospects

The preclinical and brief clinical experience with BQR has demonstrated its remarkable efficacy as a primary immunosuppressant, synergy with other drugs, potential for use in presensi-

tized patients and xenogeneic transplantation, predictable side-effects and potential for their reversal, all characteristics which make it an attractive agent for inclusion in combination immunosuppressive protocols.

15-DEOXYSPERGUALIN

In 1981 Takeuchi *et al.*, at the Institute of Microbial Chemistry in Tokyo, presented a novel antitumor antibiotic obtained from the bacterial strain BMG 162-aF2 (*Bacillus laterosporus*)[257]. The compound was named spergualin, and was noted to exhibit strong inhibitory effect against experimental mouse tumors. Over the next several years the group studied more than 400 analogs of spergualin and the synthetically dehydroxylated compound (7-guanidino-3-heptanimide group replaced by 7-guanidinoheptanimide), 15-deoxyspergualin (DSG), was selected for further development due to its greater potency and broader dose range compared to spergualin.

Mechanism of action

The precise biochemical mechanism of action for DSG has not yet been fully elucidated; however, recent studies have presented exciting hypotheses of possible intracellular targets. DSG specifically binds to Hsc70, a constitutively expressed member of the heat shock protein 70 (Hsp70) family[258]. Hsp70 plays an important role in the translocation of proteins from cytosol to nucleus in the immune response[259], and in the binding and intracellular transport of antigenic peptides within the antigen-presenting cell[260,261]. Supporting this theory are several reports suggesting that heat shock proteins (Hsp) contain a peptide groove, very similar to MHC molecules[262], and it is plausible that DSG interferes with antigen processing and presentation by interfering with the loading of peptides onto MHC molecules, hence behaving as a peptide mimetic[263].

The other likely explanation for the immunosuppressive activity of DSG may be related to the inhibition of function of Hsp essential for the immune response. It is known that heat shock proteins, Hsp70 and Hsp90, are associated in a complex with the glucocorticoid receptor, Hsp 59, and possibly the immunophilin-cyclophilin. The exact role of this complex is not well understood, but the Hsp are essential for the transformation of the glucocorticoid receptor to the high-affinity binding state[264,265]. The binding of the immunosuppressive agents cyclosporin, Tacrolimus, corticosteroids and DSG to one common receptor complex suggests a common biochemical pathway; however, the exact mechanism is still unclear.

Pharmacokinetics

DSG carries an asymmetric carbon atom in position 11, thus two isomeric forms are possible, (+) and (–); the immunosuppressive effects are connected to the latter enantiomer, though toxicity seems to be associated with both[266]. It is stable in lyophilized form but unstable in neutral solution or culture medium at 37°C. The drug is poorly absorbed from the gastrointestinal tract, with an oral bioavailability in the range 3–6%. The pharmacokinetic profile reveals a short plasma half-life in the range of 4–37 minutes, depending upon the species studied, with predominantly renal clearance[267]. Six major metabolites of DSG have been identified, but none of them is known to have any clinical activity[268].

Experimental pharmacology

The activity of DSG has been tested in several *in-vitro* models of immune function, but the results have been confounded by the fact that DSG becomes metabolized to toxic aldehydes by polyamine oxidases present in fetal calf serum. It has been shown that, in the absence of such oxidases and the toxic aldehyde metabolites, DSG has no effect on mitogen-induced T cell proliferation[269]. DSG has a partial inhibitory effect on alloantigen-driven human mixed lymphocyte responses (MLR), even when added to the culture after 24 hours, unlike cyclosporin which inhibits the response more completely but has no effect upon inhibition of lymphocyte proliferation once the immune response has been activated. It appears that DSG inhibits the secondary cytotoxic T lymphocyte (CTL) response in its early induction phase and not the later effector phase, and there is no inhibition of already induced cytotoxic T lymphocyte[270]. Furthermore, the CTL response can be restored *in vitro* by addition of IL-2 or interferon gamma[270]. DSG also inhibits the delayed-type hypersensitivity (DTH) response to sheep RBC *in vivo* when the animals are pretreated with DSG, but not when treated at the time of rechallenge, suggesting it interferes with the development of effector cells rather than the effector phase itself[271].

The immunopharmacologic profile of DSG is remarkable for extremely potent suppression of the humoral immune response which results in the inhibition of antibody response to both T cell-dependent and T cell-independent antigens. It is likely that these effects are due to suppression of B cell or antigen-presenting cell function. Studies with murine pre-B cell lines have localized the suppression by DSG to inhibition of nuclear and cytoplasmic transcription factors which regulate expression of differentiation specific genes for synthesis of immunoglobulins. The exact details of its interactions with the intracellular targets are still to be determined, and further studies are required to elucidate if other stages of B cell differentiation are also affected[263].

DSG also inhibits macrophage functions such as production and secretion of hydrolytic enzymes[272], expression of MHC class II antigens and the secretion of IL-1[273]. Other investigators have shown that DSG inhibits antigen-stimulated lymphocyte proliferation in response to conventional antigens such as tetanus toxoid or diphtheria toxin, but not in response to superantigens such as staphylococcal enterotoxin A or toxic shock syndrome toxin. The conventional antigen-induced response is inhibited by pretreatment of monocytes with DSG, but not of T cells alone. This suggests that DSG inhibits responses to antigens which require processing prior to presentation to T cells and the inhibitory process is mediated via the antigen-presenting cells[274]. There are several reports of the use of DSG in a variety of animal models including mice, rats, dogs and monkeys for prevention of rejection in heart, kidney, liver, lung, pancreas, skin, thyroid and bone marrow transplants. The murine heterotopic cardiac allograft model shows several-fold longer survival when treated with DSG

when compared with cyclosporin, and also results in donor-specific immune unresponsiveness[275]. DSG treatment of allogenic bone marrow recipient mice prevents the development of graft-versus-host disease and also encourages engraftment of the donor bone marrow[276].

In rats, DSG produces a dose-dependent prolongation of median survival of heterotopically transplanted cardiac allografts[277] and was as effective as cyclosporin or tacrolimus in extending the median survival of orthotopic liver allografts, with less hepatotoxicity than cyclosporin[278]. Similarly, survival of orthotopic renal allografts in mice is prolonged by DSG with induction of donor-specific immune tolerance, but the histopathologic analysis reveals that DSG does not prevent the infiltration of the allograft by lymphocytes. The graft-infiltrating lymphocytes from DSG-treated animals have considerably less T cell activation markers when compared to untreated controls, but it proves that DSG does not completely block the immunologic response[279]. DSG is also extremely effective in the treatment of established acute rejection in a rat orthotopic liver transplant model[278].

The experience in canine transplant models with DSG has been significantly hampered by its gastrointestinal toxicities, but in primate models of cardiac allografts it reaches the same efficacy as antilymphocyte globulin in prevention of acute rejection[280,281]. DSG has also been effective as a single agent for prolongation of graft survival in concordant xenotransplants involving hearts[282], kidneys[283], and skin[284]. Furthermore, in discordant models such as pig-to-rat islet allografts, it is effective in combination with antilymphocyte globulin[285].

Clinical trials

Clinical trials have been almost exclusively limited to renal transplantation. DSG has been used for prophylaxis, for treatment of acute rejection, and as rescue treatment for refractory rejection in over 300 patients worldwide, but the reports are non-randomized, are unblinded, and use many different immunosuppressive protocols, thus making comparisons very difficult. In the Japanese Collaborative Transplant Study, deoxyspergualin was used to treat acute and chronic renal allograft rejection in a controlled multicenter trial, and it successfully reversed all types of rejection in 80% of the patients. Early or accelerated rejection was reversed in 80–92%, chronic rejection was reversed in 67–69%, and as rescue therapy it was successful in 80–88%[286,287]. DSG, when used in induction protocols, has beneficial effects by maintaining patients on steroid-free regimens[288], and at lower maintenance doses of cyclosporin[289]. Anecdotal reports on use of DSG in liver transplantation and pancreatic islet cell transplantation have also been encouraging[290,291].

DSG has also been used in reducing the rebound HLA antibodies in renal transplant recipients after plasmapheresis[291]. There is also a report of DSG being used successfully in adjunct with cyclosporin, prednisone, antilymphocyte globulin, azathioprine and local irradiation following splenectomy as salvage therapy in incompatible transplantation with a positive crossmatch[292].

Toxicity

The toxicity of DSG is believed to be species-specific. While dogs develop serious gastrointestinal side-effects at relatively small doses[280], rodents show virtually no side-effects. Clinical trials in humans have shown mild adverse reactions with transient hematologic abnormalities, which seem to be dose-dependent and are most prominent 2–3 weeks after a 5-day induction period[286]. Evidently there is suppression of both erythrocyte and leukocyte precursors in the bone marrow with prevention of proliferation and differentiation, as seen from animal models. Besides bone marrow suppression the only other major side-effect in humans was nausea seen at a dose greater than 6 mg/kg per day.

The other reported side-effects include patchy alopecia, lethargy, diarrhea, epistaxis and elevated liver enzymes in rats treated for 3 weeks[292].

Future prospects

DSG is a promising agent with a unique mechanism of action and potential for use in prophylaxis and treatment of acute cellular re-

Table 4 Monoclonal antibodies

Antibody	*Target*	*Source*	*References*
OKT4	CD4 receptor	Murine chimerized	292–297
Anti-TAC	IL-2 receptor beta chains	Human	298–305
Anti-LFA-1	CD IIA	Murine	306–308
Anti-ICAM-1	CD 54	Murine	309, 310
Anti-IL-2R (campath)	CD 25	Murine	311
Anti-IL-1R	IL-1 receptor	Murine	312
Soluble HLA class 1	HLA class 1 molecule	Murine	313–316
CTLA-4 Ig	B7 receptor	Human	317
Xomazyme	CD5 receptor ricin toxins	Chimeric	318
DAB 486-IL-2	IL-2 receptor diphtheria toxins	Chimeric	319–323
Anti-TNF	Tumor necrosis factor	Murine, human	324–330

jection. Its potent humoral immunosuppressive effects may be helpful in the prophylaxis and treatment of acute vascular rejection in allogeneic, as well as xenogeneic, transplantation.

MONOCLONAL ANTIBODIES

Monoclonal antibody therapy (see Chapter 71) has opened new vistas in antigen-specific therapy in organ transplantation. The past decade has seen the introduction of the murine monoclonal anti-CD-3 antibody, OKT3, in clinical practice as a popular choice for early prophylaxis and treatment of corticosteroid-resistant rejection; however, it has several limitations including the first-dose cytokine release phenomenon, and development of human antimurine antibodies, and most distressing is the increased incidence of vascular rejection associated with sensitization. Since OKT3, several other agents have entered into clinical trials, but none has yet graduated into clinical practice. Table 4 lists the newer monoclonal antibodies which are currently being investigated.

The evolution of these immunotherapies has closely followed the advances in our knowledge of the various mechanisms involved with allograft rejection, the role of cytokines, MHC and adhesion molecules. Thus today we have several targets for alteration of the immune response rather than the purgative panlymphocytic depletion approach of earlier polyclonal agents.

The recent data from several trials employing monoclonals against CD4 and CD54 have suggested remarkable efficacy. The experience with these agents and other monoclonal antibodies is reviewed in detail elsewhere[331].

SK&F 105685

SK&F 105685 is a novel azaspirane with potent immunoregulatory activity and the ability to suppress autoimmune disorders in experimental animals. The exact mechanism of action of this agent has still not been clearly elucidated, but it is believed that SK&F 105685 and other related azaspiranes are able to induce 'natural' or 'non-specific' suppressor cells (SC) much like those seen after total lymphoid irradiation in absence of myelotoxicity[332]. The identity of these SC which confer specific transplantation tolerance continues to be nebulous, but their activity is clearly unrelated to classic CD8 suppressor T cells, other mature T cells, B cells, NK cells or macrophages, and they may belong to the premyeloid/monocytic lineage[333].

Total lymphoid irradiation (TLI) has been extensively studied and often used for immunosuppression in bone marrow and solid-organ transplantation for induction of relative allotolerance, especially in face of unrelenting and unremitting rejection. In experimental models TLI induces 'natural' or 'non-specific' SC, which are resistant to irradiation and confer suppression of the host immune response to antigenic challenge[334]. SK&F 105685 has the same immunological profile with respect to its ability to induce SC.

Pharmacokinetics

The paucity of detailed pharmacologic studies with SK&F 105685 makes it difficult to characterize its kinetics, bioavailability and metabolism. The few animal experiments have suggested fair bioavailability following oral doses ranging from 5 to 30 mg/kg per day. The dose–response studies reveal the 20 mg/kg per day dose to be most efficacious in prevention of acute rejection in rat heterotopic cardiac allograft models[335]. There are no data available on its pharmacokinetic profile in humans.

Experimental pharmacology

The few reported animal experiments studying the effects of SK&F 105685 in the transplantation models have suggested benefit from treatment irrespective of particular protocol. Prolongation of graft survival was nearly equal whether patients were pretreated with SK&F 105685 or received it post-transplant[335]. The effects of pretreatment with SK&F 105685 were potentiated by the use of low-dose cyclosporin, which by itself was ineffective[333].

The immunohistopathologic evaluation of cardiac allografts harvested from SK&F 105685-pretreated rats reveals marked abrogation of classic rejection with significantly reduced mononuclear cell infiltration, reduced induction of IL-2 and transferrin receptors, decreased production of IL-2 and IFN-γ, suppression of other cytokines (IL-1, IL-6, TNF-α), reduced endothelial activation factors and reduced expression of adhesion molecules such as ICAM-1 or thrombomodulin[336].

Similar findings have also been noted in rat renal allografts with decreased infiltration of mononuclear cells, and decreased eicosanoids production[337]. The drug has also been effective in inhibiting hind paw inflammation in the adjuvant arthritis model[338], and it blunts the delayed hypersensitivity responses to tuberculin (PPD)[332], suppresses paralysis in the experimental allergic encephalomyelitis model[332] and attenuates glomerular changes in the MRL(Lpr/Lpr) lupus nephropathy model[339].

Clinical trials, toxicity, future prospects

There are no clinical trials or data on toxicity available currently on SK&F 105685; however, considering its novel mechanism of action one would anticipate its early graduation into dose-ranging, safety and pharmacokinetic studies. The immunoregulatory therapeutic strategy may some day find a niche in the world of organ transplantation.

PHOTOPHERESIS

Extracorporeal photopheresis (see Chapter 72) is an apheresis-based immunomodulatory therapy which has been successfully used for treatment of patients with cutaneous T cell lymphoma, systemic sclerosis and other autoimmune disorders. The limited clinical experience with use of this immunomodulatory therapeutic modality in the realm of solid-organ transplantation has provided encouraging results, and given it the status of a promising neo-adjunct to immunosuppression.

Mechanism of action

The mechanism of action of photopheresis still remains unclear, though it is postulated that the photoactive medication

8-methoxy-psoralen is taken up by leukocytes and is activated by ultraviolet A irradiation, which leads to crosslinking of DNA and a proliferative arrest[340]. The activated or large mononuclear cells appear to be exceptionally sensitive to the effects of psoralens and ultraviolet A, possibly due to increased receptors for psoralens. It also induces cytokine elaboration from treated monocytes, some of which may play a key role in the immune response to the allograft. Its lack of effect on host response to T cell-dependent soluble antigens such as tetanus toxoid, or on delayed-type hypersensitivity response, suggests that it does not profoundly suppress cell-mediated immunity[341]. However, there is now experimental evidence in cutaneous allograft rejection models that photopheresis may stimulate an antigen-specific suppressor-T cell response, akin to active immunization against alloreactive photoinactivated T cell clones[342].

Clinical and experimental reports

Experimental rat models of skin[343] and cardiac[344] transplantation have shown significant prolongation of allograft survival. Encouraging results have also been noted in primate models of allo-and xenotransplantation[345]. Success in animal models led to early graduation of this novel immunomodulatory technique to use in clinical transplantation. Costanzo-Nordin *et al.* reported the use of photopheresis in treatment of acute rejection in cardiac allografts. In a pilot study, nine episodes of hemodynamically stable moderate rejection (ISHLT grades 2, 3A and 3B), occurring at a mean of 114.4 ± 180.5 days post-transplant, were treated in seven patients. Eight of nine episodes were successfully resolved, as assessed by subsequent endomyocardial biopsies performed 7 days after treatment[346].

In a subsequent randomized study by the same investigators photopheresis, when compared to corticosteroid therapy, was found to be equally effective and well tolerated, with no adverse effects[347]. Analyses of post-treatment biopsies revealed significant decrease in macrophages and B cells in the photopheresis group, but the interstitial T cell infiltrative response was more prevalent and persistent, confounding interpretation of endomyocardial biopsies in photopheresis-treated patients[347,348].

Photopheresis has also been used as adjunct immunosuppression for chronic maintenance, and was shown to be safe and effective, with reduction in numbers of acute rejection episodes and fewer infections than the control group who received standard triple therapy with cyclosporin, azathioprine and glucocorticoids[349]. The above approach has also shown beneficial effects in the prevention of progression of coronary intimal hyperplasia, as photopheresis-treated patients had significantly decreased coronary intimal thickening as measured by intravascular ultrasound at 1 year follow-up[349].

A multicenter trial is currently under way to assess the safety and efficacy of adjunctive photopheresis in prevention of acute rejection, and the results of this study should be available soon.

Toxicity

The wealth of clinical experience with photopheresis has been gathered with its use in patients with T cell lymphoma, and it reveals that it is an extremely well-tolerated procedure. The most common side-effect is psoralen-induced nausea, which is usually mild and transient. Hypotension may occur in the leukapheresis phase and seems to be dependent upon the intravascular volume status. Low-grade fevers have been noted in patients with cutaneous lymphoma 4–12 hours after therapy, and seem to be caused by the release of inflammatory and pyrogenic cytokines by induced monocytes[340].

Future prospects

Extracorporeal photopheresis is a promising new modality with remarkable efficacy and lack of toxicity or profound side-effects, and awaits further randomized clinical trials prior to gaining acceptance as neo-adjuvant immunosuppressant.

CASTANOSPERMINE

Castanospermine is an alkaloid isolated from the seeds of an Australian legume *Castanospermum australe*. The past decade has seen considerable advances in our knowledge of cell–cell interactions and the role of adhesion molecules in the co-stimulatory pathways mediating vascularized allograft rejection[22]. It has been theorized that, since adhesion molecules play an important role in allograft rejection, interference in their expression may alter the immune response.

Mechanism of action

Castanospermine is an α-glucosidase inhibitor which interrupts the intracellular processing of glycoproteins by preventing the removal of glucose from the N-linked carbohydrates of various glycoproteins[350]. The retardation of glycoprotein processing results in down-regulation of T cell glycoprotein surface receptors such as LFA-1, ICAM-1, MHC class II[351] and MHC class I[352], and this interferes with lymphocyte homing, lymphocyte–endothelial cell interaction and antigen-presenting-cell–T-helper-cell interaction[12,353].

Experimental pharmacology

CD54 or LFA-1 is a glycoprotein expressed on vascular endothelial cells, tissue macrophages, T lymphocyte blasts, and dendritic cells, and presents itself as a binding ligand for CD11a or ICAM-1. The treatment of these cells with Castanospermine markedly diminishes CD54 expression, and hence interferes with leukocyte migration into the allograft[354]. Castanospermine also inhibits pokeweed mitogen-induced human lymphocyte cultures, where it strongly decreases the number of IgG, IgM, and IgA secreting cells. The effect seems to be preferential on B cells suggesting alteration of their membrane oligosaccharides which interferes with T cell–B cell interaction[355].

Several animal models stand to the testimony of efficacy of Castanospermine in prevention of allograft rejection. It has been tested in pancreaticoduodenal[356], heart[357], and kidney[358] grafts with favorable results showing prolongation of allograft survival. It also inhibits the rejection of accepted thyroid allografts induced by intraperitoneal injection of donor strain spleen cells with a

reduction in lymphocyte infiltration of 25–35% when compared with untreated controls[359].

Castanospermine when used in combination with FK-506 at subtherapeutic doses was able to inhibit the proliferative response in a mixed lymphocyte reaction, and prolong heterotopic cardiac allograft survival in MHC mismatched rats, suggesting pharmacologic synergism[360].

Clinical trials, toxicity, future prospects

The lack of clinical trials makes it difficult to predict the toxicological profile and future prospects for this agent. It is well known that the seeds of *Castanospermum australe* are toxic to some animal species, resulting in gastrointestinal disturbances, but detailed studies are still to be performed. Certainly, castanospermine presents a novel approach to immune modulation, and may find a role in the multi-drug combination protocols by nature of its synergy with other agents and decreased potential side-effects.

OTHER AGENTS

Discodermolide

Discodermolide is a polyhydroxylated lactone which was isolated from a marine sponge *Discodermia dissoluta*[361], and is different from other novel immunomodulatory agents which are mainly derived from plants or microorganisms. This compound blocks cellular proliferation in lymphoid and non-lymphoid cells.

It was noted to have potent immunosuppressive properties in both murine and human *in vitro* lymphocyte stimulation assays[362]. It is capable of inhibiting mixed lymphocyte response even when added up to 3 days following initiation of cultures of mitogen-stimulated T cell proliferation, but has little or no effect on IL-2 production or IL-2 receptor expression. It suppresses the activity of lymphokine-activated killer (LAK) cells[363]. Cell cycle progression analysis reveals that discodermolide blocks the progression of cells from G_2/M phase to the G_1 phase, causing the proliferative arrest in a manner quite similar to anthracyclines. The exact point where mitosis is blocked is unclear, and is currently under investigation. Possibly interruption of DNA topoisomerase II activity[364] may represent the biochemical lesion responsible for the antiproliferative effects of discodermolide.

In vivo studies have shown it to be effective in suppression of graft-versus-host reaction[365]. Furthermore, it has no cytotoxicity, and its effects are reversible by removal of the drug. Additional studies in animal models of experimental transplantation are currently being pursued, and it may be several years before discodermolide reaches clinical trials.

LF 08-0299

LF 08-0299 is a new immunosuppressive compound, currently in preclinical development at the Laboratoires Fournier, France. It has been investigated as an agent to induce donor-specific allograft tolerance in experimental models of rat skin and cardiac transplants in MHC-mismatched recipients[366]. In this model it demonstrated ability to induce a specific state of clinical tolerance with short-term treatment, an objective which could not be achieved with cyclosporin.

Enisoprost and SC 45662

Enisoprost is a prostaglandin E analog which suppresses IL-2 production and IL-2 responsiveness. SC 45662 is a 5-lipooxygenase inhibitor which may affect the immune system by prevention of formation of leukotriene B4, a known enhancer of IL-2 production and natural-killer-cell-mediated cytotoxicity. Both these agents have been shown to possess immunosuppressive activity, with suppression of mitogen-induced mononuclear cell proliferation and inhibition of effector function of cytotoxic T lymphocytes against allogenic target cells[367]. These compounds need further studies, and may have a role as adjunctive therapy.

Bryostatin

Bryostatin is a macrocyclic lactone isolated from the marine organism *Bugula neritina*, and shares the ability of phorbol esters to bind and activate protein kinase C. There is some experimental evidence to suggest that it may induce antigen-specific non-responsiveness in human peripheral blood T cells[368].

COMMENT

The newer immunosuppressive agents possess a variety of mechanisms of action and affect diverse components of the immune response. Since allograft rejection is a complex process, to assume that one single agent could possibly prevent and control the rejection process would be ludicrous, hence combination immunosuppressive regimens with complementary mechanisms of action and without potential for unfavorable interactions or overlapping toxicities should provide the desirable outcome in clinical practice.

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New Monoclonal Antibodies

M.R. COSTANZO

INTRODUCTION

The technology for the production of monoclonal antibodies (moabs) was established 20 years ago by Kohler and Milstein[1]. The fusion of myeloma cells with immunized spleen cells produces a hybridoma. Such a cell line possesses both the immortality of myeloma cells and the ability to produce an antibody of a single predetermined specificity. In contrast to polyclonal antibodies, moabs are homogeneous, have greater potency at lower doses, and have a more predictable and consistent effect[1]. Since its introduction, moab technology has become an important diagnostic and therapeutic tool in many areas of medicine. These include the identification of phenotypic markers unique to particular cell types, immunodiagnosis, tumor diagnosis and therapy, functional analysis of cell surface and secreted molecules, and prevention and treatment of organ transplant rejection.

Moabs represent a significant advance in the immunotherapy of organ transplantation, since they are free of the main limitations of polyclonal preparations, such as undesired cross-reacting antibodies and batch-to-batch variability[2–4]. To date, the moab which has been most widely used clinically is OKT3, a murine moab of the IgG2a subclass directed against the epsilon chain of the CD3 receptor on the surface of human T cells[5]. The CD3 complex is closely associated with the T cell receptor (TCR). The CD3/TCR complex plays a pivotal role in T cell function. Antigen recognition by the TCR results in signal transduction via the CD3 molecule and subsequent T cell proliferation and activation of cytotoxic T cells. OKT3 inhibits the CD3/TCR complex, inactivating lymphocytes[6,7].

In heart transplant recipients OKT3 successfully reverses rejection refractory to intensified corticosteroid therapy and polyclonal anti-T-cell preparations[8]. While there is general agreement that OKT3 is effective for the treatment of stubborn rejection, the role of this moab in the prevention of heart allograft rejection remains controversial[9]. The use of OKT3 can be associated with significant adverse effects. These include the cytokine release syndrome resulting from OKT3-induced T cell activation which can occur after the first few OKT3 doses[10–15], the development of human anti-mouse antibodies which may limit efficacy and preclude retreatment with OKT3[16–20], an increased incidence of opportunistic infections, such as those due to cytomegalovirus (CMV)[21], and an increased incidence of post-transplantation lymphoproliferative disorders[22].

Advances in the knowledge of molecules and immune mechanisms involved in the rejection of an allograft, and the need to overcome the limitations of currently available immunosuppressive strategies have stimulated the development of new moabs, which will be the focus of the brief review that follows.

NEW MONOCLONAL ANTIBODIES CURRENTLY BEING EVALUATED

CD3 monoclonal antibodies

WT32

WT32, a murine IgG2a moab which has the same molecular target and adverse effects as OKT3, is very effective in reversing acute allograft rejection[23]. CD3 cells are eliminated by 2–4 mg of WT32 and return to normal levels a few days after the last moab dose. Antibodies against WT32 are detected within 4 days of cessation of therapy, but they are mainly anti-idiotypic[23] and their titers are usually lower than 1:100.

Anti-T-cell receptor monoclonal antibodies

T10B9.1A-31

T10B9.1A-31 is a murine moab of the IgM subtype directed against the human α/β TCR which co-modulates TCR and CD3. It is non-mitogenic to human lymphocytes because it does not bind to the Fc receptor of monocytes[24]. In clinical trials it has been effective for the prevention and treatment of renal allograft rejection and the reversal of corticosteroid-resistant heart allograft rejection[25,26]. The main disadvantage of T10B9.1A-31 is a short half-life, which requires administration every 8 hours. The main advantages include a toxicity lower than that of OKT3 and the opportunity for sequential moab therapy, since antibodies

produced against OKT3 and T10B9.1A-31 are of different isotype and idiotype[24–26].

Anti-adhesion molecules monoclonal antibodies

The binding of adhesion molecules on antigen-presenting cells to their ligands on T cells is a critical event for T cell activation and function. A large body of experimental evidence suggests that inhibition of the interaction between adhesion molecules and their ligands may promote long-term acceptance of an allograft. Important in this regard is interference with the binding of the intercellular adhesion molecule-1 (ICAM-1) on antigen-presenting cells to its leukocyte-function-associated-1 ligand (LFA-1) on T cells[27]. The ICAM-1 molecule is identified by anti-CD54 moabs. The LFA-1 molecule is a heterodimer made of an α chain, identified by anti-CD11 moabs, and a β chain, identified by anti-CD18 moabs. Since the ICAM-1 β chain plays an important role in leukocyte adhesion and function, anti-CD18 moabs may be clinically effective in reducing donor allograft ischemic injury, but not in the prevention and treatment of allograft rejection[28]. On the other hand, moabs directed against the α chain of the LFA-1 and ICAM-1 may modulate allograft rejection.

In a murine heterotopic heart transplant model between histoincompatible strains, the combined use of KBA, an anti-LFA-1 α chain moab, and YN1/1.7, an anti-ICAM-1 moab, but not the separate use of either moab, resulted in indefinite acceptance of the heart allograft[29]. In a phase I clinical trial, 18 high-risk renal transplant recipients (prolonged preservation, highly sensitized recipients of cadaver kidney allografts) received the BIRR-1 moab (anti-CD54) intravenously at a loading dose of 20–160 mg followed by a 2-week 10 mg/day course. When therapeutic BIRR-1 serum levels were achieved (>10 μg/ml) no primary non-function occurred and allograft survival at 16–30 months of follow-up was 78%. In contrast, among the recipients of the contralateral kidneys from the same donors, there were three cases of primary non-function, and survival was only 56% over the same follow-up period. Phase III clinical trials are ongoing in renal, but not yet in heart, transplant recipients[30].

Anti-interleukin-2 receptor monoclonal antibodies

Phenotypic changes in the expression of the interleukin-2 receptor occur as a result of cell activation. The IL-2R consists of a low-affinity 55-kDa subunit, termed the Tac peptide, and a high-affinity 70-kDa subunit. Anti-IL-2R moabs include the murine anti-Tac (IgG2a) and the rat 33B3.1 (IgG2b). Anti-Tac has been used in clinical renal transplantation in combination with cyclosporin, prednisone, and azathioprine[31]. Use of this agent resulted in a decreased frequency of early rejection episodes, a delayed onset of rejection, and good patient and graft survival rates[31]. 33B3.1 has been used as an induction agent in primary cadaveric renal allograft recipients. It was well tolerated, but appears to be slightly less effective than ATG in preventing acute rejection episodes. Sensitization to both antibodies occurred in 70–80% of treated patients[32].

CHIMERIC AND HUMANIZED MONOCLONAL ANTIBODIES

A major complication in the use of murine moabs has been the elicitation of anti-murine antibodies in the recipients. These antibodies, if present in sufficiently high titers, can decrease moabs' efficacy and preclude retreatment. In an attempt to attenuate sensitization against foreign antibodies, rodent antibodies have been 'humanized' by linking rodent variable regions with human constant regions (chimeric). The immunogenicity is reduced, but not eliminated, since the foreign variable region is retained[32]. Further refinement in 'chimerism' has been achieved by the development of moabs in which the only remaining murine portion of the antibody is the complementarity-determining region (CDR-grafted moabs)[33].

A chimeric anti-CD4 moab has been compared to ATG in the prophylaxis of heart transplant rejection. A trend was detected for the anti-CD4 moab-treated heart transplant recipients to have a greater delay in the occurrence of the first rejection episode, lower rejection and infection rates, and higher 1-year survival[34]. Prospective randomized trials in larger patient populations are needed to confirm the superiority of CDR-grafted moabs over conventional polyclonal preparations.

FUTURE APPLICATIONS OF MONOCLONAL ANTIBODIES

Future developments in moabs use will include: (a) therapy with the *fully* human effector portion of the moab (Fab') rather than with the whole antibody[35]; (b) induction of tolerance to allografts and xenografts[36]; (c) sequential use of moabs of different isotypes; (d) delivery of toxins to target cells[37,38].

The production of fully human Fabs' requires that human variable regions (V-regions) be isolated and assembled in a phage. Because V-regions are inserted in contiguity with a gene encoding for one of the phage's external membrane proteins, surface display of the Fab' portion of the antibody will occur. *Escherichia coli* bacteria can then be infected with the Fab'-encoding phage and secretion of a soluble fully human Fab' induced. Selection of high affinity Fab' can then be done on an antigen-coated solid phage[35].

The use of moabs to induce tolerance is likely to involve the combined administration of the moab and donor antigen. In a model of murine heterotopic heart transplantation, indefinite tolerance to the allograft was obtained only with the combined use of an anti-TCR moab and the intrathymic injection of donor antigens[36].

Immunotoxins are chimeric molecules in which cell-binding ligands, a cell-reactive moab or growth factor are coupled by a crosslinker to toxins or their subunits[37]. The murine moab Zomazyme H65 (IgG1) is a ricin-A toxin conjugate that has *in vitro* effects of an anti-pan T cell antibody. The drug has been used in preliminary studies for the prevention and treatment of graft-versus-host disease[38]. Phase I trials for the prevention and treatment of renal allograft rejection have been planned.

COMMENT

The therapeutic efficacy of moab therapy in human solid-organ transplantation has not been clearly demonstrated. Future devel-

opments must focus on the development of fully 'human' moabs. It is likely that strategies designed to induce tolerance to the allograft will involve the combined administration of moabs and donor antigens[39,40].

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Immunomodulation with Photopheresis

M.L. BARR

INTRODUCTION

Modern immunosuppressive regimens utilizing cyclosporin or tacrolimus (FK506), azathioprine or mycophenolate mofetil, and corticosteroids, with or without induction protocols using monoclonal or polyclonal antibodies, have resulted in dramatically increased survival of transplanted organs. However, in addition to suppressing the immune response to the allograft, they impair immune function in a non-specific and often toxic fashion, leaving the host susceptible to increased risk of opportunistic infections, malignancy, and the direct side-effects of these drugs. Moreover, there remain significant morbidity and mortality from organ rejection in the form of acute episodes, as well as chronic forms as seen in late-onset progressive graft failure such as transplant coronary disease.

Thus, despite the undesirable sequelae of current immunosuppressive protocols, there are still a significant number of patients dying from organ-specific under-immunosuppression. While the percentages may vary, these problems continue to plague all varieties of solid-organ transplantation. The inability of these regimens to discriminate between individual clones of pathogenic and benign T cells, and the persistent problem with ongoing chronic B cell-related humoral immunity, have led to the use of novel forms of immunomodulatory therapies.

TECHNIQUE

Photopheresis is a technique in which the patient's peripheral blood mononuclear cells, in the presence of a photoactivatable compound, such as 8-methoxypsoralen, are exposed extracorporeally to ultraviolet A light. Blood is usually removed via a peripheral intravenous line. Utilizing a cell separator the leukocyte-depleted blood is returned to the patient while the leukocyte-enriched plasma, containing either systemically absorbed or directly administered liquid 8-methoxypsoralen, is exposed to ultraviolet light in the 320–400 nm range. The buffy coat, with a hematocrit of approximately 5–7%, passes through a photocassette at a thickness of 1 mm and has an average ultraviolet A exposure of 2 J/ml. The photoexposed white cells are then returned to the patient. The entire procedure can usually be completed in a total of 4 hours.

The photoactivated 8-methoxypsoralen covalently binds to DNA pyrimidine bases, cell surface molecules, and cytoplasmic components in the exposed white cells[1]. The induction of 8-methoxypsoralen–DNA crosslinks and photoadducts results in a lethal defect, and the reinfused cells die gradually in the recipient over a 1–2-week interval. The reinfused altered lymphocytes produce an autologous suppressor response that targets unirradiated T cells of similar clones via an ill-defined mechanism.

EXPERIMENTAL AND CLINICAL EXPERIENCE

In non-transplant conditions

Edelson *et al.* first used photopheresis in humans to successfully treat cutaneous T cell lymphoma, a disease characterized by massive expansion of a single clone of helper T cells[2]. Of importance as a safety issue, general immune competence was spared in these treated patients, as demonstrated by the absence of infectious complications and the persistence of skin test reactivity. Based on work with this disease, photopheresis received FDA approval for this indication. Additional disease states in which there has been laboratory and/or clinical work[3] include scleroderma, pemphigus vulgaris, rheumatoid arthritis, systemic lupus, multiple sclerosis and, most recently, solid organ and bone marrow transplantation. All of these disease states are in part potentially mediated by expanded populations of unregulated effector T cells.

In transplantation

Based on prior experimental work with 8-methoxypsoralen and ultraviolet A light treatment in rat autoimmune encephalitis[4] and murine lupus models[5], Perez *et al.* performed a study in which CBA/j mouse donor skin was grafted onto Balb/c mice to introduce histoincompatible tissue with disparity in the H2 locus[6]. Upon skin graft rejection the spleen from the transplanted mouse

was removed, and splenic lymphocytes were cultured and then treated with 8-methoxypsoralen and ultraviolet A light. These photoinactivated recipient splenic lymphocytes were then reinfused into a naive Balb/c mouse. These immunized Balb/c mice were tested for specific T cell immunoresponsiveness to CBA/j alloantigens through the use of mixed lymphocyte cultures and cytotoxicity assays. The ability to mount a delayed-type hypersensitivity reaction and reject a CBA/j skin allograft was also tested.

Compared to controls, Balb/c mice treated with the photoinactivated CBA/j spleen cells demonstrated decreased mixed lymphocyte culture proliferation and cytotoxic activity to CBA/j antigens. *In vivo* the treated mice had significantly longer survival of CBA/j skin allografts, yet retained the ability to respond to other third-party skin grafts. This series of experiments represented an important finding in that donor-specific immunosuppression, as opposed to pan-immunosuppression, was seen.

The effects of photopheresis on primate cardiac xenografting were studied by Pepino *et al.*[7]. Utilizing a heterotopic Cynomolgus-monkey-to-baboon model, a cyclosporin- and steroid-based regimen was used. In addition, the experimental group was started on prophylactic photopheresis beginning 3 days post-transplant, and then weekly thereafter. Similarly to the mouse skin graft model the photopheresis group had increased donor-specific immunosuppression, as evidenced by inhibited mixed lymphocyte culture responses compared to controls, and decreased formation of lymphocytotoxic antibodies to the donor, with prolonged xenograft survival. In addition, resolution of an acute rejection episode was seen in one animal following photopheresis therapy, without the need for augmented conventional immunosuppression.

Based on the experimental murine and primate work, and the clinical experience and safety seen in cutaneous T cell lymphoma, our group initiated human cardiac transplantation trials. High-risk patients with elevated levels of non-donor-specific anti-HLA antibody were treated with adjuvant photopheresis. Three of the four patients were multiparous women and two of the four patients were retransplants. Oral 8-methoxypsoralen and the Therakos UVAR system (Therakos, Inc., West Chester, Pennsylvania, USA) were used in a treatment schedule of 2 days sequentially every 3–4 weeks for the first postoperative year, and every 6–8 weeks during year 2. While there was no control group, and this was a purely observational study[8], Rose *et al.* noted an early decrease in non-donor-specific anti-HLA antibody levels and a relatively low incidence of rejection.

In a study by Costanzo-Nordin *et al.*, a regimen of one photopheresis treatment was compared to 3 days of high-dose steroids in the treatment of patients with hemodynamically stable cardiac rejection[9]. Although both the success and time to complete resolution of the episode were inferior to corticosteroids, photopheresis alone was capable of reversing acute cellular rejection in the majority of patients. Of additional importance, the photopheresis treatment group had a trend toward fewer post-rejection infections.

Atherosclerosis in cardiac allografts may be a manifestation of chronic vascular/humoral rejection. Prior work has shown an association between the production of non-donor-specific panel-reactive anti-HLA antibody and the development of transplant atherosclerosis[10]. Based on these observations, and the prior animal and human work, a phase II pilot clinical study[11], was performed by Barr *et al.* to determine whether the addition of monthly photopheresis to standard triple-drug therapy with cyclosporin, azathioprine and corticosteroids is safe, and results in lower levels of panel-reactive antibodies and transplant atherosclerosis.

Photopheresis was begun within 1 month of heart transplantation and was performed on 2 successive days every 4 weeks. Oral 8-methoxypsoralen and the Therakos UVAR photopheresis system were used in this study. Patients were randomized to either adjunctive photopheresis or standard triple-drug therapy only. Mean follow-up was 1.5 years. Both groups were comparable in the following demographics: age, sex, race, pretransplant heart disease, graft ischemic time, HLA mismatch, and donor– recipient CMV status. During the follow-up period cyclosporin level, azathioprine dose, and cumulative steroid doses were similar between the two groups. No differences in average white blood cell count or cholesterol level were seen. There was no difference in infection rates. Rejection incidence and grades of rejections were also not significantly different.

Non-donor-specific panel-reactive anti-HLA antibody levels were significantly reduced in the photopheresis group compared to the control group by postoperative month 3–4 ($p<0.03$), and remained significantly lower through postoperative month 6 ($p<0.05$). Of patients reaching at least 1 year of follow-up, 20% in the photopheresis group had transplant atherosclerosis on coronary angiography (defined as any abnormality seen) compared with 36% in the control group (p=n.s.). If the patient did not have obvious transplant atherosclerosis at the time of the angiogram, intravascular ultrasound was performed to determine coronary artery intimal thickness. Intimal thickness at 1 year was 0.23 mm ($\pm$0.09) in the photopheresis group vs 0.49 mm ($\pm$0.20) in the control group ($p<0.04$). At 2-year follow-up intimal thickness was 0.28 ($\pm$0.08) in the photopheresis group vs 0.46 mm ($\pm$0.07) in the control group ($p<0.02$). There was no difference at 2 years in the incidence of death in the two groups – 20% in the photopheresis group vs 23% in the control group. Photopheresis was safe, was well tolerated, and did not increase the morbidity of triple-drug-based immunosuppression in cardiac transplant patients. Treated patients showed an early reduction in non-donor-specific panel-reactive anti-HLA antibody levels. Of greater clinical importance was the finding of a significantly decreased coronary artery intimal thickness at up to 2 years of follow-up, this being the first immunotherapy that has resulted in this finding in humans.

In a subsequent study by Meiser *et al.*[12], utilizing an earlier and more frequent treatment schedule, and using liquid 8-methoxypsoralen added directly to the extracorporeal buffy coat, rejection and infection rates were reduced compared to the control group. This study, as well as prior work by Knobler *et al.*[13], confirmed the highly unpredictable nature of oral 8-methoxypsoralen absorption and the resulting blood level, and demonstrated that the extracorporeal addition of the liquid form resulted in reliable levels in the buffy coat. Based on these prior studies an international, multicenter, randomized clinical trial utilizing liquid 8-methoxypsoralen and the Therakos UVAR system is in progress, to investigate the impact of prophylactic therapy on the incidence of acute cellular rejection and infection in cardiac transplant patients.

POTENTIAL MECHANISMS OF ACTION

There are various theories regarding the potential mechanisms of photopheresis including: (a) generation of CD8-positive clonotypic T cells, (b) increased expression and/or recognition of immunogenic peptides in Class I HLA clefts, (c) inhibition of second signal transmission from antigen-presenting cells, and (d) production of cytokines by irradiated leukocytes. Characterization of the host response will be needed to define the mechanism of action, and may lead to new drug development in the future. Further research will need to establish the optimal/minimal treatment frequency and duration, and potential synergy with other new immunoregulatory agents.

COMMENT

New transplant studies currently under way involve non-cardiac solid-organ recipients, as well as patients with graft-versus-host disease following bone marrow transplantation. This technology may be particularly important as adjuvant prophylaxis in the areas of pulmonary and small bowel transplantation, in which current clinical results with conventional immunosuppressive agents have been disappointing. Increasing anecdotal clinical experience with photopheresis for resistant rejection in cardiac, pulmonary, and renal transplant patients has led to the development of 'rescue' protocols. Dall'Amico *et al.*[14] most recently reported their experience with adjuvant photopheresis in patients with recurrent cardiac rejection and showed a decrease in the number and severity of rejection episodes, thus allowing a subsequent reduction in the doses of maintenance immunosuppressive agents required in this problematic group.

Currently, FDA monitored, open-label protocols for refractory rejection in heart and lung transplant recipients are in early phases. These current studies and future experimental and clinical trials will help to define the role of this novel, safe and non-toxic, immunomodulatory technology in the field of transplantation.

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Gene Transfer

A. ARDEHALI, H. LAKS AND A. FYFE

INTRODUCTION

Major advances in recombinant DNA technology have remarkably improved our understanding of gene expression, and heralded new therapies for inherited and acquired diseases. This progress is especially notable considering that the structural unit of the human genome was discovered only 50 years ago. Recent studies have successfully introduced foreign DNA sequences into the vessel wall of multiple experimental animals, using a variety of vectors[1-7]. These studies have stimulated hope that this technology may be used to revolutionize the practice of cardiovascular medicine. Instead of using empiric observations to form the basis of medical therapy, insight into the genetic mechanisms of disease can allow a directed approach to medical therapy. Extension of gene transfer technology to the field of solid-organ transplantation is in its infancy; however, it holds great promise in the future.

This chapter will provide an overview of the important milestones in the development of recombinant technology, review the principles and methods of gene transfer, and discuss the applications of this technology to the field of transplantation.

HISTORICAL PERSPECTIVE

In 1944 Avery *et al.* clearly established that deoxyribonucleic acid was the genetic material in bacterial cells, and was responsible for transmission of hereditary information[8]. Chargaff *et al.* subsequently demonstrated that the numbers of adenine and thymine residues of a DNA sequence were equal, as were the numbers of guanine and cytosine residues[9]. This important observation, as well as other reports, laid the groundwork for the double helical model of DNA which was proposed by Watson and Crick in 1953[10]. Isolation and purification of enzymes responsible for synthesis of DNA and RNA (DNA and RNA polymerases), identification of mRNA as the intermediate molecule between the DNA sequence and the protein product, and discovery of three nucleic acid (codon) as the genetic code for a single amino acid[11,12] were important milestones in the evolution of molecular biology. Discovery of restriction endonucleases[13], the ability to separate, purify, sequence, and then join segments of DNA, led to the development of recombinant DNA technology.

Introduction of foreign genes into mammalian cells did not occur until the 1980s, due to methodological limitations. In the past decade gene transfer into the germ line of experimental animals (transgenic animals) has resulted in the development of models for studying human diseases. Furthermore, the clinical applications of gene transfer technology are slowly being realized. The first gene therapy trial was undertaken at the NIH in 1990, and was designed to introduce the adenosine deaminase gene into bone marrow cells of patients with severe combined immunodeficiency syndrome[14]. To date, in excess of 100 gene transfer protocols have been approved by the Food and Drug Administration and the Recombinant DNA Advisory Committee of the NIH.

GENE TRANSFER TECHNOLOGY

Somatic gene transfer is the introduction of exogenous DNA sequences into host cells. If gene transfer is limited to somatic cells the altered genome is not passed to the offspring. Although the principles of gene transfer are elegantly straightforward in concept, the applications have been limited, due to technical difficulties. Several conditions must be fulfilled in order to effectively transfer a gene of interest into a host: (a) the gene of interest must be cloned, (b) a vector capable of delivering the gene to the target cells must be available, (c) the gene must enter the nucleus intact, (d) the gene must be expressed in the target tissue, and (e) the protein product of the recombinant gene should be produced at a biologically appropriate level to render its activity.

Somatic gene transfer can be utilized for two purposes: (a) as an investigative tool to study gene function, or (b) as a therapeutic tool to treat inherited or acquired disorders. The principles of gene transfer technology have proved invaluable in studying the functions of single genes. An example is the study of individual growth factors in the development of atherosclerosis. The role of growth factors such as platelet-derived growth factor B, acidic fibroblast growth factor-1, and transforming growth factor β_1

(TGF-β_1) in proliferation of smooth muscle cells and progression of atherosclerosis has been difficult to characterize *in vivo*. Nabel *et al.* have utilized plasmid expression vectors containing the cDNA of each of the growth factors to transfect porcine iliofemoral arteries using cationic liposomes[15–17]. They were able to identify the effect of individual growth factors *in vivo*. The investigative potential of gene transfer technology in transplantation, where individual gene products can be studied *in vivo*, appears very promising and valuable. For a more detailed discussion the reader is referred to several recent reports that have reviewed the general principles of experimental gene transfer[18–20].

The approaches for gene transfer may be either direct or indirect. In cell-mediated or indirect gene transfer, the target cells are harvested from the host, transfected *in vitro*, and then returned to the host. This procedure requires a syngeneic cell line in culture, and is associated with a time delay between harvest and reimplantation. An example includes harvest of endothelial cells or smooth muscle cells from an artery or vein, growth in culture, transfection with the gene of interest, selection of cells expressing the gene, and reimplantation of the genetically modified cells on denuded arteries[21]. Direct gene transfer refers to the introduction of a foreign gene directly into the tissue. Access to organs during harvest provides a unique opportunity for direct transfer of genes of interest prior to implantation.

Methods of gene transfer

The methods for introduction of foreign genes into host cells can be divided into three general classes (Table 1). Physical methods of gene transfer include electroporation and microinjection. The electroporation technique delivers a rapid pulse of high-voltage current causing cellular membrane disruption, allowing entry of DNA[22]. This method is limited only to experimental use. Microinjection refers to direct injection of a DNA sequence into target cells under the microscope, and is not practical in gene transfer to a large number of cells[23]. Chemical techniques for gene transfer include cationic liposomes, calcium phosphate, and ligand–DNA conjugates. Liposome-mediated gene transfer is a safe non-viral method of gene transfer which can be administered repeatedly. The cationic liposome components form a lipid particle which interacts spontaneously with the nucleotide[24]. This cationic liposome–DNA complex fuses with the cell membrane by receptor-mediated endocytosis, is degraded in the lysosomes, and releases some of the DNA–lipid complex in the cytoplasm, which is subsequently translocated to the nucleus. The ability to target a specific cell type *in vivo* by this method is virtually impossible. Calcium phosphate transfection involves co-precipitation of DNA with calcium phosphate, and exposure of target cells to this complex[25]. The co-precipitate is taken up into phagocytic vesicles where some enter the nucleus and become integrated into the host genome, yielding stable expression. The disadvantages of this technique include low transfection efficiency and limited targeting.

Another chemical method of gene transfer is receptor-mediated ligand–DNA conjugates, where specific ligands are conjugated to a polylysine moeity/DNA complex[26]. The complex enters the cell via ligand-receptor-mediated endocytosis and escapes the lysosomal system, and some of the nucleotide is released into the cytoplasm, which is then translocated to the nucleus. An improvement in the efficiency of this technique has been the addition of a replication-defective, chemically inactivated adenovirus which is linked to the DNA complex[27]. Once the complex is internalized into a lysosome the adenoviral coat proteins disrupt the lysosome and release a greater number of DNA molecules into the cytoplasm of the target cell. This method also leads to formation of nucleotide episome, yielding transient expression.

Viruses are ideal naturally occurring vectors for gene delivery to mammalian cells. They bind to a specific surface receptor, enter the cytoplasm and then the nucleus, and utilize the host machinery for expression and replication. Several viruses have been studied for gene transfer; however, we will focus on the two most commonly used viral vectors – retroviruses and adenoviruses.

Table 1 Methods of gene transfer

1.	*Physical*	
	(a)	Electroporation
	(b)	Microinjection
2.	*Chemical*	
	(a)	Liposomes
	(b)	Calcium phosphate
	(c)	Ligand–DNA conjugates
3.	*Viruses*	
	(a)	Retrovirus
	(b)	Adenovirus
	(c)	Others (adeno-associated, herpes, polio, vaccinia)

Retroviral vectors

Retroviruses are RNA viruses that replicate through a DNA intermediate. To construct a retroviral vector the structural and replicative genes (*gag*, *pol*, and *env*) of the virus are replaced by the gene of interest under the retroviral promoter sequence[28,29]. The retroviral vector is thus rendered replication-deficient. This vector is propagated in cell lines in which the 'missing' genes are present (packaging cell lines). Retroviral vectors stably integrate into the host genome and result in stable expression of recombinant gene. Since integration occurs at random sites the potential for insertional mutagenesis remains a concern. Integration of retroviral vectors requires division of the host cells, making this vector unsuitable for organs with a low frequency of division, such as the myocardium[30]. Other limitations of this vector include its low efficiency of gene transfer *in vivo* and its relative susceptibility to degradation by the host complement system[31].

Adenoviral vectors

The adenovirus genome is a 36 kb linear double-stranded DNA[32,33]. Deletion of the early-1 (E1) region, which encodes for a transactivator of viral gene expression, yields a replication-incompetent virus. These vectors are propagated in cell lines that contain a copy of the adenoviral E1 gene. The gene of interest usually replaces the E1 gene through homologous recombination. Replication-defective adenoviral vectors are especially attractive vehicles for gene transfer due to their high efficiency of infection. The adenoviral genome does not usually integrate into the host genome, and is maintained in an extra-chromosomal state. This

vector can be efficiently used for the *in vitro* and *in vivo* transduction of non-dividing cells.

Despite these advantages the expression of DNA transferred by adenoviral vectors is transient, generally lasting less than 3 weeks. It has been observed that generation of cytolytic T cells stimulated by adenoviral antigens may destroy the virally infected cells[34]. Generation of an immune response against virally transduced cells eliminates the possibility of repeated transduction using adenoviral vectors. There is also potential concern that recombination with endogenous adenoviruses may lead to the release of a novel wild-type virus.

APPLICATIONS OF GENE TRANSFER TO ALLOTRANSPLANTATION

The harvest of a solid organ provides a unique opportunity for direct genetic modification of the allograft at the time of implantation. Direct access to an organ allows efficient, yet targeted, delivery of a gene of interest. The application of gene transfer technology to intrathoracic organ transplantation is in its early stages. Early studies have addressed the issues of feasibility and safety of gene transfer to allografts. Wang *et al.* studied the expression of a reporter gene following direct apical injection into rat cardiac isografts[35], and observed focal and transient reporter gene expression.

Our group has been investigating the expression of a reporter gene (firefly luciferase) in a heterotopic mouse isograft model using cationic liposomes[36,37]. In an effort to reproduce the clinical scenario, donor hearts were arrested with cold University of Wisconsin (UW) solution and then perfused in antegrade fashion via the aortic root with a solution containing the reporter plasmid. The donor hearts were stored in cold UW solution for approximately 45 minutes to 1 hour, and were then implanted. After 4 days the donor hearts were removed and studied. There was no histological evidence of acute cellular rejection. Reporter gene expression was present in perivascular areas surrounding coronary arteries and veins, coronary capillaries, and the endocardia of both ventricles (Figure 1). Expression of reporter gene was sparse or focal when the plasmid solution was injected into the right atrium or directly into the cardiac apex, respectively. It was concluded that administration of plasmid solution antegradely via the aortic root localized the reporter gene expression to the endocardium and coronary vasculature, both sites of immunologic interaction between the donor organ and host blood-borne cells. This study demonstrated that direct gene transfer into donor hearts under conditions mimicking the clinical transplant setting is technically feasible, and appears safe.

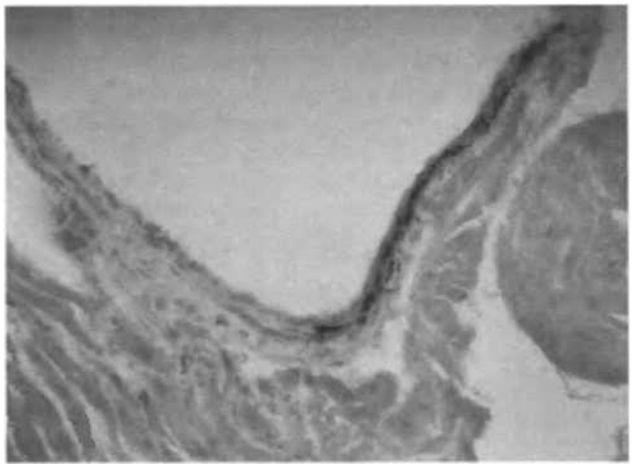

Figure 1 Cationic liposome-mediated reporter gene transfer in a mouse isograft model via the aortic root yielded reporter gene expression on the endocardium of both ventricles

We have also used this mouse isograft model to study the modification of immune responses by transfer of the human HLA-B7 gene[38]. The cDNA of human HLA-B7 was introduced into donor hearts during harvest via cationic liposomes, and the donor hearts were removed at 7–10 days for immunohistochemical studies. The donor hearts expressed human HLA-B7 sparsely throughout the myocardium, and the expression of this protein was associated with mononuclear/lymphocytic cellular infiltration. Thus, the transfer of human HLA-B7 cDNA during hypothermic preservation was followed by expression of protein and the relevant immunological response. These preliminary studies will serve as the groundwork for future studies on the investigative and therapeutic potential of gene transfer in transplantation of thoracic organs.

Experimental studies have also shown that efficient *ex vivo* gene transfer to liver allografts can be achieved under conditions mimicking the clinical scenario, such as hypothermic organ preservation. In liver transplantation, retroviral vectors have been used successfully for *ex vivo* reporter gene transfer, but with low efficiency[39]. Adenoviruses, as previously noted, are more efficient viral vectors and can be used to transduce non-dividing cells. Adenovirus-mediated gene transfer to isolated hepatocytes and to liver grafts in rats by portal vein perfusion has proven efficient and feasible[40,41].

The potential applications of gene transfer to the field of allograft transplantation can be classified under two broad categories:

(1) Modification of self/non-self identity or alteration of allograft phenotype

Ideally, an allograft may be engineered where the donor major and minor histocompatibility antigen genes are suppressed and the recipient histocompatibility antigen genes are transferred and expressed. The allograft is thus considered self; alloreactivity is abrogated and immunosuppression is not required. At the present state of technology, inhibition of constitutive or inducible gene expression is in a state of infancy. Antisense oligonucleotide technology uses complementary mRNA sequences to bind to the mRNA of interest and prevent its translation[42]. Antisense oligonucleotides have proven effective in experimental studies to inhibit inducible gene expression (smooth muscle cell proliferation in response to injury)[43]. Suppression of histocompatibility gene expression by antisense oligonucleotides may be accomplished by blocking translation of common elements of MHC antigens such as β_2 microglobulin. Experimental suppression of MHC antigen gene expression by antisense oligonucleotides has yet to be accomplished. Another approach to inhibit the transcription of a gene is to synthesize an oligonucleotide sequence that binds to a DNA sequence of interest, creating a DNA triplex, thus preventing transcription[44].

'Molecular chimerism' is another potential application of gene transfer technology to modulate the host 'self/non-self identity'. This method induces specific tolerance to MHC antigens on the donor organ by introducing the allogeneic MHC cDNA into autologous bone marrow cells, followed by transplantation of the engineered marrow. The transplanted marrow cells recognize the allograft MHC antigens as self, and prolonged specific tolerance can ensue. Prolonged unresponsiveness to skin grafts from donor mice whose class I MHC antigen gene has been transferred into recipient bone marrow cells has been reported[45]. Although appealing in concept, the application of 'molecular chimerism' technology to vascularized organ transplantation remains to be established.

(2) Modulation of the alloreactive immune response

Systemic immunosuppression allows tolerance of an allograft at the expense of increased susceptibility of the host to bacterial, fungal, parasitic, and viral infections, as well as to some malignancies. Transfer and expression of immunologically relevant molecules to the allograft can impede immune activation, while avoiding the toxicities of systemic immunosuppression. Among the immunologically important molecules, the immunosuppressive cytokines are potential candidates due to their low level and transient nature of expression. The presence of these immunosuppressive cytokines in the allograft could interfere with the early stages of antigen recognition, and potentially modulate an alloreactive immune response. An important and welcome feature of this local immunosuppressive phenomenon is a lack of systemic side effects.

TGF-β_1 is a regulatory molecule involved in wound healing, fibrosis, immunoregulation, and immunosuppression[46]. Interleukin-10 has also been reported to impair a variety of alloreactive immune functions[47]. Recently, Qin *et al.* have utilized these molecules to modulate alloimmunity[48,49]. They have demonstrated that various viral vectors can be used to transfer immunologically important genes to the hearts of neonatal mice prior to transplantation in a non-vascularized heterotopic model. This study further documented the expression of protein TGF-β_1 and vTGF-β_1, with prolongation of graft survival due to the local immunomodulatory effects of these agents. Delivery and expression of immunosuppressive cytokines by gene transfer to solid allografts to generate local immunosuppression are an area which warrants further investigation.

APPLICATIONS OF GENE TRANSFER TO XENOTRANSPLANTATION

Donor organ availability remains the major obstacle to the growth of solid-organ transplantation. Xenotransplantation is one potential solution to the scarcity of donor organs. Among animal species, pigs share a number of anatomical and physiological characteristics with humans, and appear socially and ethically acceptable as a source of xenografts. Hyperacute rejection is the major obstacle to xenotransplantation. It is characterized by activation of complement factors and histopathological stigma of organ damage. Strategies to control hyperacute rejection include genetic modification of the donor, or modification of the host, which may include removal of natural antibodies, systemic inhibition of complement factors, or injection of α-galactosyl sugars to block xenoantibodies[50]. The previously cited strategies for alteration of graft phenotype and modulation of alloreactive immune response may similarly be employed in xenotransplantation.

Access to donor species in xenotransplantation has broadened the scope of genetic manipulations for transplantation. Application of gene transfer technology to develop a transgenic pig which expresses the regulators of human complement system or 'knock-out' pigs deficient in the target of human natural antibodies (α-1,3-galactosyl) has generated much enthusiasm and optimism in xenotransplantation. Transgenic pigs have been bred that express human-specific decay accelerating factor (hDAF), which prevents the assembly of C3 and C5 convertase, and accelerates the decay of C3 convertase[51]. The organs from such transgenic animals produce hDAF at levels that are comparable to those found in human tissue[52]. Under experimental conditions, endothelial cells expressing hDAF are protected from lysis by the human complement system[53]. Histologic examination of skin from transgenic pigs has shown that hDAF is expressed on endothelial cells and smooth muscle cells[54]. The development of this transgenic pig model has stimulated interest in the creation of transgenic strains displaying other human antigens.

COMMENT

The application of gene-based therapies in transplantation will provide important insights into fundamental mechanisms of acute and chronic rejection. Study of individual genes under controlled settings *in vivo* may unveil their roles in transplantation immunology. Gene-based therapies may allow development of genetically engineered allografts which may escape host immune surveillance mechanisms, or may produce intragraft immunoregulatory molecules creating local immunosuppression. Extension of gene transfer technology to xenotransplantation may also bring this area of ongoing research a step closer to clinical reality.

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Advances in Heart Storage

W.N. WICOMB, V.F. PORTNOY AND G.M. COLLINS

INTRODUCTION

In this chapter we have chosen to focus our attention on some of the recent developments in heart preservation (both research and clinical) that we believe require additional investigation, and thus we hope to reveal new insights into the broad field of hypothermic myocardial protection. We will also discuss various interventions that influence the return of normal myocardial function at the end of the period of hypothermic heart storage. There are many unanswered questions that will continue to challenge basic research workers, including the notion that a universal preservation solution is possible.

The human myocardium is relatively resilient to ischemic injury and this has permitted it to endure both warm ischemic arrest and the adverse effects of many cardioplegic solutions. With prolonged ischemia, however, some tissue damage is inevitable, and is often exacerbated by the inotropic agents used to maintain hemodynamic stability, or masked by routine management procedures in both the donor and the recipient.

In the research laboratory, most innovative ideas and interventions are based on experimental data. However, these data have limitations because many studies are conducted in the absence of brain death (which also influences myocardial stability). Moreover, innate differences in ischemic tolerance among animal species also skew the experimental findings. Thus, the animal data may be pertinent in that setting, yet not necessarily clinically applicable.

The injury sustained by the donor heart occurs soon after the onset of brain death[1]. This results in an unstable hemodynamic response, often requiring immediate management. Management procedures do not always guarantee donor stability and may even have adverse effects. These include volume overload and the continuous use of high doses of inotropic agents. Additional myocardial damage may occur during cardioplegic arrest and preservation in ice. During this quiescent state of the heart, the injury often goes unnoticed, until the advent of reperfusion. The latter itself can promote injury when an ischemic organ encounters deleterious substances in the blood[2].

HEART PRETREATMENT

There are many studies that show the beneficial effect of proper donor management[3,4]. During these procedures the aim is to optimize the conditions of the circulatory system to provide a suitable environment for recovery of the heart. This requires that abnormalities of the donor blood pressure, pH, electrolytes, blood volume and body temperature be corrected. Pretreatment regimens focus on the administration of pharmacological agents to the donor in an attempt to increase myocardial tolerance to ischemia[5]. The list of agents available for heart pretreatment is extensive, based on their efficacy in laboratory animals. Some pretreatment protocols include the intravenous administration of glucose[6,7], lidocaine[8], nicorandil[9], propranolol[10,11], chlorpromazine[12], verapamil[13,14], nicardipine[15], dipyridamole[16], prostacyclin[17], prostaglandin[18], adenosine[19], methylprednisolone[20], and halothane inhalation[21].

More recent developments showed that the administration of halothane anesthesia improved coronary flow and contractility[22]. Halothane inhalation decreased isoproterenol-mediated norepinephrine release by inhibiting β-adrenergic receptors[23], and demonstrated an improvement in the force–frequency relationship in the failing human heart[24]. Although the intended use is to achieve protection from Ca^{2+} overload, this benefit may be overridden by the inherent vasodilatory property of halothane, resulting in hypotension. Heart pretreatment methods therefore remain an auxiliary approach to heart preservation, requiring further investigation.

Triiodothyronine (T_3) therapy

A current controversy in donor management involves whether to administer triiodothyronine as first described by Novitzky *et al.*[25]. In earlier studies these authors showed that T_3 simultaneously promoted the influx of Ca^{2+} and the efflux of potassium in tissue slices. Thus, the inotropic effect of T_3 may be due to thyroxine-induced calcium influx, and extended intravenous use of T_3 may be detrimental[26] because of possible intracellular accumulation of

Ca^{2+}. Orlowski *et al.* administered T_4 and demonstrated increased graft survival of donated hearts[27]. In a controlled study, Randell *et al.* showed deteriorating metabolic acidosis after T_4 administration; the hemodynamic response did not improve when compared to an untreated control group[28]. In two separate studies, Macoviak *et al.* and Gifford *et al.* determined serum T_3 levels in cadaver donors and observed acute hypothyroidism, but found no other association with donor hemodynamic instability[29,30].

Novitzky *et al.* specifically studied reverse T_3 because they found T_4 therapy to be ineffective[31]. In order to validate the efficacy of T_4, future studies should attempt to demonstrate the presence of an active thyroid-converting enzyme to show that T_4 is converted into T_3 in the brain-dead donor. Finally, thyroid hormone may improve hemodynamic stability at the expense of diminishing myocardial energy reserves promoted by the influx of Ca^{2+}. Future studies should be directed at controlling T_3 levels to avoid additional myocardial injury during brain death. Bittner *et al.* demonstrated an unchanged donor heart failure rate of 9–10% among various centers, despite the use of many pretreatment regimes[32]. Wheeldon *et al.*, in their survey pertaining to donor heart pretreatment protocols, noted no significant difference in the post-transplant mortality rate after 30 days of follow-up[33]. Although encouraging, the available information remains speculative, with some proponents advocating the use of hormone replacement therapy and others finding it to be of little or no benefit.

HEART RESUSCITATION

In 1976 Cooper outlined what was then known about resuscitation of the donor heart[34]. Recently, the increasing demand for donor organs has encouraged some investigators to explore more risky protocols, such as transplantation of hitherto unacceptable donor hearts, including the salvaging of non-beating hearts[35,36]. We have shown that only a 10-minute period of normothermic arrest following exsanguination resulted in poor return of rabbit heart function[37]. Illes *et al.* also exsanguinated rabbits after 10 minutes of hypotension and followed this with 90 minutes of isolated heart storage. Three different solutions were evaluated in heart preparations using the assessment technique of intraventricular balloon pressure monitoring: (a) modified St Thomas' Hospital (St Thomas'), (b) University of Wisconsin (UW) and (c) blood cardioplegia[38]. Hearts arrested using blood cardioplegia performed well, although the adenine nucleotide concentrations were significantly below control levels. The authors attributed the good function to effective buffering and oxygen free radical scavenging. In studies performed by Ferrera *et al.*[39] in the pig, 10 minutes of delay before retrieval resulted in reanimation being unsuccessful. Despite these findings the reader is reminded that variations in species can result in different ischemic tolerances in the myocardium. Studies also indicate that the immature heart is more resistant to ischemia[40,41].

Some resuscitation data, however, are encouraging, especially the work of Shirakura *et al.*, who resuscitated and successfully preserved dog hearts for 24 hours[42,43]. Gundry *et al.* resuscitated pulseless asystolic donor juvenile lamb hearts after 30 minutes of warm asystolic death[44]. In a clinical trial that followed, these investigators ventured into the salvaging of infant hearts using cardiopulmonary resuscitation. Myocardial function fared well after orthotopic transplantation, and only modest increases in serum troponin levels were noted[45]. These findings may once again reflect the human heart's resilience to injury. Although resuscitation of dead hearts will increase the donor pool, the feasibility of such protocols on a large scale remains speculative unless a reliable viability assay becomes available.

The unpredictable influence of the agonal period on donor heart function following reimplantation remains poorly understood. Other factors influencing injury include: (a) perfusate composition, (b) length of the preservation period and (c) reperfusion effects. Post-ischemic dysfunction manifested by poor myocardial performance is indicative of either reversible global ischemia (stunned myocardium) or irreversible ischemia (cellular necrosis or myocardial infarction)[46]. We propose that these mechanisms are parts of a continuum which is primarily driven by the diminished free radical protection evident following ischemia.

CARDIOPLEGIA

In the early 1970s successful application of Bretschneider's and Collins flush solutions in experimental heart transplantation was achieved, demonstrating their superiority over hypothermic immersion preservation[47,48]. In the 1980s hypothermic potassium cardioplegia became the most common technique for myocardial protection during clinical cardiac transplantation[49]. The combination of K^+ arrest and cooling reduces the metabolic rate, extending the interval of myocardial tolerance to ischemia[50]. Although many centers express satisfaction with their existing method of cardioplegia, numerous studies are ongoing in search of the optimum K^+ concentration[51]. The fundamental aim is to obtain consistently successful myocardial preservation.

Methods currently used for routine cardiac surgical procedures are usually considered safe, but occasionally spurious episodes of deteriorating myocardial performance are encountered[52]. Various factors are responsible for the altered functional state of the myocardium; these include: (a) potassium concentration, (b) temperature, (c) solution additives, and (d) length of ischemic interval. In addition to these factors we have demonstrated that the solution composition alone can adversely influence myocardial function immediately after cardioplegic flushing. We compared the function of hearts flushed with different solutions with that of freshly removed unflushed hearts (Table 1). All the unstored hearts tested immediately after flushing differed in cardiac performance. These results are, however, in conflict with earlier data reported by Hearse *et al.*, who demonstrated no loss of function in

Table 1 Functional evaluation* of unflushed (control) and flushed rabbit hearts not subjected to a period of storage

Group	*Cardiac output* ($ml\ min^{-1}\ g^{-1}$)†	*n*
Unflushed (control)	66.3 (3.2)	10
UW	54 (1.93)	6
Cardiosol II (20L)	71 (5.3)	6
St Thomas'	41 (2.8)	6

* Hearts perfused *in vitro* on a Langendorff system with a crystalloid solution.
† Mean (± standard error)

the rat heart after flushing with the St Thomas' solution following 30 minutes of hypothermic ischemia[53]. Their stable aortic output data can be attributed to the better tolerance of the rat myocardium to the St Thomas' solution. By contrast, in our rabbit heart studies, the macroscopic appearance of hearts flushed with St Thomas' solution showed a significant reduction in overall size, including a decrease in the volume of the ventricular cavity. Upon reperfusion, the stroke volume in the working heart was reduced, and we attribute this change to the absence of oxygen free radical protection, and possibly the high calcium concentration of the St Thomas' solution.

Hypothermia reduces the tissues' metabolic rate by >99%[54]. Immediately after cardioplegic flushing with no intervening period of storage, a return of >99% of the functional response might be expected. However, in our own studies this ideal was achieved only with the new flush solution containing polyethylene glycol (Cardiosol, PEG) (Table 1). We believe that the reduced performance in some groups (Table 1) is due to the absence or limited availability of oxygen free radical protection, eventually leading to calcium overload in the cold cardioplegically flushed myocardium. There was no significant difference between the St Thomas' and UW groups when comparing the absolute cardiac output values. This was due to the absence of colloidal material in the St Thomas' solution, resulting in the formation of more extensive tissue edema. The unflushed control hearts underwent immediate transfer from the animal to the Langendorff apparatus, to avoid the development of progressive ischemia and impaired function.

Formulation of an optimum cardioplegic solution requires attention to many factors including: (a) the combination of constituent chemicals and pH; (b) the solution infusion pressure; (c) direction of flow (whether antegrade, retrograde or antegrade–retrograde); (d) temperature (whether warm or cold); (e) volume; (f) whether single-dose, multidose, or continuous perfusion; and (g) intermittent warm or intermittent cold cardioplegia[55]. Further potential improvement derives from the use of pharmacological and metabolic additives. These have included: (a) oxygen free radical scavengers[56–58], (b) Krebs' cycle substrate enhancement[59–61], (c) high-energy phosphates[62–64], and (d) calcium antagonists[65], and many other agents[51]. We believe that antioxidants have a particularly important role during the period of preservation, and postulate that this subject is the central determinant of the outcome of a period of heart preservation (Figure 1). Obviously, warm blood cardioplegia most closely mimics life. If damage ensues during its use, it must result from technical error, hemodilution or unphysiological conditions such as temperature changes, contact of the blood with foreign surfaces, or altered perfusion conditions.

The use of crystalloid cardioplegic solutions introduces many physiological unknowns, including lack of essential agents (such as antioxidants) found in blood, and the potentially harmful effects of components of the artificial solution. On the other hand, there is no evidence in the extensive literature on organ preservation to show that blood is a good solution in which to preserve organs under non-perfused hypothermic conditions. Under these circumstances, cell free solutions containing impermeant anions and appropriate cation content and buffering, such as UW or Collins solutions, are the most effective. Since conventional cardioplegic solutions were not constructed with these considerations in mind, but rather were designed to quickly arrest the heart, it is not surprising how ineffective they are for protecting the heart during more than transient periods of storage. We therefore concur with the trend towards diminished clinical application of hypothermic crystalloid cardioplegic solutions in open-heart sur-

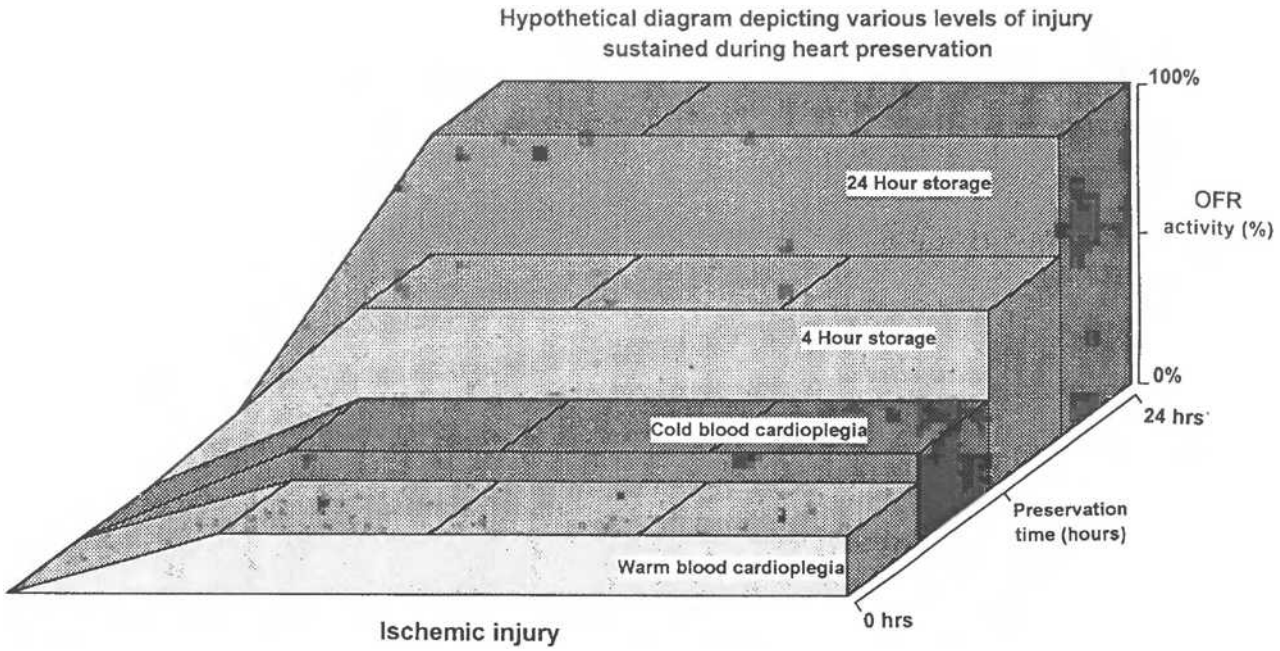

Figure 1 Diagram depicting hypothetical levels of injury sustained during heart preservation. The use of diluted blood and cold crystalloid preservation solutions for myocardial preservation results in increased production of harmful oxygen free radicals (OFR)

gical procedures within the USA[66]. However, it is possible that the situation would be quite different if one were to compare blood cardioplegia with the much more effective heart preservation solutions, such as UW or Cardiosol, which are designed for organ preservation and contain effective concentrations of antioxidants.

The use of hypothermia, although routine in organ preservation, has become a major point of contention in cardioplegia. Some investigators believe hypothermia to be responsible for eliciting rapid cooling contracture of the myocardium[67]. Firm hypercontracted hearts are associated with low cardiac output and increased mortality, resulting from calcium entry[68]. The evidence, however, is circumstantial, and dependent upon the type of solution used when the contracture occurs (Table 2). For example, in rabbit hearts we have observed the hypercontracted state after flushing with a large volume of St Thomas' solution (unpublished observation). The severity of contracture is attenuated by using less flush volume, resulting in decreased exposure time to calcium. Thus, solutions with lower calcium content permit the use of higher flush volumes[69].

PRESERVATION MODALITIES

The rationale for the use of ice storage is based on simplicity, since it requires only a single flush before placing the organ in ice. Recent developments in this field have included formulation of the University of Wisconsin solution which has become the standard for preservation of most solid organs. Initial studies suggested its suitability for heart and lung preservation in both ice storage and perfusion systems[70,71]. Recently, however, some of the enthusiasm for UW solution has waned after several deficiencies have been brought to light[72]. Viscosity was thought to jeopardize graft viability by reducing blood flow and encouraging retention of erythrocytes, leading to stasis[73,74]. Furthermore, the concentration of oxidized glutathione (GSSG) increases with solution aging by the process of autoxidation, promoting damage to the collagen network of the myocardium[75]. Fresh UW solution contains reduced glutathione (GSH) with protective properties, whereas aged (>2 weeks) solution contains predominantly GSSG[76]. Thus, the age differences in UW solutions may explain the functional discrepancies noted by various centers. (Note that the addition of fresh GSH to an already oxidized UW solution does not improve the quality of preservation.)

Precipitation is another inherent deficiency of this solution. The particulate matter consists of palmitic and stearic acid that is removable upon filtration, but the process is cumbersome, and sterility poses a further problem[77]. The preservation efficacy of the UW solution is also dependent upon storage temperature. Ambient temperatures (>18°C) result in poor myocardial performance and increased levels of GSSG (unpublished observation).

The major issue surrounding the use of UW solution in heart preservation pertains to its high potassium concentration. In 1955 Melrose *et al.*[78] noted that potassium citrate concentrations in excess of 550 mEq/l frequently caused depression of left ventricular function and led to multifocal myocardial necrosis. These high K^+ concentrations, however, raised false concerns regarding the K^+ concentrations of intracellular preservation solutions. It is true that the use of intracellular solutions promotes a transient episode of potassium–calcium vasospasm[79]. However, the spasm ceases soon after the organ temperature equilibrates with the hypothermic milieu of the flush solution. At that point the membrane pump activity stops[80]. Confusing terminologies have germinated from these observations, including vaguely defined phrases such as 'potassium burning' or 'irreversible endothelial damage'. These conclusions evolved during routine open-heart procedures in which surgical staff observed vein grafts constrict immediately after flushing with an intracellular-based solution (personal communication). Even the potassium load occasioned by the administration of 1 liter of these high-potassium solutions during routine open-heart surgery has not, in our experience, proved to be a problem, because the potassium concentration gradually decreased to acceptable levels by the end of the procedures (unpublished observations).

Recent advances in preservation technology include the development of a novel two-layer cavitary method in which an organ is kept suspended in an insoluble perfluorochemical for 24–48 hours[81]. Viability of these organs was tested by heterotopic transplantation in the abdomen of rats. The authors speculated that the beneficial feature was the oxygen delivery to the tissues by the perfluorochemical. Although passive diffusion may occur in small rodent hearts, the likelihood of oxygen penetrating the left ventricular wall of larger animals decreases as left ventricular wall thickness increases. This limitation renders the method impractical for large donor organs.

The authors propose that the benefit of increased oxygen tension during preservation may eventually prove to be something other than increased ATP production. (An alternative hypothesis is that hypothermia uncouples mitochondrial oxidative phosphorylation – the coupled process of oxygen consumption and ATP production.) The resulting combination of an oxygenated hypothermic environment and the hypothermic inhibition of superoxide dismutase (SOD) leads to increased oxygen free radical production and less ATP formation. Alternatively, oxygen may inhibit the anaerobic production of lactate and therefore diminish cellular acidosis, providing a more neutral environment for cellular survival. This is the conventional action of oxygen during normothermia, in which an abundance of oxygen inhibits the anaerobic glycolytic pathway, resulting in decreased lactate production.

Another recent development is the use of lazaroid compounds in preservation solutions. These compounds function both as calcium antagonists and as antioxidants, protecting tissues from lipid peroxidation[82]. In studies of these agents, systolic pressures were found to be uniformly less than in the control group and cardiac performance was not improved above the controls. Although lazaroid compounds have promising properties, their value cannot be compared with that of lactobionate as a basic component of organ preservation solutions[83–85].

The importance of a colloid in a flush solution will remain unanswered until such time that the disadvantages of edema formation are clarified. However, the physicochemical properties of colloids may provide some benefit from unexpected directions if, for example, they have the inherent capabilities of chelating Fe^{2+} or protecting the endothelium from lipid peroxidation.

Currently, these polymers are used predominantly for their colloidal properties and are otherwise considered chemically inert in preservation solutions. This notion may be erroneous, since col-

loids themselves are susceptible to oxidative change, which may affect their performance. Even small low-molecular-weight organic components, such as gluconate and lactobionate, undergo oxidative reactions.

Extracellular solutions (high Na^+)

'Extracellular' implies a high sodium level in the interstitial compartment. Hence, we term cardioplegic solutions with high (>100 mmol/l) sodium levels as 'extracellular'. Under hypothermic conditions the Na^+ K^+-ATPase electrogenic pump is inhibited and, as a result, the ionic distribution becomes reversed. An extracellular solution will maintain this reversed state of Na^+/K^+ ratio, with Na^+ predominantly entering the intracellular space[80]. Upon reperfusion, this disturbed gradient places a large load upon the pump, requiring expenditure of additional energy to correct the ionic disturbance. Energy reserves become depleted. The Na^+Ca^{2+} exchange mechanism is likely to participate during the realignment of the gradient. Here the high intracellular Na^+ is exchanged for extracellular Ca^{2+}, promoting further intracellular Ca^{2+} accumulation[86]. The detrimental effects of free Ca^{2+} in the tissues are well described[68].

The net effect of an increased intracellular concentration of calcium limits the duration of cold ischemic preservation. This divalent ion promotes the gradual development of a calcium-induced resting tension that ultimately impairs myocardial function. Extracellular formulations are mainly used in crystalloid and blood cardioplegia. Although these solutions are effective for use in most routine procedures, they fail to satisfy the demands of longer storage periods. During periods of extended preservation, intracellular formulations provide superior protection[87].

Intracellular solutions (high K^+)

'Intracellular' implies a high level of potassium inside of the cell. Hence, we term cardioplegic solutions with potassium concentrations above 100 mmol/l 'intracellular' (e.g. Collins, UW, Cardiosol) (Table 2). This term is occasionally loosely applied to solutions containing a low sodium concentration, without taking into account the potassium level (e.g. Bretschneider's and Stanford solutions)[51,88,89].

Intracellular solutions are largely used in the preservation of solid organs (kidney and liver)[90]. In the case of heart preservation the transition from extracellular to intracellular formulation is still in its infancy. There remains significant skepticism regarding the suitability of intracellular solutions for heart preservation because of the K^+-induced (Ca^{2+} influx) vasoconstriction (see Preservation Modalities). Despite these concerns, the application of intracellular solutions in transplantation procedures is increasing[91,92].

The benefit of an intracellular formulation lies in the maintenance of the cellular ionic gradient. Under normal circumstances the ionic gradient of the myocyte is maintained by the electrogenic pump, keeping the potassium within the intracellular compartment. Alteration of this gradient promotes the wasteful expenditure of energy required to re-establish the gradient. On the downside, however, intracellular formulations activate calcium influx, resulting in vasoconstriction during the induction of cardioplegic arrest. Furthermore, the addition of calcium to these so-

Table 2 Composition of preservation solutions (mmol/l)

Components	*SU_2*	*STH_2*	*HTK_4*	*Collins*	*UW*	*Cardiosol I*	*Cardiosol II*
Potassium	17	16	10	115	125	125	125
Sodium	14.5	140	15	10	35	40	40
Magnesium	–	16	4	30	5	5	4
Calcium	–	1.2	–	–	–	–	–
Chloride	17.4	139	50	15	–	–	–
Bicarbonate	14.5	10	–	10	–	–	–
Phosphate	–	–	–	58	25	25	25
Lactobionate	–	–	–	–	100	100	100
Raffinose	–	–	–	–	30	30	30
Glutathione	–	–	–	–	3	3	–
PEG	–	–	–	–	–	50 g/l (20 mol/l)	50 g/l
HES	–	–	–	–	50 g/l	–	–
Adenosine	–	–	–	–	5	–	–
Insulin	–	–	–	–	100 U/l	–	–
Decadron	–	–	–	–	8 mg/l	–	–
Penicillin	–	–	–	–	133 mg/l	–	–
Allopurinol	–	–	–	–	1	–	–
Desferal	–	–	–	–	–	–	7.1 μmol/l
Nitroglycerin	–	–	–	–	–	–	2.5 mg/l
Histidine	–	–	180	–	–	–	–
Histidine HCl	–	–	18	–	–	–	–
Mannitol	72	–	30	–	–	–	–
Tryptophan	–	–	2	–	–	–	–
Ketoglutarate	–	–	1	–	–	–	–
Glucose	250	–	–	140	–	–	–
pH (at 4°C)	7.8	7.8	7.1	7.0	7.4	7.4	7.8

Collins = Collins solution, UW = University of Wisconsin solution[92], STH = St Thomas' Hospital solution[91], HTK = Bretschneider's solution[93], SU = Stanford University solution[94], PEG = polyethylene glycol, HES = hydroxyethyl starch.

lutions presumably magnifies the vascular spasmodic activity and subsequent energy depletion. This negative response occurs only during temperature equilibration, or until such time as pump activity ceases. Thereafter, the vessels dilate. It should be noted that 'intracellular' organ preservation solutions have very low calcium levels, since they contain components such as lactobionate and phosphate, which tend to chelate calcium.

There are various pharmacological agents available to counteract calcium-induced vasospasm or the possible development of enhanced resting tension. These include verapamil[14], butanedione 2-monoxime (BDM)[93] and nisoldipine, although their effectiveness in a hypothermic environment remains controversial. During hypothermia numerous metabolic processes are markedly reduced, altered or inhibited. Nisoldipine may offer some benefit during reperfusion when it effectively reduces the production of lipid peroxides[94]. Recent work by Stringham *et al.* has provided evidence for the use of BDM in preservation solutions as a calcium antagonist to increase myocardial protection[95].

Perfusion methods

The benefits provided by perfusion preservation are: (a) oxygenation, (b) buffering capacity, (c) delivery of substrates and (d) removal of toxic metabolic end-products. Many questions remain unanswered, for these factors alone do not overcome ischemic injury during preservation. Does substrate delivery enhance metabolic acidosis?[96] Does oxygenation overwhelm the tissues with reactive oxygen free radicals?[97,98] Until these basic questions (and many others) are answered, perfusion technologies will remain in the laboratory setting. Perfusion techniques are usually cumbersome and complex in design, compromising opportunities for clinical application. At present no new 'state-of-the-art' technology is available besides the intra-abdominal perfusion pumps developed by Waters Inc. (Minnesota) and Belzer *et al.* (Wisconsin). New developments are usually too elaborate. Such devices are merely browsed over by the experts, leaving them to drift into the archives of scientific literature.

The major disadvantages of continuous perfusion are edema formation and the constant exposure to high levels of oxygen[99]. The absence of an oxygen-scavenging system at low temperature may increase the organ's susceptibility to free radical attack. An alternative perfusion technique involves the use of microperfusion or 'trickle' perfusion[90,100–103]. Susuki *et al.* perfused dog hearts using a trickle perfusion device at a flow rate of 20 ml/100 g per hour, and successfully preserved the organs for 24 hours[100]. We have conducted similar experiments with rabbit hearts using a syringe-type perfusion device that delivers perfusate at a flow rate of 10–15 ml/100 g per hour (Figure 2)[101]. Edema formation was negligible, and the return of function after 24 hours of storage was close to that of control values (control = 265 (12.5) ml/min versus microperfusion = 220 (11.9) ml/min).

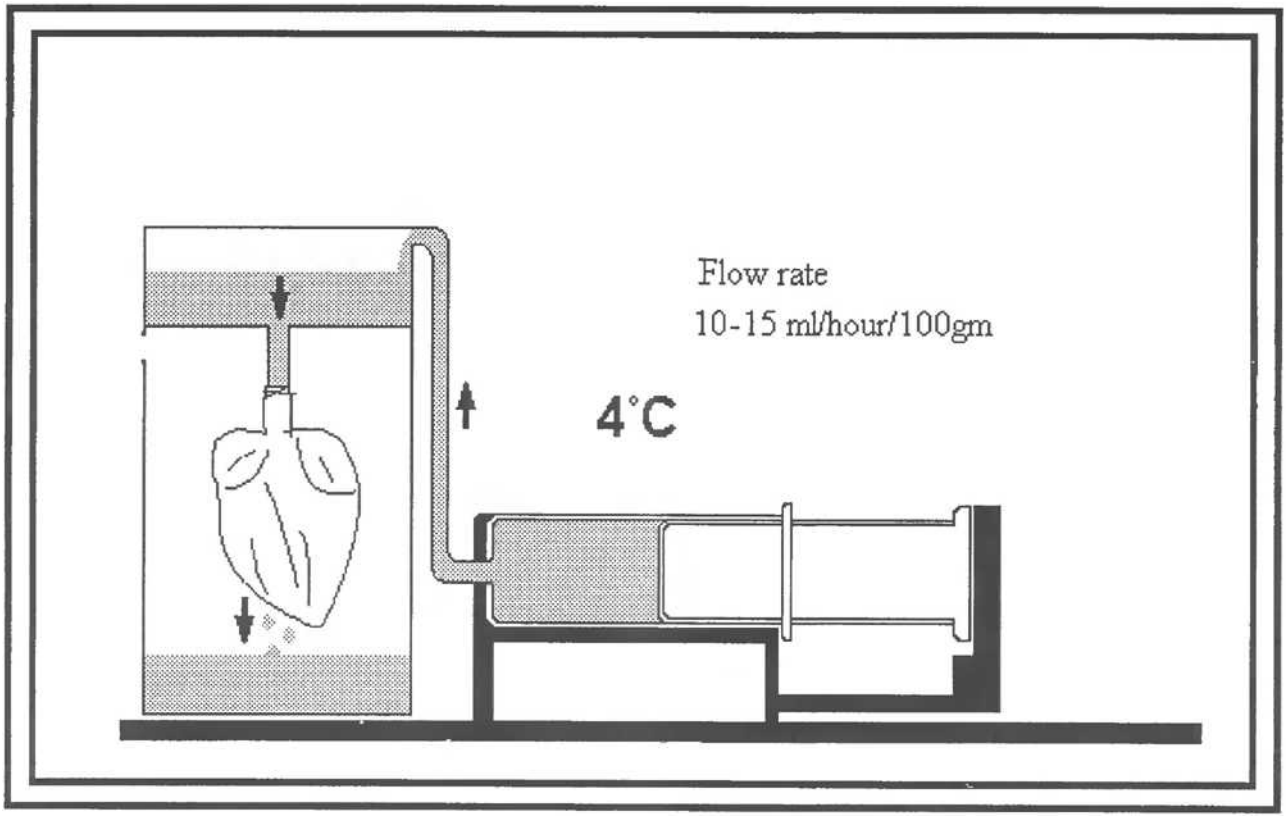

Figure 2 Microperfusion for heart preservation. A syringe pump device delivers perfusate through an airtight reservoir at 4°C via the aorta to the myocardium. The heart chamber is submerged in ice. The myocardium is slowly perfused at a flow rate of 10–15 ml/100 g per hour. Coronary venous return accumulates in the lower chamber

Storage of the human donor heart by perfusion preservation was first carried out in 1981 using an airlift pump device[104,105]. Hearts were stored for periods ranging between 4 and 16 hours, after which they were heterotopically transplanted. Initially, function was largely provided by the recipient's own heart, followed by a gradual recovery of the donor heart. Three additional patients received orthotopic grafts following periods of 4–5 hours of donor heart preservation. Both immediate and long-term function of these hearts was good.

In a clinical trial at the California Pacific Medical Center in San Francisco, in 1989, 22 hearts were preserved using Cardiosol I, eight of which were stored by microperfusion with storage periods of 3–5½ hours. These hearts were orthotopically transplanted, and demonstrated excellent immediate function[92]. All of the primary heart transplant recipients remain alive up to 6 years later.

ASSESSMENT OF MYOCARDIAL VIABILITY

Ideally, a viability assay for cardiac preservation should be simple (practical), be inexpensive and not require large tissue or fluid samples. Although orthotopic transplantation is the ultimate test, *ex-vivo* evaluation might be used as a predictor of *in-vivo* function. In experimental settings this may be less complex, and not influenced by the effect of an intact nervous system on the circulation (Figure 3). We have listed what we believe to be the most important tests in Tables 3 and 4. For the present, *in-vitro* tests are limited to laboratory studies and are, of course, not sufficiently established to be applicable in the clinical situation. Numerous large-animal versions of *ex-vivo* heart-testing apparatus have been published, demonstrating the importance of these models. The miniature model shown in Figure 3 uses a perfusor rabbit to prime the portable Langendorff apparatus. This reperfusion model resembles *in-vivo* conditions in which white blood cells and all other blood components are in contact with the heart. We used this *ex-vivo* blood recirculating apparatus to compare hearts preserved in Cardiosol I and UW solutions (Table 5). Hearts stored in Cardiosol demonstrated good function after 24 hours of ice storage, whereas UW-preserved hearts failed to achieve any measurable aortic outflow (Table 5).

The metabolic and functional assays listed in Tables 3 and 4 have served as probes to study experimental heart viability for many decades. Measurement of nitric oxide level has gained much attention recently[106]. We have studied endothelial function following a period of anoxic ice storage[107], comparing four solu-

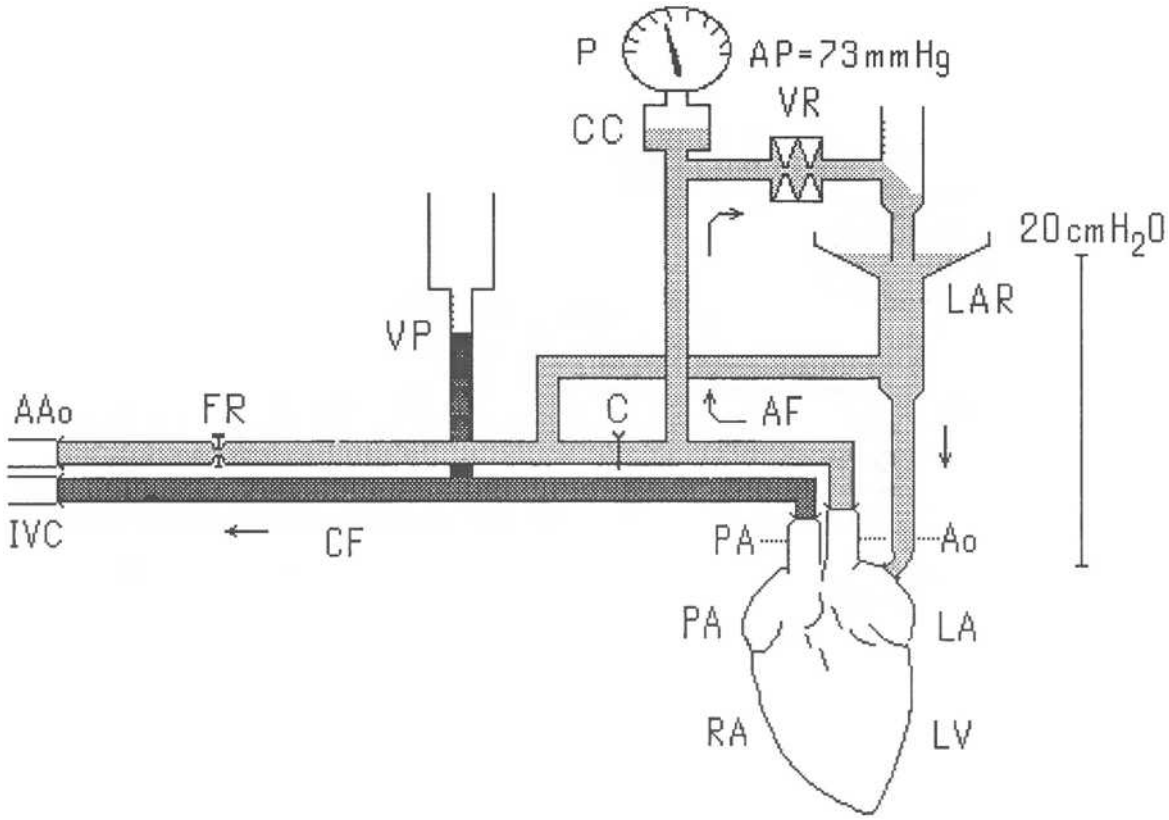

Figure 3 *Ex vivo* working rabbit heart model. Blood from the abdominal aorta (AAo) of an anesthetized perfusor rabbit provides the arterial pressure (AP) during initial reperfusion of the heart. Once myocardial activity stabilizes the arterial line pressure is clamped off (C) and heart activity is reverted to the working mode via left atrial (LA) filling. Blood is then pumped through a variable resistor (VR) (set at a resistance of 73 mmHg) via a pressure gauge (P) and a compliance chamber (CC) and returned to the LA reservoir (LAR). Aortic output and coronary flow were measured and cardiac output calculated. (IVC = perfusor rabbit inferior vena cava; Ao = aorta; PA = pulmonary artery; RA = right atrium; LV = left ventricle; RV = right ventricle; AF = aortic flow; CF = coronary flow; FR = flow regulator; VP = venous pressure)

Table 3 Tests of potential use in evaluating myocardial viability during and after heart preservation
(The tests listed have to-date been inadequate, but could prove to be reliable indicators of viability)

During preservation (predictable parameters)
Metabolic tests
Electron spin resonance (oxygen free radicals)
Nitric oxide levels
Lipid peroxidation (TBA test)
NMR (adenine nucleotides)
Cellular calcium levels
Post-preservation parameters
Metabolic tests
Metabolic plasma tests
Oxygen free radicals
Lipid peroxidation
CK-MB bands
Cardiac troponin T levels
Myosin light chain I levels
LDH
Functional tests
Morphological tests (in animal experiments)

Table 4 Models for assessing heart viability
(The models listed have been extensively investigated and are suitable only for evaluation of myocardial integrity and research studies)

1.	Orthotopic heart transplantation
2.	Isolated working heart
	ex-vivo blood perfusion
	in-vitro blood perfusion
	in-vitro crystalloid perfusion
3.	Isolated heart with left ventricular balloon
	ex-vivo blood perfusion
	in-vitro blood perfusion
	in-vitro crystalloid perfusion
4.	Heterotopic heart transplantation
5.	*In-vitro* papillary muscle
6.	*In-vitro* atrial muscle
7.	*In-vitro* myocytes
8.	*In-vitro* tissue slices

Table 5 *Ex-vivo* (blood perfused) functional evaluation of unstored fresh (control) and 24-hour stored rabbit hearts

	*Cardiac output (ml min^{-1} g^{-1})**		
Solution	*Control*	*24-h ice stored*	*n*
Unflushed fresh (control)	33 (2.0)	–	6
Cardiosol I (5%)	44 (4.3)	28 (3.3)	5
Cardiosol I (10%)	43 (4.0)	31 (2.3)	6
UW	31 (3.0)	1 (0.3)	10

*Mean (± standard error).

tions: (a) St Thomas', (b) Bretschneider (HTK), (c) UW with and without hydroxyethyl starch and (d) Cardiosol. We evaluated cardiac performance on the Langendorff working heart apparatus, and endothelial function using an *in-vitro* myograph assembly by determining the contraction and relaxation properties of rabbit aortic rings. These properties were evaluated in epinephrine-contracted aortic ring preparations by measuring responsiveness to added acetylcholine. Acetylcholine produces relaxation of the vascular smooth muscle via the release of nitric oxide. The cardiac output data matched the endothelial relaxation data, implying that quality of heart preservation is dependent on preservation of the vascular endothelium. Hearts preserved in Cardiosol suffered the least damage (Cardiosol>UW>HTK>St Thomas'). Killinger *et al.* carried out vascular functional studies on human umbilical endothelial cell cultures stored in saline, UW, PEG or Collins solution[108]. These authors similarly showed the beneficial effect of PEG (PEG>UW>Collins>saline). Jeremy *et al.* investigated the production of prostacyclin in rat thoracic aortic rings, and found minimal damage following storage in either lactobionate-raffinose or hypertonic citrate solutions[109].

In recent experiments we simultaneously evaluated hearts after 24-hour ice storage on the Langendorff apparatus and measured the concentration of nitric oxide released into the effluent via the coronary sinus. Hearts that were poorly preserved had lower levels of nitric oxide in the effluent, whereas well-preserved hearts had high levels. When inferior solutions were used for heart preservation, function improved significantly upon reperfusion if nitroglycerin was added to the preservation solution. Omission of nitroglycerin resulted in impaired myocardial function (unpublished observations). These findings suggest that nitroglycerin raised coronary effluent nitric oxide levels, resulting in the return of good myocardial function.

Mankad *et al.* studied the effects of temperature on endothelial release of 5-hydroxytryptamine and nitroglycerin in rat hearts preserved in UW solution[110]. A significant increase in coronary vascular resistance occurred at 15°C, suggesting that the UW solution produced temperature-dependent endothelial dysfunction in the isolated rat heart. At 4°C and 10°C the changes were not significant. This study, and those referred to above, provide evidence supporting the concept that a test of endothelial integrity, as measured by effluent nitric oxide production, may be useful in predicting myocardial viability.

Other potential tests include: (a) the leakage of myosin light chains from the myocardium[111], (b) the release of serum troponin and CK-MB isoenzymes[112] and (c) left ventricular contractility by ^{31}P-nuclear magnetic resonance spectroscopy[113]. By using spectroscopy, myocardial high-energy stores and intracellular pH can be measured. The changes detected by these methods have been used as markers of ischemic injury. In the case of troponin T and CK-MB isoenzymes, myocardial injury was detected during reperfusion following a short period of preservation. Viability data obtained during reperfusion do not, of course, have any predictive value as a viability assay.

Consider an example in which damage to the vascular endothelium resulted from a toxic component in the preservation solution. In this scenario, NMR spectroscopy may still detect normal high-energy phosphate levels, despite the presence of significant injury in the vascular compartment. Such limitations would render this method inadequate as an ultimate assessment of viability.

The biochemical response of the myocardium to injury may always be of a multifaceted nature and therefore no single test may be adequate to quantify the extent of injury. It is possible that a panel of tests may be sufficiently sensitive and specific to provide reliable donor heart selection. If such a viability assessment panel is discovered, the use of non-heart-beating cadaver donor hearts may result in significant expansion of the donor pool.

OXYGEN FREE RADICALS

We propose that myocardial ischemic injury following hypothermic storage or reperfusion is initiated by two mechanisms. The first mechanism is brought about by the inherent properties of the preservation solution discussed above, and the second mechanism by the spontaneous production of reactive oxygen free radicals in an altered environment, in which free radical scavenging capabilities are markedly reduced. We provide the following explanation.

During life, tissues are inherently saturated with antioxidants, many of which are physically removed or rendered inactive during hypothermic cardioplegic flushing. The loss of this antioxidant protection results in extensive oxidative tissue damage when oxygenated reperfusion occurs, or even during the preservation period. Antioxidants added to the solution or resident antioxidants, such as superoxide dismutase, catalase and glutathione reductase, may be rendered inactive by hypothermia. This would reduce their protection during the hypothermic phase, but residual activity may explain the fact that free radical scavengers have in some cases provided promising results. *In-vitro* antioxidants differ in their biological interactions with the tissues because of the altered physiochemical environment. The explanation is as follows: *in-vivo* antioxidants operate at body temperature in concert with plasma and cellular enzymes to defend the tissues. In this system the products of oxidative reactions are reduced by tissue enzymes (temperature-dependent) and other organic cellular reducing agents. In this way the redox potential of the organism is maintained. Conversely, *in-vitro* antioxidants are at a great disadvantage because the regenerating support enzyme systems are not available to the same extent as in their *in-vivo* counterparts. In other words, the oxidized intermediate species (generated by the reaction of the antioxidant with the oxidant) transforms into a new reactive redox species with destructive capabilities.

In the example below, these concepts are illustrated in the form of redox equations. GSH (*reduced*) denotes an antioxidant, and the undamaged tissue is represented by RH. A peroxy radical (LOO•) symbolizes the oxidant that oxidizes the tissue RH, yielding a peroxy radical R• and a lipid peroxide LOOH (equation 1). Next, GSH rescues the partially reacted tissue radical (equation 2), which itself becomes oxidized (GSSG), transforming into a new oxidant with free radical capabilities (GS•). Finally, the two GS• moieties associate (GSSG), imparting additional toxicity to the tissues (equation 3)[114].

$$LOO^{\bullet} + RH \rightarrow LOOH + R^{\bullet} \quad (1)$$

$$R^{\bullet} + GSH \rightarrow GS^{\bullet} + RH \quad (2)$$

$$GS^{\bullet} + GS^{\bullet} \rightarrow GSSG \quad (3)$$

This enzyme-independent oxidative process is spontaneous and occurs at temperatures below –20°C.

Intracellular or extracellular solutions do not influence oxidative reactions, but additives to these solutions, such as organic molecules or antioxidants, may participate in oxidative reactions. The above examples show how the addition of oxygen free radical scavengers or reducing agents themselves may paradoxically elicit an indirect assault on tissue integrity by promoting the production of oxidants.

OXIDATION AND POLYETHYLENE GLYCOL

In our laboratory we continue to explore the multifaceted nature of polyethylene glycol (PEG). The two forms most effective in preservation solutions are the linked PEG20M (Cardiosol I) and the 20 linear (20LPEG) (Cardiosol II). The linear species is less viscous and homogeneous compared with heterogeneous PEG20M, which is contaminated with breakdown products of the linker, including toxic phenol. Both forms of PEG are susceptible to spontaneous oxidative decay. The *reduced* form behaves like a supercharged redox-buffered species capable of preventing oxidative reactions until fully discharged. The loss of charge represents a fall-off in preservation potential and explains why random samples of this material off the shelf behave in an unpredictable manner (*reduced* versus oxidized).

By analogy, PEG loses its charge in a similar fashion to that of GSH in the UW solution[115]. For example, when GSH oxidizes (GSSG), heart function becomes impaired following preservation[115]. St Thomas' solution, on the other hand, consists mainly of salts and is therefore unable to undergo oxidative change. However, the contaminating Fe^{2+} in reagents used to prepare all solutions can interact with the tissues, promoting lipid peroxidation[116].

Reduced PEG interacts negligibly with Fe^{2+} or Fe^{3}, conserving its reducing potential. PEG renders oxidized species harmless within the vascular compartment. The superiority of PEG in preservation solutions remains widely accepted despite the unpredictable nature of this polymers' oxidation state[117-119].

The presence of trace metals plays a central role in tissue injury during storage and reperfusion. Ferrous Fe^{2+} propagates the oxidative cascade more efficiently[120,121] than ferric Fe^{3+}. Many organic molecules are susceptible to oxidative attack, including gluconate, lactobionate and dextran, although lactobionate is the least reactive (unpublished observations).

Besides the addition of antioxidants to ward off the oxygen free radical onslaught, an alternative strategy is the introduction of Fe^{2+} chelators during preservation or reperfusion[122]. These drugs can, however, distort the Fe^{2+}/Fe^{3+} ratio and trigger a pro-oxidant effect[123]. Unfortunately these agents have diffusion constraints with limited availability in remote areas within the myocardium. A low concentration of desferal can reduce oxidative stress, provided it does not interfere with the ferrous/ferric ratio during ischemia[124].

Storage in *reduced* PEG Cardiosol II solutions has permitted successful 24-hour ice storage of rabbit hearts. The performance of these stored hearts exceeded all previously tested solutions in our laboratory (cardiac output = 71 ml min^{-1} g^{-1} versus fresh controls 66 ml min^{-1} g^{-1}) (Table 1). The superior function observed in hearts preserved in PEG II was attributed to: (a) the reducing potential, (b) lack of toxic contaminants and (c) protection against tissue oxidation promoted by an altered Fe^{2+}/Fe^{3+} ratio within the vascular compartment. In this study we used 10% PEG20L, which generally offers better protection than the 5% concentration unless the PEG is available in the fully *reduced* form (Table 5). PEG20L and PEG20M also have the potential to prolong graft survival by means of an as-yet-undefined immunosuppressive effect[92,125]. The induction of tolerance by antigens covalently linked to PEG is well documented[126]. However, we remain uncertain about its immunosuppressive mode of action, and believe that

the observed lower incidence of rejection is primarily due to improvements in the quality of preservation[92].

REPERFUSION

The issue as to whether reperfusion damage is a real phenomenon or merely the outcome of a laboratory artifact is still controversial[127]. In the previous edition of this book we defined reperfusion 'as an amplification at normothermia of an event (or events) that occurred during an ischemic phase, whether normothermic or hypothermic'. An alternative definition by Jennings states that 'Reperfusion merely accelerates the funereal events of those cells doomed to die in any case'[128]. Schaper provides further support for the above definition by proposing that reperfusion of an ischemic heart is a state of *down-regulating* performance, thereby sparing energy reserves for subsequent harmful episodes. This is manifested in the form of arrhythmias and stunning[129].

Hearts preserved on ice for 4 or more hours demonstrate some loss of function when evaluated on the Langendorff apparatus using a Krebs buffer perfusate. If blood products alone were responsible for reperfusion injury, then no damage would be evident in a crystalloid buffered solution. Furthermore, the finding that little or no loss of function occurred in PEG-preserved hearts lends support to the hypothesis that reperfusion injury is a physicochemical response resulting from a prior injury.

The major tissue changes brought about by reperfusion injury include myocardial stunning (a reversible form of injury exhibited by poor contractile activity) and lethal reperfusion injury (recurrent ischemia eventually resulting in cellular disintegration)[46]. These ischemic changes are observed on histological examination as contraction-band necrosis. Many theories have attempted to explain the underlying causes of lethal reperfusion injury. The prime suspects are: (a) oxygen free radicals, (b) leukocytes and (c) plugging of capillaries by granulocytes[130,131].

The fact that most myocardial cell death occurs during reperfusion has encouraged the development of modified reperfusion protocols. Currently, there are many interventions that aim at limiting the severity of functional impairment. Despite the great variations between protocols, all have enjoyed widespread confirmation. Many of the modified reperfusion therapies focus on the vascular compartment. Pharmacological intervention includes the administration of: (a) adenosine[132], (b) thromboxane A2 receptor antagonists[133], (c) dipyridamole to limit platelet deposition[134], and (d) platelet-activating factor receptor antagonists[135], as well as (e) leukocyte-depleted reperfusion[136,137]. There is also evidence that demonstrates an association between reperfusion and damaged coronary microvessels, resulting in an impaired endothelial-dependent response[138]. The aim of modified reperfusion is to accelerate the rate of tissue recovery. Protocols include: (a) lowered reperfusion pressure[139,140], (b) substrate enhancement[141,142], (c) calcium channel antagonists[43], (d) administration of oxygen free radical scavengers[143], (e) terminal (secondary) cardioplegia[137,144] and others. Many of these reperfusion treatment strategies favorably influence the return of myocardial function[143,145,146].

COMMENT

The major forms of injury sustained by the myocardium are due to: (a) instability of the donor, (b) composition of the cardioplegic and or preservation solutions, (c) washout of inherent antioxidants and (d) duration of the ischemic period. These factors ultimately determine the magnitude of the oxygen free radical assault and the responsiveness of the myocardium to therapeutic interventions such as donor management and donor pretreatment.

In this review we stress the importance of vascular integrity, with special emphasis being placed on tissue nitric oxide levels having potential use as a viability marker. We propose that the major pathways leading to oxidative injury occur through iron-dependent oxygen free radicals that respond to the *reducing* effect of PEG. All other free radical scavengers tested in our laboratory were unable to match the effectiveness of PEG20L. Using Cardiosol II we obtained successful 24-hour ice storage in Langendorff and *ex-vivo* reperfusion testing systems. Details of the biological interactions of PEG have not been fully defined, but laboratory tests indicate that it functions as a reducing agent capable of scavenging superoxide anions. Furthermore, PEG supports reversible redox reactions, akin to an *in-vivo* oxidant-scavenging enzyme system. This system reconverts oxidized moieties into *reduced* equivalents which again have the capacity to neutralize additional oxidative processes.

We believe that an optimal heart storage method will become available in the near future. The requirements for these developments include: (a) adequate preservation of myocardial structure and function to obtain at least 24 hours of storage without the need for inotropic support following heart transplantation, (b) a practical and reliable method for transportation of the stored heart, and (c) immediate availability of preservation solutions without the need for additives or other cumbersome intervention.

Heart pretreatment will become an essential component of the preservation process, allowing for chemical manipulation of the myocardium before the induction of deep hypothermia. Ideally, 24 hours of ice storage of the donor heart will be routinely possible, with cellular integrity being maintained by controlling oxygen free radical generation and calcium flux. Currently this objective is not unrealistic, because polyethylene glycol already appears to fulfill the requirements for prolonged ice storage of the heart. The use of oxygenated perfusion techniques will also be pursued in order to reliably stretch preservation times beyond the limits of ice storage. We anticipate further developments in long-term normothermic isolated heart perfusion using artificial blood, but progress will depend on the proper antioxidant protection. These developments will eventually result in standardization of experimental procedures for evaluation of heart function following preservation. Although there continue to be advances in freezing of single cell preparations, developments in freezing of whole organs for heart storage are still limited by the toxicity of the high concentrations of cryoprotective agents that need to be introduced and removed. In the clinical setting, on the other hand, we envisage moving towards uniformity in heart preservation methods and assessment of viability. These developments, however, require a profound understanding of the mechanisms involved in ischemic injury of the myocardium.

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Advances in Lung Storage

G SPEZIALI, R.C. DALY AND C.G.A. McGREGOR

INTRODUCTION

Several informative reviews of lung preservation have been published since the landmark review by Haverich and colleagues at Stanford in 1985[1–6]. The purpose of this chapter is to outline currently used techniques of lung preservation (including those of the authors), define controversies and options that exist for these methods, and briefly describe future prospects for improved preservation.

THE NEED FOR LUNG PRESERVATION AND THE CONSEQUENCES OF FAILURE

The need for effective lung preservation is clear. After appropriate donor and recipient selection, the next key step in achieving a successful outcome for the lung recipient is good preservation of the donor lung. Lung preservation encompasses: (a) initial procurement, (b) lung storage, (c) recipient intraoperative protection, and (d) the management of reperfusion. A deficiency in any one of these areas can compromise the eventual outcome. Acceptable preservation results in good early and (possibly) late lung function, leading to improved patient survival, reduced postoperative morbidity, reduced intensive care and hospital stays, and resulting cost savings. Another advantage is the ability to use donors from a distant site with hitherto unacceptable ischemic times, therefore optimally utilizing this precious resource.

Failure to achieve adequate pulmonary preservation results in a spectrum of postoperative pulmonary dysfunction, ranging from primary graft failure (resulting in the patient's death) to a milder form of the 'reimplantation' response (which may have little clinical consequence). Primary graft failure is manifested by early (immediately or within hours), severe lung dysfunction with progressive non-cardiac pulmonary edema (sometimes hemorrhagic) and resultant hypoxia with decreased pulmonary compliance and elevation in pulmonary artery pressures. Radiologically, increasing diffuse opacification of the lung is seen. Histologically, diffuse alveolar damage is present. These changes may be irreversible, depending on the severity of the process. At the other end of the spectrum is the 'reimplantation' response, which consists of a transient hypoxemia associated with increasing radiological pulmonary opacification resembling pulmonary edema. Immediate lung function may often be excellent but it begins to deteriorate after 6–12 hours, being worst at 24–48 hours, before responding to therapy with diuretics.

The causes of this spectrum of postoperative lung dysfunction include ischemia, reperfusion injury, preprocurement lung damage, surgical trauma (e.g. coarse handling or overinflation), lymphatic interruption, and denervation. Since these changes are not inevitable, the latter two potential causes do not seem central to the causation of early postoperative lung dysfunction. Recipient factors, such as the underlying condition for which the transplant is required and its severity, may also predispose to improved postoperative lung function. Most lung transplant surgeons have occasionally seen worse lung function in the lung with the shorter ischemic time than in the other (from the same donor) with the longer ischemic time. Clearly, our understanding of lung protection (although adequate for current practice) is incomplete and necessitates ongoing research efforts.

CURRENT TECHNIQUES OF LUNG PRESERVATION

Previously clinically employed methods of lung preservation, such as topical cooling[7,8] and the use of the normothermic autoperfusing heart–lung block[9,10], have been abandoned and will not be discussed further.

The two currently used techniques of lung preservation are: (a) donor core-cooling by cardiopulmonary bypass and (b) cold single-flush pulmonary perfusion. The latter technique is by far the most commonly applied clinically at the present time, although core cooling is the method of choice at Harefield Hospital, London, currently one of the busiest cardiothoracic transplant centers in the world[11]. Donor core-cooling is slower, more cumbersome and expensive. Special equipment and bypass technicians are required. This method may also be unacceptable to other organ retrieval teams, and the possibility of cardiopulmonary-bypass-induced lung injury exists. Advantages of the technique, however, may include more uniform cooling of the lung,

particularly of the bronchi, and the beneficial properties of blood as a perfusate as it is a colloid and contains buffer, free radical scavengers, and metabolic substrates[12]. Single-flush pulmonary perfusion was the technique employed in the original successful heart–lung transplants at Stanford[13] and, with modifications, has stood the test of time. This method is simple, fast, inexpensive, acceptable to other retrieval teams, and of proven effectiveness. Distribution of the cold perfusate, however, may be uneven in the lung and may not result in optimal cooling of the bronchial tree. A large number of clinical variables in this technique makes comparison between centers difficult.

Variables in single-flush pulmonary perfusion technique

These variables include: (a) the composition of the flush solution; (b) adjunctive treatments, e.g. steroids and prostaglandins; (c) inflation or deflation of the lung; (d) the composition of gases used to inflate the lung; (e) the targeted temperature; (f) the storage medium; (g) antegrade or retrograde administration; and (h) the management of reperfusion.

The following descriptions are not intended to be exhaustive but reflect the authors' clinical views.

Composition and volume of the flush solution

Cold (4°C) Euro-Collins solution (ECS) (60 ml/kg), modified by the addition of 12mEq magnesium sulfate and 65 ml of 50% dextrose per liter, was the first solution used for distant lung procurement[14] and probably remains the most widely used technique currently[15–17]. Prostaglandin therapy is given to the donor to mitigate the vasoconstrictive effects of this solution[18].

University of Wisconsin (UW) solution has recently displaced ECS in several centers[19,20], based on improved pulmonary preservation in laboratory studies[21,22]. It remains to be seen whether the significantly increased cost of UW solution is justified by improved lung preservation clinically.

The use of cold donor blood, modified by the addition of prostacyclin, has provided satisfactory lung preservation for up to 4–6 hours[23,24].

Low potassium dextran solution has been proposed as an alternative to ECS and UW solution based on laboratory experiments[25–27], but we are not aware of current clinical application of the technique.

Adjunctive treatments

Corticosteroids are variably administered to lung donors before retrieval, based on persuasive evidence from the experimental laboratory[28–30]. The timing of administration may be critical[29].

Prostaglandins in the form of either prostaglandin E_1 or prostacyclin are widely used as part of pulmonary preservation protocols. The basis for their application has been described in detail elsewhere[31]. Other treatments or additives, such as free radical scavengers, calcium channel blockers, complement inhibitors, substrate enhancers and platelet-activating factor antagonists, have been investigated in the laboratory.

Inflation versus deflation of the lung

It has been generally accepted for a long time that lung ventilation during flush perfusion improves perfusate distribution, and that maintenance of inflation during storage prolongs the safe ischemic time of the lung[6,32–34].

Gas composition in the inflated lung

The appropriate oxygen content in the ventilated and the stored inflated lung remains controversial and clinically variable. Experimental evidence suggests that 100% O_2 is either deleterious[35,36] or beneficial[37] to lung preservation.

The ideal temperature for storage

Hypothermia clearly increases the tolerance of the lung to ischemia[38,39]. The ideal temperature for storage is unknown. Clinically, most surgeons store the lung in 4°C solutions[3–6]. Recent animal laboratory evidence suggests 10°C may be the optimal temperature of storage[27,40,41], but we are unaware of clinical storage at 10°C being employed at the present time.

Up to the present time, lungs have been stored in cold solution at 4°C or surrounded by ice slush. Buoyancy of the stored, inflated lung in the storage container can result in areas of lung lying either adjacent to air (of unknown temperature) or next to ice (with the potential for cold injury). Recently, it has been proposed that lung storage in cold air at a controlled temperature may be a superior technique[42].

Storage medium

Clinical practice is variable, but there is some experimental evidence that storage of lungs in the same solution as the pulmonary flush perfusate is beneficial, rather than in physiological saline.

Antegrade versus retrograde pulmonary perfusion

Traditionally and still most commonly applied is antegrade flush pulmonary perfusion[3–6]. Recently, retrograde flush perfusion of the lung via the left atrium has been used clinically[43], with the intention of providing better airway preservation, a concept substantiated by later experimental evidence[44,45]. However, concerns regarding distension of the left heart remain.

Management of reperfusion

The biochemistry of ischemic reperfusion injury is the focus of continuing intensive research efforts. Lung injury may be mediated by leukocyte and platelet activation, oxygen free-radical production, generation of arachidonic acid metabolites, and other metabolic effects. This subject has been well reviewed recently[3,6]. Clinically, the use of corticosteroids as potent inhibitors of phospholipase is the best example of attempts at mitigating reperfusion injury. Controlling reperfusion by initially limiting the pulmonary blood flow appears to be a potentially important concept based on recent laboratory data[46]. In the future, further methods to modify this injury will likely be applied.

Current technique of lung preservation at Mayo Medical Center

(1) The donor is pretreated with 1 g of methylprednisolone intravenously 1–2 h before lung excision.
(2) Fifteen minutes prior to inflow occlusion, an infusion of prostaglandin E_1 is begun at an initial rate of 20 ng kg^{-1} min^{-1}. This infusion is titrated to achieve a systemic systolic blood pressure of 80 mmHg or a 30% drop in the systemic pressure.
(3) The donor is heparinized (300 units/kg).
(4) The main pulmonary artery is cannulated.
(5) Gentle hand-ventilation of the donor with room air is carried out to re-expand any area of atelectasis.
(6) After inflow occlusion, aortic cross-clamp, and initiation of cardioplegia, 60 ml/kg of cold (4°C) UW solution is flushed through the main pulmonary artery over a 3–5-min period.

To each liter of solution, 200 000 IU of penicillin, 40 IU of regular insulin, 16 mg of dexamethasone, and 20 μg of prostaglandin E_1 are added immediately prior to infusion.

(7) The left atrial appendage is excised to decompress the pulmonary veins and the left heart.
(8) Concomitant topical cooling of the lungs is carried out using cold 4°C saline solution as hand-ventilation with room air continues.
(9) When the perfusion is complete, the trachea is clamped at end-inspiration.
(10) After excision, the lungs are stored inflated in cold 4°C UW solution for transportation.

During performance of the transplant, the operating room temperature is kept low to minimize warming of the graft. The lung is wrapped in sponges soaked in cold saline with adjunctive topical cooling through an external line dripping cold (4°C) saline onto the sponges enclosing the lung.

FUTURE PROSPECTS FOR LUNG PRESERVATION

We believe reasonable expectations for the future in clinical lung preservation in the next 5 years include: (a) improved preservation for longer ischemic times; (b) identification of the ideal storage temperature for the lungs; (c) application of further pharmacological techniques to reduce ischemia-reperfusion injury; and (d) the use of selected circulation-arrested donors.

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Permanent Cardiac Replacement by A Total Artificial Heart: Experimental Background and Current Problems

W.J. KOLFF

INTRODUCTION

I could not be more delighted. Nearly 13 years after the implantation of an artificial heart into Dr Barney Clark in 1982, the Utah-type artificial heart (first called the Jarvik heart, then the Symbion heart, and now the CardioWest heart) has returned to Utah. On 12 April 1995 James W. Long implanted this artificial heart in Alvin Marsden at the LDS Hospital in Salt Lake City. It was my privilege to visit with him 10 days later.

Alvin Marsden is a real-estate developer from Boise, Idaho, and already had his computer set up in his room. He told me that he had been 3 hours from death; that he had not only heart and lung failure, but also liver and renal failure. All of this has now reversed, although he required two short treatments with the artificial kidney during the first days after his heart implant. He had walked on the treadmill without any problems and was eating 'like a horse'.

I asked him several questions. 'Does the noise of the driver bother you?' 'No.' 'Does the artificial heart cause any discomfort or pain' 'No.' 'Do you still love your family now that your heart, the symbol of love, has been removed?' 'Yes. Love is in the mind.'

Indeed, many surgeons have now seen the rapid improvement of a dying patient following the implantation of a total artificial heart (TAH). The secondary organ failures (e.g. renal, liver, lung), if caused by cardiac insufficiency, disappear within hours or days. The edematous patient may even become dehydrated, and improved liver function may render the dosage of anticoagulant ineffective.

Cabrol emphasizes the importance of not transplanting a patient until his or her condition has been stabilized by the TAH. 'A transplanted heart will not perform better than a mechanical heart, and any hope that the patient will improve after transplantation is ill-founded. It is therefore mandatory that a patient placed on TAH support be thoroughly scrutinized to try to ensure that a donor organ is not wasted in a vain and unsuccessful attempt to save the patient'.

As increasing numbers of patients are supported by TAH as a bridge to transplantation, and as an ever-increasing number of them awaits a suitable donor heart, eventually such patients will ask to be sent home with the device. Thus, slowly but steadily, the permanent TAH will take its place among the accepted methods of treatment for end-stage cardiac failure.

Since the first implantation of a 'permanent' TAH was performed in Dr Barney Clark in 1982, 264 patients have received the Utah-type air-driven artificial heart. Only the first four patients received a permanent device; thereafter, the Food and Drug Administration (FDA) allowed the use of the TAH only as a bridge to transplantation. There is now good evidence that the results of a subsequent heart transplant are much better when a recipient in severe heart failure is first restored to a healthy state with a TAH. The results are discussed in this book (Chapter 21).

Peter Salisbury mentioned the artificial heart in his Presidential address to the American Society for Artificial Internal Organs (ASAIO) in 1957. In the Western world the first TAH was implanted by Tetsuzo Akutsu and the author in December of that year in an anesthetized dog that survived for 90 minutes[1].

In this chapter certain selected aspects in the development of the TAH will be discussed, as will problems that still complicate the long-term use of such devices.

SOURCES OF ENERGY

A number of different sources of energy have been used experimentally to power artificial hearts. The pneumatically powered TAH is today in vogue.

The future use of muscle- and pneumatic-powered left ventricle assist devices (LVAD) deserves to be mentioned, since these will be developed soon after this book is published. The use of piezoelectric power to convert skeletal muscle power is a very new and exciting development at the present time, although I suspect these methods will be used in LVAD rather than in TAH. I expect this technique to replace dynamic cardiomyoplasty, which involves the wrapping of the latissimus dorsi muscle around the ailing heart (Chapter 84). (Patent pending).

Pneumatic (compressed air)

While we were struggling with electrohydraulic and mechanically driven artificial hearts[2,3], Kirby Hiller of the National Aeronautics

and Space Administration (NASA) suggested the use of compressed air as a source of energy for a drive system outside the body. NASA proceeded to build a most sophisticated drive system for us which would respond to physiological needs[3–5].

The pressure curve of the driving air could be altered at will, and the percentage systole/diastole could be changed or was automatically regulated, depending on the rate. The system was, however, extremely complex to manage, and stimulated personal efforts to try to develop the simplest possible drive system outside the chest, and the simplest blood pumps inside the chest.

The result was the Detroit Driver (made by the Detroit Coil Company in Michigan), distributed by the National Institutes of Health (NIH) free of charge to laboratories interested in artificial hearts[3,6]. When larger artificial hearts were developed for larger experimental animals, Kwan-Gett enlarged the size of the valves[7].

Air-driven systems have been maligned more than they deserve, particularly with regard to their size and mass. The heavy weight of the drive system used in the first patient to receive a permanent TAH (Barney Clark) was due to compressed-air cylinders for redundancy and two drive systems instead of one (also for redundancy).

The FDA, in requiring two drive systems, showed little concern for cost. Bishop had proven in Kolff's laboratory that the second drive system can be replaced by two simple hand pumps which are ordinarily used to inflate footballs (at a cost of eight dollars each). The patient or assistant can use the hand pumps until another mechanical driver is in place. (One spare or a Datascope Driver should be in the ward.) We use the same hand pumps in our mock circulations to teach various personnel just how the TAH works.

With a minor change, the drive system of the Datascope intra-aortic balloon pump (IABP) can be adapted to drive a TAH[8]. In contradistinction to the IABP, synchronization with the natural heart is, of course, not required.

This artificial heart, once approved by the FDA for implantation into two patients at Temple University in Philadelphia, is no longer available at Cardiac Systems. A modification is still being made at Kolff's laboratory at the University of Utah.

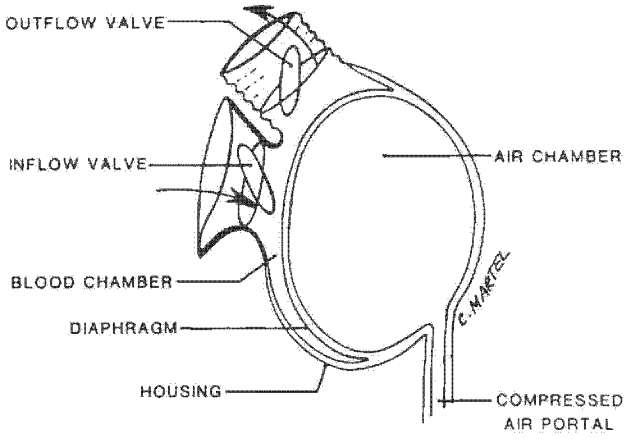

Figure 1 Diagram of basic design of the Philadelphia total artificial heart (TAH). (After ref. 9)

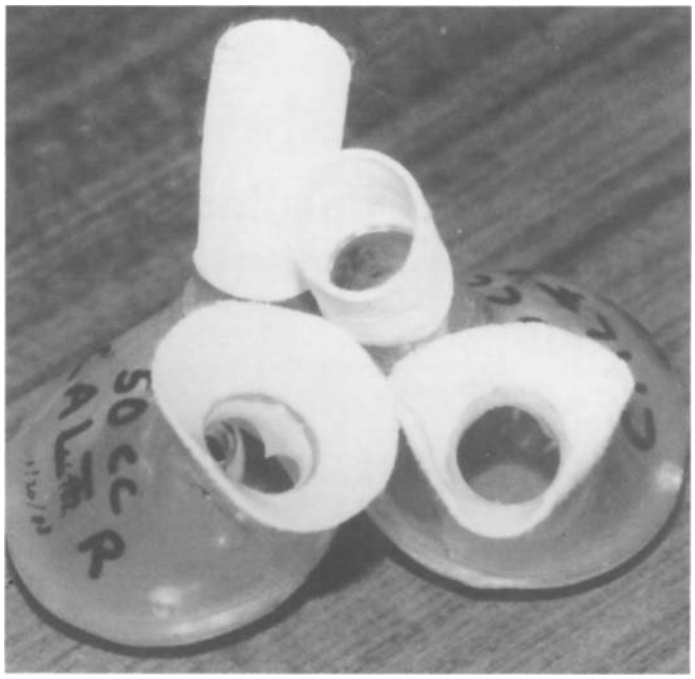

Figure 2 The Philadelphia TAH has no connectors (quick-connects). The atria and aorta are one piece with the housing. The surgeon can sew in the valve of his or her choice on the operating table

The basic design of a pneumatically powered TAH is illustrated in Figure 1, which is based on the Philadelphia TAH (Figure 2)[9]. The implantable portion of the Philadelphia TAH is composed of two ventricular chambers. The volume within each chamber is divided by a flexible diaphragm, separating the chamber into compartments for blood and air. When the blood chamber is completely filled, the air chamber is nearly empty. Introduction of pressurized air into the air chamber causes the ejection of blood from the ventricle, and removal of air allows the blood compartment to fill once again. The air conduits, or drivelines, connect the ventricles with the external control console via the chest wall; the console provides the pneumatic driving energy. The Philadelphia TAH driver has a gentle dp/dt. The pneumatic drive source was designed to produce a gentle, pulsatile, pumping action that closes the inflow valve before the more powerful ejection phase occurs[8,9].

This was accomplished by a small air valve that opened just before the large air valve did. Stephen Topaz at Kolff's laboratory can accomplish the same with an air vortex. The air enters at an angle at the periphery and exits at the center; instead of a separate vacuum pump he uses an inexpensive Venturi. The high-pressure air at the end of systole is released by a plastic sheet flap valve. (A limited number of these devices can be obtained from Kolff's laboratory for our collaborators abroad.)

Air-driven TAH can be monitored with the COMDU (Cardiac Output Monitor and Diagnostic Unit), which incorporates a flow meter in the driveline. Usually only the diastolic flow of air is recorded[10,11]. The amount of air that leaves the TAH during diastole is equal to the amount of blood that enters the ventricle and, if we assume that the ventricle is completely emptied with each stroke, this also represents the stroke volume. If multiplied by

rate, the cardiac output of both right and left sides can be measured with an accuracy of 10% without need for transducers inside the chest. The COMDU only considers the inflow volume, and does not automatically compensate for regurgitation or other losses. The shape of the curve, however, gives valuable information regarding: (a) whether or not the ventricle is sufficiently filled, (b) the presence of a broken valve, or (c) air between the diaphragms when a multiple layer diaphragm is used, and (d) 'valving' (occlusion of the airline entry point into the ventricle by a distended diaphragm).

Portable air-drive systems

Portable air-drive systems situated outside the chest have some obvious advantages: (a) they are small and no heavier than the oxygen tank that many people with emphysema walk around with; (b) the system can be replaced, reducing the demand on durability; (c) they can be repaired without opening the chest; (d) their batteries, which can run for 6–8 hours, can be recharged or replaced. (Leif Stenberg, a patient in Stockholm, Sweden (Figure 3), walked with this drive system to a restaurant, served himself four times at the smorgasbord table, and sent a telegram to the United States saying, 'I am the happiest man in Europe.')

Figure 3 Leif Stenberg with his portable drive system

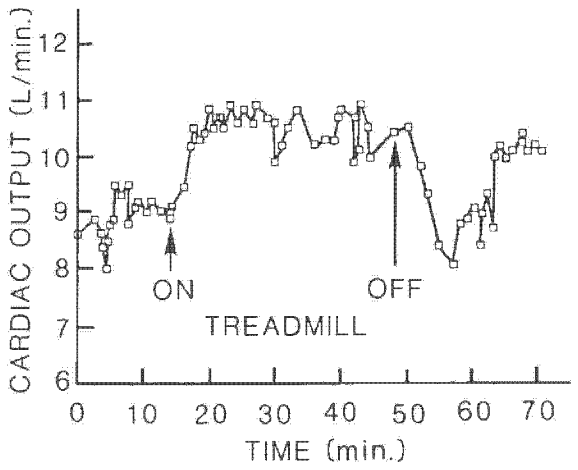

Figure 4 Tracing (from Heimes portable heart driver) shows the cardiac output of a calf, 'Albert', 50 days after implantation of a TAH. Before the animal is on the treadmill the cardiac output is about 9 l/min; during exercise the cardiac output automatically increases to 10.5 l/min; when the treadmill is stopped the cardiac output falls to 8 l/min within minutes. The rise 10 minutes later occurs when the animal walks off the treadmill back to its cage

The portable drive system used by Leif Stenberg was designed and built by Heimes[12], who built a newer version around 1988 which provides continuous readouts of cardiac output and pressures and can be connected to recorders (Figure 4). Another portable drive system has been build by Affeld and his associates in Berlin[13].

Electrohydraulic

The first electrohydraulic heart was built by Norton, together with my laboratory personnel, in 1963[2] (Figure 5). Five mechanically coordinated electromagnets compressed hydraulic fluid, which bathed both right and left ventricles. The important principle that not only mechanical energy, but also heat, is conveyed by the hydraulic fluid, was established. The heat radiates into the blood, and the body serves as a radiator.

Our present electrohydraulic artificial heart (1995) has definite advantages over others. The motor-impeller sits between the two ventricles. It can be reversed within 14 thousandths of a second from a top speed of 1200 revolutions in one direction to top speed in the other direction. Small turbine blades on the rotor propel hydraulic fluid from left to right and vice-versa. It should be emphasized that the blood does not go through the impeller, but the hydraulic fluid does; therefore the blood is not damaged by the impeller.

From the onset, Kolff's laboratory tried to use back-electromotive force for the reversal of the motor. Years ago I sent Robert Jarvik to Jim Isaacson, President of NuTech Industries, Inc., in Dayton, Ohio, which at the time was the only manufacturer that used back-electromotive force. (Abiomed does not reverse the motor but uses a sleeve around the centrifugal-pump–motor

Figure 5 Five solenoids are arranged in a rosette. When energized they compress a hydraulic fluid that is within the housing. On the top of the artificial heart one sees the atria, the pulmonary artery, and the aorta. These vessels are made of corrugated polyurethane so they can bent without kinking

aggregate to rock back and forth in order to shift the fluid from right to left and vice-versa. It is hard to believe that such a system will be durable.)

The blades of our impeller are designed according to the recommendations of experts in 'small pump' technology at NASA. Other small impellers at high speed get backflow through the space between the impeller and the housing. Stephen Topaz avoids this by providing a shroud around the impeller blades; this also prevents cavitation.

We use polymer hydrodynamic bearings which cost 29 cents each. These bearings are normally used in the bottom of deep wells where they 'eat' fine sand or gravel; they last 15 years. *We use saline as our lubricant and hydrodynamic fluid* – no yuyuba oil or silicone fluid (as was used in breast implants). The hydraulic film reaches all surfaces that would otherwise be in contact, and does not break during reversal. Even if it did break, such as after standing still, it would immediately be restored by the centrifugal force of the liquid.

Stephen Topaz and David Jones have returned to timing and reversing of the motor by pure back-electromotive force. No transducers for pressure or Hall effect devices (which inside the body have poor longevity) are needed. The switch-over becomes simple when two chips are used: one for rotation to the left and one for rotation to the right. The switch-over can be selected after one or many revolutions. Each rotation dispenses 2 ml of hydraulic fluid. Blood flow meters are unnecessary.

The difference in cardiac output between the left and the right ventricle is considerable and must be provided for. In calves we have seen it to be 2 l/min. There are at least two sources for the difference in cardiac outputs: (a) the bronchial circulation, which comes from the left side and returns to the left side, and (b) the higher pressures on the left side, which result in greater regurgitation through valves and increased loss by distension of the ventricle. (There is some additional loss in an air-driven system due to the compressibility of the driving air, but it becomes important only when the volume of the driving air is large.)

In the electrohydraulic heart the difference between right and left cardiac output can be compensated by creating a leaking pulmonary valve, so that part of the blood which is pumped out returns during diastole. If the pulmonary artery valve is purposely made insufficient, during a long diastole a large backflow from the pulmonary artery will occur; during a short diastole a smaller backflow will occur. This allows regulation of imbalance by varying the ratio between systole and diastole[14]. An unfortunate consequence is that, since the right and left ventricles are hydraulically coupled, a longer diastole on the right side results in a longer systole on the left, and we need a relatively longer diastole for adequate filling.

There is another solution, which is not a compensation with blood but with the driving fluid. This can easily be accomplished if a small extension for the driving fluid is provided on the right side (Figure 6). During systole, part of the hydraulic fluid goes into the extension, not into the right ventricle.

The anti-vacuum bellows will be described later (under Regualtion of Cardiac Output) and can also be used to compensate for differences in cardiac output (see Figure 7). When the blood is not present to fill a ventricle, the bellows prevent excessive suction, and less blood will be available to be pumped out in the next systole on that side.

A soft part in the ventricular wall can serve the same function, but in a less sensitive way. The soft part must be flexible but not distensible. We have used this in our latest version of the electrohydraulic heart.

Jack Kolff demonstrated, in his experiments with brain-dead cadavers (neomorts), that any imbalance of atrial pressure can be easily compensated by a hole in the atrial septum. The danger of right heart failure, which might occur in the natural heart, does not exist with artificial ventricles[15,16].

Alternatively, one can decouple the right and left sides making them quite independent, and then provide a compliance sack for each ventricle during diastole. It has been well substantiated by Nose's laboratory[17], and others, that compliance sacks covered

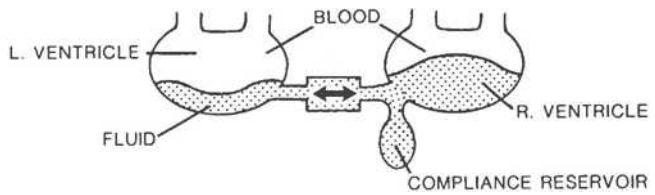

Figure 6 Diagram of electrohydraulic heart. A reversible pump moves fluid (not blood) from left to right and vice-versa. Some of the fluid can be diverted to a compliance reservoir to reduce the stroke volume of the ventricle

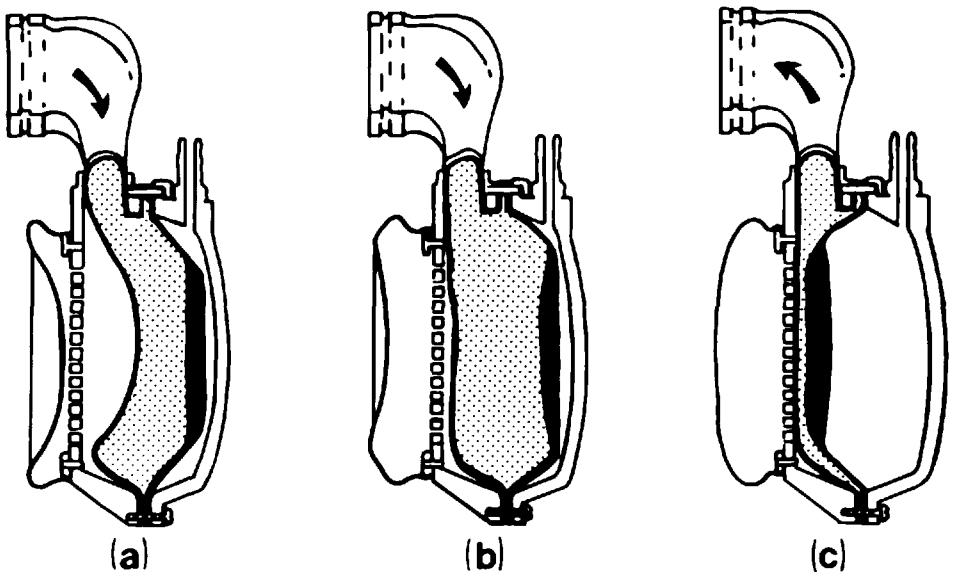

Figure 7 Diagram of an artificial heart (TAH) with anti-vacuum bellows. If a simple reciprocating pump is used to drive a TAH with compressed air or fluid, then undue suction might be generated during diastole if there is insufficient blood available to fill the ventricle. The *left* cross-sectional diagram (**a**) demonstrates what happens if insufficient blood is available. The left wall of the ventricle, which is supported by a screen, is sucked into the ventricle, and the bellows on the outside are drawn inward. The *middle* diagram (**b**) shows what happens if there is enough blood to fill the ventricle during diastole. The ventricle is entirely filled, and the left side of the ventricular wall remains against the screen. The *right* diagram (**c**) shows what happens during systole. The ventricle is compressed so that the blood is expelled. The screen provides support for the flexible left side of the ventricle

with fibrils can maintain their flexibility for years. The porous silicone surface made by Dr William Seare promised to stay flexible with less fibrous tissue for even longer periods[18].

Atomic energy

The first totally implanted TAH was actually built for the Atomic Energy Commission between 1971 and 1974[19]. An atomically driven motor fueled by plutonium-238 (built by North American Philips) was placed in the abdomen, and a flexible driveline passed through the diaphragm to the artificial heart. The mechanical drive was built by Westinghouse, and our laboratory built the blood-handling mechanism (called the soft-shell artificial heart). Atomically driven TAH are not being pursued at present, from a fear of radiation hazard. For the same reason, atomically driven pacemakers have also disappeared from the market.

Electricity

Using an electric motor (instead of the Sterling hot-air engine driven by atomic energy), a calf was maintained alive in a reasonable condition for 35 days. This record for a mechanically driven pump, achieved in 1975, stood for almost 10 years until it was broken by Pierce's group in Hershey, Pennsylvania[20].

With improved batteries, and methods to transfer energy through the intact skin, atomic sources of energy are no longer necessary. Pusher-plate hearts have been brought to a considerable degree of sophistication and reliability, yet remain heavy and cumbersome[21]. Novacor's pusher-plate has been used to power an LVAD in humans. If the drive system is mounted between the ventricles, and moves back and forth, as in the pendulum heart[22], the space required by the system is smaller. The most elegant pusher-plate drive system to date has been built by Heimes in Aachen, Germany (unpublished).

The Milwaukee group device has a flat plate that moves back and forth between the two ventricles. The ventricles are not connected but bathed in fluid. The activation is the same as it was in the pendulum artificial heart – an eccentric device, driven by planetary gears on a small electric motor. The whole heart is very compact[23]. Another variation of the pendulum heart is the Korean heart[24].

Atsumi's group has come up with an undulating plate that forces the blood out of the cavity. They use two ventricles with an undulating plate or one ventricle that alternates the pumping of blood to the right or left side. A special membrane valve takes care of separation of red and blue blood[25]. Four of Imachi's 'jellyfish' valves were used, which have proved to be excellent valves. [Since this design is being perfected by Dr Imachi, we had better pay attention![26].]

REGULATION OF CARDIAC OUTPUT

Starling's Law of the heart assumes that the innervation of the pulmonary and peripheral systemic vascular systems is intact, and that if each ventricle (of the TAH) pumps out all of the blood that is delivered to it, the natural regulating systems of the body will suitably adjust pulmonary and peripheral systemic arterial pressures. Thus, when the venous or atrial pressure rises, our artificial ventricle is more fully filled, and automatically pumps out more

blood. This also ensures a balance between the pulmonary and systemic circulations.

One might anticipate that, if the right heart delivers more blood to the left side, then the left heart will pump out more; consequently, the right side would pump out more, and so on. Fortunately, this does not happen – neither in a mock circulation nor in the experimental animal.

An air-driven TAH usually vents into the atmosphere, but if one applies a small amount of suction during diastole, Starling's curves shift to the left. This simple system requires heart valves that offer little resistance, and pumping diaphragms or sacks that are thin so that they can move easily; with a heavy diaphragm, other methods must be used.

The most sensitive, purely mechanical application of Starling's Law is possible with a TAH that incorporates anti-vacuum bellows[27] (Figure 7).

If one has a non-thinking, reciprocating drive system, Starling's Law can be accommodated by making part of the ventricle collapsible, but not distensible (Figure 8). If insufficient blood is available during diastole, then part of the ventricle simply collapses; during the next stroke only that amount of blood that fills the non-collapsed ventricle is pumped out[19].

PROBLEMS WITH ARTIFICIAL HEARTS

Placement within the chest

The major problem in the development of the TAH has been to design it so that it would fit satisfactorily, within the chest. My personal design was of a flat 'pancake' TAH (Figure 9). A calf, in which this heart was inserted, was the first calf that did not show an increase in venous pressure over a period of time[28]. I asked Robert Jarvik to redesign the heart, which later became the Jarvik III. The dimensions were such that it would fit inside a calf's chest without compromising the venous return of the right and left atria. In the course of developing a larger heart with a larger cardiac output, the Jarvik VII heart was developed, and was the heart implanted in Barney Clark in 1982[29,30].

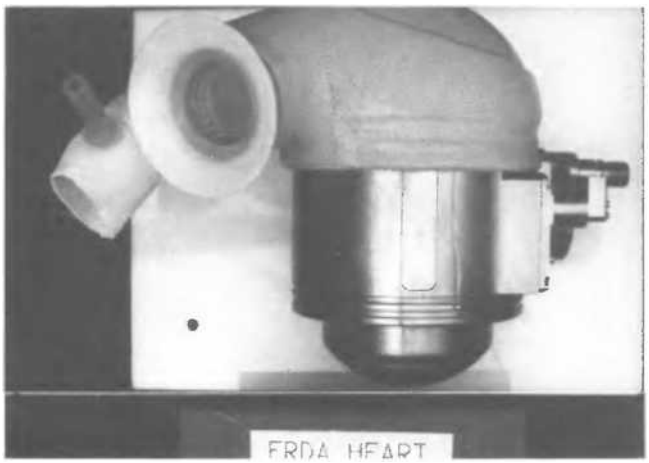

Figure 8 The ERDA (Energy Research and Development Administration) heart. Only one ventricle is shown. The blood ventricle has a collapsible part (top of the figure). The drive shaft is to the far right. Blood handling parts are made of silastic

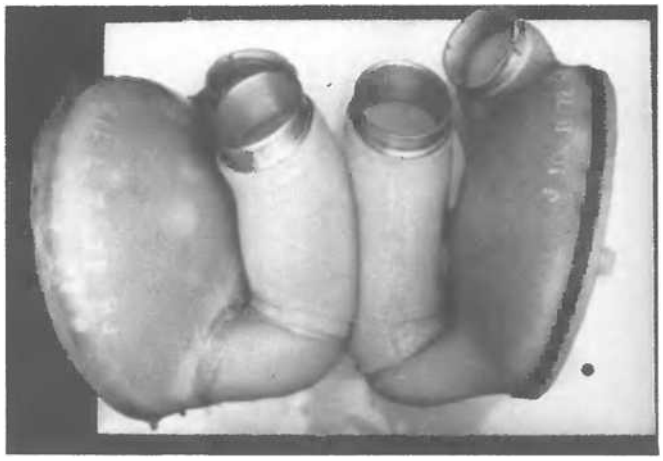

Figure 9 In the 'pancake' artificial heart the ventricles lie against the rib cage, thus leaving the area between sternum and vertebral column available for the connections to the atria, aorta, and pulmonary artery

Before the first clinical implantation, Jack Kolff and coworkers demonstrated convincingly (in brain-dead cadavers) that it was preferable to place the left ventricle more to the left, or the right ventricle more to the right, so that the narrow space between the sternum and the vertebral column was not overcrowded[31]. During the implantation in Barney Clark the pericardium on the left was slit to allow space for the left ventricle.

To facilitate positioning of the TAH, with its rather rigid driveline, in the chest, the surgeon can experiment with a dummy ventricle (Figure 10), which has the exact size of the ventricle to be implanted. When he has determined the best possible location, he can use a flexible driveline to plan the point of exit from the chest. He then feeds the rigid driveline through the incision, and the artificial ventricle will fit satisfactorily in place.

Although I have had 'kits' with dummy parts available (for use to assess choice of size or 'fit' of the TAH) for many years, neither Symbion, Inc., nor its successor, CardioWest Technologies, Inc., have made them available to heart surgeons.

Thromboemboli

Of the first six patients who received the Jarvik-type TAH, five had thromboemboli. The unfortunate alternative to such thromboemboli is hemorrhage from anticoagulant therapy. Thrombus formation is most common: (a) on the suture lines, (b) in the connectors (so-called quick-connects), (c) around the valves, and (d) at the junction between the diaphragm and the housing (DH junction)[32–34].

Fortunately, as of May 1995, we can report that none of a consecutive series of 40 patients implanted with the Utah-type heart now made by CardioWest Technologies, Inc., has had any evidence of a stroke.

Finding a small thrombus in a crevice in the TAH is not necessarily a bad omen; it should be considered as part of the natural repairing process of the body. Sooner or later the equilibrium between thrombus-producing and thrombus-removing

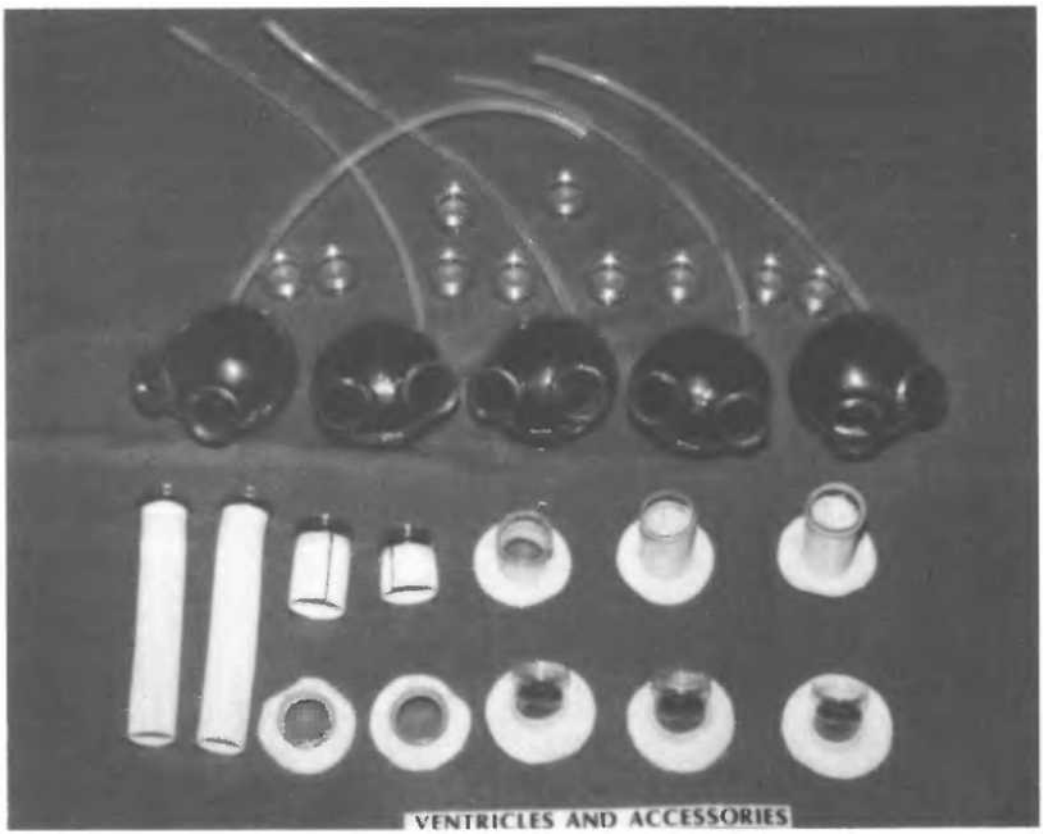

Figure 10 Set of dummy ventricles and accessories that the surgeons may use to estimate ideal size and position in the chest. The drivelines are not connected. There is a choice of atria and shape of ventricles

factors will be established, and a small thrombus in a crevice may be smoothed off at the surface and even overgrown with endothelium.

Jack Kolff has suggested doing away with the quick-connect system used heretofore in nearly all TAH implants. The left atrium, left ventricle, and aorta are now manufactured in one piece, as are the right atrium, right ventricle and pulmonary artery (Figure 2). The surgeon can now sew in (on the operating table) the valve of his choice; St Jude, Bjork-Shiley or Hall-Kaster valves can be used. If he or she is particularly concerned with the risk of thrombosis, tissue or polyurethane valves can be sewn in (Figure 11).

A promising and compact solution has been suggested by Olsen and his associates[35]; it consists of axial flow pumps magnetically suspended in the bloodstream, thus negating the need for bearings, which are notorious for causing thromboemboli.

In general, to avoid thrombus formation in the TAH or in the patient's atria, one of two approaches can be used.

The first is the use of a rough intima, accepting that fibrin formation will occur, but trusting that it will not be dislodged as emboli. This rough intima can consist (a) of: small titanium balls, or, (b) on moving diaphragms, of Dacron fibrils firmly anchored with a second layer of polyurethane, or (c) of a fascimile of the Dacron fibrils but consisting of the same kind of polyurethane (as used by the Thermedics device)[36,37]. Rough intimas can be successfully coated with what Nose has called 'a biolyzed surface', which basically is pure gelatin crosslinked with glutaraldehyde[16]. This highly hydrophilic surface has proved very successful.

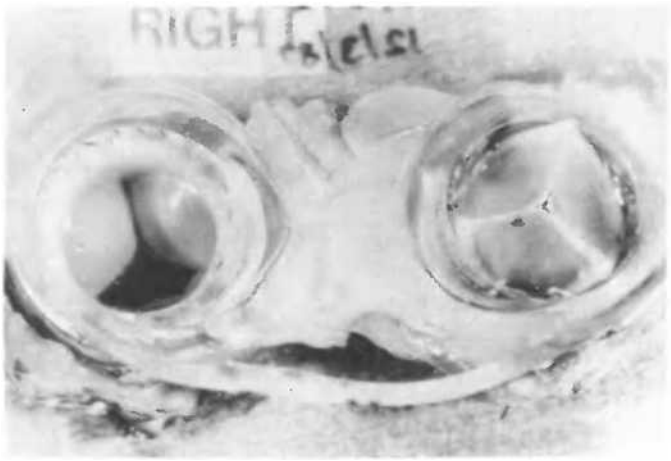

Figure 11 Polyurethane valves, with sewing cuffs removed, 28 days after implantation in a TAH. One leaflet of the inflow valve has been cut for study. The leaflets were clear of thrombus except for one small speck

The second – and, to date, more popular – approach is to use smooth elastomer surfaces[38]. (Smooth intimas are used by CardioWest, the Berlin group, Thoratec, and Abiomed.) For a long time it was believed that air-dried polyurethane was to be preferred, though this is probably not so. The ideal is for thrombus never to form, and therefore embolization never to take place, but this ideal is rarely achieved. Jack Kolff's group in Philadelphia has demonstrated that there is basically no difference when one looks with the scanning electron microscope at the smooth intima of a TAH implanted for 2, 10 or 30 days (J. Kolff, unpublished). This suggests that small thrombi are formed all the time, but are then dissolved. This appears to be harmless, as long as the thrombi do not become too large.

The treatment of the smooth intima with heparin, prostaglandin, heparin–prostaglandin compound, albumin, or albumin IgG is aimed at making the smooth surface even less thrombogenic. At present there is competition between two concepts: (a) incorporating the substances in the polyurethane so that they leach out, which, of course, results in a limited active life; and (b) grafting them on to the surface. It has been well substantiated that if heparin, for example, is grafted to the surface with a long chain of carbon atoms, so that the heparin can wave back and forth in the bloodstream, it provides high protection against thrombus formation[39–41].

Some of the heparin-coating techniques have used DMAC (dimethyl acetamide) as a solvent for polyurethane and heparin, but DMAC has a tendency to destroy the polyurethane membranes and valves. Chisato Nojiri (Terumo Corp.) prefers to use ozone or a novel photoreactive phenyl azide-derived hydrophilic polymer to graft the heparin[42].

The inertness of smooth surfaces can be further enhanced by coating them with pyrolytic carbon[43,44]. The pyrolytic carbon surface may be the most inert surface known to man. Coating the inert surface with compounds in which water is incorporated (hydrophilic coating), such as is used in contact lenses, is another possibility. Owen (at the Biosouth Research Institute in New Orleans) is using polyhydroxyethylene oxide acrylate to coat artificial hearts for our group at the present time.

In summary, it seems to this author that ultra-smooth surfaces grafted with heparin will have the best future.

Infection

It is believed that even a small thrombus may be a place where bacteria can proliferate; the number of local infections (often around the valves of the TAH) seen in experimental animals is high. Once a vegetative bacterial endocarditis has developed, it is usually impossible to ascertain whether or not its origin was a pre-existing thrombus. Gristina has ascertained that bacteria, which are innocuous while circulating, will proliferate and become clinically significant when they find a surface on which to settle (e.g. elastomers). Indeed, specific bacteria appear to have a preference for specific elastomers, and other bacteria for other elastomers[45]. The possibility of making the surface of the elastomer less attractive to bacteria is one of the challenges of the future. It may possibly be achieved with antibiotics or antiseptic agents, as long as they are not damaging to the blood components.

Infection along the drivelines or wires that pass through the skin is a well-recognized danger. To a large extent it can be avoided by using special entry tubes provided with a subcutaneous flange[46] (Figure 12).

Figure 12 A one-piece transfer-molded double-skin button (Elastomer HP-100 by Dow Corning) allows two drivelines to pass through the skin and reduces the penetration area by 44%. The flange, which is covered with Dacron velour, keeps the button from being pulled out, and is a barrier against infection

Aging of polyurethane

For many reasons, polyurethanes are the easiest and most desirable material with which to make artificial hearts and artificial heart valves, but all polyurethanes age. A TAH which has been in a mock circulation for many years shows brittleness of the diaphragm. We can return to the use of silicones, which do not age. The newer silicones (e.g. Silastic HP 100) are stronger than those used around 1970, have a greatly reduced tendency to tear, and can be reinforced with fibers, such as carbon fibers, if needed.

Unfortunately, none of the large American companies, such as DuPont, Dow Chemical, Dow Corning, or American Cyanamide, are willing to deliver their elastomers for artificial hearts or implantable medical devices for fear of litigation. Feeble attempts are being made to pass a law to hold them harmless. Some small companies are trying to fill the void. One small company, Corvita Corporation (8210 N.W. 27th St, Miami, FL 33122, USA), has produced a copolymer of polyurethane with polycarbonate called Corethane, which is thought to be better resistant to body fluid.

FINANCIAL CONSIDERATIONS

An air-driven TAH need not be exorbitantly expensive. The production of a TAH by vacuum-forming techniques requires only simple molds, and is a rapid process compared to that of solution-casting. Cardiac Systems Inc. (1027 Conshohocken Rd, Conshohocken, PA 19428, USA) had a license from the University of Utah for the production of such TAH, and FDA approval was obtained for two implantations in human patients.

Currently, similar ventricles can be obtained from Kolff's laboratory at the University of Utah in Salt Lake City.

Neither need the drive system be expensive. One such system at our center (a slightly modified two-cylinder gasoline engine driven by an electric motor) costs less than $1000, and has been pumping every night for several years to test hearts and valves for durability. Other more sophisticated reciprocating pumps, which can be regulated, were designed by Norton and were relatively inexpensive[47]. Other types of drive systems for air-driven hearts are available for approximately $12 000. They are usually operated by solenoid-driven valves, and need a source of compressed air. For collaborating laboratories, Kolff's laboratory can provide a simple drive system for $6000.

Drive systems which use air-actuated valve systems, such as are commonly used in respirators and diving equipment, are extremely inexpensive, but have a tendency to drift.

If we wish to provide an inexpensive TAH it makes no sense to incorporate four commercially available valves, knowing that their costs range between $1800 and $4200 each. Copies of the Bjork Shiley valve can be bought for $100 each in India and in China. During the last 6 years we have concentrated our efforts on building elastomer valves. My former co-worker, Long Shen Yu, can do this with either a vacuum-forming or a solution-casting technique, or by spraying on simple male molds. The valves in our artificial ventricles are being tested for durability. We prefer biflap inflow valves because of their low resistance and tricusp semilunar valves with sinus valsalvae for outflow valves because they are so easy to manufacture.

COMMENT

It has been estimated that some 35 000–50 000 people per year in the United States alone will need some kind of replacement of their failing heart[48]. It is unlikely that human donor hearts will be found for more than a very small proportion of them. At most, approximately 2000 human donor hearts are available per year. Xenografts may become possible, but the most appropriate donor, the pig, has a very short lifespan even when he does not meet the butcher's knife (a 3-year-old pig is a very old pig.)

There is, therefore, a great incentive to persist in our efforts to develop the perfect TAH. If we do not squander our money on such items as SDI ('Star Wars'), there should be plenty of money in the United States to take care of its citizens in need. We must convert our military-directed industry towards peaceful goals, and our production-oriented society toward a service-oriented society. Now is the time to tell our political representatives where our priorities are.

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Early Clinical Experience with Permanent Cardiac Replacement by a Mechanical Device

D.K.C. COOPER

INTRODUCTION

Cooley (Figure 1) and colleagues[1] implanted the first total artificial heart (TAH) in a human in 1969. This attempt was intended as an interim measure until a suitable human heart could be located and transplanted. This group performed a second bridge-to-transplant procedure in 1981[2,3]. Since then the TAH has successfully served as a bridge to transplant in many patients.

The first intended permanent implantation of a TAH was performed by DeVries (Figure 2) and his colleagues in 1982[4–8], who used the device to prolong the lives of four patients. This initial clinical experience was based to a great extent on pioneering work by Willem Kolff (Figure 3) and his colleagues in Utah, whose bioengineering research did much to advance the artificial heart to the point where its clinical use could be considered (Chapter 76). The clinical experience of DeVries and his colleagues (initially at the University of Utah and subsequently at Humana Hospital Audubon, Louisville) will be briefly reviewed[4–9].

DEVICE

The Jarvik-7-100 (Figures 4 and 5) TAH consists of right and left ventricles and four tilting-disk valves with anatomical communications to the atria and great vessels. Each ventricle contains a flexible diaphragm constructed of multilayered polyurethane. At the base of each ventricle is a 30-F polyvinylchloride connecting tube that is tunneled under the skin and exits the body in the left lateral abdominal area through Dacron felt skin buttons. The connecting tubes are attached to polyvinylchloride drivelines, 1.6 cm in external diameter and 2.2 m in length. The drivelines are attached to an external pneumatic pump (the Utahdrive System II console, Symbion, Inc., Salt Lake City). The lines may be connected to a portable heart driver during periods of patient mobility.

The maximum stroke volume of each Jarvik-7-100 ventricle is 100 ml. The ventricles are pneumatically 'driven'. During diastole, blood fills the ventricle on the upper side of the diaphragm.

Figure 1 Denton Cooley, who attempted the first temporary implantation of an artificial heart (the Liotta heart) in 1969

During systole, air is pulsed on the underside of the diaphragm to eject the blood from the ventricle. The heart functions in response to circulatory needs (Frank–Starling law); thus, with increased venous return, stroke volume is increased without a change in heart rate.

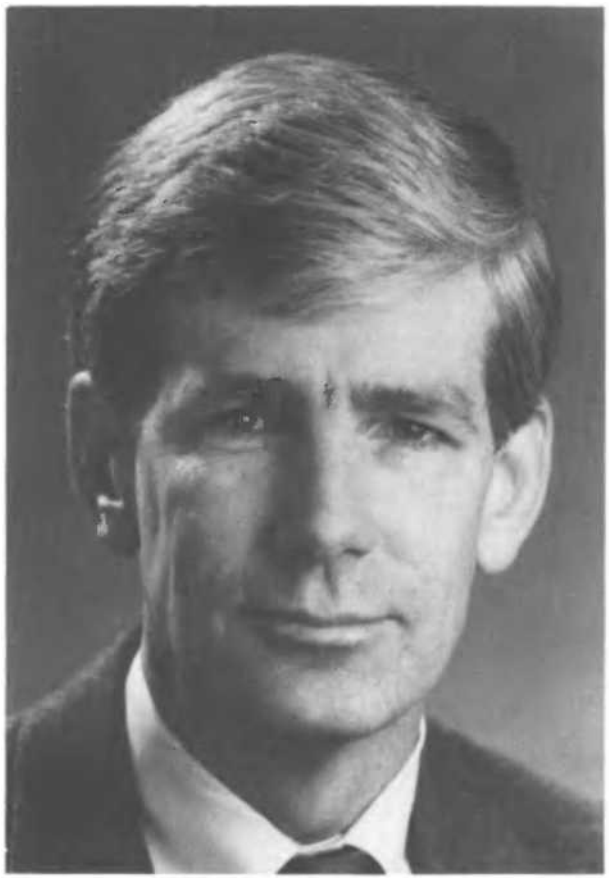

Figure 2 William DeVries led the surgical team that carried out the first trial of permanent replacement of the heart by an artificial device

PATIENT SELECTION CRITERIA

The four potential recipients of the TAH were categorized as New York Heart Association class IV, and had each been rejected as candidates for cardiac transplantation by at least three programs. All revealed stable psychological profiles and had strong, reliable family support systems. They were all unanimously approved by an evaluation committee, and gave informed consent.

Details of the four recipients are given in Table 1[8]. The first patient to undergo this procedure was the first human subject in whom the Jarvik-7-100 TAH was implanted; the operation was performed on 1 December 1982.

Table 1 Pre-implant clinical data of the four recipients of permanent total artificial hearts

Patient	*Age (years)*	*Underlying cardiac pathology*	*Reason transplantation denied*
1	61	Dilated CM, COPD	Advanced age
2	54	Ischemic CM	Insulin-requiring diabetes mellitus
3	58	Dilated CM	Advanced age
4	62	Ischemic CM, mild COPD	Advanced age

CM = Cardiomyopathy; COPD = chronic obstructive pulmonary disease

Figure 3 Willem Kolff, of the University of Utah, whose pioneering work contributed much to the development of the artificial heart

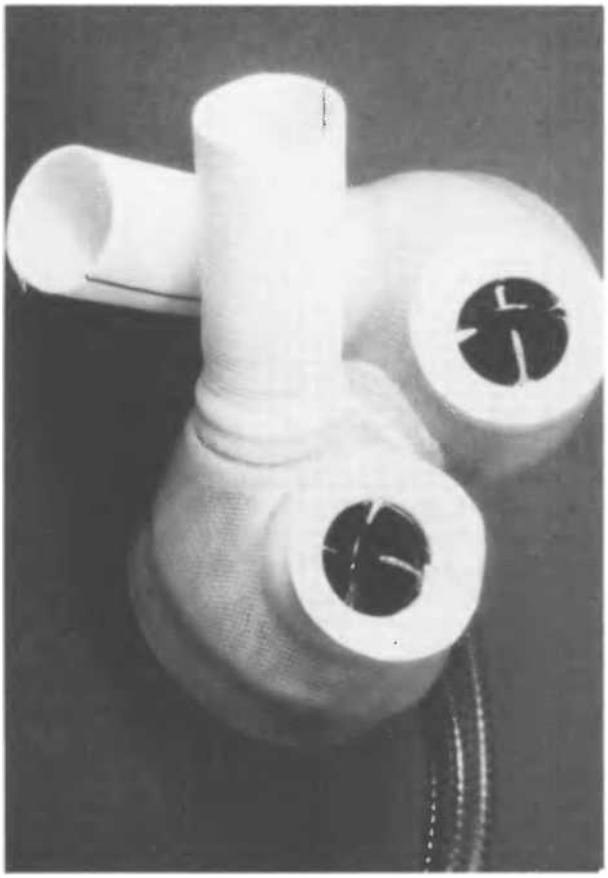

Figure 4 Jarvik-7-100 total artificial heart

Figure 5 Robert Jarvik, who was a member of Willem Kolff's research team, holding one of the devices named after him.

SURGICAL TECHNIQUE

The technique of implantation has been reported by DeVries previously[9].

MONITORING

A computerized bedside monitor–terminal stored and displayed systemic and pulmonary arterial and right and left atrial pressures. A cardiac output monitoring diagnostic unit (COMDU) displayed and stored left and right filling volumes, left and right cardiac outputs, and heart rate[10]. Left and right drive pressure wave forms were also recorded. These data were stored on a tape-recorder for later analysis[11,12].

CLINICAL PROGRESS

These patients lived for periods of 112, 620, 488, and 10 days respectively. The Jarvik-7-100 TAH functioned well, and hemodynamic stability was achieved in all patients[10,11]. Only one device failure was experienced – a broken mitral valve prosthesis (patient 1)[4]. Their postoperative course was, however, eventful in every case (Table 2), the major problems being hemorrhage, acute tubular necrosis, embolic phenomena, and infection, all of which occurred in all three long-term survivors. Detailed reports of their clinical courses have been described elsewhere[8]. Only one patient (patient 2) was able to be discharged from the hospital, this patient being able to live in an apartment close to the hospital after day 133.

Table 2 Chronological order of significant post-implant events and complications in patient 2*

Days	
1	TAH implanted
1	Exploration of mediastinum for bleeding
3–6	Renal failure
19	CVA (thromboembolic)
68	Neutropenia
94	CVA (hypoperfusion)
133	Discharged from hospital
150–620	Subacute bacterial endocarditis
163	CVA (hemorrhagic)
202	Changed to new Utahdrive console with low dp/dt
352	CVA (thromboembolic)
444	Liver biopsy – microabscesses
590	Feeding gastrostomy
612	Tracheostomy
620	Respiratory failure, sepsis, death

* Based on ref. 8
CVA = cerebrovascular accident (stroke)

Hemodynamic observations

All of the patients demonstrated a remarkable degree of autoregulated hemodynamic homeostasis for prolonged periods[12]. Patient 1 was maintained with high cardiac outputs (6–8 l/min) that were associated with the onset of seizures. In the cases of patients 2–4, cardiac outputs were initially maintained at 3–4 l/min and, over a 7-day period, were increased and stabilized at 5–6 l/min.

The cardiac output was readily altered by elevating the heart rate[12], which was usually set initially at 50 beats/min, and increased gradually over a 1 month period to 80 beats/min. Systolic and diastolic blood pressures could be maintained within normal ranges with heart rate set at 75 to 80 beats/min. After approximately 45 days an autoregulation of the vascular system, in response to changes in device parameters, was noted in all patients. At this time, attempts to increase cardiac output by increasing heart rate led to vasodilatation, resulting in a return of cardiac output to the original level. Cardiac output was extremely stable. Light exercise activities on the non-resistant exercise cycle, achieved by patients 2 and 3, were associated with an increase in cardiac output of 1–2 l/min.

Complications

Hemorrhage

All four patients required re-operation for bleeding. The surgical team's experience led them to conclude that postoperative bleeding in these anticoagulated patients should be treated by prompt reoperation rather than by repeated blood transfusion and observation.

Hemolytic anemia

All patients developed a significant hemolytic anemia. After a change in the Utah drive consoles to provide a lower d*p*/d*t* (on days 116 and 202 for patients 3 and 2 respectively), transfusion requirements decreased. This was paralleled by a fall in the lactate dehydrogenase and plasma free hemoglobin levels.

Acute renal failure

Depressed preoperative cardiac output and poor renal perfusion undoubtedly increased the risk of postoperative renal failure. Patient 4 had the greatest compromise in renal function before operation, and was the only patient to require dialysis postoperatively. The etiology of acute tubular necrosis in the early postoperative course in patients with implants was considered to be multifactorial. The combination of high transfusion requirements and postoperative hemolysis probably played a significant role, and the toxic effects of long-term aminoglycoside therapy were also considered to be a possible contributing factor.

In the light of this experience, DeVries and his colleagues believed that in future patients several therapeutic approaches could be used to prevent the development of renal failure. Patients at other centers who received TAH in which the low d*p*/d*t* Utah drive System II was utilized have not developed severe renal failure[13,14]. Therefore, this drive system, or the Heimes driver, would seem preferable. Improved hemostasis would minimize the need for multiple transfusions in the early postoperative period. Renal blood flow, already reduced in such patients, could be maintained by low-dose dopamine in the intraoperative period, and by the use of mannitol and furosemide at critical times.

Thromboembolism

One of the greatest concerns in the care of the TAH patient is the prevention of thromboembolism. The use of anticoagulants, however, is not without risk, and requires careful monitoring of the thrombotic and fibrinolytic systems.

In this small series, anticoagulation policy varied from patient to patient[8]. Patient 1 had no thromboembolic events, but his course was complicated by recurrent bleeding episodes. Patient 2 experienced several thromboembolic episodes, though patient 3 experienced only minor transient episodes. At autopsy, both of these patients had prominent infected thrombi (subacute bacterial endocarditis) on all of the valves of the prosthesis.

DeVries' group pointed out that, in assessing the thrombogenic potential of the artificial heart, several factors must be considered, including: (a) the effects of activation of both the intrinsic and the extrinsic pathways of the coagulation cascade, (b) activation of platelets and of (c) the fibrinolytic system, and (d) the antithrombotic/antiplatelet regimen used. None of these systems acts in isolation; they interact with one another, as well as with other enzymes and cellular systems, such as complement and kinin. Whether the coagulation cascade proceeds to completion depends on the adequacy of the antithrombotic/antiplatelet regimen that is used.

As a result of the experience with these four patients, several changes were recommended in the anticoagulation protocol to be used in the future. Once hemostasis has been achieved in the surgical wound, heparin should be administered by continuous infusion; heparin kinetic studies should be employed to estimate dosage, with the goal being a partial thromboplastin time increased 50% above control. When the indwelling catheters have been removed and prophylactic antibiotics discontinued, heparin administration should be by subcutaneous injection every 8 hours.

Since the patients in this study demonstrated thromboembolic problems after the diagnosis of bacteremia, concern was expressed that subacute bacterial endocarditis accounted for some or all of the thromboembolic events, and that this would not be responsive to antithrombotic therapy. In view of this possibility, in future cases treatment of infectious problems should have the highest priority as an antithrombotic measure.

Infection

After TAH implantation the blood is in continuous contact with synthetic materials; it was considered possible that blood material interactions impacted adversely on the immune status of the recipient. Infections severely compromised these patients, necessitating multiple and long courses of antibiotics for infections caused by urinary tract, bowel, respiratory, and skin normal flora and contaminants. Many of the organisms that caused chronic problems were detected in the early postoperative period[15].

Infection arising from the drivelines, with spread to the mediastinal periprosthetic space, was the major limiting factor in long-term use of the device. Intensive antimicrobial therapy for prolonged periods seemed to suppress but not to eradicate infection, and was accompanied by the appearance of multiresistant bacterial strains. Complications of antimicrobial therapy included diarrhea secondary to overgrowth with *Clostridium difficile* in two patients[15].

The surgical group concluded that the prevention of infection needed to be of foremost importance in the future development and utilization of the artificial heart. Of particular concern for TAH development was the finding of culture-negative 'skip areas' between the prosthesis and the skin; this was believed to imply a blood-borne infectious source of origin in some cases, rather than infection ascending along the drivelines from the skin[16]. Infection could therefore be a problem even with a future device that was fully implantable.

It was concluded that the incidence of infection could possibly be reduced in future cases by: (a) improved selection of patients to exclude those with a predisposition to infection, (b) perioperative antibiotic prophylaxis with the use of narrow-spectrum antibiotics whenever possible, (c) particularly careful aseptic urinary bladder catheterization, (d) the use of non-invasive hemodynamic monitoring techniques, (e) frequent surveillance cultures, (f) the use of full antithrombotic and antiplatelet therapies with the subcutaneous (rather than intravenous) administration of heparin, and (g) improved protective isolation and wound dressing procedures.

COMMENT

The complications of thromboembolism and infection were considered to be the most significant limiting factors to the use of the Jarvik-7-100 as a long-term cardiac replacement; it was thought

that avoidance of these complications might prove difficult. Exposure of circulating blood to foreign surfaces appeared to induce changes in both humoral and cellular immunity. Such changes complicated efforts to avoid blood-borne infection of the TAH, or ascending infection along the drive lines.

Those associated with this initial clinical research program are to be commended on clarifying many of the problems that need to be overcome before permanent replacement of the heart by a mechanical device can become a totally successful and routine procedure.

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Long-term Cardiac Support with the HeartMate® Vented Electric Left Ventricular Assist System

T.J. MYERS AND O.H. FRAZIER

INTRODUCTION

Over the past 30 years extensive research and development efforts have been aimed at chronic cardiovascular disease, a major health problem in the United States. Although numerous advances have been made in medical and surgical therapy, as well as in preventive medicine, finding a definitive treatment for chronic end-stage heart failure continues to be a great challenge. Current therapies are mostly palliative, and the manifestations of heart failure progress until death. Because a cure for cardiovascular disease is not likely in the near future, the goals of therapy should be to extend life and to improve the quality of life. To achieve these goals the therapy must be reliable, cost-effective, relatively easy to implement and maintain, and able to provide a near physiologic level of support.

Many surgical therapies for end-stage heart failure have evolved over the past 40 years. Heart transplantation and mechanical circulatory support systems have become fairly common therapeutic modalities, but each has its limitations. Other therapies, such as cardiomyoplasty[1] and cardiac xenotransplantation[2], still require considerable refinement before they can be commonly used. In the United States alone it is estimated that nearly 60 000 people each year could benefit from some form of cardiac replacement or long-term circulatory support[3]. Heart transplantation has become a good therapeutic option for patients with end-stage heart failure, but its effectiveness is limited by the number of donor hearts available. A variety of mechanical circulatory support systems are being developed and tested, but their use is generally confined to relatively short periods. These devices require refinement and continued clinical testing before they can become a reasonable long-term therapeutic alternative.

Research into artificial heart development intensified in the 1960s, and in 1969[4] a total artificial heart was used clinically as a bridge to heart transplantation. Clinical experiences during the 1980s with both total artificial hearts and left ventricular assist systems demonstrated that these devices were useful as a bridge to heart transplantation[5,6]. Although the use of total artificial hearts has declined in recent years, various left ventricular assist systems are being used clinically with increasing frequency throughout the world. Total artificial hearts and left ventricular assist systems have been used almost exclusively as short-term bridges to heart transplantation; there have been few attempts at long-term use. When the total artificial heart was used as a permanent system it provided sufficient circulatory support; however, its limitation on mobility and the eventual thromboembolic and infectious complications led to the abandonment of this program.

The HeartMate® left ventricular assist systems (Thermo Cardiosystems Inc., Woburn, MA) were designed during an era when heart transplantation was not yet a clinical reality. The device was conceived for long-term use and not as a bridge to heart transplantation. The present HeartMate® system is the product of more than 20 years of research and development at Thermo Cardiosystems Inc., its parent corporations, Thermedics and Thermo Electron, and the Texas Heart Institute. The HeartMate® implantable pneumatic left ventricular assist system (IP-LVAS) and vented electric left ventricular assist system (VE-LVAS) have undergone extensive clinical evaluation as bridges to transplantation during the past 10 years. The HeartMate® systems were tested clinically in this capacity (that is, as bridging devices), because there was a great need for mechanical circulatory systems in patients awaiting transplantation and because a close relationship existed between the transplant population and the target population for the HeartMate®; that is, patients with chronic heart failure who were not candidates for transplantation.

The clinical research to date with both the electrically and the pneumatically powered HeartMate® systems has demonstrated that these devices are useful as a bridge to heart transplantation[7,8], and that they may be employed as a therapy for chronic heart failure patients[9]. For example, patients who have end-stage heart failure but are not candidates for heart transplantation may benefit from long-term mechanical circulatory support as an alternative to conventional medical therapy. Also, in a selected group of patients, long-term left ventricular support may lead to recovery of a substantial degree of native cardiac function, thereby allowing for removal of the left ventricular assist system. The clinical evaluation of long-term support with the left ventricular assist system is a vital next step in mechanical circulatory support research. Because we cannot reasonably expect a cure for heart failure, or a

solution to the disparity between the number of available donor hearts and the number of waiting recipients, there is a profound need to pursue long-term mechanical circulatory support. The experience and knowledge gained by using the HeartMate® left ventricular assist system as a bridge to transplantation have validated the concept of possible long-term or permanent implantation. The experience to date in the improvement of native left ventricular function following long-term implantation has also given hope that long-term rest of the ventricle in a heart failure patient might enable complete recovery of ventricular function.

DESIGN AND DEVELOPMENT OF THE VE-LVAS

Early in the development of the HeartMate® left ventricular assist systems it was determined that a long-term device must be reliable, implantable, easy to operate, and constructed of biocompatible materials[10]. An implantable system would reduce infectious complications and enhance the patient's mobility. Ease of operation was vital because patients would be expected to operate and maintain the system with little assistance from medical or engineering personnel. Biocompatibility was essential to prevent thromboembolism and keep anticoagulant therapy minimal. These design requirements were fully incorporated into the current VE-LVAS and have been demonstrated to be clinically acceptable.

Two important backup characteristics are also incorporated into the design of the VE-LVAS. Because the native heart remains in place it can function as a backup pump in most cases in which pump malfunction might occur. Also, a vent is externalized from the motor chamber and functions primarily to equilibrate air pressure as the pumping diaphragm moves; in the event of power loss or motor failure, however, the vent provides a means by which the pump can be actuated pneumatically. With the native heart as a backup pump, and the vent readily available for pneumatic pumping, the VE-LVAS can be used safely outside the hospital.

The relatively uncomplicated design of the internal and external components make the system both durable and easy to operate. The simplicity of the electromechanical actuator results in a high degree of reliability[10]. There are few moveable parts, and the motor functions at physiologic rates. Extensive *in-vitro* testing of the blood pump and electromechanical driver has demonstrated that the VE-LVAS implantable components are highly reliable for extended periods. The external system components, which include a system controller and a 12-volt direct current (DC) power source, are of durable construction and can be readily exchanged if necessary. Routine maintenance of the system does not require special equipment or tools.

The blood/biomaterial interface is made biocompatible through a counterintuitive approach to thromboembolism[11,12]. All blood-contacting surfaces within the HeartMate® blood pump (except the porcine valves) are textured both to attract circulating blood cells and to promote their adhesion to the surface. A thin, uniform and well-adhered tissue lining is established throughout the blood pump and is maintained during support[12,13]. This biologic lining resists thrombus development and bacterial colonization. Consequently, patients can be supported with the HeartMate® for extended periods using minimal anticoagulant therapy and with a low risk of thromboembolism and device-related infection. The biocompatibility of the system is enhanced by the blood flow characteristics within the pump and by the materials of which it is made. The blood pump and actuator are placed within the abdomen or preperitoneal space; this placement avoids the problems associated with thoracic implantation, namely, space limitations and infections[14]. The implanted pump components are relatively easy to place[15,16] and are accommodated well by patients[17]. A thick fibrovascular capsule that develops around the pump when placed in the abdomen[18] is believed to prevent migration of the device and to resist infection within the pump pocket.

DESCRIPTION OF THE VE-LVAS

The VE-LVAS (Figure 1) provides pulsatile blood flow by means of a flexing polyurethane diaphragm and a pusher-plate[19]. An electromechanical actuator beneath the diaphragm provides the power to move the diaphragm. The main body of the blood pump weighs approximately 980 g, measures 11.2 cm in diameter, and is 4 cm thick. The metallic components of the pump are fabricated of a titanium alloy containing 6% aluminum and 4% vanadium. The inlet and outlet conduits each contain 25-mm porcine valves within a woven polyester Dacron graft and a titanium cage. A 19-mm textured inflow tube, which is located in the left ventricle, is attached to the proximal end of the inflow valve conduit. A 20-mm woven polyester Dacron graft is attached to the distal end of the outflow valve conduit, and the opposite end is anastomosed to the ascending aorta. A double-lumen percutaneous tube from the motor chamber contains an air vent and a set of electric wires.

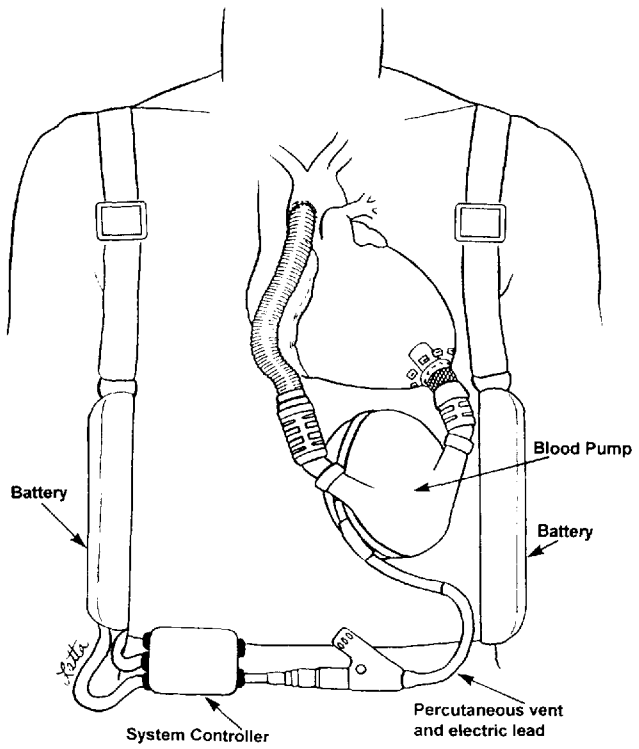

Figure 1 Major components of the HeartMate® vented electric left ventricular assist system include the implanted blood pump, the percutaneous vent and electric leads, the system controller, and a pair of batteries

The total blood volume of the pump is 218 ml with a maximal stroke volume of 83 ml.

The actuator consists of an electronically commutated, low-speed, torque motor and a pair of helical face cams that are attached to the pusher-plate (Figure 2). The motor causes the rotation of two diametrically opposed ball bearings that push against the helical face cams. Lubricants are not required for these internal moveable parts. The rise of the helical cams results in the conversion of rotary motion to linear motion. The motor operates at physiologic speeds, and one rotation of the motor causes one stroke of the pump. When the motor receives a start signal the rotor completes one revolution and then stops. The rotor remains in a stop position, or standby phase, until another start signal is received. Passive pump-filling occurs during the standby phase because the motor and the pusher plate are not attached to one another. The fact that these two components of the pump are not attached is an important design feature, because it allows backup pneumatic actuation in the case of motor or power failure. The external noise of the motor is minimal; air movement through the vent can be heard in a very quiet environment. Patients and family members rarely complain of the noise created by the device.

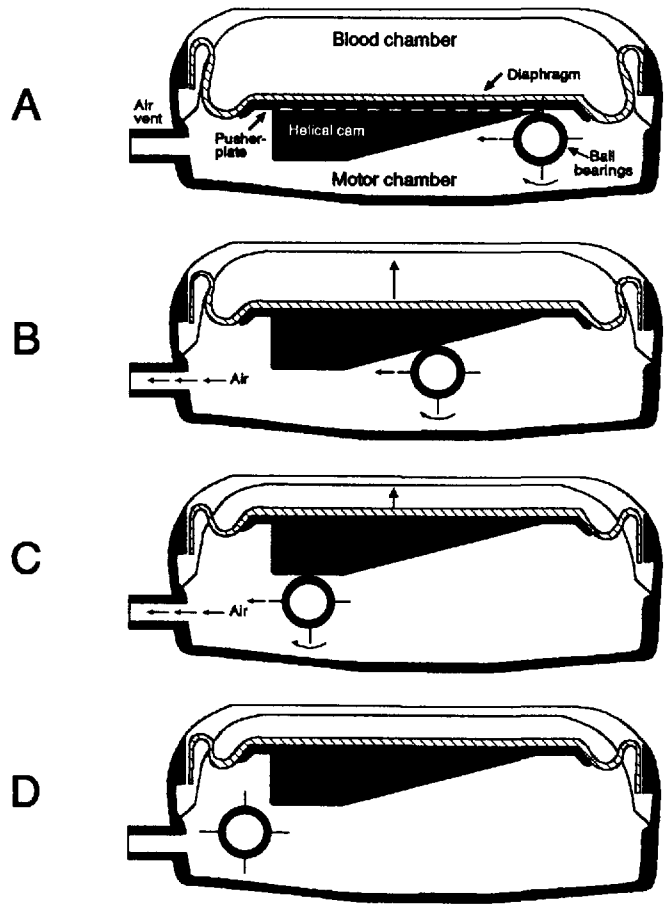

Figure 2 A cross-sectional schematic of the HeartMate® vented electric left ventricular assist system. The blood chamber is separated by a flexing polyurethane diaphragm that adheres to a pusher plate attached to helical cams. Part A represents the standby position of the rotor and ball bearings. In parts B and C the ball bearings push against the helical cams on the pusher plate, causing the diaphragm to move upward. Part D represents the end of ejection and the beginning of the filling phase

The microprocessor-based system controller is the central control and monitoring unit for the VE-LVAS. On one end the system controller is connected to the implanted motor by a series of wires within the externalized percutaneous cable. On the other end there is a dual 12-volt DC power connection that allows the power supply to be exchanged without interrupting pump function. The system controller measures 8.5 × 8 × 2 cm and is normally worn on the patient's belt or in a pouch on a waist belt (Figure 1). This unit controls the mode of operation and monitors the function of the motor. A button on the system controller allows the operator to toggle between a fixed rate and automatic mode. The unit has LED displays and an audible alarm to alert the operator of battery voltage level, motor malfunction, or broken electrical leads.

Power to the implanted motor is provided via the system controller from either a pair of lead-acid-gel cell batteries or a 20-foot power cable. A table-top power base unit functions as a battery charger, an interface between a system monitor and the system controller, and a continuous 12-volt power supply from a 120-volt alternating current (AC) source. Up to six batteries can be charged while power is supplied via the power cable or another pair of batteries. While the batteries are charging they are monitored for the proper charge and voltage maintenance. A pair of batteries can provide up to 8 hours of continuous operation of the system. The actual length of time that a pair of batteries will last depends largely on the pumping rate – that is, the amount of work the motor is required to perform. During periods of rest, patients normally use the cable power source while recharging all batteries. The power cable also provides a communication link between the system controller and a system monitor through the power base unit.

A system monitor is used during implantation, in the intensive care unit, or at any other time that pump operation must be monitored. The system monitor continuously displays the pump operating parameters (mode of operation, pump rate, stroke volume, and total pump flow), and an operator may change parameters using the touch-screen menu. As appropriate, messages will be displayed to alert the operator of any pump malfunctions or low pump flow. The system monitor also enables pump performance data to be stored on a computer disk or sent to a remote computer via modem.

PATIENT SELECTION

As of July 1995 the VE-LVAS has been used only as a bridge to heart transplantation. In the clinical trials in the United States all patients must be active candidates for heart transplantation and meet a set of specific inclusion criteria (Table 1). These criteria identify patients with severe end-stage heart failure who are receiving maximal medical therapy and mechanical support with an intra-aortic balloon pump. Other criteria include the expected length of the waiting period for a donor organ and the patient's risk of sudden death. Because donor organs are especially scarce for patients with an O-positive blood type who weigh more than

Table 1 Patient inclusion criteria for the HeartMate® vented electric left ventricular assist system clinical trials

Approved candidate for heart transplantation
Circulation supported by IABP and inotropic drugs
LAP or PCWP ≥ 20 mmHg and
Cardiac index ≤ 2.0 l min^{-1} m^{-2} or
Systolic blood pressure ≤ 80 mmHg

IABP, intra-aortic balloon pump; LAP, left atrial pressure; PCWP, pulmonary capillary wedge pressure

Table 2 Patient exclusion criteria for the HeartMate® vented electric left ventricular assist system clinical trials

Body surface area < 1.5 m^2
Age > 70 years
Chronic, irreversible renal, hepatic, or pulmonary disease
Pulmonary infarction or hypertension
Right ventricular failure with an ejection fraction < 10%
Intractable ventricular tachycardia or fibrillation
Cerebral vascular disease with stroke and transient ischemic attack
Unresolved malignancy
Positive HIV
Severe blood dyscrasia
Long-term high-dose steroid therapy

200 lb, such a patient who is critically ill should be promptly considered for VE-LVAS implantation. Likewise, a patient who meets the inclusion criteria and has a serious uncontrolled arrhythmia should immediately be evaluated for implantation. A separate set of exclusion criteria (Table 2) are also used with potential VE-LVAS candidates. The exclusion criteria are intended to standardize the study population and to maximize the probability for long-term survival. The inclusion and exclusion criteria in the current protocol reduce the number of patients in the study; they may also limit the number of patients who might benefit from this technology.

The timing of the LVAS implantation is important but difficult to define. Most chronic heart failure patients have some degree of multiple organ dysfunction when they are being considered for VE-LVAS implantation. The duration and severity of multiple organ dysfunction must be taken into account because deteriorating renal, hepatic and pulmonary function must be reversible if the patient is to survive beyond the short term. Most patients with mild (and some with severe) end-organ dysfunction will recover when normal circulation is resumed. Some patients, however, despite the resumption of normal circulation, continue to deteriorate into severe, non-reversible, multiple organ failure resulting in death. Improved patient selection should minimize this outcome, as it appears to be related to the overall clinical status of the patient at implantation.

Right heart function is an important consideration in determining whether to implant a VE-LVAS. Most cardiomyopathy patients have some degree of right heart dysfunction. This can usually be treated medically during the short period of accentuated right-sided dysfunction following implantation. A patient with clinical right heart failure and an elevated pulmonary vascular resistance is considered to be at high risk for problems following implantation of an LVAS. Preoperative and intraoperative pharmacologic control of the pulmonary vascular resistance is critical in preventing right heart failure and the need for right heart mechanical support. The prognosis for patients who require right heart mechanical support after having been implanted with a left ventricular assist system is generally poor.

IMPLANTATION OF THE VE-LVAS

The technique for implanting the VE-LVAS is similar to that for implanting the IP-LVAS, which has been previously described in detail[16,17]. After complete cardiopulmonary bypass is established, the apex of the left ventricle is cored with a circular knife. A Silastic and Teflon sewing ring is attached to the opening in the left ventricular apex with 12 pledgeted interrupted sutures. The main body of the pump is placed in the left upper quadrant of the abdomen, either in a preperitoneal pocket or within the abdominal cavity. The inflow tube is passed through the diaphragm and inserted into the left ventricle which is then secured by tying a heavy ligature around the sewing ring and inflow tube. The outflow graft is anastomosed to the ascending aorta and then attached to the outflow valve assembly. Finally, the percutaneous lead is externalized through the left anterior abdominal wall, just above the iliac crest.

Once the entire pump assembly is in place, and all connections are complete, a gradual transition from cardiopulmonary bypass to VE-LVAS support is begun. The aortic crossclamp is removed and the heart is allowed to resume beating. Inotropic support and pulmonary vasodilators are given as necessary. After the heart beat has been restored, and while the distal end of the outflow graft is crossclamped, the system is de-aired by placing a 19-gauge needle in the proximal end of the outflow graft while blood flows through the blood pump. As cardiopulmonary bypass is reduced, blood volume is returned to the patient. After cardiac function is stabilized and de-airing is complete, the crossclamp is removed from the outflow graft and slow, manual, pneumatic pump actuation is begun. Finally, cardiopulmonary bypass is terminated and electrical actuation of the pump is begun in the fixed rate mode at a minimum rate of 50 beats per minute. The automatic mode is selected after it has been determined that there is an adequate pump preload. Successful implementation of the VE-LVAS is enhanced with adequate support of right heart function, maintenance of a low pulmonary vascular resistance, and control of bleeding. During the intraoperative and immediate postoperative periods an appropriate intravascular blood volume must be maintained using replacement blood products as necessary. Surgical bleeding sites are meticulously controlled, followed by routine closure of the chest and abdomen.

OPERATION OF THE VE-LVAS

The VE-LVAS has a stroke volume capacity of 83 ml and a maximum pump output of approximately 10 l/min. The pump rate can be varied from 50 to 120 strokes/min. The system may be operated in one of two modes: fixed rate or automatic. In the fixed rate mode the operator sets the pump rate, and the stroke volume varies depending on the pump preload from the left heart. The fixed rate mode is used during the intraoperative pump start-up or at any time when a limited pump flow is desired. The microprocessor-controlled automatic mode functions by adjusting the

pump rate to achieve an average stroke volume that is approximately 95% of capacity. As pump filling increases (as, for example, during exercise), the pump rate and total flow increase. Conversely, as pump filling decreases, the pump rate decreases to maintain the 95% stroke volume. The automatic mode is the setting used most often, because it provides maximal cardiac support and responds to the patient's physiologic demand for blood flow.

Once the patient is stabilized intraoperatively the system operator only rarely needs to change a pump setting while the system remains in operation. Mode selection and a fixed rate setting are the only pump functions that can be adjusted.

The system controller continuously evaluates the function of the implanted pump and the external power supply. Audible and visual alarms alert medical personnel or the patient to conditions of low voltage, low blood flow, or a broken electrical lead. A low battery voltage has three levels of alert. At the first level an intermittent beep and an indicator light inform the operator that the batteries need to be exchanged. If the batteries are not exchanged the second level of alert is triggered: the beeps increase in frequency. At the third level of alert the beeps again increase in frequency, and the pump rate slows to 50 strokes/min to conserve power. The low blood flow alarm has a fixed threshold of 1.5 l/min. When the pump flow rate falls below that threshold a continuous beep occurs, and a red light on the controller is illuminated. The low flow condition is the equivalent of the pump being off as a result of malfunction. If this critical condition occurs, an internal clock displays the accumulated time of low flow (or no flow) on the system monitor. If an electrical lead breaks, a yellow light on the controller is illuminated at each stroke of the pump, and an intermittent beep sounds every 30 seconds.

An alarm system also functions when the pump is being powered from the power base unit via the power cable (rather than by batteries). Redundant audible alarms will sound from both the system controller and the power base unit.

The VE-LVAS can be used conveniently in either the hospital or a patient's home. In the intensive-care unit a patient's VE-LVAS is connected to the power base unit and system monitor via the power cable. This power source enables pump function to be monitored continuously, and system changes may be made readily. After leaving the hospital a patient (and his or her family) must maintain only a few pieces of equipment: a power base unit, a set of six batteries, an emergency power supply, and an emergency response kit. The emergency power supply is a single-use pair of batteries that will provide up to 48 hours of power. This backup battery supply is in addition to the 24 hours of power from the set of six batteries used routinely. A patient will normally have up to 72 hours of battery operation available should a power failure occur at home. The emergency response kit is a small camera bag that contains four spare batteries, a pneumatic hand pump, a spare system controller, and an identification card. Because of its reserve batteries the emergency response kit enables a patient to be away from home for many hours at a time. Because the VE-LVAS home equipment is easy to operate, patients can resume a relatively normal lifestyle.

Patients and family or friends are trained to maintain and operate all of the external system components. During the training sessions, emphasis is placed on recognizing problems and knowing the emergency backup measures. All trainees practice pneumatic pumping with the hand pump, making system controller changes, and exchanging power supplies. Patients and their companions are also taught appropriate communications and emergency transport measures.

CLINICAL EXPERIENCE WITH THE HEARTMATE® SYSTEMS

The IP-LVAS and the VE-LVAS have both been undergoing clinical trials approved by the United States Food and Drug Administration (FDA). Trials of the pneumatic system began in 1986, followed by trials of the vented electric system in 1991. All LVAS implantations in both trials have been in patients awaiting subsequent heart transplantation. The FDA approved the IP-LVAS for commercial use as a bridge to transplantation in October 1994, after determining that it was safe and effective when used for its intended purpose. Continuing clinical trials of the VE-LVAS will probably also lead to an application to the FDA for commercial approval of the vented electric system as a bridge to heart transplantation. In addition, clinical protocols currently being developed in both the United States and Europe are intended to study the use of the VE-LVAS as an alternative to heart transplantation and conventional medical therapy.

Although more patients have received an IP-LVAS than a VE-LVAS, the clinical results of the two HeartMate® systems are similar. The primary difference between the two systems is the method of pump actuation, but both systems are implanted and function almost identically. The IP-LVAS requires an external control console that generates pulses of air to move the flexing diaphragm. The implanted electromechanical actuator of the VE-LVAS makes this system more portable. The portability of the VE-LVAS enables patients to resume relatively normal physical activities, and it is this characteristic that enables patients to live for extended periods outside the hospital.

Between January 1986 and July 1995 the IP-LVAS was implanted in 422 patients worldwide. In the FDA-approved clinical study that was conducted in the United States the IP-LVAS was evaluated in 116 heart transplant candidates[20]. This study included 46 retrospective control patients who met the criteria for an IP-LVAS but in whom the device was not implanted. The primary end-point of this study was a comparison of survival rates 60 days after heart transplantation. The IP-LVAS group had a survival rate of 65%, whereas the control group had a survival rate of 30%. In addition, many more IP-LVAS patients (71%) than control patients (36%) survived to undergo heart transplantation. We believe that the higher survival rate in the IP-LVAS group is partly a result of the physiologic rehabilitation that results from circulatory support with the LVAS. Throughout the duration of support the average pump flow index (pump flow [in l/min] divided by body surface area [in m^2]) was 50% greater than the pre-implant cardiac index. The greatly improved hemodynamics often normalized end-organ function, and such patients could resume physical activity. Many patients participated in exercise programs while their circulation was supported by the IP-LVAS, and this physical conditioning enabled them to undergo heart transplantation in a normal physiological condition.

Complication rates in the 116 study patients were acceptable. The overall device failure rate was 0.9%: one event in 116 pa-

tients. The single device failure occurred because of a loosened connector. The only moveable components that are implanted, the valves and the flexing diaphragm, have not failed or shown apparent signs of wear. The device-related thromboembolic rate was 3% during the cumulative support time of 26 patient-years.

The FDA-approved clinical trials of the VE-LVAS began at the Texas Heart Institute in January 1991[20,21]. Since that time the VE-LVAS has been implanted in 46 patients in the US and one in Europe. As of July 1995, 28 (59%) patients have been transplanted, 13 (28%) are still being supported by the VE-LVAS, and six (13%) died while being supported on the system. The average duration of support has been 100 days, with a range of 1–503 days. As with the IP-LVAS experience, most patients who survive beyond the immediate perioperative period have a significantly improved end-organ function and a greatly enhanced physical capacity. In a subgroup of nine VE-LVAS patients the hemodynamic effectiveness of VE-LVAS support was demonstrated by a 57% increase in the cardiac index and a significant reduction in the pulmonary capillary wedge pressure (Table 3). The average pump flow index over the duration of support was 2.72 l/min per square meter.

Patients who have an uncomplicated recovery from the implant operation and participate in rehabilitative programs generally feel well and have a positive emotional state. During hospitalization before the operation, patients generally become very anxious; afterwards they have a strong desire to resume as normal a life as possible. In response to that desire, VE-LVAS patients who qualify for discharge (on the basis of a patient release protocol) are allowed to leave the hospital to await a suitable donor heart. The protocol was developed to provide a controlled method for ensuring the safety of VE-LVAS patients outside the hospital[22,23]. After patients have met a set of physiological criteria, they (and the companions they have chosen) are given training in LVAS operation and emergency procedures. After completing the training, patients are allowed to leave the hospital on day trips for increasing periods. When they have successfully completed five 3-day trips they may be discharged from the hospital; they must return to the hospital weekly for brief visits. During periods away from the hospital, patients and their companions maintain records of their activity and health care. Patients may live only a limited distance from the hospital, and the patient and hospital personnel maintain close communication through pagers or cellular telephones.

Since September 1992, 21 patients have participated in various phases of the patient release program. Patients have left the hospital on 380 day-trips, and 12 patients have been discharged from the hospital for extended periods. Patients have been away from the hospital for a total of 1021 days (2.8 years) in a 3-year period. No device has failed while a patient has been away from the hospital. Minor technical problems have been resolved either by the patient or by hospital personnel during routine visits to the hospital. Because the pumps have functioned successfully while patients were away from the hospital, there has been no opportunity to evaluate emergency measures.

The preliminary experience with outpatient VE-LVAS therapy has led to two positive results. First, daily maintenance costs have been dramatically reduced in all discharged patients. In some instances patients have incurred costs as high as $5000 per day while waiting for heart transplantation in the intensive-care unit before VE-LVAS implantation. VE-LVAS patients at home have costs of less than $50 per day, because they require only minimal medical care. Patients typically take few oral medications and require only a few medical supplies. The cost of medical care is significantly reduced, and some patients have been able to resume employment (Figure 3). Second, use of the VE-LVAS for outpatient therapy has positively affected the emotional status of patients. Patients who feel well but are confined to the hospital often become depressed, and their anxiety over heart transplantation is profound. Patients who have returned to their home and work are more consistently positive about life and the possibility of transplantation. Many of the VE-LVAS outpatients are enthusiastic about participating in exercise programs, and they generally practice good health care. Frequent participation in social, work, and family activities enables patients to better tolerate the long waiting periods that are often required before a suitable donor organ becomes available. Many VE-LVAS patients become active members of organ transplant support groups and other community service programs. Because the VE-LVAS outpatients have a good attitude toward their life, and are also in good physiological condition, their heart transplantation can occur under nearly ideal circumstances.

COMMENT

Clinical experience with both HeartMate® systems has demonstrated that these devices can reliably and safely provide physiologic levels of circulatory support for extended periods. Continued research with this technology will answer many important questions. One of these questions is: Which patient populations can benefit most from this technology? The use of mechanical circulatory support systems is expanding rapidly

Table 3 Hemodynamic results in nine patients who were implanted with a HeartMate® vented electric left ventricular assist system

	Cardiac index ($l\ min^{-1}\ m^{-2}$)			*PCWP* (*mmHg*)			*Pump flow index* ($l\ min^{-1}\ m^{-2}$)	*Pump flow* ($l\ min^{-1}$)
	*Base**	*24 h*	*48 h*	*Base**	*24 h*	*48 h*		
Mean	1.77	3.11†	3.18†	26.7	11.6†	14.5†	2.72	5.47
Range	1.44–1.99	1.98–4.43	2.33–4.26	21–35	7–17	9–18	1.7–3.4	2.8–7.0
SD	0.22	0.93	0.72	4.8	4.8	4.0	0.35	0.75

* Base values are those before implantation.
† Statistically significant when compared to values before implantation.
PCWP, pulmonary capillary wedge pressure; SD, standard deviation.

Figure 3 This patient, whose circulation is supported by the HeartMate® vented electric left ventricular assist system (VE-LVAS), began day trips away from the hospital 30 days after the VE-LVAS was implanted. He returned to his work as an oil and gas trader approximately 6 weeks after implantation

throughout the world and, as a result, a vast amount of experience will accumulate in the near future. This new experience will determine the future use of the VE-LVAS and systems like it. The goals must be to explore the use of this technology in new patient populations, while refining its use as a bridge to heart transplantation. The clinical trials with the VE-LVAS as a bridge to heart transplantation are currently in the early stages; however, if we extrapolate from the experience with the IP-LVAS, the VE-LVAS, too, is likely to be judged a safe and reliable device. The only functional difference between the two systems is portability. The need for a safe, reliable bridge device may possibly be met by both HeartMate® devices. We also anticipate that these devices may someday be used long-term as an alternative to transplantation in selected patients.

The portability, safety and effectiveness of the HeartMate® systems have transformed the physiologic condition of chronic heart failure patients[24,25]. It has been well documented that the ability to undergo physical rehabilitation during mechanical circulatory support positively affects outcome over the long term[7,20,26,27]. Patients often recover some degree of native heart function during chronic circulatory support by a left ventricular assist system[28,29]. Patients who are in good physical condition, who have a reliable circulatory support device, and whose native heart functions well enough to provide a backup to the mechanical system can safely and reasonably be discharged from the hospital[22,26,30,31].

Patients in whom the VE-LVAS has been implanted have resumed nearly normal lives while waiting for heart transplantation. By returning to their home, patients can participate in most usual activities with their families. They have returned to work, traveled across the United States by airplane, attended social events, and resumed other normal physical activities, including lawn mowing, basketball, and sexual intercourse. Patients who faced imminent death have returned to their home and work within 6 weeks after implantation of the VE-LVAS.

As a result of their illness many patients believe that they are a burden to their family. Unfortunately this is so in many families, especially when financial hardship occurs and caring for routine family matters becomes almost impossible for the healthy spouse. Therefore, a patient's ability to resume his contributions to the family's well-being is important to maintain himself in a reasonable emotional state. VE-LVAS support greatly enhances the quality of life for many patients, which in turn enhances their outcome after transplantation.

Another important question that VE-LVAS research will answer is: How cost-effective is such technology? All mechanical circulatory support systems and their implementation are costly; however, the total cost of this treatment is unknown. Potential cost savings exist in the use of the VE-LVAS both as a bridge to transplantation and as an alternative to transplantation. Most patients who receive mechanical circulatory support therapy are critically ill before implantation and are incurring daily hospital charges of $2000–5000. Early reports on costs for outpatients implanted with a left ventricular assist system (as a bridge to heart transplantation) indicate that the daily maintenance cost is significantly reduced when patients leave the hospital[26,32]. Patients living at home with VE-LVAS circulatory support generally take few medications, require few medical supplies and, most importantly, do not require attendance by specially trained personnel. If VE-LVAS therapy is used as an alternative to transplantation the potential cost savings may occur through a reduction in the number of medications and in the frequency of hospital and clinic admissions. The current high maintenance cost of heart failure patients may be reduced simply because fewer medical problems would occur during VE-LVAS circulatory support.

Long-term mechanical circulatory support systems also have the potential for cost savings to our society as a whole. Most chronic heart failure patients are unable to work; therefore they can only consume medical financial resources. Patients who return to work, either while their circulation is supported by a mechanical system or after transplantation, become contributors to the financial system by resuming the payment of taxes and insurance premiums. If such patients can be converted from consumers to contributors this phenomenon will have a profound positive effect on the overall cost of caring for heart failure patients. Long-term mechanical circulatory support systems have the potential for being an investment with a reasonable return[33].

The morbidity and mortality associated with cardiovascular disease continue to be a major problem in the United States and in other industrialized nations. Because our population is aging, and the incidence of cardiovascular disease remains fairly constant, the number of heart failure patients requiring cardiac replacement is likely to grow in the coming years. The current 5-year mortality rate for heart failure patients is as high as 75%[34], and little hope exists that the number of human heart donors will increase. The potential solutions to this problem include transplantation of transgenic hearts, xenotransplantation, cardiomyoplasty, reliable long-term mechanical circulatory support systems, and prevention. Of these solutions, it appears that mechanical circulatory support systems are currently the most reasonable. Mechanical circulatory support systems are reliable, are cost-effective, and can be manufactured in the numbers necessary to make them readily accessible worldwide.

Recent clinical experience with various mechanical circulatory support systems has created excitement and optimism, because it appears that such technology might positively affect the survival and quality of life for millions of people in the coming years. However, numerous challenges must be met before mechanical circulatory support systems are in widespread use. The current economic, regulatory, and acceptance barriers must be overcome so that mechanical circulatory systems may be used appropriately[35]. Research into, practice with, and education about mechanical circulatory support systems should continue to expand in hopes of extending the lives (with concomitant quality) of millions of heart failure patients worldwide. The HeartMate® systems now offer great hope for many relatively young people who in the recent past had no hope of returning to a healthy life.

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Temporary Support of the Lungs – the Artificial Lung

W. FEDERSPIEL, P. SAWZIK, H. BOROVETZ, G.D. REEDER AND B.G. HATTLER

INTRODUCTION

The adult respiratory distress syndrome (ARDS), described first by Ashbaugh and his colleagues[1] almost 30 years ago, continues to present a dilemma to the clinician, for the mortality associated with the syndrome has remained exceedingly high in spite of a better understanding of the mechanisms that contribute to this pulmonary disease process. For the patient with ARDS, maintaining gas exchange at a level consistent with survival is associated frequently with progressively increasing levels of respiratory support. The final outcome in these patients is contributed to not only by the degree of pulmonary injury, but also by the level of dysfunction in multiple other organs[2]. Recognizing these facts, and that any therapy for ARDS involves more than just providing a setting where the lungs have the potential for recovery, we will concentrate this discussion on new therapies with intracorporeal devices under development for the treatment of ARDS.

Intracorporeal devices for oxygenating venous blood are based on well-established bioengineering principles that will be reviewed to allow a better understanding of the theory behind this form of respiratory support. Although the information is largely experimental, progress in this field has been dramatic over the past 10 years. There is therefore every reason to believe that a device will be available within the next 5 years, that, when implanted in the body, will provide clinically significant support while the lungs themselves recover from a spectrum of injuries. Once temporary support is a clinical reality, significant progress will already have been made towards a more permanent implantable device.

BACKGROUND

During the early twentieth century, names that are legendary to medicine and surgery advanced the development of artificial support for the lungs[3–8]. Sauerbruch initiated work that was eventually utilized in the development of the cuirass, and later the tank ventilator. Drinker's refinement and advancement of these concepts led to the tank ventilator in which patients with poliomyelitis were treated at the Peter Bent Brigham Hospital in Boston. The modern area of artificial ventilation was furthered by Frenckner and Crafoord[5,6], who perfected a compressor-powered volume generator to either assist or control ventilation. The administration of predetermined volumes – rather than pressures – was advanced by Engstrom with his ventilator designs. The possible advantages of negative pressure during expiration were delineated by Maloney and Whittenberger[9,10]. The classic work of Cournand and Werko demonstrated the effect of intermittent positive-pressure ventilation on the circulation, and recommended the use of artificial ventilation for a wide range of medical and surgical conditions[11,12]. The continuing design, development and practical applications of ventilators for respiratory support have supplanted many of the devices in use over the past 40 years which have been largely replaced by volume-cycled, positive-pressure ventilators in conjunction with cuffed endotracheal tubes.

The acute trauma sometimes associated with the institution of mechanical ventilation was recognized by these early twentieth-century innovators. However, these were the consequences of over-zealous efforts which sometimes led to ruptured alveoli, pneumothoraces, and death. Many of the more subtle findings of respiratory barotrauma and pulmonary damage were not recognized, and did not become apparent until the early 1960s with the establishment of the first pulmonary intensive-care units[13]. An understanding of the role of oxygen in health and disease also had to await the introduction of methods for delivery of high concentrations of oxygen in clinical situations. The value of supplemental oxygen in the treatment of hypoxemia is widely recognized. Oxygen in high concentration, however, is toxic and can lead to progressive respiratory failure and death. Damage to the lungs is most pronounced when oxygen is delivered at high partial pressures, where oxygen can act as a biochemical reactant with the generation of cytotoxic byproducts. Among these, oxygen free radicals have recently attracted intense interest. Thus, oxygen has the potential to damage any and all cells of the lung, with the alveolar capillary membrane being particularly sensitive to this effect[14–16].

Clinically, one sees a picture of non-cardiogenic pulmonary edema, which has been associated with ARDS. In experimental

and clinical settings a return to reduced inspired oxygen concentrations and eventual air breathing before a lethal stage is reached can result in healing, survival, and avoidance, in the later stages, of pulmonary fibrosis. In addition, newer forms of respiratory care, such as positive-pressure controlled inverse ratio ventilation, have improved the outlook for patients with severe respiratory failure[17]. Patients who survive ARDS and its treatment do well, and resume normal levels of activity.

With prolonged mechanical ventilation and high partial pressures of oxygen, sequential changes in the lungs of primates and experimental animals have been well documented. Initial damage occurs at the epithelial and endothelial cell level with mitochondrial swelling, cytoplasmic disruption, and nuclear degeneration.

Platelet plugging and polymorphonuclear leukocyte infiltration become increasingly prominent – as does alveolar edema. This provides a framework for continuing cellular damage, increased capillary–membrane fluid permeability, and bacterial invasion. Pulmonary microthrombosis and vasoconstriction raise pulmonary artery pressures. Decreased ciliary action and tracheobronchial particle clearance can be observed as early as 6–12 hours after 100% oxygen breathing. This is followed at 24 hours by depressed protein synthesis and altered endothelial function. By 48 hours alveolar edema and surfactant inactivation are pronounced, and by 60 hours ARDS is well established[15,16,18,19]. Thus the pathologic stages of ARDS can be reproduced experimentally by the overzealous use of the very therapy intended for its treatment[20,21].

In the human, making a clear differentiation of the various phases of pulmonary deterioration is difficult, because the underlying lung involvement already present with ARDS leads to the institution of mechanical ventilation and oxygen therapy. However, it does appear that changes similar to those in experimental animals occur in humans. Available studies of humans indicate a similar pathology to that observed in animals. In the absence of any specific biochemical tests of practical use to diagnose the threat of impending ARDS, the clinician must rely on patient evaluation. Clinically, the findings in humans have been well documented and include a progressive pulmonary interstitial edema, decreased pulmonary compliance, pulmonary vasoconstriction and hypertension, decreased diffusion capacity and a widened alveolar–arterial gradient, all leading to respiratory failure and death unless the cycle can be broken and healing allowed to occur[22–24].

In clinical practice a wide variety of factors and mechanisms contribute to the development of ARDS. Among these are severe trauma and its associated multiple transfusions, septicemias of various etiologies, aspiration, and drug overdoses. The incidence of inciting causes in any published report may be important in evaluating results since trauma as a cause of ARDS has been reported to have a higher rate of survival[25]. Thus, a heterogeneous group of disorders results in damage to the lung parenchyma, the pulmonary capillaries, and the epithelium. Young and healthy individuals, as well as those in various chronic disease states, may be equally susceptible. This phenomenon, most frequently encountered in intensive-care units, has an incidence of over 150 000 cases per year in the United States, with a fatality rate of over 50%[26,27]. The reported incidence of ARDS, however, can vary according to the criteria used to define it[84]. Even in settings where ARDS is not a primary consideration, hospital-acquired pneumonia has been reported in 10–70% of patients requiring prolonged mechanical ventilation, where lower airway colonization and infection can occur[29–32,84]. The fatality rate in these nosocomial pneumonias is often as high as 60%. Further, they pose a great expense to hospitals and insurance carriers because of the prolonged length of stay and cost of treatment[33,34]. Although many of these patients are chronically ill and demonstrate multiple factors that contribute to their deteriorating pulmonary status, it is the act of mechanical ventilation and oxygenation itself that, while initially sustaining the patient, can eventually become a major factor in the further loss of pulmonary function and create a downhill course leading to a fatal outcome.

These often-disappointing results with the use of ventilators and oxygen therapy provided the experimental and clinical impetus for development of other forms of support. Membrane oxygenators and improved devices for circulatory assistance were now available, and culminated in the first success for extracorporeal membrane oxygenation (ECMO)* in a patient with acute respiratory failure following severe trauma[35]. A review of worldwide experience (1972–1975) with ECMO, however, reported a disappointing 15% success rate[36]. The need for a systematic evaluation of this new form of therapy led to a multi-center randomized trial from 1975 to 1977, sponsored by the National Institutes of Health (NIH). Results of the NIH study demonstrated 90% mortality in patients managed with best-case ventilatory support, and a similar mortality in patients receiving joint ECMO and mechanical ventilation[37,38].

As Kolobow has pointed out, however, a careful reading of the published report indicates that 'lost in the study was the observation that the severest interstitial pulmonary edema when confined to a small part of the lung most commonly healed without mechanical ventilation. In contrast the same disease process with more wide-spread involvement so as to impair gas exchange and require mechanical ventilation had a distinctly worse prognosis – even while on ECMO'[38,39]. The indications were, therefore, that relatively unaffected lung with near-normal compliance was interspersed among diseased segments and was preferentially overventilated during respiratory support for ARDS[40,41]. The high inspiratory pressures and volumes, and the partial pressures of oxygen which were required, produced not only a worsening involvement of the diseased lung, but also rapid deterioration in those relatively unaffected areas.

The barotrauma and volotrauma associated with mechanical ventilation alone was underscored by Kolobow and his colleagues. They demonstrated that high peak inspiratory pressures (50 cmH_2O) at an F_IO_2 of 0.4 produced in normal sheep all the main variants of ARDS, with few animals surviving the 48-hour study period[42]. Similar results have been reported in healthy dogs ventilated for from 22 to 70 hours at high peak inspiratory pres-

* In more modern terminology ECMO is now included under the broader term of extracorporeal life support (ECLS), which includes both veno-arterial and veno-venous access to the patient.

sures (34 cmH_2O) with room air[43]. These studies have stressed the need for treatment strategies for ARDS that rest the lung, not only from barotrauma and volotrauma, but also from high partial pressures of oxygen.

To this effect, extracorporeal CO_2 removal ($ECCO_2R$) has been contrasted with extracorporeal membrane oxygenation (ECMO). Both therapies are included under the broader encompassing term extracorporeal life support (ECLS). Thus, adult ECMO refers to high flow (>50% of the cardiac output) veno-arterial bypass with emphasis on arterial oxygenation, whereas $ECCO_2R$ employs low flow (20–30% of the cardiac output) veno-venous bypass with emphasis on CO_2 removal. In fact both therapies provide oxygen and remove carbon dioxide. With ECMO, patients are usually supported with continuous positive-pressure ventilation and other conventional means. Low-frequency ventilation (three to four cycles per minute) and reduced peak inspiratory pressures characterize $ECCO_2R$[44]. In practice $ECCO_2R$ has resulted in improved patient survival. The juxtaposition of $ECCO_2R$ to ECMO, however, suffers from limitations in the use of any historical comparative groups, and emphasizes the importance of randomized clinical trials and concurrent controls.

Indeed, Morris, in a detailed computer-constructed approach to the care of patients with ARDS, has shown that meticulous attention to respiratory support based on a specific algorithm without the need for extracorporeal intervention can yield results similar to those with $ECCO_2R$[45], and a 42% survival rate[17]. Nevertheless, encouraged by recent reports and anticipating enhanced survival in the future, the results with $ECCO_2R$ have rekindled support for adult extracorporeal pulmonary support and a continuing enthusiasm for neonatal ECMO. Both employ low-frequency, low-volume, and low-airway-pressure ventilatory support. With respiratory management that maintains healthy lung parenchyma while allowing the remaining diffusely diseased lung to begin to heal, expected survival has increased from 10% to 80%, and from 10% to 50% in neonatal and adult respiratory disease respectively[39,44,46–49].

In over 80% of cases of ARDS, compromised pulmonary gas exchange is the primary cause of death[17,37,38]. This failure of the alveolar capillary membrane represents a major obstacle, therefore, to therapeutic approaches that rely solely on mechanical ventilation, and presents to the bioengineer a major challenge to duplicate synthetically the O_2 and CO_2 exchange that occurs in the native lung.

BASIC PRINCIPLES AND THEORY OF GAS EXCHANGE

Artificial lungs range from extracorporeal oxygenators, being currently used clinically, to intracorporeal oxygenators under development for future clinical application. The physical principles underlying gas exchange in all artificial lungs are remarkably similar. Artificial lung devices are designed to replace or augment the gas exchange function of the lungs by adding sufficient oxygen and removing carbon dioxide from blood. 'Arterializing' venous blood presents a challenging task. Sufficient gas exchange must occur to supply basal O_2 consumption (VO_2) requirements of the body and to eliminate the CO_2 produced (VCO_2) by all metabolic processes. With these criteria as guides, mean resting exchange rates required of an artificial lung designed to replace lung function would be about VO_2 = 270 ml/min and VCO_2 = 240 ml/min[50]. Furthermore, O_2 and CO_2 exchange rates can increase several-fold even for mild stress or exercise. An artificial lung designed to supplement rather than supplant lung function may need to exchange gas at rates at least one-half those of basal consumption and elimination.

That the healthy lung easily provides for basal gas exchange with sufficient reserve to meet exercise demands provides an elucidating lesson in basic gas exchange concepts. The simplest relevant paradigm to introduce these concepts is that of gas exchange across a membrane interposed between a well-mixed liquid (blood) phase and a gas phase. Here, 'membrane' refers to any medium separating the liquid and gas phases, and in which the relevant gas species (O_2 and CO_2) are soluble and can diffuse. In real lungs the 'membrane' consists predominantly of the epithelium of alveolar sacs and the endothelium of pulmonary capillaries. In artificial lungs the 'membrane' is usually the wall of a porous hollow fiber within a fiber module (ensemble). The overall rate of oxygen exchange across the membrane, VO_2, is given by[51]

$$VO_2 = K_M A\,(P_{O_{2g}} - P_{O_{2l}}) \qquad \text{(Equation 1)}$$

where $P_{O_{2g}}$ and $P_{O_{2l}}$ are the oxygen partial pressures in the gas and in the liquid, respectively, A is the total surface area of the membrane, and K_M represents the permeability of the membrane to oxygen.* The membrane permeability quantifies the ease of gas diffusion across the membrane and is given by

$$K_M = \frac{\alpha D}{\delta} \qquad \text{(Equation 2)}$$

where α and D are the solubility and diffusion coefficients of the gas within the membrane, and δ is the membrane thickness. Thus, this simple gas exchange paradigm indicates that designing an artificial lung for maximum exchange rate requires, from a geometrical design perspective, maximizing membrane area while minimizing membrane thickness.

The normal human lung possesses a remarkable geometrical design. Total membrane area is 90–95 m^2, with a mean thickness of about 1 μm or less[52]. This represents an area comparable to a tennis court, with a thickness appreciably less than that of airmail stationery. To be effective an artificial lung need not necessarily duplicate the geometrical design parameters of a real lung. Real lungs have a substantial reserve capacity for handling maximal exercise exchange rates, and current artificial lung technology can focus primarily on meeting or augmenting basal gas exchange requirements. Furthermore, an artificial lung can at least partially compensate for membrane area and thickness limitations by increasing the partial pressure difference which drives exchange (Equation 1), for example by using pure O_2 near atmospheric pressure as the gas phase. Nevertheless, a principal goal of artificial lung development is to maximize the exchange area separating gas and blood phases, while simultaneously minimizing its thickness.

*Oxygen is used as an example, but analogous relationships can be written for any gas which can diffuse across the membrane.

The earliest artificial lungs sought to maximize exchange area by bringing direct contact between blood and gas phases, e.g. as in the bubble oxygenator. While no explicit membrane between blood and gas phases existed, most of the resistance to exchange occurred in small unmixed liquid diffusional boundary layers adjacent to the gas bubbles. The thickness of these layers was minimized by increasing the intensity of gas bubbling within blood, but not without increasing overall trauma to blood, producing unacceptable levels of red cell hemolysis and protein denaturation at the blood–gas interface. Artificial lungs currently in use and under development increase exchange area and decrease membrane thickness, while avoiding direct contact between blood and gas phases, by using hollow fiber membranes. Hollow fiber membranes are simple, generally porous-walled polymer tubes, where exchange occurs by diffusion through the porous walls of the tube. The hollow fiber membrane is the basic exchange element of present-day artificial lung devices.

Hollow fiber membranes (HFM) are tubular polymer membranes with internal diameters generally less than 500 μm. An HFM can be made from nearly any polymer which is soluble in a solvent, by transforming the polymer in a controlled manner from a liquid to a solid state[53]. In the process, several HFM characteristics pertinent to gas exchange can be controlled, including the size and wall thickness of the fiber, and its wall morphology. Porous fiber membranes are the most common HFM used in artificial lung devices. These fibers have fixed submicron-sized pores that are contiguous across the fiber wall and form tortuous paths for gas diffusion. Gas exchange across the wall occurs by diffusion through the pores themselves, rather than in the polymer material. Thus the polymer material does not generally determine exchange performance and behavior as much as the characteristics of the pores themselves (e.g. pore size and tortuosity)[53].

One polymer property of importance is a fiber's hydrophobic versus hydrophilic nature. Hydrophobic hollow fibers (e.g. polytetrafluoroethylene or Teflon, and polypropylene HFM) are used in artificial lung applications because these fibers resist fluid wetting under normal transmural pressure differences. Nonwetting of fibers is important to optimal gas exchange performance, since liquid within pores would markedly reduce the rate of gas diffusion and the amount of gas species per unit pore volume. Thus, the exchange permeability of a wetted porous fiber may be several orders of magnitude smaller than that of a fiber whose pores remain gas-filled[54,55]. Even with hydrophobic hollow fibers, the membrane permeability may actually lie between the limits of gas-filled and liquid-filled pores[56]. Hydrophilic hollow fibers (e.g. polycarbonate or polysulfone HFM) are wettable, and are used predominantly for exchanging chemical species which are soluble only within liquids, and for which liquid within the membrane is required for transmural solute movement (e.g. hemodialysis and hemofiltration).

Hollow fiber membranes can also be manufactured with nonporous and/or composite wall structures[53]. Non-porous HFM have walls consisting of a homogeneous, dense polymer. These fibers are often referred to as 'true' membranes because they provide

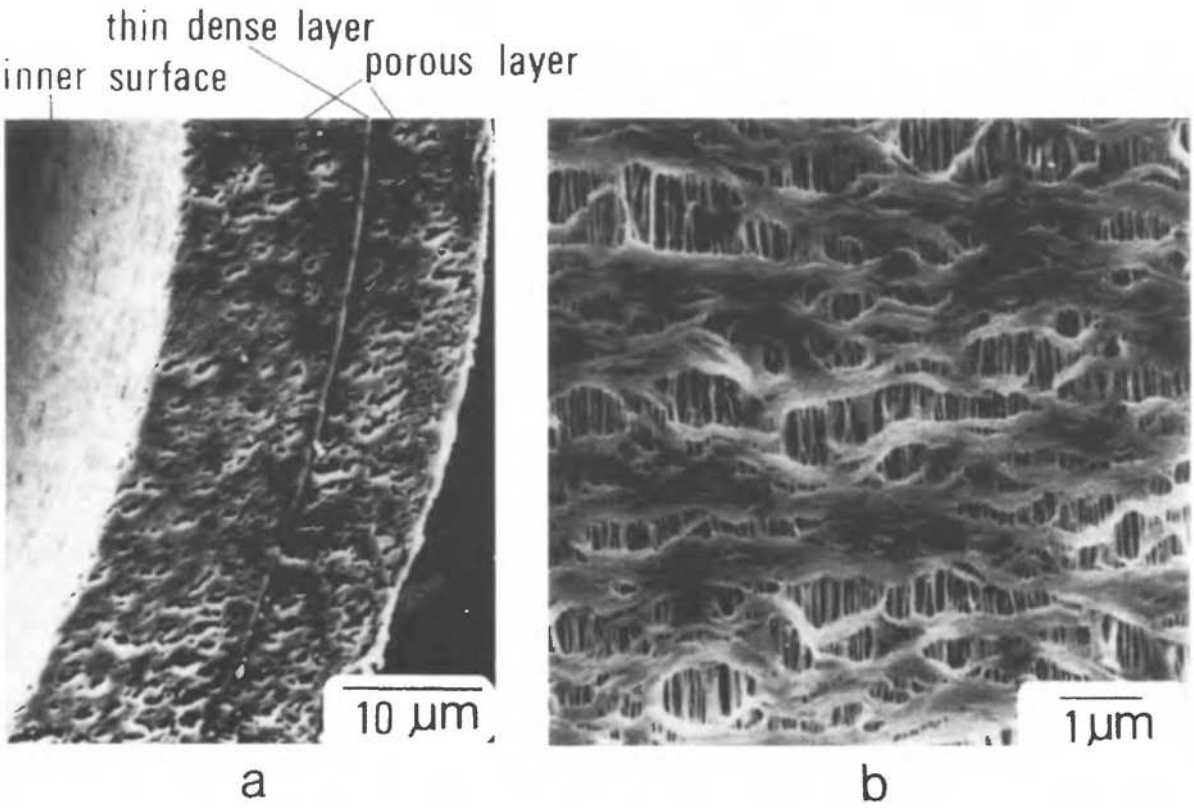

Figure 1 Electron micrographs of a composite hollow fiber membrane. (**a**) Cross-section showing porous outer and inner layers sandwiching a non-porous, true membrane. (**b**) Inner porous surface. (From ref. 57, reprinted by permission from Blackwell Science, Inc.)

absolute segregation of the liquid and gas phases. A true membrane fiber with walls thick enough for structural support would have a permeability much smaller than a porous fiber of similar size, and would not be generally suitable for an artificial lung[55]. Thus, composite hollow fiber membranes have been developed which combine a thin true membrane for liquid and gas segregation with a porous wall for structural support and optimal gas permeability. The Mitsubishi MHF composite fiber incorporates a non-porous polyurethane membrane sandwiched between two porous polypropylene layers, as shown in Figure 1[57]. Diffusion of a gas species across the non-porous polymer layer involves absorption of gas molecules on the polymer surface, diffusion through the polymer molecular matrix, and desorption of the gas species at the opposite surface. Thus, the permeability of a composite HMF is largely dictated by the permeability of the non-porous polymer layer used as the true membrane[54], and is generally smaller than the permeability of a porous membrane of comparable wall thickness[54,57].

The gas exchange capacity of any artificial lung will generally not be limited by the permeability of hollow fiber membranes. For example, measurement and theory suggest that the O_2 permeability for porous hollow fiber membranes ranges approximately from 10^{-2} to 10^{-4} ml $O_2/cm^2/s/cmHg$[54,56,57]. Placing these permeabilities in perspective, an artificial lung with 2 m^2 of membrane surface area (representative of extracorporeal oxygenators), and with a 70 mmHg average P_{O_2} difference between the gas and blood phases, could theoretically exchange from 8.4 l/min to 840 l/min of O_2, which are rates substantially larger than actually arise in extracorporeal oxygenators. The principal reason for the smaller realized exchange rates is that substantial diffusional resistance to gas exchange resides within the blood phase, in small unmixed diffusional boundary layers adjacent to the fiber wall surfaces. These liquid-side boundary layers reduce the overall exchange permeability of artificial lung devices to levels substantially below the ideal exchange capacity associated with the hollow fiber membranes themselves.

Artificial lungs employ large numbers of hollow fiber membranes in ensemble arrangements which vary with device and application. Because of the ongoing exchange processes, the partial pressures of oxygen and carbon dioxide vary along the blood and gas flow pathways. Convective mixing within blood and gas phases is not perfect; hence gas exchange involves diffusion not only through the fiber wall, but through the gas and liquid phases as well. For practical purposes diffusion through the gas phase occurs readily. The principal resistance to diffusion, therefore, is associated with the fiber membrane and the liquid (blood) phase, and the overall gas exchange permeability, K, of the artificial lung is given by

$$\frac{1}{K} = \frac{1}{K_m} + \frac{1}{K_l} \qquad \text{(Equation 3)}$$

where K_m and K_l are the permeabilities of the fiber membrane and liquid phase, respectively. The reciprocal relation arises because resistance to transport is the inverse of permeability, and the membrane and liquid phase represent diffusional resistances in series, which add directly to determine overall resistance. Thus, as indicated by Equation 3 the overall gas exchange permeability, K, is always less than the smallest of the permeabilities, K_m, and K_l. In simpler terms the smallest permeability controls overall exchange in an artificial lung.

The liquid (blood) phase permeability, K_l accounts for diffusion from the fiber surface through the imperfectly mixed liquid boundary layers which arise adjacent to the fiber surfaces. These layers always exist because providing sufficient convective mixing adjacent to the fiber surface is difficult, and even a micron-size unmixed liquid layer can reduce K_l appreciably below the fiber K_m, and dictate overall gas exchange. The liquid phase permeability depends inversely on the size of the diffusional boundary layers (see Equation 2). Thus, an effective artificial lung maximizes convective mixing within the blood phase so that the diffusional boundary layer is minimized. Nevertheless, in practice the overall artificial lung permeability is generally dictated by the liquid diffusional boundary layer, and

$$K \cong K_l \qquad \text{(Equation 4)}$$

Only under conditions of markedly reduced K_m, as would accompany fiber wetting, would oxygenator exchange performance be affected by the permeability of hollow fiber membranes.

There are two principal phenomena relative to gas exchange that relate predominantly to flow around the hollow fiber membranes. First, the gas exchange permeability increases at increasing rates of blood flow. The flow dependence arises because, all else constant, increasing blood flow velocity through the hollow fiber ensemble decreases the diffusional boundary layer thickness, and increases the effective exchange permeability. Also, changing the character of the flow from a laminar state, as occurs in the vena cava, to a more disturbed pattern conducive to secondary currents would reduce the boundary layer and increase exchange permeability. The second principal phenomenon is that the gas exchange permeability of hollow fiber ensembles is substantially greater with blood flow perpendicular (cross-flow) rather than parallel to fibers. Wickramasinghe *et al.*[58] looked at oxygen exchange in hollow fiber bundles using both perpendicular and parallel flow to the fibers. At equal values of flow per fiber surface area, the O_2 exchange permeability with cross-flow was about 22 times greater than that with parallel flow. These substantial differences can be attributed to smaller diffusional boundary layers around individual fibers with perpendicular flow.

The theoretical basis for gas exchange with an artificial lung device is, therefore, well founded and supported by extensive experimental data. Since in humans the oxygen dissociation curve predicts that in going from a P_{O_2} of 45 mmHg to a P_{O_2} of 60 mmHg one approaches 90% oxygen saturation, an increase in arterial oxygen tension of 15 mmHg from an intracorporeal artificial lung is desired, which corresponds to an oxygen transfer of approximately 127 ml/min in a 70 kg man. Through computer modeling in our laboratory[54] we have determined that a sufficient membrane surface area can be attained and housed in the inferior vena cava, right atrium and superior vena cava of humans to meet this gas exchange requirement without obstructing venous return to the heart.

INTRACORPOREAL ARTIFICIAL LUNGS

More recent reports of mortality with extracorporeal lung support in the adult with respiratory failure continue to be excessive at the 50% level[59–61]. Complications with this therapy are significant, with over 80% of patients experiencing bleeding and

requiring transfusions[61]. Mechanically related malfunction, secondary to circuit clotting and loss of gas exchange usually following severe plasma leakage within the membrane fibers, is a constant concern. In the early 1980s work was begun utilizing a new approach to the treatment of reversible pulmonary failure. Here the oxygenator would be introduced into the body without the need for any mechanical support to circulate the blood.

This approach was founded on the original experiments of Bodell[62], who in 1965 designed an implantable artificial lung – not as a lung replacement, but as a 'ventilatory booster'. The device was constructed of silicone rubber capillary tubing, housed within a Teflon graft connecting the pulmonary artery and left atrium. Pulsatile flow passed through the graft and around the capillary tubing. The ends of the capillary tubing passed through the graft at sites which were sealed by an adhesive, and were brought out through the chest wall, where oxygen could be added at one end and CO_2 and any residual oxygen exhausted at the other end. In experiments using dogs and sheep, oxygenation was demonstrated, as well as a decrease in CO_2 tension. A major problem with the device was clotting within the prosthesis. Bodell suggested an alternative would be to 'suspend the capillary tubing directly within a blood vessel such as the vena cava'[62]. Here, therefore, were the foundations for both an intrathoracic and an intravenous oxygenator in support of the failing lung.

Intracorporeal artificial lung technology comprises lung assist and replacement devices designed for intravenous or for intrathoracic placement. Unlike its extracorporeal counterpart, intracorporeal artificial lung development is still in its infancy. No intracorporeal devices have been brought to standard clinical practice, although clinical trials of an intravenous device have recently been concluded[63]. After introducing some of the earlier pioneering work, this section overviews recent developmental work in the areas of intrathoracic and intravenous artificial lungs.

INTRATHORACIC ARTIFICIAL LUNGS

The intrathoracic artificial lung developed at the Northwestern University Medical School (under the direction of Bruce Bodell, nearly 30 years ago) was remarkable in gas exchange efficiency when tested in animals, given some of the design limitations of this early device[62]. The wall thickness of the non-porous silicone fibers was about 150 μm, which is about 3–4-fold more than the thickness associated with porous hollow membrane fibers available today. Furthermore, the combined surface area of the fiber ensemble was only about 0.06 m^2. Extracorporeal devices typically present at least 2 m^2 of exchange area. Current intravenous prototypes can achieve up to about 0.5 m^2 of surface area. The average increase in P_{O_2} with the Bodell device varied among animals from 79±23 mmHg to 120±28 mmHg. The P_{CO_2} behavior was not as consistent but was generally reduced.

The group of Awad *et al.* at Laval University in Quebec also contributed substantively to early artificial lung work. One of their prototypes involved an intravascular oxygenator composed of bundles of silicone rubber tubing placed within the vena cava[64]. The tubing size was the same as used within the Bodell intraluminal device, and the bundles consisted of 16–20 tubes of 1.1 m length. The tubes were imbedded at one end into an arterial catheter, with the other end free and open. The insertion procedure involved threading a polyethylene catheter from a femoral vein insertion site, through the vena cava, to the jugular vein, where the catheter was exteriorized. The free ends of the silicone tubing were tied together and secured at the femoral end of this catheter, and the catheter pulled through until those ends appeared at the jugular site and were exteriorized. The ends were subsequently cut free to provide gas exhaust. At the end of the insertion procedure the 1.1 m intravenous oxygenator lay within the superior and inferior venae cavae.

A total of nine dogs were studied with the device. The dogs were respirated with an O_2 mixture, and the O_2 concentration lowered until arterial P_{O_2} fell to 50 mmHg. Subsequently, pure O_2 was passed through the fibers at 1–2 l/min for 4 h. The resulting arterial P_{O_2} increases were only 5–10 mmHg, and CO_2 levels continually increased. Interestingly, this intravenous device used the same fibers as the intraluminal device of Bodell *et al.*[62], and had comparable surface areas (0.04 m^2 versus 0.06 m^2), yet the intraluminal device of Bodell accomplished an apparently better overall oxygen exchange performance, possibly related to the pulsatile blood flow from the pulmonary artery.

The artificial organs group at Brown University, headed by Galetti and Richardson, has done pioneering work towards developing an implantable booster lung[65]. Scaled-down artificial lung prototypes were made of woven or coiled Teflon tubes. In the internal blood perfusion mode the tubing curvature associated with weaving or coiling induced secondary convection currents which aided mixing of the blood phase[66]. In the area of device development, three implantable booster lungs were constructed incorporating 12–24 tubes and total areas of 0.13–0.16 m^2, with different manifolding and weaves among devices[65]. The woven tubes were placed in polyethylene bags which were ventilated with pure O_2. *In-vitro* water tests examined exchange under steady and pulsatile perfusion conditions. VO_2 increased from 2 to 6 ml/min as pulsation frequency increased from 0 to 100 bpm. The *in-vivo* studies involved a pulmonary-artery-to-vein implantation in anesthetized sheep with right pneumonectomy. The implanted booster lungs were in parallel to the remaining lung and competed with it for pulmonary flow. Blood flow to the prosthesis was varied by altering inspired O_2 concentration to the natural lung, which altered pulmonary vascular resistance and redistributed flow to the booster lung. The implanted booster lungs achieved O_2 exchange rates of from 2 to 8 ml/min as blood flow varied from 200 to 600 ml/min. In general, artificial lungs that have relied on blood flow within (rather than around) the hollow fiber membranes have demonstrated limited gas exchange capabilities[67].

Current developmental work towards an implantable intrathoracic artificial lung focuses primarily on a device which can serve as a bridge-to-transplant for patients with chronic respiratory failure. As is the case for heart transplantation, demand exceeds supply for donor organs. The wait for a suitable lung donor exceeds any time frame suitable for extracorporeal support. The efforts towards developing an intrathoracic artificial lung lie more in infancy than those focusing on intravenous oxygenation. Current developmental efforts have been centered at the Department of Surgery, University of Michigan, and the Departments of Biomedical Engineering and Surgery at Northwestern University.

The University of Michigan's implantable gas exchange device (IGED)[68] is designed for pleural placement without the need for

pneumonectomy. The IGED is implanted in series with the native lung by placing it in-line with the pulmonary artery. Thus, this intrathoracic artificial lung may potentially be useful for treatment of acute respiratory failure, since the lungs remain intact for potential healing and ultimate removal of the device. IGED implantation is done by exposing the pulmonary artery (PA) through a left lateral thoracotomy. End-to-side Dacron grafts are sewn under partial occlusion of the PA close to the pulmonic valve, with a second graft being placed on the more distal PA. The IGED is placed between the grafts and application of a tourniquet to the PA diverts total pulmonary flow through the artificial lung.

In addition to the advantage that all flow goes through the device, a PA to PA circuit also has other advantages. Perfusing the lung with oxygenated blood reduces pulmonary vascular resistance and may promote healing of the lung. The serial arrangement means that the lung can act as a filter for particulate and embolic material shed from the device, which otherwise would travel to the systemic circulation. Since a partial occlusion can be used to attach grafts, the IGED can be implanted without cardiopulmonary bypass. Furthermore, PA implantation preserves left atrial anatomy for subsequent lung transplantation. The principal disadvantage to the IGED's serial disposition to the natural lung is that its blood flow resistance must be minimal. At 5 l/min blood flow, the pressure drop engendered by the IGED device should be less than 15 mmHg to minimize the potential for right ventricular failure.

The IGED device is composed of a hollow fiber woven membrane placed within a compliant shell to enhance any mixing induced by pulsatility of PA flow. The membrane surface area of prototypes made varies from 1.2 m^2 to 1.95 m^2. The inlet and outlet ports are 20 mm in diameter to minimize flow resistance, and are configured to produce a radial flow from shell to center, cross-wise with respect to HFM orientation. The devices made are relatively compact, measuring 30 cm by 8 cm by 5 cm, and together with their compliance/conformability allow for pleural placement without pneumonectomy. Figure 2 shows an IGED device in position on the main PA.

Several IGED devices have been manufactured and tested in anesthetized adult sheep. Sodium heparin was used to maintain activated clotting times from 180 to 320 s. After implantation, the evaluation procedure begins with occlusion (snare tourniquet) of the PA segment between the end-to-side grafts, and with clamping of the endotracheal tube used for ventilating the animal during surgical implantation. Arterial P_{O_2} went from a mean value of 290 mmHg pre-device to one of 200 mmHg post-device (IGED supplying all gas exchange), but variations across animals appear large (50 mmHg to 300 mmHg). Overall, VO_2 did not differ significantly pre- and post-device use, being 1.9 and 2.1 ml/min per kg respectively. Arterial P_{CO_2} went from a pre-device mean of 30 mmHg to a post-device mean of 35 mmHg, but was also highly variable, an effect attributable to gas flow rate sensitivity and its effect on CO_2 removal. The hemodynamic indices of cardiac output and mean arterial pressure showed no significant pre- and post-device use differences. The longest *in-vivo* test period of the device was 8 h prior to animal sacrifice.

The developmental efforts at Northwestern University focus on an intrathoracic artificial lung device to be placed in parallel with the native lung, as a bridge-to-transplant in chronic respiratory failure or a bridge-to-healing in acute respiratory failure[69]. This particular artificial lung device may be unique in that its design was based on extensive, semi-empirical mathematical modeling for optimizing fiber bundle parameters. The device consists of 380 μm polypropylene HFM matted into a fiber bundle with a frontal area of 128 cm^2, a blood flow path of 3.5 cm, and fiber bundle porosity of 0.53. The membrane surface area of the design is 2.2 m^2, with a predominant blood flow path which is cross-wise to the attendant fibers. The device is intended for placement between the main PA and the left atrium, in parallel with the natural lung. This device has been tested through *in-vitro* blood experiments and in a few *in-vivo* tests. The mean O_2 and CO_2 exchange rates *in vitro* varied from 50 to 200 ml/min and 150 to 300 ml/min, respectively, as blood flow rate was varied from 1 to 5 l/min. *In-vivo* tests in pigs demonstrated an O_2 transfer rate of 99 ml/min and CO_2 transfer rate from 86 to 144 ml/min as gas flow rate was varied from 3.2 to 5 l/min. The maximum blood flow rate obtained through the device *in vivo* was 1.2 l/min, and the maximum pressure drop was 10 mmHg. The tests were designed primarily to establish implantation methodology and to preliminarily assess gas exchange. Further tests are continuing.

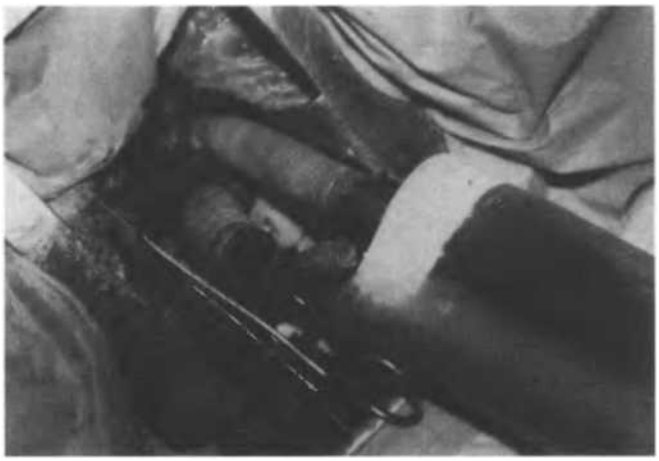

Figure 2 The IGED intrathoracic gas exchange device in position on the main pulmonary artery. (From ref. 68, reprinted by permission from J.B. Lippincott Co)

INTRAVASCULAR ARTIFICIAL LUNGS

The present objective of this innovative effort is to augment compromised gas transfer in patients by intravenous oxygenation, providing an approach that will be less expensive, less personnel-intensive and easier to operate than extracorporeal lung assistance, and less invasive than intrathoracic artificial lungs. In comparison to ECMO this approach would also maintain normal pulmonary blood flow while markedly reducing biomaterial–blood interfaces. The potential exists, therefore, for reducing mortality and morbidity in these critically ill patients.

Clinical experience with extracorporeal membrane oxygenation has provided ample proof that adequate oxygen delivery and carbon dioxide removal can be obtained with artificial lung devices that have a membrane surface area up to 6.0 m^2 and a pressure drop across the device of 100–300 mmHg. In this

context the challenges of intravascular oxygenator development are defined by the anatomical and physiological constraints of device placement in one of the major blood vessels of the human body.

All of the intravascular devices currently under development are intended for insertion either percutaneously or by venotomy through the femoral or jugular vein and require total or partial placement in the inferior vena cava (IVC), superior vena cava (SVC), and right atrium. One device also uses the PA. Convenient, practical implantation is a clinically desirable feature but limits the insertion diameter of the device to 10–15 mm. Once the oxygenator is deployed in the vena cava, the optimum configuration is one which allows the membrane fibers to be distributed in the available space, maximizing blood–fiber interaction and minimizing the ability of blood to shunt away from the gas exchange surfaces. Intravascular oxygenators are thus required to be compact for insertion, yet maintain the potential for an expanded configuration upon implantation.

In order to avoid impeding return of venous blood to the heart, devices that reside in the vena cava are restricted to providing no more that 10–15 mmHg in flow resistance. In addition, blood flow rate over the gas exchange surfaces is not a controlled variable and is dependent on native flow. The adult human IVC ranges on average from 2.2 cm to 3.3 cm in diameter along its length, and the SVC averages from 1.5 cm to 2.2 cm. The usable length for device placement in the IVC, right atrium, and SVC is approximately 40–50 cm[70].

Given the physical constraints of size, shape and flow resistance, one of the primary design considerations of intravascular oxygenation is miniaturization. These devices must be significantly smaller than their counterparts which are used clinically for extracorporeal membrane oxygenation. Most of the intravascular oxygenators under investigation do not exceed 0.5 m^2 of membrane surface area. It is generally agreed that, in order to be clinically useful, intravascular oxygenators need to provide at least half of the basal gas exchange requirements of an adult human, i.e. 125 ml/min of oxygen delivery and carbon dioxide removal. Thus arises the second design consideration of improving efficiency for membrane gas exchange performance by increasing cross-flow and convective mixing of blood as it interacts with membrane surfaces. The third design challenge in intravascular oxygenator development is to improve biocompatibility. Even for short-term (<24 h) implantation of membrane devices, full heparinization is required.

Since the mid-1980s four research groups have taken on the challenges of intravascular oxygenation, and each has produced innovative device designs that attempt to accommodate the constraints and requirements for a clinically useful product. Mortensen and colleagues at CardioPulmonics, Inc. (Salt Lake City, UT) developed the IVOX, the only intravascular oxygenator to date that has undergone human clinical trials[71–73]. The IVOX, shown in Figure 3, is intended for placement in the IVC, right atrium and SVC, through a femoral venotomy. The device consists of a bundle of microporous hollow membrane fibers which are joined at the distal end of the device to the inner lumen of a dual-lumen gas conduit, and at the proximal end to the outer lumen of the gas conduit. An oxygen source is attached to the inner lumen and a vacuum pump attached to the outer lumen of the gas conduit, thus drawing oxygen through the hollow fibers.

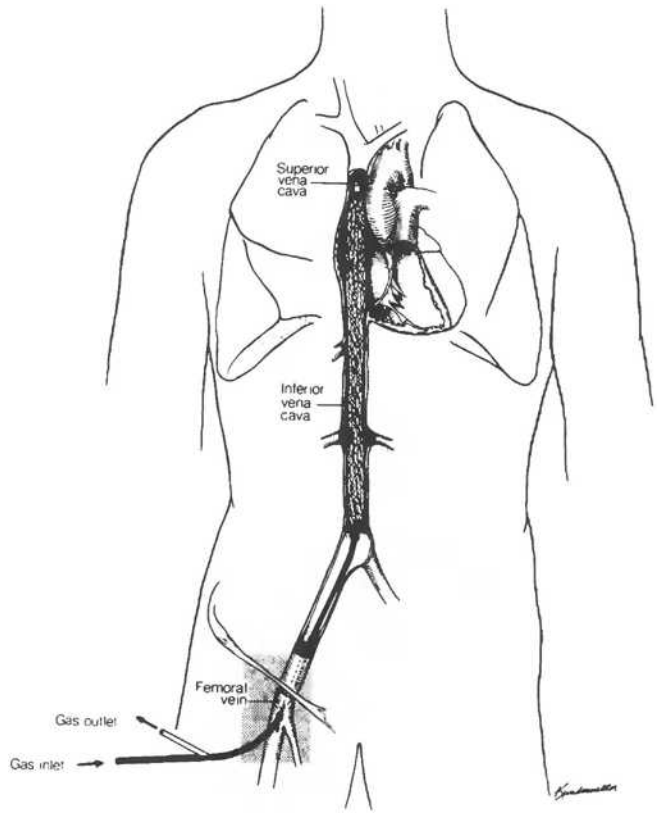

Figure 3 IVOX. Drawing illustrating position of IVOX within a patient's venae cavae. The IVOX device in this illustration has been implanted through a right common femoral venotomy. The right internal jugular vein is an acceptable alternative access route for insertion of the device. (From ref. 73, permission given by Blackwell Science Inc.)

Oxygen diffuses through the fiber membranes into the blood where it is exchanged for carbon dioxide, which in turn diffuses back across the membranes and enters the exhaust stream. CardioPulmonics has produced IVOX devices in a range of sizes, with varying fiber length, number, and total surface area. Devices that have been tested clinically range in membrane surface area from 0.21 to 0.52 m^2.

The IVOX incorporates a mechanism for furling the hollow fiber bundle to reduce its diameter for insertion and subsequent unfurling and expansion for better exposure of the hollow fibers to blood upon implantation. The hollow fiber membranes of the IVOX are crimped along their length, a process which permanently bends a straight fiber in the pattern of a sine wave. The undulations of the crimped hollow fibers allow them to maintain a spaced relationship to one another when they are deployed in the vena cava, and to some extent inhibit the tendency of membranes to clump together when introduced into the blood flow. In addition, the crimping provides an opportunity to produce a disturbed blood flow pattern around each hollow fiber, a condition which will improve gas exchange efficiency of the membranes.

The polypropylene microporous hollow membrane fibers of IVOX devices have two coatings. The first is an ultra-thin (less than 1 μm) siloxane coating which functions as a true membrane. It is permeable to oxygen and carbon dioxide but impermeable to

water, thus preventing the membrane pores from being fouled by serum leakage. This type of true membrane 'skin' on the outer surface of a porous membrane fiber is expected to extend the lifetime performance of membrane gas exchange devices that contact blood. The second coating is applied to all components of the IVOX, and is a covalently bonded heparin derivative that is intended to increase the thromboresistance of the device.

Extensive clinical trials with the IVOX device will be summarized in a subsequent section of this chapter. The gas exchange performance of the device varied widely, with average rates of oxygen and carbon dioxide transfer into and out of circulating venous blood ranging from 40 to 70 ml/min, as reported in a summary of 160 IVOX clinical trial patients[63,74].

Hattler and colleagues at the University of Pittsburgh Medical Center are collaborating with Medtronic, Inc. (Minneapolis, MN) to develop the intravenous membrane oxygenator (IMO)[75–77] Several prototype IMO configurations have been developed and tested. Their unique and common feature is a centrally positioned balloon, around which microporous hollow membrane fibers are arranged in various configurations. The polyurethane balloon is inflated and deflated with helium by an intra-aortic balloon pump console. This pulsation provides a means for regularly moving the fiber membranes, changing their configuration and minimizing the tendency for fibers to clump. In addition, the balloon motion enhances convective mixing around the fiber membranes and thus improves gas exchange performance.

The most promising IMO design to date consists of a bundle of microporous hollow membrane fibers which are potted into manifold units at the proximal and distal ends. A central tube delivers oxygen to the distal manifold where it is distributed to the fiber bundle. Vacuum pressure is applied to the proximal manifold unit and oxygen is thus pulled through the hollow fibers. Oxygen diffuses through the pores of the membrane fibers into the blood, where it is exchanged for carbon dioxide which diffuses back through the membrane wall and is exhausted in the exit stream. Centrally located in this fiber bundle is the rhythmically pulsating balloon (Fig. 4). The IMO is intended for percutaneous insertion through the femoral or jugular vein, and will reside in the IVC, right atrium, and SVC.

The central gas delivery tube is used as a mechanism around which the fibers can be twisted or furled for a compact insertion configuration, and subsequently unfurled after placement in the vena cava. Typical investigational prototypes range in length from 34 to 40 cm and have fiber surface areas which range from 0.12 to 0.59 m^2. Several different prototypes which incorporate a variety of membrane fiber configurations have been tested *in vitro* using fresh bovine blood at flow rates of 2–3 l/min. Oxygen and carbon dioxide exchange rates range from 30 to 250 ml/min per m^2. Design challenges for the future include optimization of the balloon inflation volume and rate, optimal fiber selection, and incorporation of biocompatible fiber coatings[77].

Mockros of Northwestern University (Evanston, IL) and his colleagues have developed several innovative prototype intravascular lung assist devices (ILAD)[78–80]. Their most recent efforts are focused on a dynamic intravascular lung assist device or D-ILAD. The D-ILAD is made up of sheets of short microporous fibers. The ends are potted in two tubular manifolds which are disposed lengthwise along the device and perpendicular to the fiber axes. The fiber sheets are stacked, folded, and then twisted

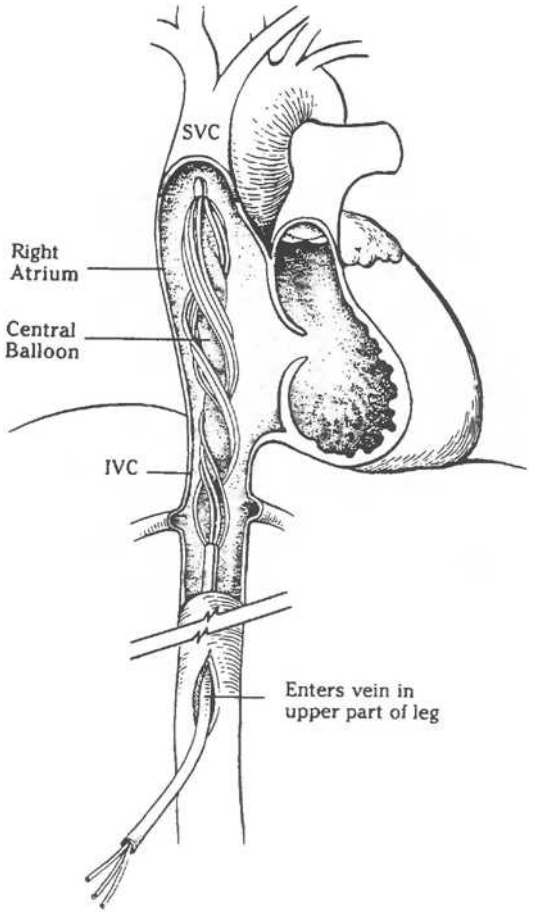

Figure 4 IMO. Anatomic placement of the intravenous membrane oxygenator (IMO). SVC = superior vena cava; IVC = inferior vena cava. (From ref. 76, permission given by J.B. Lippincott Co.)

around a central shaft. The result is a helical or screw-like fiber arrangement with the fiber membranes representing the threads of the screw and the manifolding tubes residing at the axial core (Figure 5). The inlet oxygen is delivered through the length of one manifold tube and the outlet gas, containing carbon dioxide, is exhausted through the other. Gas is pulled through the D-ILAD under vacuum pressure. Several different fiber folding and stacking arrangements have been incorporated into prototype devices.

The entire D-ILAD device can be rotated to enhance mixing of blood around the fibers, thus improving gas transfer performance, while the pumping motion can also augment blood flow. In addition, this group has introduced yet another means of increasing the relative velocity between membrane fibers and the blood, i.e. oscillation of the fiber sheets. In initial experiments, oscillatory motion was shown to be superior to rotational motion in enhancing gas exchange performance. The Northwestern group has fabricated prototypes with membrane surface areas ranging from 0.1 to 0.5 m^2. Oxygen transfer fluxes of 208 ml/min per m^2 and carbon dioxide transfer fluxes of 310 ml/min per m^2 have been reported for *in-vitro* tests with bovine blood at flow rates of approximately 2 l/min.

One of the potential problems of the D-ILAD is the effect of fiber motion during rotation or oscillation on the wall of the vena

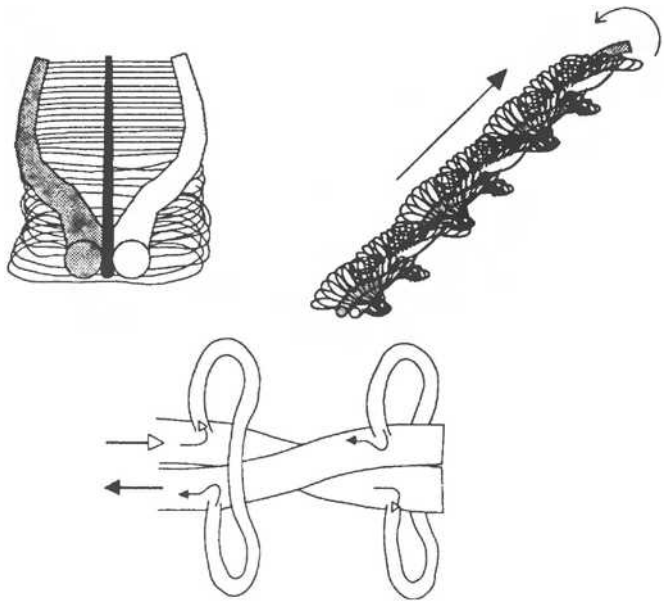

Figure 5 D-ILAD. Screw-type artificial lung, shown before folding and twisting (upper left). Finished configuration (upper right). Schematic showing gas flow path (bottom). (From ref. 79, permission given by J.B. Lippincott Co.)

cava. Current efforts are under way to develop a stationary sheath that will surround the oscillating device during operation and protect the vasculature from possible damage. The sheath will be formed from microporous membrane fibers, and will function in a dual capacity as a gas exchanger and protective covering.

Snider and colleagues at the University Hospital, Penn State University (Hershey, PA) are developing the Penn State intravascular lung (PENSIL)[81–83]. This device differs from the others described above in that its implantation position is intended to include the vena cava, right atrium, right ventricle, and PA, with intended percutaneous insertion through the femoral vein. The PENSIL (Figure 6) has, as its central core, a balloon tipped, flow-directed, PA catheter. Blind-ended microporous hollow membrane fibers are potted into manifold sleeves which are placed concentrically around the PA catheter. The central catheter serves as the spine of the prototype devices, as well as the conduit for gas delivery and exhaust. Gas is conveyed to the manifold sleeves through holes cut into the walls of the catheter. Gas delivery to, and exhaust from, the hollow fiber membranes can occur in two different ways. One mode oscillates gas in and out of the catheter and fiber membranes by pressure changes (760 mmHg absolute for flow in, and 80 mmHg absolute for flow out) at a frequency of approximately 40 cycles per second. The second mode provides gas continuously under pressure (760 mmHg absolute) to some of the fibers and vacuum (80 mmHg absolute) to another group of fibers through separate lumens of the catheter. The pressurized fibers deliver oxygen and the vacuum fibers exhaust carbon dioxide.

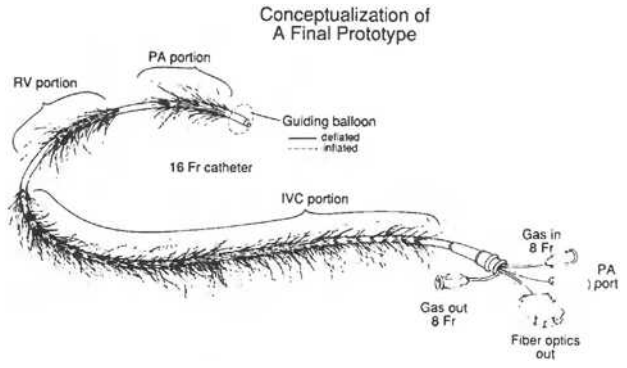

Figure 6 Pensil. Proposed intrapulmonary arterial lung *ex situ*. (From ref. 81, permission given by J.B. Lippincott Co.)

PENSIL prototypes have been fabricated which range in size from 0.04 to 0.38 m^2. Oxygen transfers as high as 140 ml/min per m^2 at a blood flow rate of 7.5 l/min, and carbon dioxide transfers as high as 350 ml/min per m^2 at a blood flow rate of 4.5 l/min have been reported. One of the advantages of the PENSIL design is that it resides in the right atrium and right ventricle, which allows exposure of the device to all of the returning venous blood, including that from the coronary sinus. However, as prototypes are scaled up in size, attention to safe insertion through the right heart will become an important design consideration.

CLINICAL TRIAL OF THE IVOX

All intracorporeal oxygenators under development for placement within the venous system provide only temporary and partial augmentation of gas transfer while the natural lungs recover from acute respiratory failure. None of these devices is intended for fixed, non-reversible pulmonary pathology. The intravascular oxygenator (IVOX) is the only intracorporeal device to have undergone phase I and phase II human clinical trials[63]. The phase I trials established the relative safety for introducing an intravascular oxygenator in humans, and the phase II trials examined the clinical efficacy and gas exchange performance for this device. A total of 160 patients from the USA and elsewhere were studied. Criteria for inclusion or exclusion in the phase II trial have been reviewed and are given in Table 1.

Table 1 Criteria for IVOX trial

Inclusion
$F_{IO_2} \geq 0.50$ for 24 h or more with Pa_{O_2} <60 torr and one or more of the following:
1 Positive end-expiratory pressure ≥10 cmH_2O
2 Peak inspiratory pressure ≥45 cmH_2O
3 Mean airway pressure ≥30 cmH_2O
4 Minute ventilation 150 ml/min per kg with Pa_{CO_2} >40 torr
Exclusion
1 Uncontrolled multiple organ failure or patient *in extremis*
2 Contraindication to systemic anticoagulation
3 Uncontrolled bacteremia/fungemia
4 Low cardiac output refractory to inotropes
5 Lack of usable access vein (jugular or femoral)
6 Existence of thrombi in major veins or vena cava
7 Abnormal anatomy of the access veins or vena cava

Once entry criteria have been met, the right internal jugular or femoral vein is used for device implantation following a cutdown

and surgical exposure. The largest sized IVOX (0.21–0.51 m^2 membrane surface area) is chosen according to the anatomical constraints of the vena cava size (determined by pre-insertion ultrasound) and suitability of the access vein. The IVOX is furled during insertion and guided over a wire into position in the IVC/right atrium and SVC, where unfurling occurs to fully expose the hollow fiber membranes to the returning venous blood. Heparinization (ACT 180–200 s) is maintained during use of the device. Once the IVOX is activated attempts are made to reduce F_iO_2 and minute ventilation as long as blood gases can be maintained at pre-IVOX levels with oxygen saturations over 90%.

The IVOX clinical trials have confirmed the earlier animal experiments in demonstrating that gas exchange does occur when hollow fiber membranes are introduced into the venous system of humans, where the average rates of O_2 and CO_2 transfer vary between 40 and 70 ml/min. Manipulation of the patient can improve gas exchange. Thus, permissive hypercapnea, and maintaining an adequate hematocrit and cardiac output, are important. These values provide approximately 30% of projected needs. Nevertheless, at this level of gas exchange clinical benefits are frequently difficult to document. During IVOX utilization some clinical trial patients showed improvements in blood gas partial pressures and in the ability to reduce the intensity of mechanical ventilation.

Moderate to severe complications considered to have produced adverse clinical sequelae, or to have contributed to the patient's death, were recorded in 24.5% of the patients. Bleeding during IVOX insertion or explantation was the most common complication, and 17.7% of the devices had significant mechanical or performance malfunctions during utilization. Conversely, the IVOX functioned in some patients for weeks without adverse effects. In these desperately ill patients, 60% survived to have the device removed, but only 30% improved to a point where mechanical ventilation could be discontinued. These same patients were hospital survivors and were discharged, for an average 30% survival rate. The IVOX clinical experience suffers, however, from the lack of concurrent controls and randomization in the trial. Nevertheless, as the first and only clinical trial of an intravascular oxygenator, the IVOX has provided evidence that gas exchange (O_2 and CO_2) occurs in humans, but at a level that meets only 30% of patients' needs and with a significant rate of complications and device malfunction.

COMMENT

Although further trials of the IVOX are not being pursued at present, methods to improve safety and gas transfer capability, and to reduce the need for anticoagulation, are actively being investigated by scientists both in the USA and elsewhere. The materials and technology presently available, and as reviewed in this chapter, more than meet those needs for a device that will provide clinically significant, temporary, reliable and safe support for patients with reversible lung failure. Continuing improvement in device development should yield a clinically useful intracorporeal oxygenator before the turn of the century.

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Xenotransplantation of the Heart

D.K.C. COOPER

INTRODUCTION

It is generally accepted that there is a shortage of suitable human organs for purposes of transplantation[1]. The total number of patients on the United Network for Organ Sharing (UNOS) waiting list in the USA is now in excess of 45 000, over double the number registered in 1988. Over 3000 patients are awaiting a heart transplant. These data indicate that the demand for organs in the USA is increasing by about 10–15% each year. Worldwide, this number can be increased by at least a further 50%, and possibly 100%. Furthermore, the median waiting period for several types of donor organ has increased significantly in recent years. In particular, patients in need of a liver or heart wait approximately twice as long today as they did 5 years ago.

The above estimate of the current worldwide need for organs each year, however, does not take into account other important factors. The first of these is whether the number of patients on the waiting list fully reflects the number who might benefit from an organ transplant. A large percentage of patients are unsuitable, for one or more relative contraindications. Much of our reluctance to accept the borderline patient is based on the knowledge that the number of donors is strictly limited, and we feel obliged to utilize this scarce resource as carefully and responsibly as possible. Many believe it is no longer justified to transplant an organ into a patient considered to be at high risk of not surviving the first few postoperative days. If there were an unlimited supply of donor organs, as would be the case if xenotransplantation were successful, this ethical barrier would be largely removed. The very sick or borderline patient could be given his or her chance without jeopardizing the future of the more stable candidate. The decision regarding retransplantation of organs, which with heart or lungs is less frequently successful, would similarly prove less of an ethical dilemma.

The second factor relates to the current status of allografting in countries where, for religious or cultural reasons, cadaveric organ donation is rare or nonexistent. Japan is the prime example of a country with advanced medical technological skills and yet where, for cultural reasons, cadaveric allotransplantation is virtually nonexistent. The impact of successful xenotransplantation in such a society would be enormous.

The advantages of xenotransplantation are obvious. Not only would the supply of donor organs be unlimited, but these organs would be available electively when required. Transplant operations could therefore be carried out on routine operating lists, and no potential recipient would need to die for lack of a suitable organ. Of equal importance would be the possibility of pretreating either the donor or the recipient to enhance acceptance of the graft, as the transplant procedure could be planned for a specific day. In addition, donor organs would not be subjected to the effects of brain death, which can be damaging, particularly to the heart. Chronic infection is proving an increasing problem in regard to human donors, particularly with regard to the transfer of hepatitis and HIV; animals could be bred and maintained to ensure that no infection is present that could be transferred to the recipient.

DEFINITIONS

Xenotransplantation refers to the transplantation of organs or tissues from an animal of one species into an animal of another species[2]. With regard to humans it clearly refers to the use of a donor other than humans. The terms concordant and discordant xenografting[3] are used frequently (and loosely) to refer respectively to transplantation between closely related animal species (e.g. baboon-to-human) and between distantly related animal species (e.g. pig-to-human).

This suggested nomenclature is helpful, but experimental and clinical experiences have demonstrated that there are not just two distinct groups of xenograft, but gradations, inasmuch as an organ from one animal species, when transplanted into an animal of a different species, may be rejected in some pairs by a cellular mechanism (acutely) and in others by a humoral (or vascular) mechanism (often hyperacutely). Furthermore, features of both cellular and vascular rejection can occur within the same xenografted organ[4].

It would therefore seem that additional nomenclature is required to define the immunological similarity or disparity between two species. There are few truly concordant pairs (in

which rejection is purely cellular), as a degree of vascular rejection is likely to be seen in many cases. In contrast, however, discordant pairs virtually always reject hyperacutely by a humoral mechanism.

With regard to the histopathology of the rejection that takes place, we should probably confine our terms to (a) cellular rejection, (b) vascular (denoting antibody-mediated or humoral) rejection, and (c) mixed rejection[4]. Vascular rejection may be hyperacute (in that it occurs within minutes or a few hours after transplantation) but may be delayed, and can occur several days or even weeks after transplantation.

BASIC IMMUNOBIOLOGY

Concordant xenografting

When xenotransplantation is carried out between closely related species, there are usually no or very low detectable levels of anti-donor species (xenoreactive) antibody in the host at the time of transplantation[5,6]. The antibody titer may rise during the first few days after transplantation. In a proportion of recipients, rejection will be cellular and will follow the normal sequence of events seen after allografting. In a proportion, however, rejection will be vascular or of a mixed nature.

The relative proportion of cases in which cellular (rather than vascular) rejection will result varies, depending on the two animal species involved and on the organ transplanted. For example, in chimpanzee-to-human renal transplants, rejection was mainly of a cellular nature[5], whereas vascular rejection was reported in a chimpanzee-to-human cardiac graft[4]. In vervet-monkey-to-baboon cardiac transplants, 80% of the hearts showed features of vascular rejection with or without cellular rejection[6]. In contrast, following vervet-monkey-to-baboon liver transplants, cellular rejection predominated[7].

Discordant xenografting

Rejection between widely differing species is uniformly vascular and generally hyperacute[4,8,9]. There is increasing evidence that vascular rejection of discordant xenografts in humans is largely a result of antibody-mediated complement activation through the classical pathway, although the alternative pathway may also play a role. Histopathologically, the features of vascular rejection consist of massive capillary destruction with severe interstitial hemorrhage and edema[4] (Chapter 81). Intravascular thrombosis resulting from platelet and/or fibrin thrombi is relatively rarely observed by light microscopy, but can be documented on electron microscopy. Degenerative changes are evident in the myocytes and contraction band necrosis may be present.

CHOICE OF AN ANIMAL DONOR FOR HUMANS

If a human were to be the recipient, and using currently available immunosuppressive therapy, xenografting from a concordant animal species such as the chimpanzee, or possibly the baboon, would have a much greater chance of success than would a graft from a discordant animal. Pioneering work in the use of primate kidneys as donor organs for humans showed clearly that the closer phylogenetically the donor to man, then the longer was the donor organ survival time. Reemtsma (Figure 1, Chapter 82) obtained longer survival using chimpanzee kidneys as donors[5,10-13] than did Starzl (Figure 2, Chapter 82)[14,15] or Hitchcock *et al.*[16], who used baboons; rhesus monkey kidneys fared even worse[10].

Immunological similarities and differences between various primate species have been studied by Sarich[17], using microcomplement fixation, which has been shown to be a sensitive and reliable technique to measure the degree of immunological cross-reactivity between species. There were a number of considerations which led to albumin being the molecule whose evolutionary changes were studied. The very close relationships existing among all hominoid albumins, and particularly among the higher primates, are shown in Table 1. There are, however, a number of problems in the use of non-human primates as donors for humans.

The baboon does not grow to a size large enough to make it a suitable donor of some organs, for example the heart, for adult humans, though there may be a role for this animal as a donor for children. The rationale for baboon transplantation in infants and small children is that few human donors can be found in this age group, and the immature immune system of newborn infants may have less competence to reject foreign tissue[18]. There is good experimental evidence that growth of xenograft anastomoses occurs, but less conclusive evidence that it will be possible to control rejection well enough to expect even medium-term survival.

Other higher primates are, in general, endangered species, and would not be available in sufficient numbers unless extensive and costly breeding programs were initiated. Even the chimpanzee heart may not be large enough to support the circulation alone in a large human adult[19] (Chapter 18). There would, in addition, almost certainly be ethical and moral objections to the use of such animals; many of these ethical considerations have been discussed by Kaplan[20] and Veatch[21]. It would appear, therefore, that xenografting between concordant species will not provide the final answer to donor supply in humans.

Certain discordant animals, such as the pig or sheep, would provide organs of a suitable size and anatomy for humans, but transplantation would be greatly complicated by the development

Table 1 Reactivity in the microcomplement fixation procedure of sera from various species with a pool of three antisera directed to human serum albumin*

Species	*Index of dissimilarity*
Hominoidea (humans and apes)	
Homo sapiens (human)	1.0
Gorilla gorilla (gorilla)	1.09
Pan troglodytes (chimpanzee)	1.14
Pongo pygmaeus (orang-utan)	1.22
Hylobates lar (gibbon)	1.28
Symphalangus syndactylus (gibbon)	1.30
Cercopithecoidea (Old World monkeys)	2.23–2.65
Ceboidea (New World monkeys)	2.7–5.0
Prosimii (Prosimian, e.g. lemur)	8.6–18
Non-primates	
Bos taurus (bull)	32
Sus scrofa (pig)	> 35

* Adapted from ref. 12.

of hyperacute rejection, which is not prevented or modified by the currently available immunosuppressive drugs[8,9]. If this immunological problem could be overcome, however, such animals as the pig or sheep would provide a readily accessible, continuous supply of donor organs. Moral and ethical objections would almost certainly be few and easily overcome as such animals are, in any case, slaughtered in large numbers on a daily basis to provide food for human consumption.

CONCORDANT CARDIAC XENOGRAFTING

From an extensive review of the experimental literature, it would seem that, with regard to transplantation of organs between some closely related species, there is the probability that, with the currently available immunosuppressive drugs, rejection can be delayed significantly. There is increasing evidence that immunosuppression with cyclosporin prolongs graft survival when xenografting is performed between two closely related animal species, such as wolf and dog[22], fox and dog[23], hare and rabbit[24], sheep and goat[25], and Cynomolgus monkey and baboon[26–28]. Rejection in these models appears to be primarily cellular; humoral factors, which might lead to vascular rejection, generally play a less important role, though this is not always so, as found in the closely related vervet-monkey-to-baboon model[6,29,30].

Rejection between such animal species is, in general, more vigorous than when allografting is performed, and is therefore more likely to result in early graft failure from accelerated acute rejection; humoral factors would also appear to play a role in many cases. In addition, rather higher doses of immunosuppressive drugs have to be administered than would be necessary after allografting, making the recipient more susceptible to the complications of such therapy, in particular, infection.

As grafts between closely related primate species are rejected acutely in some models[26–28] but hyperacutely in others[6,29,30], it is difficult to predict accurately the outcome of a transplant in humans using any one primate subgroup as donor. For example, Cynomolgus monkey hearts inserted heterotopically into baboons are rejected by a cellular (acute) rejection response, which can be successfully overcome by combination immunosuppressive therapy using cyclosporin, azathioprine, corticosteroids, and antithymocyte globulin (ATG)[26,27,31]. Hearts from African green (vervet) monkeys, on the other hand, transplanted into baboons, are sometimes rejected hyperacutely and frequently by a delayed vascular mechanism, and survival is not greatly prolonged by combination immunosuppressive therapy[6,29], though if ATG is added to the regimen, and rejection episodes vigorously treated with bolus steroid therapy, some prolongation can be achieved[30]. Pretransplant total lymphoid irradiation, in collaboration with pharmacological immunosuppression, does result in longer survival of some hearts but, in this experimental model, has been associated with a high mortality[29].

Whether pharmacological immunosuppression including cyclosporin or tacrolimus will delay or prevent rejection of transplanted primate hearts in humans remains uncertain, but, from early (precyclosporin) clinical studies using chimpanzee kidneys in humans[5,10–13], it would seem reasonable to expect that chimpanzee hearts will function for at least some weeks, or even months or years, under these circumstances. The outlook for baboon hearts, again based on early renal transplantation in humans[14–16], would seem less optimistic, though function for 3 weeks has already been shown to be possible (Chapter 82).

If clinical concordant xenografting is to be performed, the success of the procedure will almost certainly be increased if donor and recipient are of compatible ABO blood group. This conclusion is supported to some extent by experimental work using the vervet monkey as donor and the baboon as recipient[6], and by a single clinical experience (Chapter 82). In the experimental studies, early hyperacute rejection (within the first 60 minutes) was not seen in those cases where ABO compatibility was present between donor and recipient, but was seen in a significant proportion of cases (three of eight) where incompatibility was present. Mean donor heart survival was also shorter in baboons receiving ABO-incompatible vervet monkey hearts These observations were noted both in non-immunosuppressed recipients, and in recipients immunosuppressed with a combination of pharmacological agents.

The role of truly concordant xenografts in providing temporary support for patients with grossly inadequate function of important organs, such as the heart or liver, though at present uncertain, may well warrant clinical investigation within the near future. Permanent long-term function of such grafts would appear to be less likely, as almost certainly recurrent acute rejection episodes and early chronic rejection (graft vasculopathy) would develop, leading to relatively early graft failure.

Several new pharmacologic immunosuppressive agents are currently under investigation that may prove more efficient in preventing not only the cellular rejection that takes place in concordant xenografting but also the antibody-mediated rejection that can occur following the production of new antibody by B lymphocytes. Several of the drugs currently under investigation, such as brequinar sodium, mycophenolate mofetil (RS61443) and 15-deoxyspergualin, have been shown to have relatively potent anti-B-cell activity (Chapter 70).

For example, infant baboons (age 9–19 months), splenectomized and immunosuppressed with a combination of antilymphocyte globulin, FK506 (tacrolimus), and methotrexate, with methylprednisolone being used as rescue therapy for rejection, survived for up to 127 days with an orthotopically transplanted rhesus monkey heart[32]. Mean graft survival in four surviving animals was 80 days, but three other animals died between 35 and 96 days from pulmonary infection or renal failure associated with drug therapy.

A combination of total lymphoid irradiation and cyclosporin-based immunosuppression has led to survival of heterotopically placed rhesus monkey hearts in baboons for periods in excess of 1 year[33]. Whether such regimens will be well tolerated by ill patients awaiting heart transplantation remains uncertain, but clearly such heavy immunosuppressive programs are likely to be associated with a higher incidence of infection and *de novo* malignancy than is associated with allografting at the present time.

However, there have been five clinical heart transplants using non-human primates as donors for humans, the most recent being in 1984 (Chapter 82). Survival of concordant grafts has been for a maximum of only 20 days.

DISCORDANT CARDIAC XENOGRAFTING

For a number of reasons the pig has been identified as a suitable potential donor for humans[34]. These reasons include: (a) availability in large numbers, (b) inexpensiveness of breeding and maintaining, (c) suitable size for the smallest or largest of humans, (d) availability of pathogen-free (gnotobiotic) animals, and (e) considerable similarities of anatomy and physiology with humans.

Four heart transplants have been carried out using the pig or sheep as donors for humans, the most recent being in 1992 (Chapter 82). Maximum survival has been 24 hours.

The problem of hyperacute vascular rejection has to date proved insurmountable, and is yet to be resolved. Progress is taking place in the laboratory, however, and it would seem that the most likely solution to the problem will come from one (or a combination) of the following approaches.

Human anti-pig antibody depletion or inhibition

One promising approach would appear to be the depletion or inhibition of xenoreactive (anti-pig) antibodies in the host. If the xenoreactive antibody titer can be temporarily significantly or totally depleted, or in some other way 'neutralized', then an organ grafted during this critical period may not undergo vascular rejection even when the antibody titer returns to its normal level. The period of time during which antibody depletion or neutralization is required remains uncertain, but may be as short as 1–3 weeks. The resulting state that is achieved, termed 'accommodation'[35], enables survival of an organ graft in the presence of specific antibodies directed against antigens expressed on the surface of the organ. Normal levels of complement are also present.

Although this state has not been fully achieved after discordant xenografting, it has been clearly documented after the transplantation of ABO-incompatible organs, both experimentally[36,37] and clinically[38], where the mechanism of antibody-mediated rejection is very similar.

Antibody depletion can be carried out by extracorporeal immunoadsorption utilizing columns of specific immunoadsorbents that are directed only against the specific antibody whose removal from the plasma is desired. These immunoadsorbents must, therefore, consist of: (a) the antigen itself (or a cloned or synthetic antigen), (b) a cross-reactive antigen[39], or (c) an anti-idiotypic antibody[40].

There is now conclusive evidence that the pig epitopes against which human anti-pig antibodies are directed are carbohydrate structures, namely galactose structures in the α configuration (αGal1-3Gal)[41–44]. Pretransplant extracorporeal immunoadsorption using an αGal1-3Gal immunoadsorbent (or the continuous intravenous infusion of αGal1-3Gal to bind the anti-pig antibodies for a period of several days) may therefore be successful in allowing accommodation to develop. When this form of therapy is combined with pharmacologic immunosuppressive therapy, prolonged xenograft function might be achieved.

Genetically engineered donor pigs

An alternative approach is the development of a pig that might prove a universal donor of organs for humans[44–47], namely by the genetic engineering of a pig that does not express αGal on its vascular endothelium. The expression of terminal α-galactose depends on the proper function of a single gene encoding for the enzyme α1, 3 galactosyltransferase[48]. If this gene were 'knocked out' by homologous recombination, then there would be no target for the human anti-αGal antibodies. Although this 'knockout' technique is not yet possible in the pig, it has been established in the mouse[49,50] and, with the current rate of advance in the field of genetic engineering, it is likely that it will prove feasible in the pig within a few years.

McKenzie's group in Australia, however, has demonstrated that, as a result of knocking out the gene, subterminal sugars are exposed against which other natural antibodies exist (McKenzie I.F.C., personal communication). They, and others[45], have suggested an alternative strategy which they have termed 'transferase dominance', whereby the same substrate (in this case, lactosamine) can accept either galactose (under the influence of α1, 3 galactosyltransferase) or fucose (under the influence of the H transferase). After isolating and transfecting the H transferase, they were able to demonstrate that its activity dominates over that of the α1, 3 galactosyltransferase and that the αGal1-3Gal epitope is essentially not made, but its place is taken by fucose. Most humans have no natural antibodies against fucose (unless they are of the rare Bombay phenotype). Transgenic mice expressing the H transferase demonstrate little αGal expression and, if this work can be duplicated in pigs, the genetically engineered pig may prove to be an acceptable donor for humans.

The recent development of genetically engineered pigs that express certain human complement-inhibiting proteins[51,52] results in their resistance to the effects of human complement on pig tissues. This is proving a significant step forward in our efforts to overcome the hyperacute rejection that destroys discordant animal grafts within minutes or hours.

For example, decay accelerating factor (DAF, CD55), membrane cofactor protein (MCF, CD46), and CD59 (protectin, homologous restriction factor) are membrane inhibitors of complement that are present on a wide variety of cell types. These inhibitors block the activity of autologous complement but not of xenogeneic complement from a distantly related species. Lysis of human cells by human complement occurs when these regulatory proteins are deficient. Recent results show considerable prolongation of pig heart function after heterotopic transplantation into primates if the organ is taken from such a genetically engineered pig[51,52].

There is some evidence, however, that even if the complement cascade is inhibited (and hyperacute rejection avoided), delayed vascular rejection might still occur within the first few weeks after transplantation, and that this may be associated with other mechanisms involving xenoreactive antibodies and cellular infiltration[53–56]. It would therefore seem that our efforts must also be directed towards discovering some means of abrogating the effect of these antibodies, both to prevent activation of the complement system and also to inhibit other mechanisms dependent on the antibody–antigen interaction.

UNRESOLVED PROBLEMS

The major unresolved immunological barrier is clearly the problem of antibody-mediated rejection, but the severity of the

cellular response to a discordant organ remains unknown, and may prove to be a more significant barrier than has been anticipated[57].

There is the risk that, even if rapid rejection can be overcome, the early development of graft vasculopathy might occur. There is some optimism, however, that this may not develop, as in long-functioning ABO-incompatible renal allografts in patients in whom accommodation has been achieved, there does not appear to be a higher incidence of graft atherosclerosis[38]. However, there is little significant experience in this field following heart transplantation[58].

The use of a heterotopic or orthotopic non-human primate heart as a 'bridge' to allotransplantation would seem feasible for, as already mentioned, it seems likely that non-human primate hearts will function satisfactorily for possibly weeks or months. This would allow time for a suitable human heart to be located and inserted (with removal of both the non-human primate organ and the recipient's native heart, if it is still *in situ*).

The use of a xenograft as a bridge to allotransplantation would seem to be indicated particularly in infants and children with complex congenital heart disease, as left ventricular assist devices and artificial hearts have not yet been developed of a size whereby they can be implanted easily in these small patients. The small volume of the thoracic cavity may prohibit the insertion of an auxiliary (heterotopic) heart, and orthotopic transplantation may prove necessary. The complex abnormal anatomy of the recipient's own heart may be another factor making heterotopic transplantation difficult or impossible, again necessitating orthotopic siting of the xenograft. Retransplantation would be performed when a suitable human donor organ became available. Performing this form of experimental surgery initially in children, who clearly cannot give informed consent, however, may be seen by some as a major ethical hurdle.

An animal heart inserted as a bridge to transplantation would have some advantages over a mechanical support device, the most important and obvious being the fact that the animal heart can be totally enclosed within the thoracic cavity, thus reducing the risk of infection. There is similarly a reduced risk of thromboembolism when mechanical parts are not implanted. If the xenograft is sited heterotopically, it would seem essential to anticoagulate patients just as it is in patients with heterotopic allografts, as there remains a possibility that thrombus formation will occur in the patient's poorly contracting native right or left ventricle, and that embolism to the pulmonary or systemic circulations may take place.

Three aspects of this procedure, however, remain for discussion. The first has already been alluded to, and concerns the ethics of using a non-human primate for this purpose[20,21]. The use of primates, with their close relationship to humans, stimulates a considerable emotional response in the public, a significant percentage of whom may object to their use for this purpose. Objections would probably be particularly vociferous if such hearts were used in adults (as opposed to infants and children), as adequate mechanical devices for the support of adult patients with failing ventricles are readily available and, indeed, in many cases insertion has been followed by successful allotransplantation.

A second feature of the use of a xenograft heart as a 'bridge' has not yet been fully explored. It remains uncertain whether subsequent transplantation with an allograft could then be successfully achieved, as there is conflicting evidence as to whether antibodies will develop that might cause early failure of the subsequent allograft. Recent experimental experience in primates, however, is that this does not occur[59].

The third aspect of the implantation of a primate heart in humans (whether temporary or permanent) that requires very careful consideration is the risk of transferring pathogenic agents that may result in serious infection or the development of neoplasia. Primates captured in the wild, and to a lesser extent colony-bred animals, are known to harbor a host of pathogenic agents[60], of which viruses probably represent the greatest risk to humans[61-63]. This concern regarding the transfer of pathogens is reinforced by the consideration that the transfer of an agent that causes no morbidity in the non-human primate may result in serious disease in an immunosuppressed human patient. The Simian retroviruses, which include viruses related to the human immunodeficiency virus type-1, which can cause the acquired immune deficiency syndrome (AIDS) in humans, and the herpesviruses, in particular, must be considered dangerous if transferred to humans.

There is some evidence that primates bred under suitable conditions of management exhibit a lower tendency towards viral infection than do wild-caught animals[62]. The use of non-human primates as organ donors may be possible, therefore, provided that they are at least free of those infectious agents that are known to pose a serious threat to human health, e.g. *Mycobacterium tuberculosis*, herpesviruses, exogenous retroviruses, and Marburg virus. In this regard the feasibility of breeding and maintaining specified pathogen-free animals may be worthy of investigation.

Finally, questions have been raised regarding whether the metabolic environment of the human host will allow normal function of a pig (or even baboon) organ[3,64]. Will an organ from one species of animal metabolize and function satisfactorily in the different metabolic environment of a host animal of another species? In 1970 Calne[3] pointed out that, because no discordant organ grafts have functioned for long periods, we cannot answer this question. The question remains unanswered. Minor differences in, for example, pH or serum hormone levels could have profound and unfavourable effects on the function of the graft.

Organs from animals closely related to humans would seem more likely to function satisfactorily when used as xenografts. Chimpanzee and baboon kidneys, for example, have clearly functioned adequately in the human metabolic environment, and chimpanzee and baboon hearts have functioned satisfactorily in humans until rejected (Chapter 82). Chimpanzee and baboon livers have also shown reasonably good function after transplantation into humans[65,66]. It is unlikely, however, that all the enzymes and hormones that show species variation will function with equal efficiency in xenogeneic recipients. 'One of the exciting side products of successful xenogeneic transplantation would be the new insights inevitably gained into the normal processes of physiology'[64].

Pig kidneys and hearts have functioned in non-human primates for several weeks[52,55,67] and it seems likely that function of these organs will not be a major problem. Clinical experiments in which blood from patients in hepatic failure has been perfused through pig livers have demonstrated that the pig liver has the capacity to perform at least some of the functions necessary to support human life[68-70].

COMMENT

The problem of overcoming xenograft rejection is proving more difficult than predicted by no less an authority than Sir Peter Medawar who, in 1969, made the following remarks: 'A new solution is therefore called for: the use of heterografts – that is to say, of grafts transplanted from lower animals into man. Of the use of heterografts I can say only this: that in the laboratory we are achieving greater success with grafts *between* species today than we achieved with grafts *within* species 15 years ago. We shall solve the problem by using heterografts one day if we try hard enough, and maybe in less than 15 years'[71]. Over 25 years later his optimistic prediction has unfortunately not been fulfilled.

We would, however, appear to be at the threshold of an exciting era in organ transplantation where the use of xenografts is explored, initially perhaps as bridging devices, particularly in infants and young children. In adults and larger children it would seem wise to utilize the heterotopic position for xenografts when inserted as temporary assist devices. Experience gained in this area may lead to developments which allow xenografts to be used on a more permanent replacement basis.

In an interesting editorial, Chen and Michler[72] discussed the difficult question of when to initiate a clinical heart xenotransplantation program. They suggest (quoting the work of Fox and Swazey[73]) that three questions need to be answered, 'namely, (i) in the laboratory, what defines success of a sufficient level to warrant advancement to the clinical arena, (ii) under what clinical condition should this advancement proceed, and (iii) in the clinical arena, what defines success of a sufficient level to warrant further evaluation.' They do not provide conclusive answers to these questions, but clearly believe that 'the question that currently remains is not how, but rather *when* should heart xenotransplantation advance to the clinical arena?'

In line with the scientific developments outlined in this chapter, there would appear to be a growing acceptance of xenotransplantation among the public. A Partnership for Organ Donation Survey in the USA confirmed that whereas 80% of those questioned said they would accept an organ allograft, 50% said that they would accept an organ transplant from an animal if a suitable human organ was not available[74].

Just as the early pioneers of open-heart surgery from the 1950s did not envisage heart surgery on the scale it is performed today, I believe we do not envisage the role of xenotransplantation as it may be in 40–50 years time. The ready availability of a new organ to replace a diseased one will prove too great a temptation to the average patient or physician to allow either to persevere with inadequate medical therapy that maintains the patient in a suboptimal quality of life.

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81
Pathology of Cardiac Xenograft Rejection

A.G. ROSE

INTRODUCTION

Rejection of cardiac xenografts between closely related species may be exclusively acute (cellular) (e.g. Cynomolgus monkey to baboon) or it may be due to a mixture of vascular (hyperacute or delayed vascular/humoral-mediated) and acute rejection (e.g. vervet monkey to baboon). Rejection of xenografts between distantly related species (e.g. pig to baboon) is always by a hyperacute mechanism in the untreated model. Though the acute response can be delayed, or even prevented, by currently available immunosuppressive agents and techniques, hyperacute rejection (Figures 1–7) has proved extremely difficult to prevent or delay. Cardiac xenografts have very occasionally been used in humans in a desperate attempt to save the life of a patient for whom no human donor heart was available[1–3] (Chapter 82). Xenogeneic (and allogeneic in sensitized hosts) cardiac transplants, which have been performed in a wide range of experimental animals, have helped to further our understanding of the mechanism of hyperacute rejection[4–36] (Chapter 80).

PATHOPHYSIOLOGY

Hyperacute rejection[4], which by definition occurs within 24 hours following transplantation, is characterized by the immediate or early failure of graft function, and is accompanied by the development of typical morphological changes (Figure 1–4). Macroscopically the heart rapidly becomes cyanotic, turgid and edematous with epicardial ecchymoses and loss of function[5]. Histology shows interfascicular and/or interstitial edema, microvascular thrombi, destruction of capillaries, and interstitial hemorrhage. This is in sharp contrast to acute rejection, which is relatively rarely encountered within the first 5 days after transplantation. Delayed vascular rejection is a term that is sometimes used to refer to hyperacute rejection occurring more than 24 hours after transplantation (Figure 8).

The reason for this difference in the rapidity of onset is believed to lie in the different mechanisms involved in these two types of rejection. Thus, acute rejection is based on the development of cellular immunity, which takes several days to develop following exposure of the recipient's immune system to the foreign antigens contained in the graft. Vascular rejection may possibly be brought about by one of three mechanisms:

1. The classical pathway of complement activation is operative in humans and non-human primates. In discordant xenografting preformed antibodies activate donor endothelial cells, which in combination with complement achieve rapid graft destruction. In concordant xenografting there are no or low numbers of preformed antibodies. Subsequent production of cytotoxic antibodies may lead to graft destruction in a few days.
2. The alternate pathway of complement activation, which operates in some discordant species (e.g. rodents), is triggered by xenograft foreign endothelium without antibody. The complement cascade initiates platelet aggregation and cell lysis.
3. Direct activation of recipient thrombocytic aggregation and adherence is initiated by activation of the donor vasculature which produces platelet-activating factor. Neither complement nor antibodies are needed.

Since it is the vascular endothelium of the graft that comes into initial contact with the host, interacting with the host's circulating blood, it is the donor organ's microcirculation that undergoes maximal damage during vascular rejection. Formation of immune complexes may lead to endothelial cell necrosis. In keeping with the theory of immune-mediated endothelial cell destruction, it is not surprising that the ill-effects of the immune damage are most apparent in the capillaries, since they are composed of only a single layer of endothelial cells resting upon a basement membrane.

The formation of fibrin–platelet thrombi may precede the phase of recognizable endothelial cellular damage, but thrombus formation is also believed to be triggered by immune complexes within the graft microcirculation. A personal review[6] (see below) of a large number of both experimental and clinical xenografts and allografts leads one to conclude that coronary venular thrombosis (Figure 1) is a key event in hyperacute rejection. The interstitial hemorrhage that is such a characteristic feature of hyperacute rejection occurs in the distribution of the occluded venous drainage.

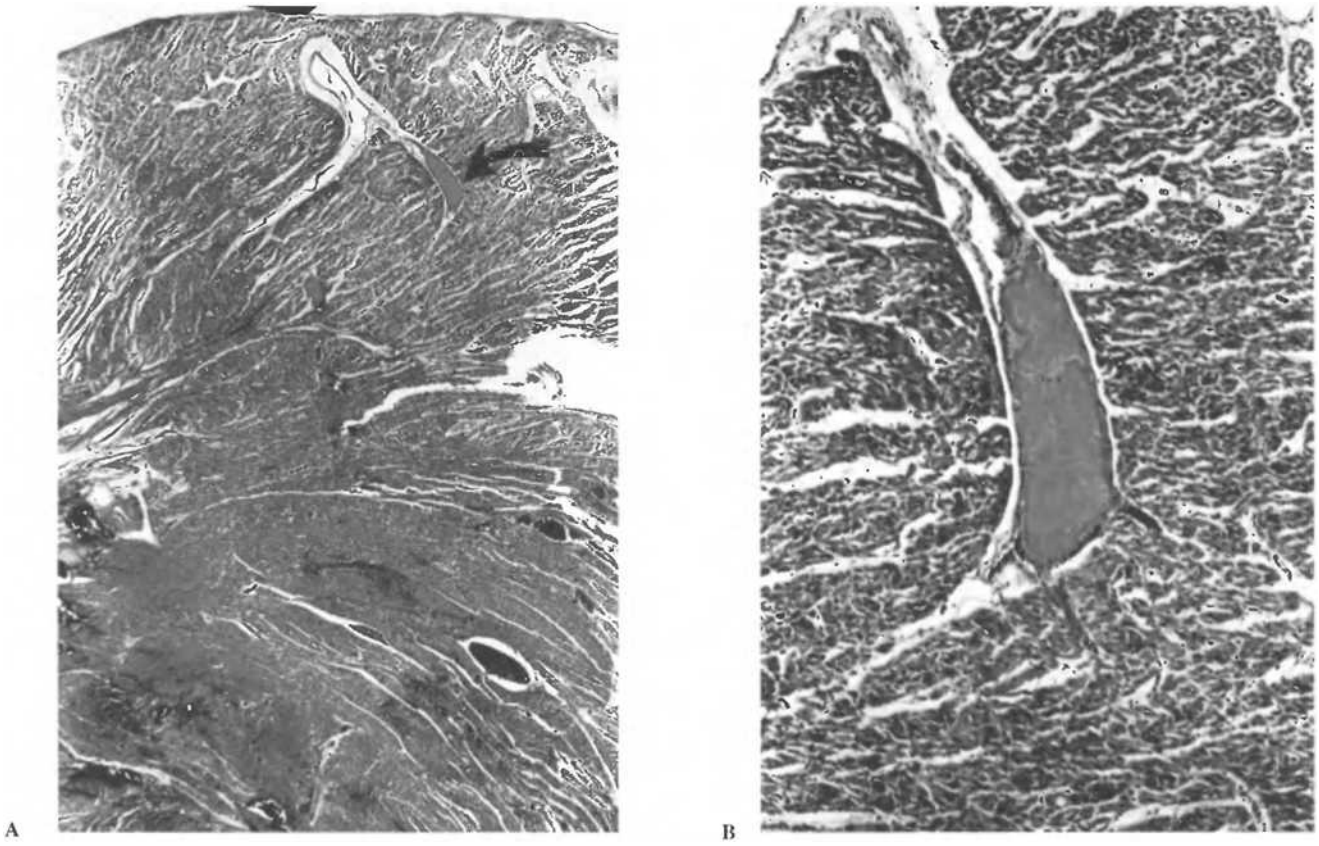


Figure 1 **A:** Low-power view across the left ventricle of a hyperacutely rejecting xenograft shows thrombosis (arrow) of a subepicardial vein and congestion plus hemorrhage within the subendocardial myocardium (bottom). **B:** Higher-power view of the same thrombosed vein

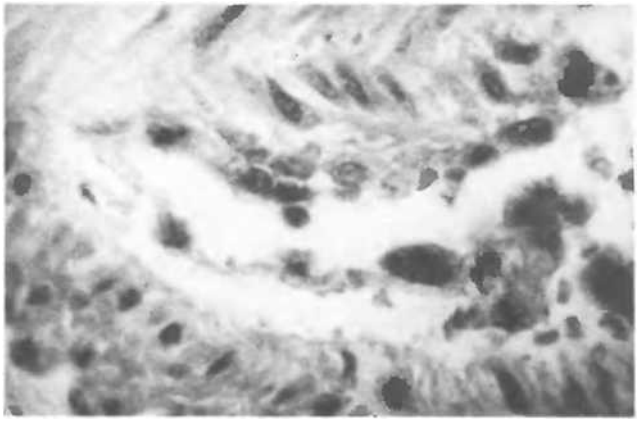


Figure 2 Detaching, partially necrotic endothelial cells (mitotic figures) within a chimpanzee heart which had been transplanted into a human patient. Delayed vascular rejection occurred on the fourth post-transplant day

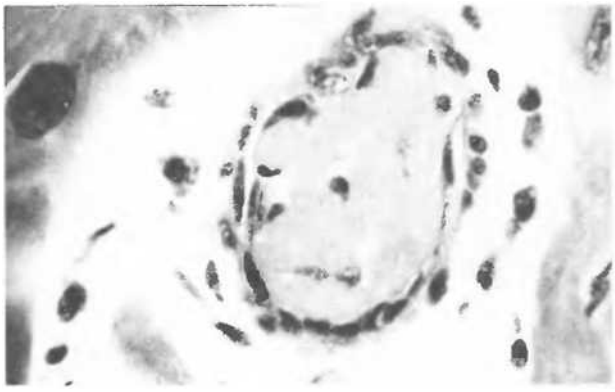
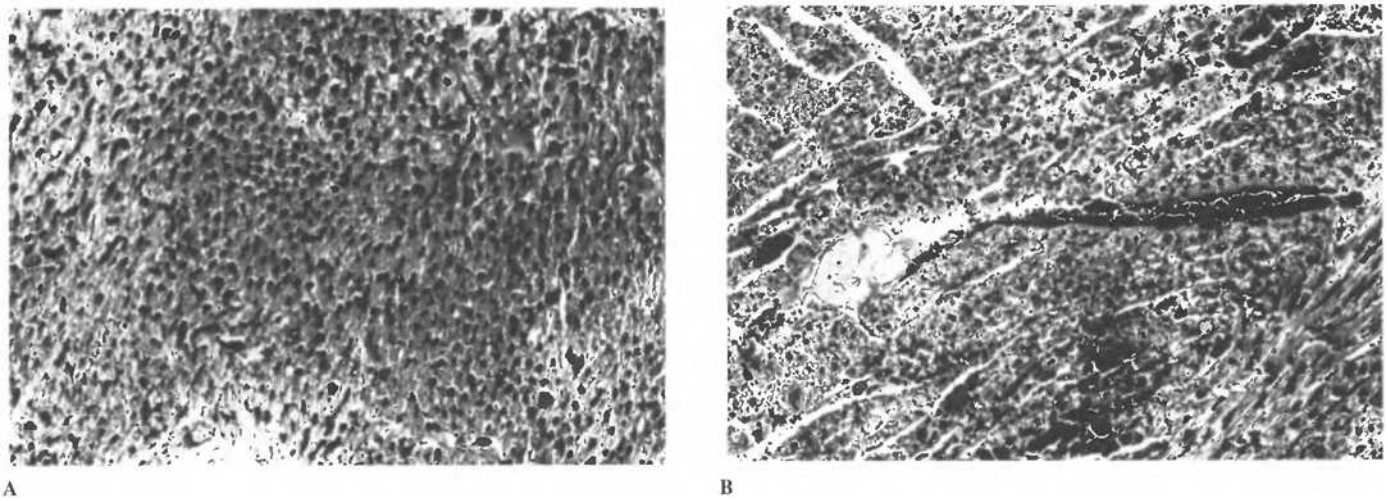


Figure 3 Post-capillary venule occluded by platelet–fibrin thrombus in the same rejected cardiac xenograft shown in Figure 2

Severe congestion precedes capillary disruption. The histological appearances of hyperacute rejection correspond to a form of venous sub-infarction of the heart. This concept would explain the lack of homogeneity of the histological findings in such hearts. The reason for the particular involvement of coronary venules in the early thrombotic process is unknown. The resultant vascular obstruction and capillary disruption rapidly lead to serious malfunction of the graft, and ischemic changes become recognizable in the myocytes. In some instances the xenograft may become totally necrotic.

The presence of neutrophils within the xenograft has been regarded as a characteristic feature of hyperacute rejection, and analogies have been drawn to the Arthus reaction, in which neutrophils are essential mediators of tissue damage. Different strains of rat have been used as a highly reproducible and rigidly controlled model of hyperacute cardiac allograft rejection. In this



A B

Figure 4 **A**: Intense capillary congestion in early hyperacute cardiac xenograft rejection is a consequence of venular thrombosis. **B**: Interstitial hemorrhage soon follows the severe congestion. A pale thrombus is present (left) within a portion of the venule running across the middle of the picture

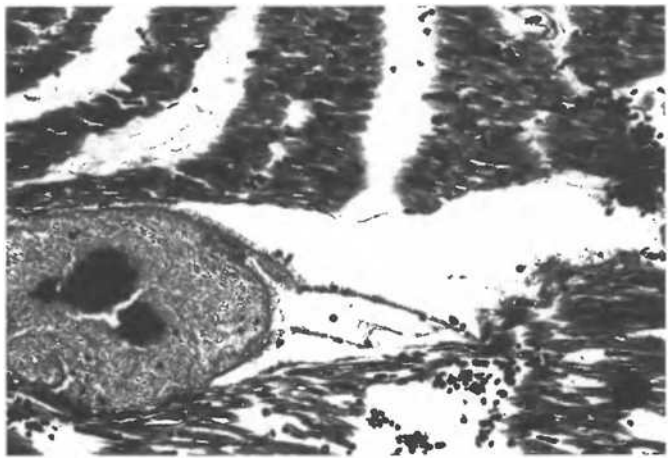


Figure 5 High-power view of thrombus within a venule in hyperacute rejection. Interfascicular edema and interstitial hemorrhage are also evident

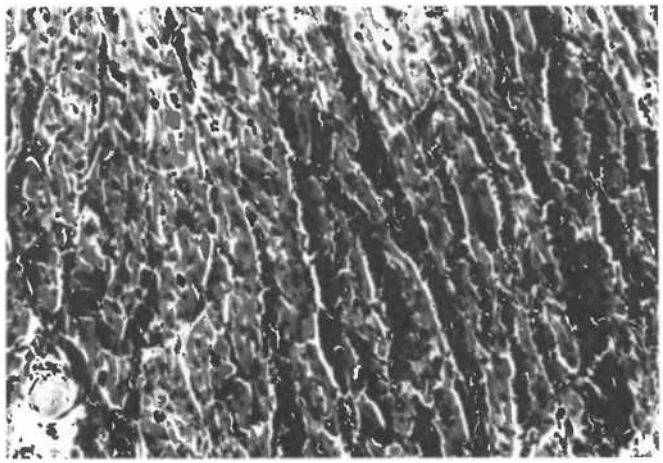


Figure 7 Intense interstitial hemorrhage between the myocytes obscures the pre-existent edema

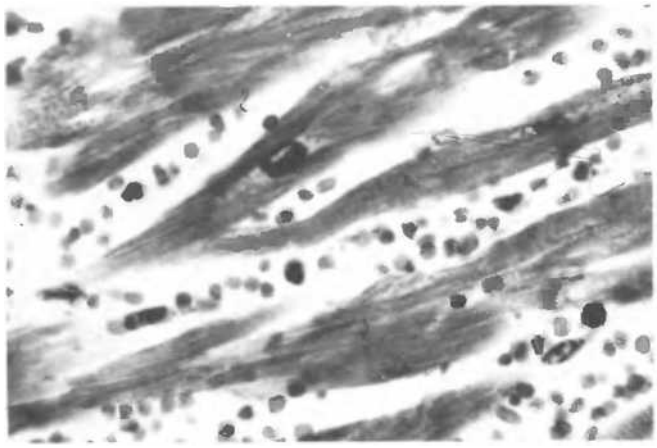


Figure 6 Vervet (African green) monkey cardiac xenograft in a baboon recipient shows diffuse capillary destruction with resultant massive erythrocyte extravasation and prominent interstitial edema characteristic of hyperacute rejection

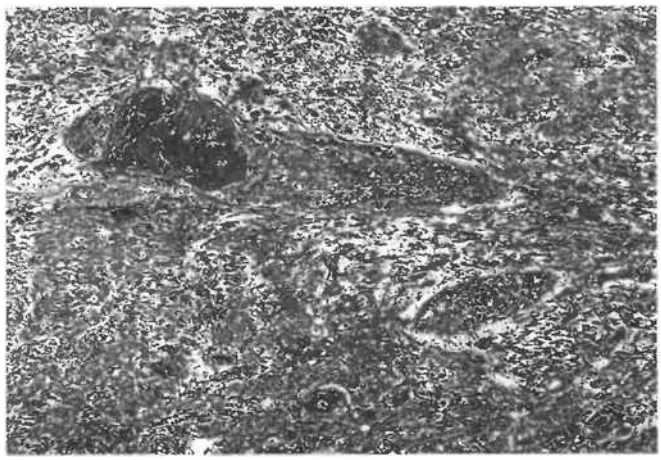


Figure 8 Delayed vascular rejection of several days duration showing organizing thrombi within subepicardial veins. The myocardium is diffusely hemorrhagic and edematous

model, Forbes *et al.*[7] presented experimental evidence to show that the absence of neutrophils from the graft produced no alteration in the characteristic pattern of vascular and myocardial damage in hyperacutely rejecting rat allografts. They concluded that neutrophils are neither essential nor specific participants in hyperacute allograft rejection in the rat model. It should also be borne in mind that interstitial hemorrhage will release leukocytes into the myocardial interstitium. Such leukocytes will be present in the same proportion to interstitial erythrocytes as is seen in the individual's peripheral blood. Forbes *et al.*[8] do not exclude a possible role for neutrophils in the production of myocardial injury in the later stages of hyperacute rejection. Neutrophils were not a prominent feature in our own material, either experimental or clinical.

The initial morphological event that characterizes hyperacute rejection in rat cardiac allografts is platelet aggregation in the vasculature of the graft, in the presence of a largely intact endothelium. This is followed by widespread endothelial damage. However, hyperacute rejection proceeded in the usual fashion in cardiac allografts in a rat strain with a hereditary platelet function defect[8].

O'Regan *et al.*[9], in a study of C6-deficient rabbits, showed that sufficiency of the sixth component of complement is required for hyperacute xenograft rejection in this model. In a study using cobra venom factor as a means of depressing recipient hemolytic C3 activity, Forbes *et al.*[10] further demonstrated that complement activation by graft-bound alloantibody is a critical effector mechanism of hyperacute rejection in an inbred rat model. Kobayashi *et al.*[11,12] have reported survival of <25 days of discordant (pig-to-baboon) complement-depleted cardiac transplants.

Another report suggests that the presence of antibodies against vascular endothelial cells may be related to hyperacute rejection in human cardiac allografts[13].

HISTOPATHOLOGICAL FEATURES OF REJECTION IN CONCORDANT AND DISCORDANT XENOGRAFT MODELS

In a personal review[14] of the histopathological changes seen in 75 experimental cardiac xenografts (52 between closely related (concordant) and 23 between distantly related (discordant) species) in which the roles of ABO-incompatibility, xenograft concordance, and the effects of immunosuppression were evaluated, the following histological patterns of rejection were observed (Table 1). Hyperacute rejection was seen in all 23 discordant xenografts (Figure 3) and in 13 concordant xenografts (Figure 4). Acute cellular rejection was seen in 20 concordant xenografts, and a mixture of acute and vascular rejection in 10 concordant xenografts (Figure 5). Nine concordant xenografts showed no rejection at the time of death of the recipient baboon; death was believed to result from the side-effects of heavy immunosuppressive drug therapy in the majority of cases.

It would appear that, if hyperacute rejection can be averted in the early period following xenotransplantation, subsequent rejection may take one of three morphological forms: (a) acute cellular rejection, (b) delayed vascular rejection, or (c) mixed acute and delayed vascular rejection.

The mixed form of rejection (Figure 9) appears superficially similar to severe acute cellular rejection with interstitial hemorrhages, but mixed xenograft rejection is characterized by a lymphocytic response which does not approach the severity of that seen in severe acute cellular rejection. The microvascular destruction is thus the dominant factor, and is disproportionate to the lymphocytic infiltration. Sharma *et al.*[15] experimentally prevented hyperacute rejection in cardiac transplants performed in presensitized dogs, but these grafts were later rejected by what was regarded as primary cellular rejection. These authors made a clear separation between hyperacute and acute rejection, and did not

Table 1 Histopathology of rejection in heterotopic cardiac xenografts in the baboon

Group		*n*	*Type of rejection*			
			None	*Acute*	*Mixed*	*Hyperacute*
Concordant (vervet monkey to baboon)						
(1) No IS*		9	0	0	4	5
(2) ABO incompatible, no IS		9	0	4	2	3
(3) CsA, AZA, MP		6	0	5(2)	0	1
(4) ABO incomp., CsA, AZA, MP		5	0	3(1)	1	1
(5) CsA, AZA, MP	i.v. MP	5	1(1)	3	0	1
(6) RATG, CsA, AZA, MP	therapy for	6	3(3)	2	0	1
(7) 15-DS, CsA, AZA, MP	rejection	7	4(4)	2	0	1
(8) 15-DS, CsA, MP	episodes	5	1(1)	1	3	0
Discordant (pig to baboon)						
(9) No IS		4	0	0	0	4
(10) Splenectomy		3	0	0	0	3
(11) CsA, AZA, MP		5	0	0	0	5
(12) Antibody adsorption		7	0	0	0	7
(13) Antibody adsorption, CsA, AZA, MP		4	0	0	0	4(1)

IS = immunosuppression; CsA = cyclosporine; AZA = azathioprine; MP = methylprednisolone; RATG = rabbit anti-human thymocyte globulin; 15-DS = 15-deoxyspergualin
*All concordant xenograft pairs were ABO blood group compatible except where stated
Figures in parentheses denote recipient died

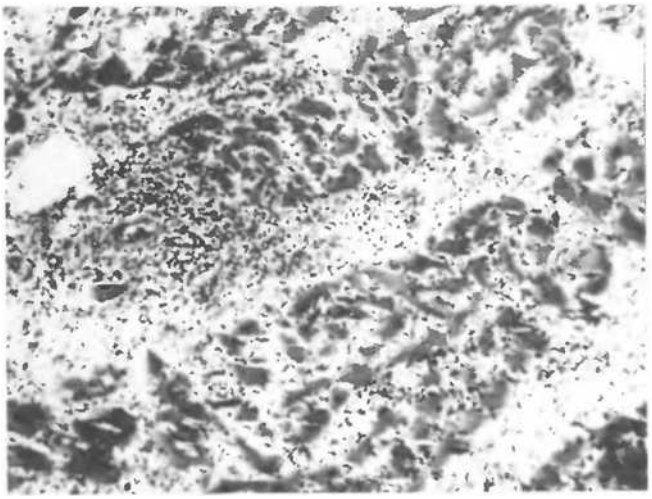


Figure 9 Vervet (African green) monkey cardiac xenograft in a baboon recipient, demonstrating a mixture of acute rejection (indicated by moderate lymphocytic infiltration, top left) and vascular rejection (indicated by massive interstitial edema and erythrocytic extravasation, elsewhere)

describe a mixed form. In our experience, however, this distinction between acute and delayed vascular (hyperacute) rejection is not always so clear-cut, and mixed forms occur. The late onset of the delayed vascular component of mixed rejection also differs from the classical concept of early-phase hyperacute rejection.

The clinical significance of the above observations is that the grading system(s) currently used to measure acute allograft rejection may have to be modified once xenografts are introduced into clinical use. Furthermore, vascular rejection may lead to graft failure at a relatively late stage after transplantation, as demonstrated by graft failure occurring as late as 25 days in the studies outlined above. Again it should be emphasized that the term 'hyperacute' is applied to vascular rejection occurring within 24 hours after grafting and 'delayed vascular rejection' is the term used for vascular rejection that occurs after the first 24 hours. A grading system for hyperacute and delayed vascular rejection is described below.

ROLE OF VENULAR THROMBOSIS IN THE PATHOGENESIS OF HYPERACUTE AND DELAYED VASCULAR REJECTION

A review of the pathologic features of 112 experimental and clinical cardiac allografts and xenografts (serial biopsies and excised grafts) revealed a common sequential development of histological changes in grafts showing vascular (hyperacute or delayed vascular) rejection[6]. Thrombosis of venules, particularly in the subepicardium and outer half of the myocardium, is the key initial event (Figure 1). This leads to interfascicular and, later, interstitial (intermyocyte) edema, which is especially obvious in the outer half of the myocardium. In parallel with these changes is the development of congestion in the subtended venules and capillaries (Figure 4). Subsequently, focal or diffuse interstitial hemorrhage (Figures 4B, 6 and 7) affecting the subendocardium, sometimes extending in time to involve the inner half of the ventricular myocardium, is observed.

Hyperacute rejection in its various temporal modes appears to be analogous to venous subinfarction of the heart. The observed pathology (in which venular thrombosis plays a key role) favours a thrombogenic hypothesis as the basis for the histological features of hyperacute and delayed vascular rejection (namely edema, vascular thrombi and interstitial hemorrhage) and is less supportive of an antigen–antibody reaction which activates complement by the classical pathway. The pathology thus fits with either (a) activation of the alternate pathway by the graft foreign endothelium in the absence of antibody or (b) the direct activation of the donor vasculature leading to platelet aggregation and adherence. The suggested key role for venular thrombosis would explain the non-uniform distribution of the histological changes in vascular rejection, and may hold the potential for finding means of preventing the development of hyperacute rejection of xenografts.

HISTOPATHOLOGIC GRADING OF HYPERACUTE AND DELAYED VASCULAR REJECTION

If future attempts to introduce xenografting into clinical practice prove successful, it will be essential to have a clinically relevant, reproducible grading system for vascular rejection. No previous formal attempt has been made to grade hyperacute or delayed vascular rejection based upon a review of both experimental and clinical material. In an attempt to define a microscopic grading system for hyperacute and delayed vascular rejection of the heart, the present author reviewed the clinical and histologic findings in 112 (previously personally studied) experimental (n=109) and clinical (n=3) cardiac xenografts and allografts, most of which showed hyperacute rejection. The study material comprised 44 discordant xenografts, 41 concordant xenografts, and 27 allografts. Hyperacute rejection was present in 75 instances, delayed vascular rejection in five, acute cellular rejection in 13, and mixed vascular and acute cellular rejection in 19. The detailed histopathologic features were analyzed together with the clinical data. Delayed vascular rejection (i.e. that occurring later than 24 hours post-transplantation) has many histologic features in common with the usual form of hyperacute rejection, the major difference being the time frames in which the two develop.

The following grading system[16] was devised:

Grade A: hyperacute rejection, which is characterized by venular thrombi, generalized edema and interstitial hemorrhage. The latter is most prominent in the inner layers of the ventricular myocardium.

Grade B: mixed delayed vascular and acute cellular rejection (which is usually encountered more than 7–10 days post-transplantation) is usually characterized by the features of hyperacute rejection plus a modest lymphoid infiltration.

Both Grades A and B vascular rejection can be subcategorized into three stages: (1) mild (initial), (2) moderate (intermediate) or (3) severe (late). *Stage 1* (early, mild phase): shows fresh venous thrombi, swelling of capillary endothelial cells, relatively normal myocytes and intact capillaries. *Stage 2* (intermediate, moderate phase): shows, in addition to the above, congestion of capillaries and interfascicular edema. Capillaries show sludging of erythrocytes and occasional thrombi. Focal interstitial hemorrhage is

seen in the inner half of the myocardium. *Stage 3* (late, severe phase): shows all of the above plus disruption of capillaries, widespread interstitial hemorrhage with most severe involvement subendocardially, and variable amounts of myocyte necrosis. If hyperacute rejection has been delayed by therapeutic means (delayed vascular rejection), some thrombi may show signs of organization.

The proposed grading system provides a basis for meaningful pathologic evaluation of vascular rejection.

COMMENT

Despite the large amount of experimental work that has already been undertaken[18-42], the successful use of animals as sources of organs for transplantation in humans awaits a solution to the problem of hyperacute rejection. If this can be overcome, then the currently available pharmacological agents may well be able to prevent or treat the subsequent acute rejection that may develop. It has also been suggested, however, that accelerated atherosclerosis will eventually present a significant problem in cross-species transplantation[43]. If this proves to be the case, the longevity of such xenografts may clearly be limited.

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82
Clinical Experience with Cardiac Xenotransplantation

S. TANIGUCHI AND D.K.C. COOPER

INTRODUCTION

There have been nine reported clinical investigations of cardiac xenotransplantation into humans (Table 1). Both concordant and discordant donor organs have been used.

Case 1

By the mid-1960s the increasing success of experimental cardiac transplantation (Chapter 18), and the experience gained by Reemtsma (Figure 1)[1–3], Starzl (Figure 2)[4,5] and others[6,7] in their initial attempts at renal xenotransplantation in humans, led Hardy (Figure 9, Chapter 18) and his colleagues to perform the first cardiac xenotransplant in a human[8]. Their attempt, in 1964, to transplant the heart of a large chimpanzee into the chest of a 68-year-old man has been outlined in Chapter 18, and will not be discussed again here. Suffice it to say that the heart was too small to support the circulation adequately, and the patient died after 1 hour.

Table 1 World experience in clinical heart xenotransplantation*

Year	*Surgeon*	*Institution*	*Donor*	*Type*	*Outcome*	*Reference Source*
1964	Hardy	University of Mississippi, Jackson, Mississippi, USA	Chimpanzee	OHTx	Functioned 2 hours (heart too small)	8
1968	Cooley	Texas Heart Institute, Houston, Texas, USA	Sheep	OHTx	Immediate cessation of function (? vascular rejection)	9
1968	Ross	National Heart Hospital, London, UK	Pig	HHTx	Cessation of function within 4 min (? vascular rejection)	10, 11
1968	Ross	National Heart Hospital, London, UK	Pig	Perfused with human blood but not transplanted	Immediate cessation of function (? vascular rejection)	10, 11
1969	Marion	Lyon, France	Chimpanzee	?OHTx	Rapid failure (? raised pulmonary vascular resistance)	12
1977	Barnard	University of Cape Town, Cape Town, South Africa	Baboon	HHTx	Functioned 5 hours (heart too small)	13
1977	Barnard	University of Cape Town, Cape Town, South Africa	Chimpanzee	HHTx	Functioned 4 days (probable vascular rejection)	13
1984	Bailey	Loma Linda University, Loma Linda, California, USA	Baboon	OHTx	Functioned 20 days (vascular rejection)	15
1992	Religa	Silesian Academy of Medicine, Sosnowiec, Poland	Pig	OHTx	Functioned 24 hours (cause of failure uncertain)	16

*Based on ref. 17.
OHTx = Orthotopic heart transplantation; HHTx = Heterotopic heart transplantation.

Figure 1 Keith Reemtsma was the first to explore xenografting in humans in a scientific manner. Between 1963 and 1965, while at Tulane University in New Orleans, he transplanted a series of chimpanzee kidneys into patients with advanced renal failure; kidney function was obtained for periods up to 8 months

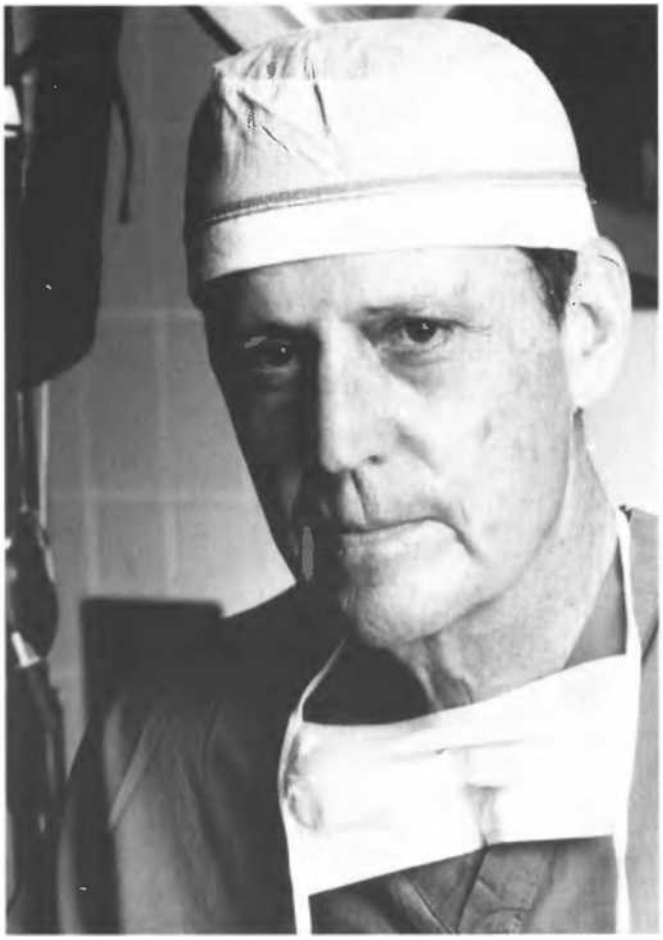

Figure 2 Tom Starzl was the first to transplant baboon kidneys and chimpanzee and baboon livers into humans. One baboon liver functioned moderately well until the patient died from overwhelming sepsis after 70 days

Case 2

The probable second attempt at clinical xenotransplantation was by Cooley (Figure 1, Chapter 77) and his colleagues in Houston in 1968, who transplanted a sheep heart into a 48-year-old man who had advanced coronary artery disease and whose circulatory status was deteriorating several hours after resuscitation following a cardiac arrest[9]. The lymphocytotoxic crossmatch was positive, and the heart was immediately hyperacutely rejected. Even today, with currently available immunosuppressive agents, such an attempt using a discordant xenograft would be doomed to early failure.

Cases 3 and 4

Further experimental steps were taken by Ross (Figure 3) and his colleagues in London, England, in early 1968, which are worthy of note even though they were unsuccessful. Ross's group was faced with the unusual circumstance of having two patients at the same time in adjacent operating rooms, neither of whom could be weaned from cardiopulmonary bypass support following open heart procedures. They reasoned that an animal heart inserted as an auxiliary pump might be able to maintain the circulation until either the patient's own heart recovered or a human donor heart became available to allow orthotopic transplantation. In one of the patients a pig heart was therefore anastomosed in parallel as an auxiliary heart transplant (Figure 4) but, within 4 minutes of reperfusion, it was hyperacutely rejected[10,11].

Following this experience, in the second patient a preliminary test was carried out by inserting the coronary perfusion lines from the pump-oxygenator into the coronary arteries of a pig heart to see whether the same response would occur. The reaction was identical, so this heart was not transplanted.

The problem of hyperacute rejection of non-primate organs in humans has still not been resolved, more than 25 years later. Nevertheless, Donald Ross and his colleagues must be credited with the concept of using an animal heart as a 'bridging' device towards transplantation with a human heart, an idea that was taken up subsequently by Barnard (see below) and has gained further support and interest in recent years. The exact date of Ross's procedure remains obscure, but it almost certainly took place in the first 6 months of 1968, possibly before Cooley's operation.

Figure 3 Donald Ross, who was the first to attempt to use an animal heart as a 'bridging' device towards cardiac allotransplantation

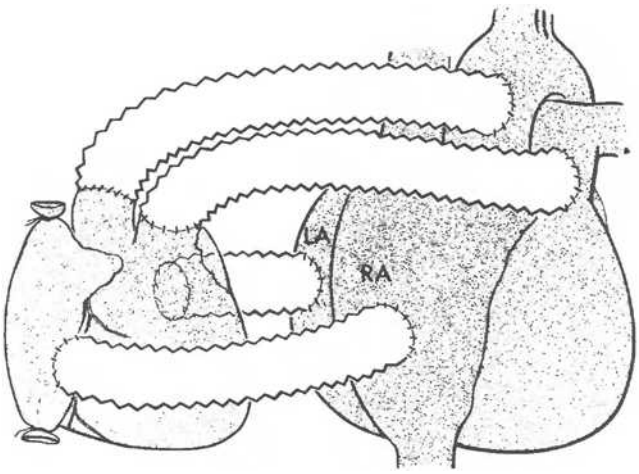

Figure 4 Drawing of the auxiliary pig heart transplant performed by Donald Ross and his colleagues. Dacron grafts were used to connect the donor and recipient atria and great vessels

Both Ross and Cooley have since indicated to us that they feel mildly embarrassed about these attempts at discordant cardiac xenotransplantation, as they were highly premature. Although with the aid of hindsight this is clearly correct, the frustration of a surgeon faced with a dying patient sometimes leads him (or her) to take desperate measures. 'Diseases desperate grown, By desperate appliance are reliev'd, Or not at all', as Shakespeare has written (Hamlet IV.iii. 9).

Case 5

In 1969 Marion in France inserted a chimpanzee heart, but few details are known[12].

Cases 6 and 7

The next two reported attempts were in 1977, when Barnard (Figure 10, Chapter 18) and his colleagues used concordant cardiac xenografts on two emergency occasions at Groote Schuur Hospital in Cape Town[13]. On both occasions the patient's native left ventricle failed to support the circulation when attempts were made to discontinue cardiopulmonary bypass after surgical procedures. Intra-aortic balloon pump support was unsuccessful in the first case and not available in the second. Both transplants were placed heterotopically and were intended as temporary cardiac assist 'devices', to support the patient until the native ventricles recovered.

The first of these two patients received a heterotopic graft from a 30 kg baboon. This small heart proved insufficient to support the circulation in the presence of repeated attacks of ventricular fibrillation which affected the patient's own heart. The patient died some 6 hours after transplantation.

The second patient was supported successfully by a heterotopic chimpanzee heart until rejection occurred 4 days later; the recipient's own heart failed to recover sufficiently to support the circulation alone. Higher doses of immunosuppression (azathioprine, corticosteroids, and antithymocyte globulin) were used than would be the case with a human donor. Although the initial report suggested that severe acute rejection was the cause of graft failure, a review of the specimen by Rose has confirmed that vascular rejection was a major feature[14]. However, at the time, this experience suggested that a heterotopic transplant, using a suitable xenograft and heavy immunosuppression, might be a successful bridge to allotransplantation, or might sustain life if the patient's own myocardial function would recover within 2–3 days.

Case 8

In 1984, Bailey (Figure 5) *et al.*[15] transplanted a baboon heart into a neonate who had hypoplastic left heart syndrome. With the advantage of cyclosporin, in addition to other immunosuppressive therapy, the recipient survived 20 days. Death was from progressive graft necrosis, complicated by acute renal and pulmonary insufficiency. Hyperacute rejection did not occur. Autopsy findings showed only traces of cell-mediated rejection in the cardiac graft. Graft failure appeared to have resulted from a progressive humoral response, unmodified by immunosuppression.

The donor selection process in this initial baboon-to-newborn-human clinical trial was concerned with the presence of preformed donor-specific lymphocytotoxic antibody, and with the degree of homology between donor and recipient tissues. The recipient was erythrocyte type O. Type O baboons are exceedingly

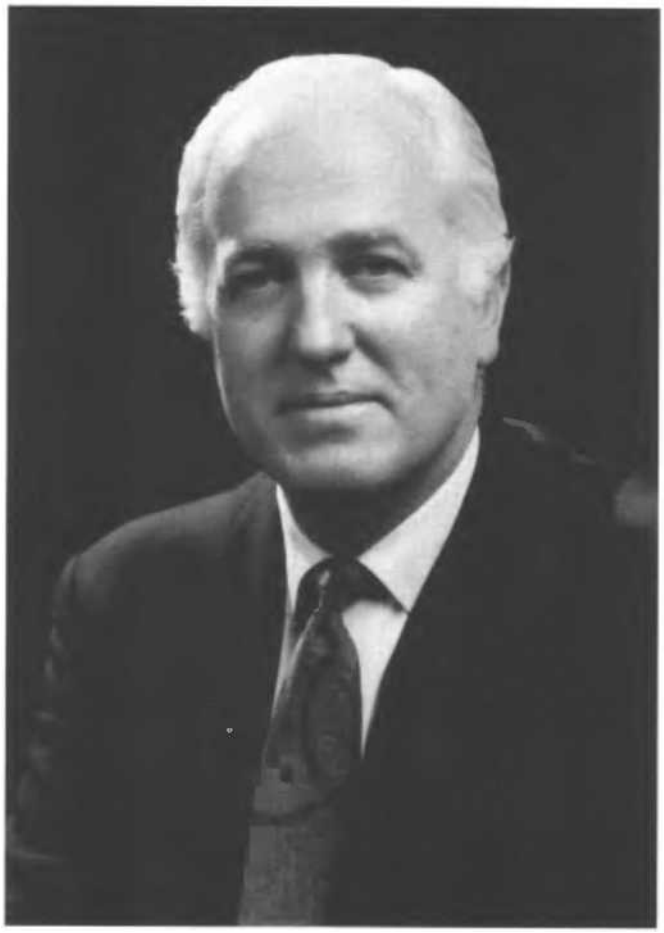

Figure 5 Leonard Bailey, who led the team that carried out orthotopic transplantation using a baboon heart in 'Baby Fae' in 1984

rare, and none was available for use as a donor. Blood group matching, therefore, could not be achieved. Anti-A and anti-B isoagglutinins to human erythrocytes and low-titered heteroagglutinins to baboon erythrocytes were present in the patient's circulation before transplantation, but disappeared afterwards. It was unclear whether the patient selectively failed to produce hemagglutinins postoperatively or, more likely, whether the hemagglutinins were immediately and continuously adsorbed on to the baboon graft vascular endothelium.

On the basis of this case, in which cardiac histopathological features appeared to be those of antibody- and/or complement-mediated injury despite immunosuppression, this group felt that rejection of the xenograft might have been due to ABO hemagglutinins and/or species-specific cytotoxic antibody, among other factors. The exact role of each type of antibody remains uncertain. It seems likely, however, that ABO antibodies and/or anti-baboon antibodies were gradually adsorbed by the graft, producing injury to the largest endothelial bed, namely the microcirculatory vessels. This phenomenon resulted in widespread microvascular lumenal narrowing. Circulatory sludging, thrombosis, cellular hypoxia, and myocyte injury and/or necrosis followed.

Case 9

The final and most recent attempt at cardiac xenotransplantation in a human was reported in 1992 by a Polish group, headed by Czaplicki, and involved the transplantation of a pig heart[16]. The patient was a 31-year-old man with Marfan's syndrome and severe aortic valve insufficiency. He was treated by an experimental regimen of homogenized embryonal and early fetal calf thymuses in capsules administered orally for 14 days prior to the transplant. He was also given cyclosporin by mouth at a daily dose of 2 mg/kg body weight and azathioprine in a daily dose of 75 mg. For the last 8 days before operation he was injected intramuscularly with 150 mg of an agent called Thymex L and also with TFX-thymomodulate every second day.

His condition was exceedingly poor, and it was decided to go ahead with a xenograft as no allograft was available. Two 90 kg pigs had been identified, and for 18 days before their use as donors they were also given homogenized embryonal and early fetal calf thymuses in capsules by mouth, and also by intramuscular injection, together with intramuscular embryonal and early fetal thymic calf extracts. Again, for 8 days before transplantation, each pig received 100 mg of cyclosporin and 50 mg of azathioprine by mouth daily and 150 mg of Thymex L daily and 10 mg of TFX-thymomodulin every second day intramuscularly.

At the time of surgery a non-treated pig heart was perfused with the patient's blood through the heart–lung machine, and the pig heart underwent typical hyperacute rejection after 20 minutes. In the opinion of the surgical team this control pig's heart served to partially eliminate antibodies against the donor species' antigens from the recipient's blood.

The heart of the first pretreated pig was then connected to the patient's blood circulation. After 80 minutes it was disconnected. There were no histopathological features of rejection in this heart. The second pretreated pig's heart was then transplanted orthotopically. The patient was weaned from cardiopulmonary bypass and, after 2 hours of cardiac function, the patient's chest was closed. For 4 hours the patient's condition was good, with his blood pressure being recorded at 90/50 mmHg. There was then a deterioration in blood pressure and the cardiac index was measured at only 1.75 l/min per m^2. Kidney function began to deteriorate and acidosis developed. A hemofilter was connected after 5 hours, but the patient died from a low cardiac output syndrome just under 24 hours from the time of perfusion of the heart. Histopathologic examination did not show any features of rejection of the transplanted heart.

The authors of this report attribute failure of the pig heart to the large size of the recipient, but provide no supportive evidence for this conclusion. In fact, the donor pig and human recipient were of very comparable sizes.

COMMENT

Though there has been increasing continuing experimental work involving both concordant and discordant xenotransplantation since 1992, no further clinical attempts have been reported.

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83
Xenotransplantation of the Lung

R.N. PIERSON III

INTRODUCTION

Lung and heart–lung transplantation are now established as therapeutic interventions for many terminal conditions affecting the pulmonary system. Paradoxically, our increasing clinical success exacerbates the donor organ shortage by broadening indications for transplantation and increasing referrals of appropriate patients at an earlier stage in their disease. The most acute need is among patients with either congenital heart disease or cystic fibrosis, for whom heart–lung or double lung transplantation is at present the only viable option. Appropriate organs are often not available for this group of generally young patients; many of those listed for transplant die waiting[1]. Additional patients with other end-stage pulmonary disorders might benefit if more lungs were available.

One potential source of organs is a xenogeneic donor[2]. In choosing a donor species for man one would intuitively choose a donor phylogenetically closely related to man; but while relative physiologic, biochemical, and immunologic similarity might favor primates, there are several important factors militating against this option. Cardiothoracic organs of a size appropriate for adult humans would be available only from large adult male chimpanzees, gorillas, or similar endangered species. These animals harbor epizootic viral infections likely transmissible to man, breed poorly in captivity and slowly in the wild, bear small litters, and take decades to reach usable adult size. Appropriate ethical concerns further weigh compellingly against their use. Several investigators are currently preparing to use baboon hearts clinically for children and small adults. However, even if successful, the use of baboon organs will not address the needs of the vast majority of potential lung recipients.

SPECIFIC ISSUES RELATED TO DISCORDANT LUNG TRANSPLANTATION

Historical background

Campbell *et al.*, in the 1950s[3], reported using dog lungs as oxygenators for seven patients; high pulmonary vascular resistance limited flow to 400 ml/min. Waldhausen *et al.*[4] similarly found maximal flows through dog lung of 200 ml/min. Bryant *et al.* perfused pig lungs with human blood using an *ex-vivo* perfusion system[5]. They achieved flow rates of less than 10% of normal human levels. Blood retrieved from the cardiopulmonary bypass machine and stored gave better results than fresh blood, suggesting that formed blood elements, which are depleted and defunctionalized by storage, contribute to the pace of the rejection response. Rapid elevation of pulmonary vascular resistance and parenchymal edema occurred promptly in all of these situations, and in a number of other experimental models of lung xenotransplantation[6–15].

Role of complement

In general, a central role for complement has been shown conclusively in the hyperacute (minutes to hours) dysfunction of vascularized organs transplanted between discordant species; its importance has been confirmed in the pig-to-primate combination[14–23]. Whether the lung is privileged with respect to complement-mediated damage is the focus of ongoing controversy.

Recently, Kaplon *et al.* reported short-term (1–3-day) pig lung survival in the baboon, with evidence of only modest levels of antibody deposition and complement activation relative to hyperacutely rejected pig hearts[24]. Flow probes around the main pulmonary artery and the transplanted lung suggested that 10–40% of the cardiac output was perfusing the xenograft. Blood gas samples from the pulmonary vein of the transplant had a high P_{O_2}. However, the orthotopic single lung grafts were unable to support the recipient when the contralateral native pulmonary artery was transiently occluded; very high pulmonary vascular resistance in the graft resulted in right heart failure and circulatory collapse.

Similarly, this group recently reported that discordant double lung transplant primate recipients could not be weaned from bypass due to right heart failure[25]. A modest decline in anti-endothelial antibody, coupled with patchy deposition of IgM and complement pathway components, were interpreted as consistent with the absence of hyperacute rejection. However, these observations might also be explained by hypoperfusion of the grafts

due to lung injury. A high pulmonary venous P_{O_2} and demonstrable pulmonary artery flow apparently do not correlate with a clinically meaningful level of graft function.

Using an *ex-vivo* working heart–lung model to address this issue, we find that pig lung is rapidly damaged by human blood[14]. The injury is characterized by a rapid, profound rise in pulmonary vascular resistance (within 5 minutes) and subsequent severe pulmonary capillary leak. With rare exceptions, oxygen transport function is lost within 30 minutes. Immunohistochemical staining shows immunoglobulin deposition (IgM>IgG) as well as deposition of complement components from both classical and alternative pathways[26]. Prevention of antibody binding and complement activation (by antibody absorption combined with heat treatment) results in graft function similar to that obtained when the graft is perfused with pig blood. Both features of lung injury (vasoconstriction and capillary leak) are significantly blunted by strategies which prevent complement activation, demonstrating that these phenomena are in large measure complement-mediated[15,26].

These findings suggest that the pig lung is susceptible to traditional hyperacute rejection, and that the process can be modulated by specific intervention directed at regulation of complement activation. In our estimation, the claims of Kaplon *et al.* (that the lung dysfunction they observe does not represent complement-mediated hyperacute rejection, and thus that the lung is privileged with regard to hyperacute rejection) are thus refuted. In fact, their physiologic and histologic observations are in large measure consistent with our own, and support the conclusion that primate anti-pig antibody and complement trigger rapid injury to the lung.

Complement-independent mechanisms

Prevention of complement activation alone permits prolonged survival of discordant heart grafts for days; survival may be extended to weeks if additional immunosuppression is used[16,17,23]. It is possible, however, that complement-independent mechanisms, driven either by xenospecific antibody or by other effectors of the immune response, such as neutrophils and platelets, will render protection of the lung by complement-directed strategies alone incomplete.

We have attempted to define the role of factors other than complement in discordant lung transplant dysfunction using a traditional model, depletion of recipient complement with cobra venom factor (CVF)[27]. CVF acts as a C3 convertase, consuming C3, and thus depleting the complement component common to both the classical and alternative pathways. Pig lungs were perfused with human blood depleted of complement by pretreatment of plasma with CVF. Neither the elevation of pulmonary vascular resistance nor capillary leak was prevented. Even when antibody absorption was added to CVF treatment, hyperacute lung injury and vasoconstriction occurred.

This result might be taken as evidence for complement-independent mechanisms governing hyperacute lung rejection. However, while CVF depletes C3, in the process it generates high levels of the neutrophil attractant and anaphylatoxin C3a; in other models CVF causes neutrophil-mediated, P-selection-dependent pulmonary capillary leak[28]. We suspect that C3a is responsible for the vasoconstriction and pulmonary injury observed in these experiments, thus simulating complement-mediated hyperacute rejection, and obscuring the role of complement-independent mechanisms in discordant lung xenograft dysfunction.

Two groups have recently achieved significant prolongation of pig heart survival in primates using hearts from pigs transgenic for human complement-regulatory proteins[19,20]. Parallel experiments used lungs from animals transgenic for human decay accelerating factor (hDAF), testing for protection from hyperacute rejection by *ex-vivo* perfusion with fresh human blood[21]. None of these transgenic lungs was protected from the development of high pulmonary vascular resistance. Only two of seven lungs expressed significant levels of hDAF on the pulmonary endothelium; in one of these two cases the rise in vascular resistance resolved spontaneously, and graft function (as measured by oxygen transport function) persisted for 90 minutes (vs <20 minutes for controls and other transgenics with low hDAF expression).

This preliminary experience suggests that strategies directed at complement regulation may contribute importantly to prolongation of discordant lung xenografts, but that other factors may also be crucial to eventual clinical success. Resolution of the relative importance of complement-dependent and complement-independent mechanisms to dysfunction of discordant lung xenografts awaits results of experiments using either soluble complement receptor 1 or pigs with a higher pulmonary endothelial expression of human complement-regulatory proteins.

COMMENT

In general the lung appears to be more sensitive than the heart to a variety of systemic insults, as manifested by the pulmonary capillary leak and ARDS syndromes, which sporadically occur during sepsis or following cardiopulmonary bypass. These insults have in common not only complement activation but the activation and intrapulmonary sequestration of neutrophils, platelets and macrophages. Supporting this general concept of lung injury are experiments demonstrating salutary effects for neutrophil depletion, adhesion molecule blockade, and anti-TNF antibody[28–32]. These observations suggest that complement-independent mechanisms, driven either by xenospecific antibody or by other effectors of the immune response such as neutrophils and platelets, may render protection of the lung incomplete, even with effective control of complement activation.

Preliminary work identifies thromboxane as the central mediator of pulmonary vasoconstriction, and a contributor to capillary leak, in pig lungs perfused with human blood (Pierson RN III, Parker RE, unpublished). We believe that platelet or tissue macrophage activation triggers thromboxane production; whether thromboxane production occurs consequentially to or independently of complement activation is an unresolved question of major importance to programs hoping to use transgenic lungs for clinical discordant lung xenotransplantation. Addition of antibody absorption to thromboxane blockade yields impressive protection of lung function, approaching 4 hours in some preliminary experiments. Thus, there is reason to suspect that, once the several interconnected mechanisms governing hyperacute rejection of the lung are elucidated, clinically important function across the discordant species barrier may be achieved.

In summary, concordant pulmonary xenografting is unlikely to be clinically important for a variety of ethical, infectious disease, and logistical reasons. Discordant lung xenotransplantation is cur-

rently prevented by hyperacute rejection. Pig-to-human lung transplantation may prove more difficult than heart or kidney transplantation due to susceptibility of the lung to non-xenospecific or complement-independent insults. Nonetheless, donor modifications to prevent complement injury, and significant advances in our understanding of hyperacute rejection of the lung, may facilitate clinical application in the foreseeable future.

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Cardiomyoplasty – Skeletal Muscle Assist

JA. MAGOVERN AND R.C. REDDY

INTRODUCTION

Congestive heart failure (CHF) is a physiologic situation in which the heart is unable to maintain a sufficient cardiac output to meet the metabolic requirements of the patient. Several diseases are responsible for CHF, including coronary artery disease, hypertension, and idiopathic cardiomyopathy, but the mortality is high regardless of the etiology. The prevalence of this disease in the Unites States is approximately 2 million cases, and the incidence is 350 000 new cases per year[1,2]. The Framingham study showed that the mean time to death after the onset of CHF symptoms was 4 years[3]. Patients with New York Heart Association (NYHA) class IV symptoms have a much higher mortality than those with less severe symptoms. Survival with medical therapy has improved with the addition of angiotensin-converting enzyme inhibitors and vesnarinone, but the magnitude of the change has been small and the prognosis for patients with advanced heart failure remains poor[4].

Cardiac transplantation has become a standard therapy for terminal heart failure. The shortage of donor organ availability, the need for life-long immunosuppression, and cost issues have prompted the search for alternative surgical means of therapy. Ventricular assist devices provide a tremendous potential therapy, and this field is reviewed elsewhere in this volume. For now, implementation of these devices as routine therapy for CHF will be hampered by high cost and regulatory issues. Renewed interest has therefore focused on the use of skeletal muscle as an autologous cardiac assist system.

This chapter will trace the history and development of this procedure, review the physiology of chronic skeletal muscle stimulation, and summarize the techniques and recent results of cardiomyoplasty (CMP). Alternative approaches, such as skeletal muscle ventricles and diastolic aortic compression, will also be discussed briefly.

HISTORY

The concept of skeletal muscle replacement or augmentation of the heart dates back to 1931, when DeJesus used a muscle graft to repair a traumatic defect of the left ventricle[5]. In 1933 Leriche proposed the use of free muscle grafts to replace infarcted myocardium, and in 1939 Griffith and Bates repaired an iatrogenic right ventricular defect with skeletal muscle[6,7]. Several authors were able to show vascular ingrowth from various muscle grafts to the myocardium[8,9].

The first dynamic utilization of skeletal muscle to perform circulatory work was in 1959, by Kantrowitz and McKinnon in a canine model[10]. They wrapped the descending aorta with pedicled diaphragm muscle and stimulated it via the phrenic nerve during diastole. Diastolic augmentation was demonstrated, but the effects were transient due to muscle fatigue. Nakamura and Glenn demonstrated atrial augmentation using pedicled diaphragm and stressed the importance of an intact neural pedicle[11]. Petrovsky (1966) reported the use of diaphragm in the repair of ventricular aneurysms[12]. Termet *et al.* in 1966 first reported the use of the pedicled latissimus dorsi (LD) for myocardial support[13]. Christ and Spira in 1982 used the latissimus to cover a partial thickness defect of the left ventricle[14]. Chachques, Carpentier and coworkers reported experimental dynamic CMP with fatigue-resistant LD, thereby paving the way for clinical CMP as performed by the same group in France and by Magovern in the USA[15–19].

CHOICE OF THE LATISSIMUS DORSI

Several muscles have been used for experimental circulatory support, but the LD has several important advantages which have made it the muscle of choice[20,21]. It is a large muscle in close proximity to the heart and it has a single major neurovascular pedicle. These properties make it simple to move the LD into the thorax without compromising function of the arm or shoulder. Other skeletal muscles that have been used for circulatory support in experimental animals include the pectoralis major, serratus anterior, and rectus abdominus.

SKELETAL MUSCLE TRANSFORMATION

Skeletal muscle is an available, autogenous and viable myocardial substitute. The biggest hurdle to its use as a biomechanical assist

is its propensity for rapid fatigue. Muscles are composed primarily of two fiber types[22]. Type I fibers are also called slow-twitch fibers; they contract and relax at a slow rate, are fatigue-resistant, and rely on oxidative metabolism. Type II fibers are faster but more fatigue-prone; they rely on glycolytic metabolism. Postural muscles consist predominantly of type I fibers, while muscles that contract intermittently contain a majority of type II fibers. Chronic electrical stimulation of skeletal muscle results in a gradual transformation of type II fibers to the type I fatigue-resistant form[23,24]. The change in muscle fiber type is accompanied by several metabolic changes, including increases in aerobic metabolism, mitochondrial and capillary vessel density, and blood flow[25–29]. There is a reduction in myofibril size and in the activity of calcium-ATPase and calcium uptake by the sarcoplasmic reticulum[30]. Transformation of the LD muscle has been shown in dogs, sheep, and goats.

Controversy exists over the ideal stimulation pattern needed to transform the LD. Muscle transformation is associated with a reduction in power output, especially when the fibers are all transformed to type I fibers. It may be that transformation to a fiber type intermediate between type I and type II will produce the best combination of power and fatigue-resistance. There is also recent evidence that dynamic training, which allows the muscle to shorten during contraction, preserves muscle power during muscle transformation to a greater degree than isometric contraction[31]. Long-term studies have shown a disturbing fibrosis and loss of muscle function in both experimental and clinical applications[32,33]. This is probably related to overstimulation of pedicled skeletal muscle grafts resulting in ischemia and subsequent fibrosis. It is our clinical impression that stimulation of the muscle with every other heart beat, and daily periods of non-stimulation (muscle rest), provide better long-term muscle performance.

SURGICAL TECHNIQUE

A longitudinal skin incision extending along the posterior axillary fold from the axilla to just above the iliac crest is used to mobilize the LD muscle. Multiple perforating vessels supplying the distal two-thirds of the muscle are divided, and the origins of the muscle from the iliac crest and spine are divided. The thoracodorsal pedicle is identified and traced into the axilla. The serratus anterior and circumflex scapular branches are divided to improve the mobility of the pedicle and to avoid stimulation of chest wall muscles. Two intramuscular stimulating leads are placed into the muscle, one near the proximal portion of the thoracodorsal nerve and one 6 cm more distally (Figure 1). A 5-cm portion of the anterolateral portion of the second rib is removed and the muscle, with the attached stimulating leads, is placed into the thorax. The tendon of the LD is then transected from its humeral insertion and anchored to the bed of the resected second rib, taking care not to twist the vascular pedicle. The flank incision is then closed in layers over a subcutaneous drain.

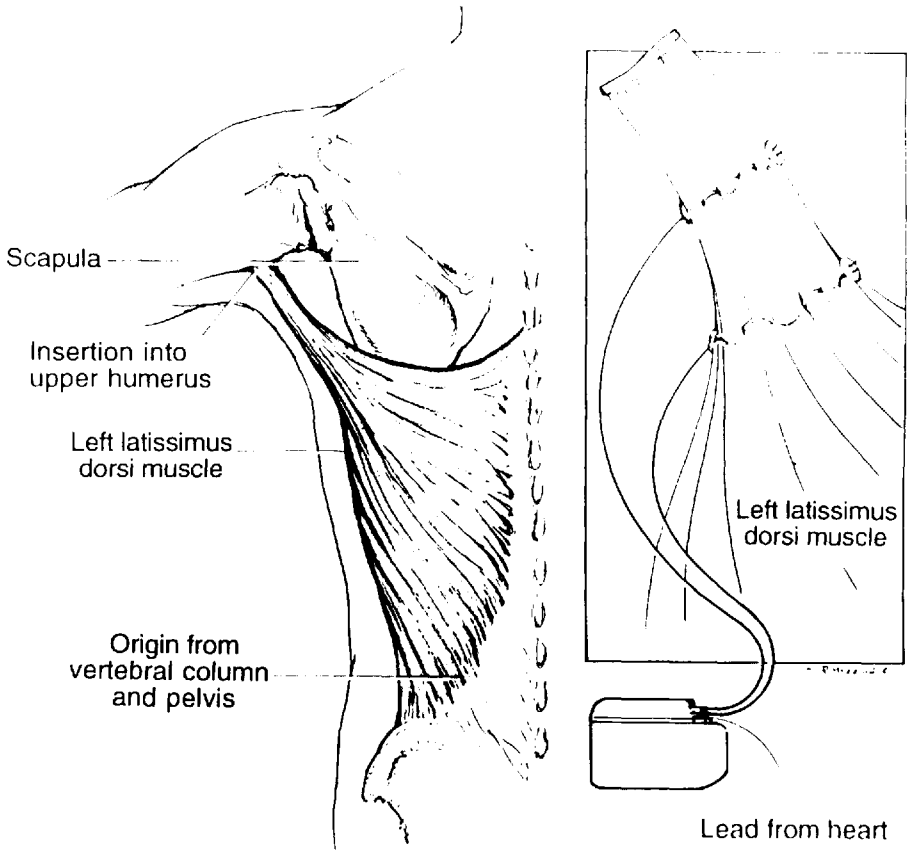

Figure 1 Left latissimus dorsi from a posterior view. Inset shows the position of the intramuscular stimulating leads and their connection to the cardiomyostimulator

A median sternotomy incision is made to complete the operation. The muscle can be applied to the heart in several ways, but the most frequently used orientation is a posterior-to-anterior wrap (Figure 2). Several principles are important. The pericardium should be opened just medial to the left phrenic nerve to create a broad-based pericardial flap. All anchoring sutures are placed into the pericardium, and not into the myocardium, in order to avoid bleeding. The heart should be lifted as little as possible during the procedure, to avoid arrhythmias and acute cardiac deterioration. Instead, the muscle can be slid under and behind the heart. An epicardial sensing lead is then attached to the right ventricle. The stimulating and sensing leads are attached to the cardiomyostimulator, which is then implanted in a subcutaneous pocket in the anterior abdominal wall. After a delay of 2 weeks, stimulation of the muscle is begun. A gradually increasing program of muscle stimulation over 12 weeks results in the following chronic stimulation parameters: amplitude of 3–4 V, heart rate to muscle stimulation ratio of 2:1, pulse train frequency of 30 Hz, and a pulse train duration of 125 ms.

Most of the world experience has been with the left LD muscle, but our institution has also described right LD CMP[34,35]. The only major difference from left CMP is the orientation of the muscle with respect to the heart. The muscle is brought anteriorly across the right and left ventricles and secured to the posterior pericardium. This means that the heart is covered with skeletal muscle, but is not circumferentially wrapped. Contraction of the LD displaces the apex and lateral walls of the left ventricle towards the right shoulder, which is approximately the net vector of flow through the aortic valve.

INDICATIONS FOR CARDIOMYOPLASTY

The indications for CMP are still evolving. Initially, CMP was used as a myocardial substitute when ventricular mass was removed, such as in resection of a left ventricular aneurysm or tumor. Subsequently, the operation was used to reinforce diseased myocardium in patients with CHF. Currently, patients with NYHA class III and IV symptoms of CHF are considered candidates for this procedure if they do not qualify for, or they decline, cardiac transplantation. Approximately half of the patients undergoing CMP have had ischemic cardiomyopathy and most of the others have had idiopathic cardiomyopathy[36,37]. The procedure is not indicated for patients with hypertrophic cardiomyopathy or Chagas' disease.

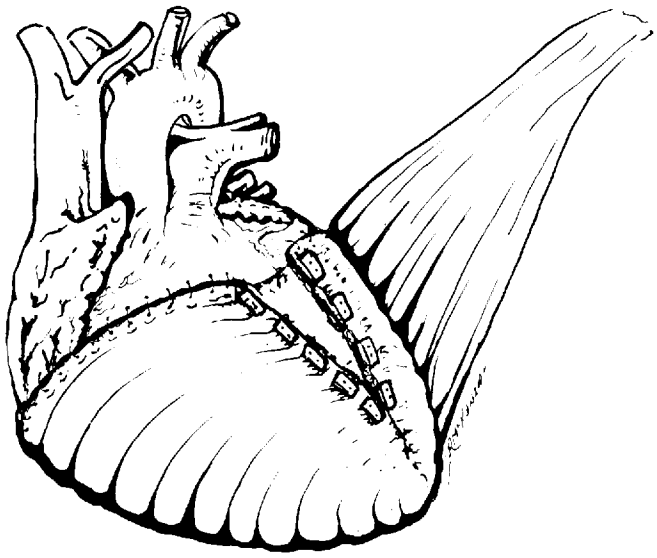

Figure 2 Left posterior cardiomyoplasty wrap. The latissimus dorsi is brought behind the left ventricle and sutured to itself anteriorly. Often the muscle is not long enough to achieve complete coverage of both ventricles. In this case a piece of pericardium is used to bridge the defect

Clinical results from the phase I and phase II trials of CMP show very high operative mortality and poor long-term survival in patients with persistent class IV symptoms or biventricular heart failure. This same situation occurs for patients with left ventricular ejection fraction (LVEF) <20% and maximal oxygen consumption <10 ml kg^{-1} min^{-1}. The best results have been obtained in patients with NYHA class III symptoms, LVEF >20% and maximal oxygen consumption >15 ml kg^{-1} min^{-1}. Patients who meet these requirements are ideal candidates, but cardiologists are reluctant to refer such patients because many of them remain stable with medical therapy for years before deteriorating. From the surgeon's perspective the situation is analogous to that of surgery for mitral regurgitation. The best long-term results are obtained in patients who have surgery before left ventricular dysfunction becomes severe, but this can take many years to develop. Earlier surgical referral for patients with cardiomyopathy will improve the long-term results of CMP, just as it does for patients with mitral regurgitation.

Current contraindications for CMP are irreversible renal and/or hepatic dysfunction, severe pulmonary dysfunction, dependence on intravenous inotropes, dependence on diastolic counterpulsation, and a history of symptomatic ventricular arrhythmias, including sudden death. Patients requiring concomitant procedures, such as coronary artery bypass or valve surgery, are not candidates. Relative contraindications are previous cardiac surgery, atrial fibrillation, class IV symptoms, and pulmonary hypertension. The indications and contraindications will continue to evolve as the procedure develops, and each case should be considered individually before making a decision.

MECHANISMS OF ACTION

The original hypothesis was that CMP would augment systolic function of the left ventricle[38]. This has been confirmed in experimental studies using animals with normal heart function and in various models of heart failure, including rapid pacing, coronary ligation, and adriamycin toxicity[39–44]. Patients in the phase II clinical trials have shown a modest but consistent increase in stroke volume, left ventricular stroke work index, and LVEF at 6 and 12 months[37]. Jegaden *et al.* were able to show improved exercise capacity, LVEF, and end-diastolic pressure at 2 years after surgery[45]. Magovern *et al.* reported an increased ejection fraction and reduced left ventricular volume in early follow-up of patients undergoing the right latissimus CMP, but this effect decreases over time[35,46]. Thus, there is evidence that CMP can improve left ventricular function.

A second benefit of CMP is the reduction of left ventricular volume. Capouya and co-workers reported that CMP prevented left ventricular dilatation from rapid pacing, and Nakajima *et al.*

showed a similar result in a model of multiple coronary ligations[43,44]. Kass *et al.* showed striking reductions in left ventricular volume in several patients studied with pressure–volume analysis using a conductance catheter[47]. Thus, the LD appears to function as a dynamic girdle that limits left ventricular dilatation. Some authors have raised the concern that the wrap interferes with the diastolic function of the ventricle[48,49]. This can occur if the ventricle is wrapped too tightly, but is not universal. In fact, some improvement in diastolic function has been shown in animals with cardiomyopathy induced with adriamycin[40]. Constrictive physiology has not occurred in patients after CMP, but impairment of diastolic relaxation can occur if the stimulator is not timed properly, especially when the heart rate is fast.

Chiu and co-workers have recently stressed the concept of functional hypertrophy from CMP[50]. The wall stress of the left ventricle is inversely proportional to its thickness. Therefore, CMP may reduce myocardial wall stress because of the effective increase in the thickness of the left ventricle produced by the LD. It is postulated that the reduction in wall stress of the left ventricle reduces oxygen consumption, which indirectly improves performance of the diseased heart.

SUMMARY OF CLINICAL RESULTS

Several hundred patients have undergone CMP in the past 5 years. Improved functional status has been a consistent finding in all centers doing the procedure. Operative mortality was 22% in the phase I clinical trial published in 1991, but was reduced to 12% in the phase II trial reported in 1994. Established centers with considerable experience in CMP have an operative mortality of 5–10%. Late mortality has been high for patients with NYHA class IV symptoms prior to surgery. Overall 1-year survival in the phase II American trial of CMP was 68%. The major causes of death were sudden death (presumed arrhythmia) and progressive CHF.

The improved functional status reported by patients has also been confirmed with formal patient interviews and questionnaires. Borghetti-Maio *et al.* found prospectively that there was an improvement in the overall quality of life, including the capacity to undertake the activities of daily living[51]. Improvements in activities of daily living and social interactions were also documented in the phase II American trial of CMP. Enhanced cardiac function has been shown in studies from South America, Europe, and North America, but the changes have been small to moderate in magnitude.

Despite improved functional status and cardiac function, late mortality has continued to be a problem. The majority of deaths have occurred suddenly in patients who were feeling well. Progressive heart failure has also occurred, but has been less common than sudden death. Arrhythmia is a common fatal event for patients with CHF, and has not been reduced by CMP. Simultaneous implantation of an automatic internal defibrillator and/or empiric therapy with amiodarone have been suggested as means to reduce sudden death after CMP. These approaches may be incorporated into future trials of CMP.

OTHER FORMS OF SKELETAL MUSCLE ASSIST

Diastolic counterpulsation of either the ascending or the descending thoracic aorta has shown promising experimental results[52–56]. Lazzara and co-workers have shown experimentally that diastolic aortic compression using a strip of LD muscle produces similar elevations in aortic diastolic pressure, and reductions in left ventricular end-diastolic pressure, to counterpulsation with an intra-aortic balloon pump[57,58]; it has also been shown to improve regional left ventricular dysfunction caused by myocardial ischemia[9].

The skeletal muscle ventricle is another possible application of skeletal muscle assist[60]. The LD muscle is fashioned into a neoventricle using a plastic mandrel. After a training period the neoventricle is connected to the circulation, where it has been able to pump approximately 50% of the normal cardiac output. Another approach is to use a muscle energy convertor to translate linear muscle contraction into hydraulic energy, which can power a blood pump[61]. Another interesting development has been the use of the LD muscle as a source of extracardiac collateral blood supply in myocardial ischemia[62].

COMMENT

It has been 10 years since the first clinical CMP procedure, and the field is still in a developmental stage. Clinical results have improved, but further refinements are necessary, especially in muscle stimulation protocols and patient selection criteria. It is estimated that 14 000 patients are added to the transplant list each year, but only 2000 will receive new hearts. If the eligible age is raised to 65 years, the number of candidate patients would quadruple. The long-term results of transplant are excellent if solely patients who receive transplants are analyzed. However, most patients who are referred for transplants are never listed, and many of those who are listed never receive a heart. A more realistic approach would include all patients referred for transplant, utilizing an intention-to-treat analysis.

The long-term results with CMP are not as good as those for patients who receive transplant, but they are at least as good as transplant if the intention-to-treat analysis is employed. With improved patient selection, continued technical refinement, and reduction of ventricular arrhythmia, it is feasible that CMP will become a realistic alternative to transplant for the management of patients with CHF. In the meantime, it remains a fertile area of surgical research and innovation.

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Blood Pumps Constructed from Skeletal Muscle

K.A. GREER, D.R. ANDERSON AND L.W. STEPHENSON

INTRODUCTION

Skeletal muscle is capable of transforming chemical energy into mechanical energy with extraordinary efficiency. We and others have shown that skeletal muscle has the capacity to become fatigue-resistant and to adapt to new patterns of work. These changes occur when skeletal muscle is subjected to low-frequency electrical stimulation for a period of several weeks.

In laboratories worldwide, two approaches have been developed in attempts to utilize skeletal muscle to augment cardiac function. Skeletal muscle grafts have been applied directly to the beating heart in hopes of aiding the function of the failing myocardium and, in some cases, to directly bolster cardiac contractile function[1-12]. The other avenue of investigation has been the formation of skeletal muscle pouches or ventricles which, when stimulated to contract, provide their own pumping function.

These skeletal muscle pouches have been shown to provide effective support of the right and left heart using a variety of configurations[13-19]. As surgical techniques have improved, the complications associated with their use in the circulation long-term have steadily declined. Substantial improvement in cardiac parameters has been demonstrated in the normal and failing canine heart, the longest animal surviving for 836 days[20]. Current efforts are focusing on developing a laboratory model that would be clinically applicable. Earlier problems with thrombus formation have largely been solved[21-23].

SKELETAL MUSCLE PUMPING CHAMBERS – HISTORICAL REVIEW

Pumping chambers have been constructed from skeletal muscle by a number of investigators. The ultimate goal of these endeavors is to use these 'ventricles' to supply power for mechanical cardiac assist devices or to use them in the circulation directly as blood pumps. Skeletal muscle ventricles (SMV) have been constructed from a variety of different muscles including the rectus abdominis, diaphragm, quadriceps femoris, pectoralis major, gluteus maximus, psoas, and latissimus dorsi. In our laboratory SMV have been constructed primarily from the latissimus dorsi muscle. We prefer the latissimus dorsi muscle because of its relatively large size, single motor nerve, single main blood supply, ease of harvesting, minimal donor disability, and close proximity to the heart.

Kantrowitz, in 1959, wrapped the left leaf of the canine diaphragm around the descending thoracic aorta, creating a muscular tube[24,25]. The diaphragm was synchronously stimulated during diastole via the phrenic nerve. Diastolic augmentation of 15 mmHg was achieved, as well as a 26 mmHg increase in mean arterial pressure. These effects were maintained for only a few cardiac cycles, however, apparently due to muscle fatigue.

Kusserow, in 1964, was the first to use skeletal muscle as the power source for a mechanical assist device[26]. He did not construct a ventricle for this purpose, but instead used the rectus femoris in a linear configuration to exert force on a lever mechanism which served as the actuator for a bellows-type blood pump. The blood pump was connected to a hydraulic circuit which allowed calculation of flow and determination of outflow resistance. These pumps functioned in four dogs for 2–8 hours, generating flows of 600–720 ml/min against an afterload of 20 mmHg.

Spotnitz *et al.*, in 1974, constructed skeletal muscle pouches from canine rectus muscle[27]. They found that their physical characteristics were similar to those of the heart. This observation should not have been too suprising since Otto Frank, a German physiologist, in 1895, had recognized that the response of isolated frog heart to alterations in tension just prior to contraction was similar to that of skeletal muscle[28]. The transmural pressure, developed during active tension, increased as the resting wall tension (preload) was increased. The rectus muscle appeared to be less compliant than cardiac muscle. However, in his experiments with filling pressures of 50–150 mmHg, systolic pressures of greater than 500 mmHg could be obtained.

In 1975, Vachon *et al.* wrapped denervated pedicle grafts of diaphragm around a fluid-filled balloon pressure transducer, and allowed this ventricle to pump against a specified outflow resistance, allowing measurements of flow, power output, and isovolumic pressure–volume relations[29]. The pouches were stimulated via stainless-steel electrodes sewn into the muscle. With stimulation voltages of 30 volts the muscle pouch generated up to

176 mmHg, the pressure decreasing proportionately with decreasing voltage. Peak pressure increased with increasing filling pressure. During these experiments the pouches were able to generate a power output of 0.05 watts. Vachon *et al.* calculated the power output of the left and right ventricles as 0.335 watts and 0.0335 watts, respectively. Stimulation of these pouches was sustained for up to 3.5 hours.

Von Recum, Kantrowitz, and co-workers in 1977 also constructed skeletal muscle pouches from diaphragm and stimulated the muscle directly[30]. In an attempt to minimize fatigue, stimulation voltages of 3–7 volts were employed. These ventricles were stimulated to contract isovolumetrically with a filling pressure of 18 mmHg. Peak pressures of approximately 60 mmHg were obtained in most experiments, and the pouches contracted continuously for up to 20 hours, although they fatigued rapidly during the initial 1–2 hours. Since the decline in pressure generation was progressive, these authors concluded that construction of an auxiliary ventricle from diaphragm was not feasible.

Juffe *et al.*, in 1977, constructed pouch-like skeletal muscle pumping chambers from gluteus maximus muscle dissected free from its insertions[31]. A balloon transducer was introduced into the pouch and the muscle was stimulated via the gluteal nerve by a pacemaker. Pressures as high as 170 mmHg were recorded initially. Animals were sacrificed at regular intervals up to 26 days for histological studies. After 26 days of continuous stimulation, muscular atrophy occurred, probably as a result of nerve damage.

CONSTRUCTION OF SKELETAL MUSCLE VENTRICLES

The surgical technique for constructing a skeletal muscle ventricle is fairly straightforward[13–19]. The latissimus dorsi muscle is a large flat muscle overlying the back and flank; its principal function is adduction of the forelimb. The muscle has attachments to the thoracic spine, the eleventh rib, the platysma, the trapezius, the teres major, the triceps, and inserts into the humerus. The blood supply comes from the thoracodorsal artery as well as numerous small arterial branches from the intercostal arteries. The thoracodorsal nerve supplies motor innervation.

The animal is anesthetized and the latissimus dorsi is mobilized through a flank incision extending from the axilla to the tip of the eleventh rib. The lesser blood supply from the overlying skin and the chest wall is divided. The attachments (as mentioned above) are also divided, leaving only the thoracodorsal neurovascular pedicle intact. A specially modified Medtronic (Minneapolis, MN) pacing lead is placed around the proximal aspect of the thoracodorsal nerve. This lead is connected to a permanent implantable nerve stimulator.

The muscle is then wrapped or rolled around a previously machined Teflon mandrel of a given size and shape. The mandrel is either conically shaped when forming pouch-like ventricles, or cylindrically shaped to produce tube-type or flow-through ventricles. The mandrel also has a Teflon felt collar on one or both ends, which ultimately acts as a sewing annulus. The wrapped muscle layers are sutured to each other as well as to the felt collar. The direction of the wrap is always such that the neurovascular pedicle is on the outside. Generally $1\frac{1}{2}$–$2\frac{1}{2}$ muscle wraps are obtained. The skeletal muscle ventricle can be placed inside the thoracic cavity or left on the chest wall under the skin and subcutaneous tissue. The skeletal muscle ventricle is sutured to the surrounding tissues to prevent migration or kinking of the pedicle. The subcutaneous tissue and skin are then closed over the skeletal muscle ventricle.

The skeletal muscle ventricle is allowed to rest for a 3-week vascular delay period. Although the neurovascular pedicle supplies an adequate blood supply to prevent immediate muscle necrosis at the time of mobilization, division of the blood supply from the intercostal arteries during construction renders the muscle relatively ischemic. Immediately following skeletal muscle ventricle construction, the distal half of the muscle is unable to increase its blood flow in response to the increased demands of exercise. A 3-week vascular delay period, however, allows for recovery of resting and exercise-induced increases in blood flow[32]. Using radiolabeled microspheres, Mannion *et al.* demonstrated that all layers of the skeletal muscle ventricle receive substantial blood flow following the vascular delay period while the ventricles are pumping in the circulation[17].

SKELETAL MUSCLE VENTRICLE PRECONDITIONING

Skeletal muscle can be divided into two basic types, one of which has relatively fatigue-resistant properties. Slow-twitch (type I) fibers are characterized by prolonged contraction time, a large mitochondrial volume, aerobic metabolism even under prolonged periods of stimulation, and a specific complement of 'slow' contractile proteins. Muscles composed of predominantly slow-twitch fibers are usually postural in function and fatigue slowly. Fast-twitch (type II) fibers are characterized by brisk contraction time, a relatively small mitochondrial volume, anaerobic metabolism during stimulation, and a fast set of contractile proteins. Typical fast muscle, such as an eye muscle, is used for quick, intense episodic activity and fatigues rapidly with prolonged stimulation.

During the past 20 years a considerable amount of biochemical research on the adaptive capabilities of skeletal muscle has been performed. Skeletal muscle changes its physiologic, biochemical, and structural characteristics in response to intense exercise and electrical stimulation. The basic experiments of Butler *et al.* showed that the nerve was able to modulate the properties of the muscle[33,34]. In those experiments the motor nerve of a fast-twitch muscle was switched with the motor nerve of a slow-twitch muscle. When the nerves regenerated, the fast muscle became slow and the slow muscle became fast. In 1969 Salmons and Vrbova determined that it was the pattern of stimulation of the motor nerve which governed the muscle fiber type[35]. When a fast-twitch muscle was stimulated for several weeks at 10 Hz the fibers were converted to a population of slow-twitch, fatigue-resistant type I fibers. These transformed, fatigue-resistant fibers more closely resemble cardiac muscle than ordinary type I fibers, being high in terms of mitochondrial content and oxidative enzyme complements[36,37].

To understand fatigue resistance induced by chronic stimulation we used phosphorus nuclear magnetic resonance spectroscopy to study the bioenergetics of electrically conditioned canine latissimus muscle *in vivo*. We found that increased resistance to fatigue is related to an increased capacity of oxidative phosphorylation, which is most likely due to increased mitochondrial volume[38,39]. Since the capacity of oxidative phosphorylation is increased, adenosine triphosphate (ATP) production by the

muscle can match a sustained increase in ATP utilization. The decline in phosphocreatine and accumulation of adenosine diphosphate (ADP) and inorganic phosphate, which usually accompany muscle fatigue, are absent.

As in the heart, resistance to fatigue of the conditioned muscle appears to derive from a highly developed aerobic capacity that supports efficient recycling of ADP to ATP and prevents the accumulation of inorganic phosphate. The increased ability to utilize oxygen during isometric exercise also contributes to the fatigue resistance of electrically conditioned skeletal muscle[40]. Conditioned muscle is homogeneously slow, and cross-bridge cycling, and therefore ATP consumption, of slow-twitch fibers is significantly slower than that of fast-twitch fibers. The rate of cycling of cross-bridges determines the energy cost for the maintenance of tension, and a given isometric tension is therefore maintained with less hydrolysis of ATP, and less oxygen is consumed with electrically conditioned canine muscle than with control muscle for identical isometric tension[40].

Mannion *et al.* showed that the combination of a vascular delay period and electrical preconditioning allowed construction of fatigue-resistant skeletal muscle ventricles[16]. Fatigue resistance can be achieved using 2–10 Hz continuous stimulation, or using a 25 Hz burst stimulation pattern, more suited to cardiac-type work[13,35,41–45]. In addition, we have shown that SMV fashioned from the latissimus dorsi muscle can be rendered fatigue-resistant while performing useful work *in vivo*[14,15]. Thus a preconditioning period may not be absolutely necessary before these ventricles can be put to work in the circulation.

SKELETAL MUSCLE VENTRICLES *IN VIVO*

Contractility reflects the intensity of the active state of the muscle. Unlike the heart, which is an electrical and mechanical syncytium, skeletal muscle is modulated by the number and rate at which the fibers are activated[46]. It has been shown by Dewar and associates[47], as well as by our laboratory[16], that a single electrical stimulus, resulting in a single muscle twitch, does not normally generate sufficient force to augment cardiac function. However, rapid repetitive stimuli, delivered before the muscle fiber completes its relaxation, result in mechanical summation (until fusion occurs), which thereby causes the muscles to generate substantial force[48]. The burst stimulation frequency of the SMV governs the cumulative duration of the active state of the skeletal muscle and produces an effect similar to the contractility of the heart. Increasing the burst frequency of the SMV produces more work.

SKELETAL MUSCLE VENTRICLES IN MOCK CIRCULATION

In earlier studies SMV were constructed as described above using a 17 ml cone-shaped mandrel. These SMV underwent a 3-week vascular delay period followed by 6 weeks of electrical preconditioning. The SMV were then connected to a totally implantable mock circulation device[13]. This device allowed control of both preload and afterload, and measurement of SMV output. No wires or tubes crossed the animal's skin barrier. The animals were able to move about freely with no apparent discomfort or physical impairment. The muscles were stimulated via the thoracodorsal nerve with a 25 Hz burst frequency (312 ms on, 812 ms off), resulting in 54 contractions per minute. These SMV pumped continuously against an afterload of 80 mmHg with a preload of 40–50 mmHg. At the initiation of pumping, mean systolic pressure was 134 mmHg and flow was 464 ml/min. After 2 weeks of continuous pumping, systolic flow was 206 ml/min. Two SMV pumped for 5 and 9 weeks, respectively.

In a subsequent study[14], SMV were constructed with a vascular delay period but without preconditioning. These SMV were connected to the mock circulation system and stimulated via the thoracodorsal nerve at a 25 Hz burst frequency, as in the previous study. Preload and afterload were again set at 40 mmHg and 80 mmHg, respectively. After 2 weeks of continuous pumping the mean stroke work of the SMV was 0.4×10^6 erg. The stroke work of these SMV was intermediate between that of the canine left and right ventricles. Two dogs continued to produce significant stroke work after 2 months. Using SMV constructed from canine rectus abdominus muscle, Stevens and Brown measured similar heart and SMV outputs during acute studies[49].

Later, the mock circulation model was used in several experiments in our laboratory[50,51]. Acute studies were performed on a series of SMV constructed as described above. Six of these were rested for 4 weeks and conditioned for 6 weeks, and five were rested for 18 weeks without conditioning. In these studies, using the mock circulation set-up, the group which had rested for 18 weeks was able to generate a maximum of 194% of left ventricular stroke work with a preload of 25 mmHg and an afterload of 80 mmHg. For the first time this study demonstrated the ability of skeletal muscle to surpass the work of the heart. In 1993, Niinami *et al.* used the same design to demonstrate a better compliance, as well as better systolic and diastolic function of intrathoracic SMV compared to those in the (at that time) traditional subcutaneous position[51].

SKELETAL MUSCLE VENTRICLES AS ARTERIAL DIASTOLIC COUNTERPULSATORS

Working in our laboratory, Mannion *et al.* showed that properly conditioned SMV with a vascular delay were able to function in the circulation for up to 14 hours as diastolic counterpulsators in the descending aorta[18,19]. SMV function eventually deteriorated, however, due to anemia, hypoxia, hypotension, and other complications inherent in prolonged acute experiments of this type. The stroke work of these SMV was 0.68×10^6 erg after 4 hours, roughly 3 times the stroke work of the right ventricle and nearly half the left ventricular stroke work. Neilson, Chiu and associates had shown an improvement in subendocardial viability ratio when SMV were used in the circulation as aortic counterpulsators[52].

Acker *et al.*[15] subsequently constructed SMV in five mongrel dogs, which differed from the design of previous chambers in that these had a cylindrical geometry with inflow and outflow on opposite ends of the chamber, as depicted in Figure 1. These ventricles functioned chronically in the circulation as aortic diastolic counterpulsators for up to 11 weeks. All animals had functional SMV at the time of death, and it was shown that these were capable of providing a substantial augmentation of forward blood flow with an increase of 29%, 40% and 63% at 25, 43, and 85 Hz of thoracodorsal nerve stimulation, respectively. Two-dimensional short-axis echocardiograms of the SMV obtained

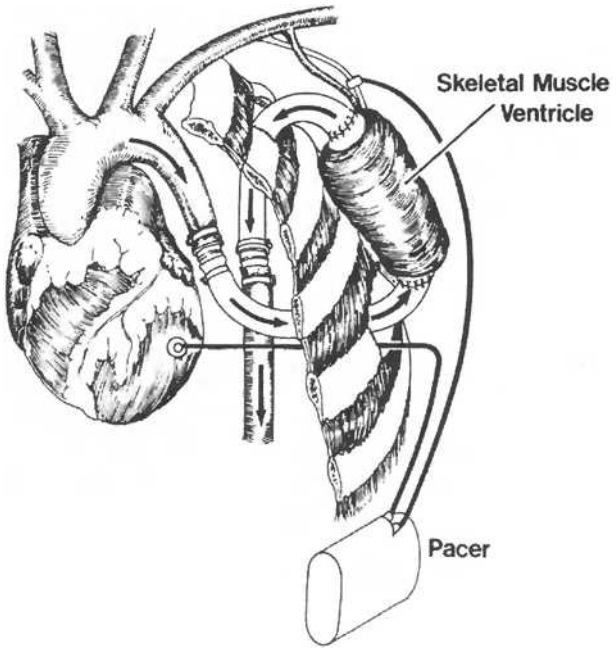

Figure 1 SMV with cylindrical geometry interposed into the descending aorta for use as a counterpulsator. Note the pacemaker stimulating electrode connected to the thoracodorsal nerve and the sensing electrode on the left ventricle

from one dog after 12 days of continuous counterpulsation are shown in Figure 2. During these measurements the burst frequency was altered from the chronic setting of 25 Hz to 43 Hz and then to 85 Hz. These echograms demonstrate a 70%, 90% and 100% decrease in cross-sectional area at the midpoint of the SMV at 25, 43 and 85 Hz, respectively, during SMV contraction. Arterial pressure tracings obtained from the same dog after 14 days of continuous pumping at these three burst frequencies are shown in Figure 3. However, this study also demonstrated the thrombotic complications associated with these pumping chambers. The two longest-surviving dogs, 5 and 11 weeks, demonstrated multiple renal and splenic infarctions. Neither of these animals, however, showed evidence of cerebrovascular or cardiac embolization.

Further studies have used the conically shaped SMV connected to the descending thoracic aorta by two Gore-tex grafts (W.H. Gore & Associates, Flagstab, AZ), as shown in Figure 4. Ligation of the aorta by umbilical tape obligated the flow of blood through the SMV. The data from these experiments showed that SMV were capable of providing long-term ventricular support with the ability to augment diastolic pressure for up to 836 days[20]. Subsequent studies were performed using propranolol-induced heart failure. The investigators were able to show a greater increase in several hemodynamic parameters for the failing heart when compared to the normal heart. Mean diastolic pressure increased 27.6% vs 12.9%, and the endocardial viability ratio increased 28.7% vs 11.2%[53].

Additional studies have been performed in an effort to decrease the incidence of SMV thrombosis. Studies comparing the tradi-

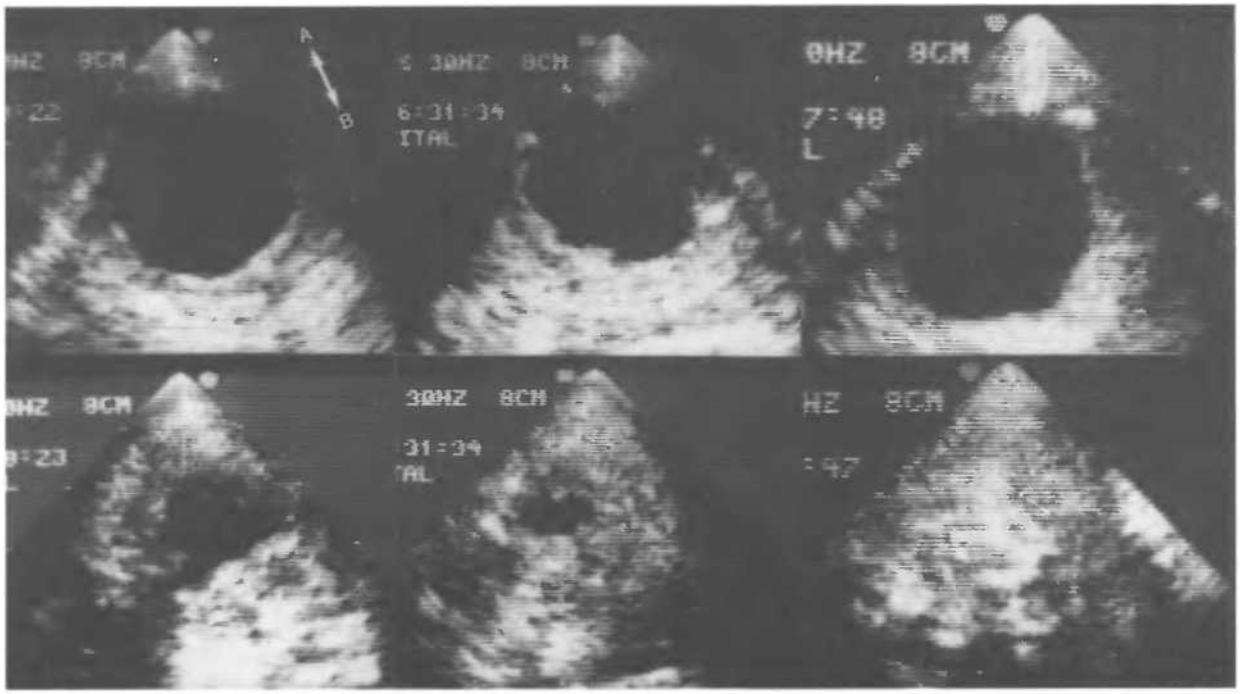

Figure 2 Two-dimensional echocardiogram, short axis view, of midpoint of SMV obtained from one dog on postoperative day 12. Top row illustrates the cross-sectional area during relaxation. Bottom row illustrates the cross-sectional area during contraction. Increasing the burst frequency (25 Hz, 43 Hz, 85 Hz) leads to increasing ejection fraction

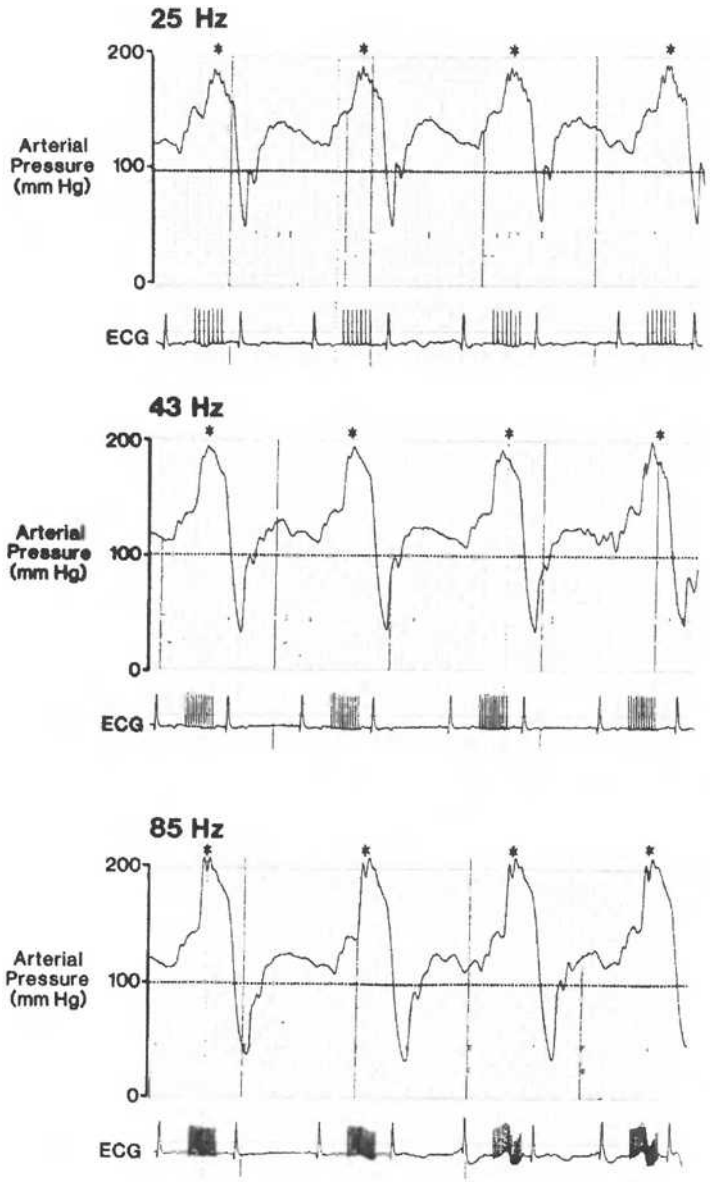

Figure 3 Distal aortic pressure and electrocardiogram tracing in the same dog as Figure 2 on postoperative day 14 at burst frequencies of 25 Hz, 43 Hz, and 85 Hz. Diastolic augmentation of 130 mmHg occurs at 85 Hz burst frequency. (*) indicates diastolic augmentation. Note corresponding superimposed burst pattern on electrocardiogram

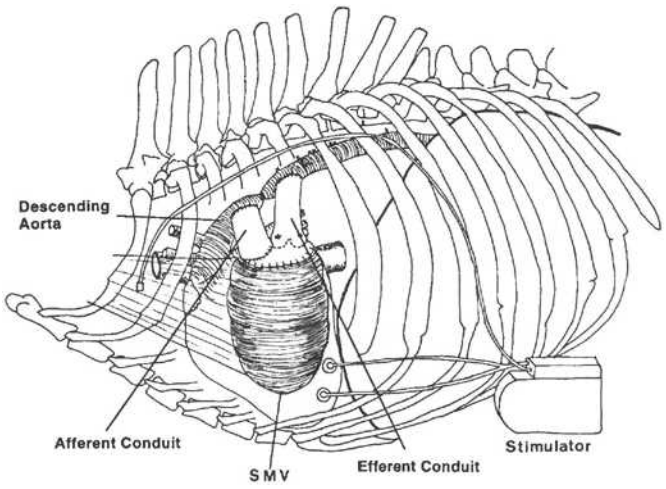

Figure 4 SMV connected in circulation as aortic counterpulsator in standard configuration

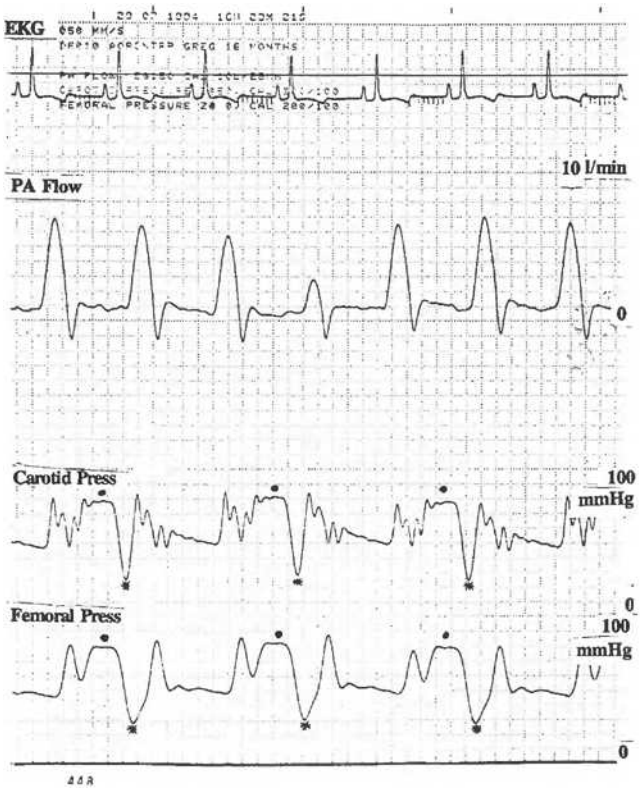

Figure 5 Diastolic augmentation over time at greater than 16 months. In this tracing shown, diastolic augmentation averaged about 32%. Dots indicate diastolic augmentation while asterisks indicate presystolic unloading (Ao presystole)

tional SMV to autologously lined SMV (with pleura or pericardium) have shown decreased thrombosis with the autologous lining[54]. Figure 5 demonstrates a typical tracing from an autologously lined SMV after over 16 months in the circulation. Most recently, a method has been developed in our laboratory for percutaneously seeding the lumen of the SMV with autologous endothelium. Preliminary studies have recently been published by Thomas *et al.*, who demonstrated that it is possible for SMV to retain the endothelium after 3 hours in the circulation[55]. However, it remains to be demonstrated if this will decrease the rate of thrombosis long-term.

RIGHT HEART BYPASS

Studies have also been done which show it is possible to totally replace the right heart[56]. SMV were constructed in mongrel dogs, and, after a 3-week vascular delay period, these SMV were electrically conditioned at 2 Hz for 4–6 weeks. These ventricles were

constructed using large (55 ml) Teflon mandrels, resulting in higher compliance SMV – ideally suited for low-pressure work. During the second operation, after electrical preconditioning, total systemic venous return was routed to these high-compliance SMV, and SMV outflow was delivered into the pulmonary artery. These SMV were stimulated using a synchronous burst pattern as described above, and generally contracted at a rate of 1:2 with cardiac systole. An ultrasonic flow probe in the SMV outflow circuit was used to monitor cardiac (and SMV) output. Complete right heart bypass was documented by the absence of flow and extreme hypotension with the SMV circuit occluded. Opening the SMV circuit allowed passive flow from the venous system through the pulmonary bed, which is somewhat analogous to the physiology with the Fontan procedure. With passive flow, however, systemic blood pressures were only 50–60 mmHg.

With stimulation of the SMV, systolic blood pressure increased to 100–110 mmHg and CVP decreased. After 2 hours of continuous pumping, SMV stroke work was 169% of normal canine right ventricular stroke work and, after 4 hours, stroke work was 174% of canine right ventricular stroke work. Total right heart bypass was accomplished in some dogs for periods of up to 8 hours, at which time the experiment was terminated.

Although these initial studies showed promise, it has been demonstrated that SMV function is substantially improved if the preload is higher than that which can be achieved by the venous system alone. In an effort to exploit the higher pressure generated by the right ventricle, a model was developed where an SMV is connected in series with the right ventricle using valved conduits. The pulmonary artery is subsequently ligated proximally to obligate flow through the SMV[57]. This uses the right ventricular pressure as preload for the SMV. Acute studies performed at 1 and 4 hours demonstrated an increase in cardiac output of 27% at 1 hour and 30% at 4 hours. Systemic arterial pressure showed an increase at 1 hour of 12% and at 4 hours of 13%. Peak pulmonary pressure was increased at 1 hour by 35% and at 4 hours by 37%. Further studies have shown that effective assistance may be obtained for periods of up to 16 weeks[58].

LEFT HEART BYPASS

Two additional configurations of SMV have been constructed and evaluated in our laboratory. These have focused on using the pressure generated by the left atrium and the left ventricle to serve as preload for the SMV. The first system involves using two valved conduits, the first between the left atrium and the SMV, and the second between the SMV and the aorta[59]. The SMV in this configuration then contracts during cardiac diastole. If it contracted during cardiac systole it would be pumping against left ventricular ejection. In acute 3-hour experiments it was shown that left atrial pressures remained in the range 14–16 mmHg and that the SMV were able to pump between 21% and 27% of the cardiac output. We have not tested this model, however, in the circulation long-term because we feel this model needs higher filling pressures to function optimally. It is possible that, with a stable left ventricular failure model, in which pressures would presumably be higher, this configuration would function effectively for longer periods.

In an effort to exploit the higher pressures of the left ventricle for SMV preload, a configuration of left ventricular apex to aorta has been developed as shown in Figure 6[60]. In addition to the improved

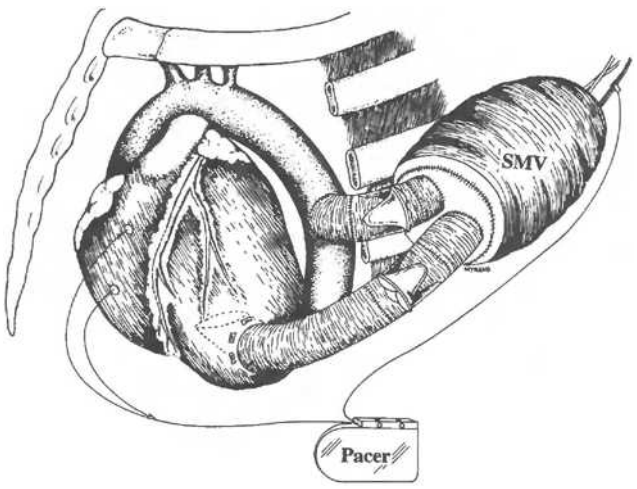

Figure 6 Left ventricular apex-to-aorta configuration, demonstrating the use of two valved conduits. Note the extrathoracic positioning of the SMV

preload there is likely to be improved blood flow in the muscle layers of the SMV when compared to that of the aortic diastolic counterpulsator, because the SMV cavity pressure is equal to LV end-diastolic pressure during a portion of the cardiac cycle[61,62]. Acute 3-hour experiments have demonstrated a significantly lower systolic tension–time index with the SMV on (versus off). The endocardial viability ratio was also significantly increased, showing an improvement of 68%, 66%, 62%, and 63% at initial measurement and at 1 hour, 2 hours, and 3 hours, respectively.

There was little improvement in cardiac output noted in this experiment. However, it was demonstrated that the SMV pumped 47% of the systemic blood flow, substantially reducing the oxygen requirement of the left ventricle. Although function gradually deteriorated over the 3 hours, there was a single animal followed for 10 hours that showed the deterioration appeared to stabilize at 3–4 hours. This study was limited by its short period of evaluation, and by the fact that the animals all had normally functioning hearts. Stevens and colleagues, however, have shown a substantial increase in systemic blood flow of 31% using a similar configuration in animals that were in heart failure, as compared to the increase in 6% seen in our study[61]. Current work in our laboratory is focusing on developing this system into a chronic model, with one dog at present having survived over 2 months with a functional SMV (unpublished).

COMMENT

The extraordinary capacity of skeletal muscle to respond adaptively to an increased level of use allows construction of pumping chambers capable of performing cardiac work. The potential applications of this phenomenon are enormous. Studies have already shown the improvement in cardiac function and decrease in myocardial oxygen requirements provided by the SMV, which has been shown to be effective in a variety of configurations. Recently developed models have shown the effectiveness of the SMV in the right and left heart assist configurations, and long-term studies have shown the effectiveness of the SMV as an

aortic diastolic counterpulsator. Clinical application may improve the outlook for infants with complex congenital heart defects as well as for patients with end-stage heart failure.

Current work in our laboratory is focusing on developing a clinically relevant left heart assist model. This will bring the clinical application of the SMV for cardiac assistance one step closer to reality.

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Dynamic Cardiomyoplasty: Multicenter Clinical Trials

B.D. MOTT, L.L. AUSTIN AND R.C.-J. CHIU

INTRODUCTION

Patients with chronic heart failure suffer from variable degrees of abnormal cardiac performance reflected in their exercise intolerance, fatigue, and dyspnea. It is estimated that approximately 400 000 people develop heart failure each year in the United States, adding to the nearly 3 million patients already suffering from this syndrome[1]. Patients with mild to moderate severity of disease can usually be managed medically with today's arsenal of drugs, including angiotensin-converting enzyme (ACE) inhibitors, diuretics, and inotropes. Furthermore, patients with correctable valvular or coronary lesions increasingly can be managed by standard cardiac surgery. Despite this, mortality in this population remains extremely high. The overall 1-year and 5-year survival rates are 57% and 25% for men, respectively, with slightly better rates seen in women[2].

Many patients progress to severe heart failure, and it is in this group that cardiac transplantation has played such a major role, often rescuing them from terminal heart failure[3]. Unfortunately, due to the limited availability of donors and strict selection criteria employed, this therapeutic option is available to only a minority of patients. Successfully transplanted patients face the risks of rejection, infection, graft coronary artery disease, and the side-effects of immunosuppressive drug usage. With these limitations in mind, alternatives to traditional cardiac transplant, such as xenotransplantation and mechanical artificial hearts, have been aggressively investigated, but are still unsuccessful in any long-term application.

Dynamic cardiomyoplasty (DCMP) has been proposed as both an alternative and a bridge to cardiac transplantation. The advantages are that there is no need for immunosuppressive drugs, and no risk of thromboembolic complications as seen with artificial prostheses. The patient's own skeletal muscle is used to create the cardiomyoplasty wrap with little, if any, loss in shoulder function. Finally, DCMP is likely to cost significantly less than any of the above-mentioned procedures.

DEVELOPMENT OF 'SKELETAL MUSCLE POWERED CARDIAC ASSIST'

Concept

The idea of using skeletal muscle to replace or repair a damaged myocardium dates back to the 1930s[4]. The initial application of skeletal muscle flaps was to act as patches to cover defects in the ventricular wall after the excision of aneurysms or tumors[5]. Soon thereafter, investigators tried to stimulate skeletal muscle wrapped around the heart with electrical current[6], but two major biological constraints impeded progress: firstly, skeletal muscle fatigues easily upon repeated stimulation; and secondly, the use of a single electrical impulse was inadequate to recruit all the motor units of the skeletal muscle flap to produce adequate contractile force.

The fatiguability issue was overcome by the discovery that skeletal muscle can be transformed by chronic low-frequency electrical stimulation, delivered over a period of weeks, from a fatigue-prone type II muscle fiber to a fatigue-resistant type I muscle fiber[7]. The mechanisms behind this process involve genetic phenotype alterations which result in an increase in the energy production of the muscle, a switch to aerobic metabolic pathways, and a change in the type of myosin protein found[8]. Secondly, skeletal muscle does not contract in an 'all-or-none' fashion in response to a single electrical impulse the way heart muscle does in response to a cardiac pacemaker stimulus. Skeletal muscle is composed of distinct individual motor units whose contractile force and duration depend on how many units are recruited by a given electrical stimulus. In order to generate prolonged contraction and power output comparable to the myocardium, it was necessary to deliver a pulse train, or burst stimulus, to the nerve supplying the skeletal muscle to recruit more motor units and summate the contractile forces produced[9].

Once these two problems had been solved, cardiomyoplasty was launched into the clinical phase of development.

TECHNIQUES FOR DYNAMIC CARDIOMYOPLASTY

In 1985, Carpentier performed the first clinical cardiomyoplasty after removing a 1.4 kg fibroma from the ventricles of a female patient[10]. Since then, more than 600 patients have undergone this procedure at over 50 centers around the world. The surgical technique has been described extensively elsewhere[11–13]. Briefly, the most common approach is to raise the left latissimus dorsi muscle (LDM) as a rotational flap, and then, through a sternotomy incision, wrap the ventricular surfaces of the heart with the flap in a posterior clockwise direction. If the muscle length is inadequate to cover both ventricles, a patch of pericardium is sewn to both ends to provide continuity around the heart. Sensing and pacing epicardial leads are placed on the heart and stimulating electrodes are sewn near the thoracodorsal nerve prior to the muscle wrap to deliver the burst stimulus to the skeletal muscle. These electrodes are then attached to a cardiomyostimulator.

Postoperatively, the skeletal muscle is allotted a period of approximately 2 weeks to undergo conformational change, adhesion to the epicardium, and revascularization. Conformational change is a process whereby the LDM adapts to its new resting tension and new geometrical orientation around the heart in order to optimize its force-producing capabilities[14,15]. The muscle flap 'conforms' to the myocardium and acts as another layer of the myocardium. The LDM accomplishes this by adding or deleting sarcomeres in order to maintain optimal sarcomere length and resting tension, so that it can deliver maximum power output. In summary, the repositioned LDM adapts to its new working configuration around the heart.

Revascularization is needed because the collateral vessels to the distal portion of the LDM flap are ligated during LDM dissection. In addition, the LDM must undergo transformation to induce fatigue resistance in the muscle. The LDM is progressively stimulated to transform according to a protocol[16]. Once the patient's muscle is transformed, the cardiomyoplasty wrap is usually stimulated to contract with every second heart beat.

PHASE I CLINICAL FEASIBILITY STUDY

Medtronic, Inc. has coordinated clinical research in DCMP since 1985, with Medtronic cardiomyostimulators and intramuscular leads implanted in over 480 patients by mid-1995. A phase I study was started in July of 1985 and continued to April of 1991 to explore the feasibility of the procedure, define case selection criteria, observe patient outcome, and determine the safety of the first-generation Model SP1005 cardiomyoplasty stimulating system. A published report on the first 78 patients[17] was followed by a presentation of results on all 118 phase I patients from 14 centers[18].

Over 80% of the patients surviving past 3 months showed improvement in their New York Heart Association Functional Class (NYHA) regardless of their preoperative NYHA class (average improvement for those improved was 1.6 classes). However, hemodynamic augmentation was inconsistent in this group. Patients who were in NYHA class IV preoperatively had a significantly greater mortality rate during initial cardiomyoplasty hospitalization than those in NYHA class III preoperatively (38% vs 12%, respectively). Survival at 12 months was 78% for NYHA III and 34% for NYHA IV patients.

The conclusions drawn from the phase I study were that the procedure is feasible and reproducible, and the cardiomyostimulator system is safe. Additionally, it appeared that functional status could be improved with DCMP, but that the patient's preoperative condition affected survival. The phase I study had several limitations, including evolving inclusion criteria and surgical techniques, varying follow-up, and a high number of patients undergoing concomitant cardiac procedures. This introduced confounding factors and made it difficult to interpret the results.

PHASE II CLINICAL TRIAL

Even with the above limitations there was still convincing subjective evidence that DCMP made significant improvements in clinical outcome (i.e. NYHA class) to warrant further, more rigorous, clinical trials. This led to the approval by the US Food and Drug Administration (FDA) to allow Medtronic, Inc. to begin a phase II clinical trial[19]. The lessons learned from the phase I feasibility study were used to improve patient selection criteria and standardize patient follow-up. Patients enrolled in the trial were evaluated preoperatively and then every 6 months with subjective and objective tests (Table 1). Data were prospectively collected and compared to pre-DCMP values using paired *t*-tests, Fisher's exact tests, and actuarial survival analyzes. Results from the first year of follow-up, initially presented in November 1994[19], are reported here (n=49 at 6 months and n=32 at 12 months). Ongoing analysis of an additional year's follow-up data is showing similar results.

A total of 68 cardiomyoplasty patients from five centers in the US, two in Canada, and one in Brazil (the American Cardiomyoplasty Group) were enrolled in this phase II clinical trial. The trial began in May 1991, and enrollment was concluded in September 1993. Major inclusion and exclusion criteria are listed in Table 2. The treatment group was also compared to a

Table 1 Subjective and objective tests for the phase II clinical trial

MUGA scan
Metabolic exercise test
Left and right heart
Catheterization
Echocardiography
Pulmonary function test
24-hour Holter monitor
Chest X-ray
Electrocardiogram
Quality-of-life questionnaire

Table 2 Inclusion and exclusion criteria for the phase II clinical trial

Inclusion
NYHA class III, refractory to medication
LVEF < 40%
LVEDP or PCWP > 15 mmHg
Age 18–80 years
Exclusion
Arrhythmias needing implantable device
Preoperative dependence on intravenous ionotropes
Vital capacity < 55%
Non-cardiac life-threatening disease

prospective, non-randomized, concurrently enrolled reference group of 58 medically treated patients[20]. There were no statistical differences between the two cohorts when comparing age, sex, etiology, duration of symptoms, NYHA class, left ventricular ejection fraction (LVEF), or quality-of-life evaluation.

Patients in the DCMP group had a mean age of 57 years, most were males (78%), and almost all were NYHA III (94%). They had been symptomatic for a mean of 3.6 years. More than one-half had a history of ventricular arrhythmia and about one-fifth had a history of atrial fibrillation. The primary etiology was idiopathic dilated cardiomyopathy (69%), while ischemic cardiomyopathy accounted for the rest (31%). All patients were on digoxin and diuretics, 90% were taking ACE inhibitors, 31% vasodilators, and 21% antiarrhythmics prior to their surgery. Hemodynamic data measured preoperatively revealed depressed cardiac function in most, with a mean LVEF of 22%, cardiac index (CI) of 2.3 l/min/per square meter, pulmonary capillary wedge pressure (PCWP) of 20 mmHg, and peak oxygen consumption (V_{O_2}) of 15 ml/kg per minute. Patients also had rather large hearts with an average left ventricular end diastolic diameter of 74 mm measured by echocardiography.

Intraoperative analysis revealed an anesthesia time averaging 7.0 hours. Only three patients (4%) had concomitant procedures (two with coronary bypass and one with valve repair). In 55 patients the left LDM was used, and in the remaining 13 patients from one center, the right LDM was used. Since preoperative characteristics and long-term outcomes for the right LDM and left LDM cohorts were found to be statistically similar, data were subsequently pooled.

The mortality rate of 12% during initial cardiomyoplasty hospitalization remained the same as that for NYHA III patients in the phase I study. Three-fourths of these deaths were from cardiac failure. In order to determine what variables, if any, were predictive of hospital mortality, data from this trial were pooled with data from 98 other cardiomyoplasty patients worldwide who followed a similar phase II protocol[21]. Univariate and multiple logistic regression analyzes of these 166 patients' data showed that patients with low peak V_{O_2} and low LVEF were found to have a significantly higher in-hospital mortality. Supporting the multicenter analysis, one single center publication identifies low peak V_{O_2} as a risk factor for early death[22], and several identify LVEF as a risk factor for early[22,23] and/or late death[23-25]. Patients with very high pulmonary vascular resistance (PVR) were also found to be at higher risk in the multicenter analysis; further implication of PVR as a notable risk factor has been published by Moreira *et al.*[26,27], who concluded that patients with elevated PVR have poorer long-term survival after cardiomyoplasty. Pulmonary hypertension has been identified as a risk factor for early[28,29] and/or late death[23,30,31] in several other publications.

Overall mortality data to date, for the treatment and reference groups, show nearly equivalent survival (Figure 1). However, there was a difference in causes of death between reference patients and cardiomyoplasty patients. Only about one-quarter of the reference patients who died had sudden cardiac death, whereas approximately two-thirds of the cardiomyoplasty patients who died following initial hospitalization had sudden cardiac death. Thus, the proportion of deaths from cardiac pump failure was lower in the DCMP patients. In this relatively small series, cardiomyoplasty appeared to have little effect on the tachyarrhythmias common in heart failure patients that usually account for their sudden deaths. It has been suggested that the development of automatic implantable cardioverter defibrillator capabilities in future cardiomyostimulators may help lower the incidence of sudden death in this group of patients, and improve their survival[32].

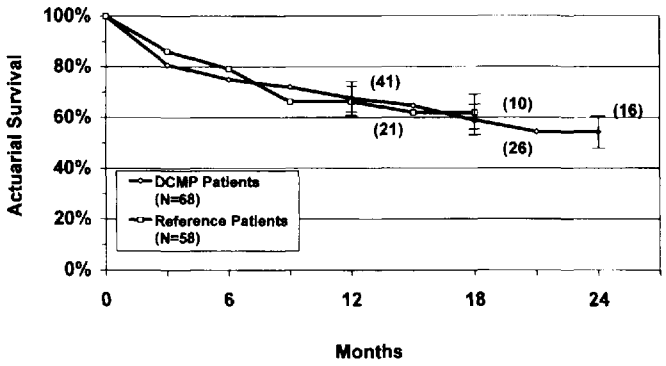

Figure 1 Survival of phase II patients. The reference patient survival curve incorporates three reference patients who crossed over to the DCMP group. These patients are censored in the reference curve and counted in the DCMP curve beginning on the date of surgery. There was no significant difference between the two curves

Cardiomyoplasty patient evaluation at 6 and 12 months showed modest but statistically significant improvements in some objective test results (Figure 2). LVEF increased from 23% to 26% at 6 months, representing a 15% increase. At 12 months, stroke volume increased from 48 to 57 ml/beat, an 18% increase, and left ventricular stroke work index increased from 25 to 32 g/m^2 per beat, which is a 27% increase. There was no statistically significant change in heart rate, PCWP, CI, or PVR. Maximal exercise performance neither deteriorated nor improved significantly at 6 and 12 months. However, evidence of submaximal exercise improvement may be inferred from the statistically significant increase in the 'daily activities' score noted in the quality-of-life questionnaire (Figure 3).

While objective measures showed modest improvement, subjective measures continued to show the significant improvement evident in the earlier phase I study. Of the 32 patients with 12-month follow-up data, 94% were in NYHA class III or IV preoperatively, but only 12% remained in NYHA III at 12 months, with the rest of the patients in NYHA class I or II. This change was highly significant ($p = 0.0001$), and resulted in a mean improvement from 3.0 preoperatively to 1.7 at 12 months (Figure 4). The reference patients also improved their NYHA at 6 and 12 months, but the cardiomyoplasty patients' improvement was greater. The mean change in NYHA for all treatment patients was 1.2 classes at both 6 and 12 months, while for all reference patients it was only 0.5 classes at 6 months and 0.6 classes at 12 months. The proportion of DCMP patients improving was also greater (Figure 5). At the 6-month follow-up, 79% of DCMP patients had shown improvement, while only 49% of reference patients had improved ($p = 0.003$). Similarly, at 1 year, 84% of DCMP patients had shown improvement compared to only 52% of reference patients ($p = 0.009$).

These NYHA class changes were reflected in scores for quality of life. A quantifiable survey[33] was used to provide objective data

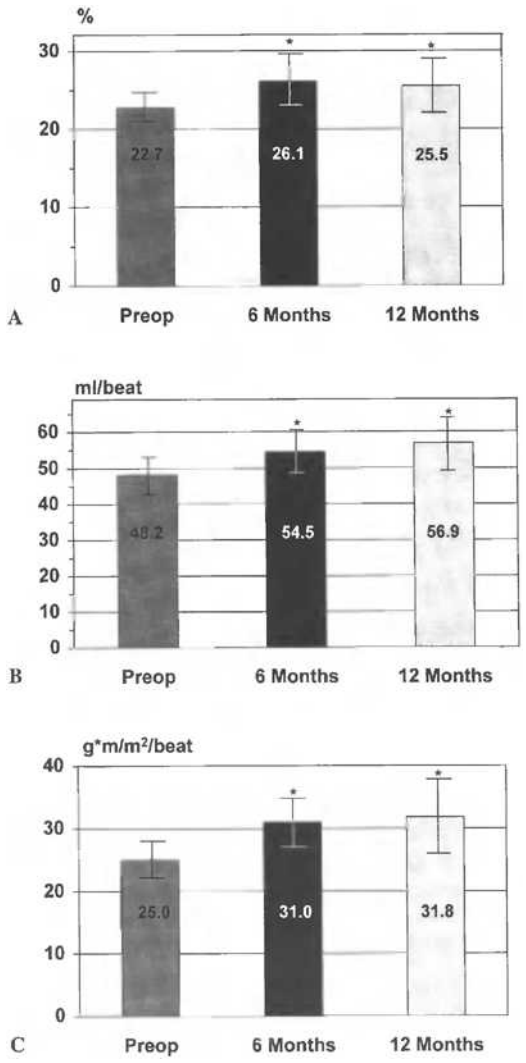

Figure 2 Hemodynamic measurements in the DCMP patients. (a) Left ventricular ejection fraction (LVEF), (b) stroke volume (SV), and (c) left ventricular stroke work index (LVSWI) are significantly higher at 6 and 12 months follow-up ($^*p < 0.05$)

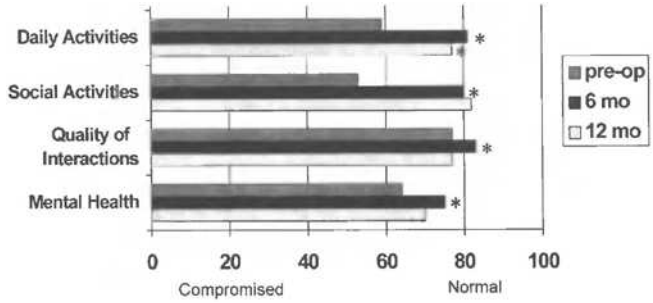

Figure 3 Quality-of-life changes in DCMP patients. Daily activities, social activities, quality of interactions, and mental health were significantly increased at 6 months in the DCMP patients ($^*p < 0.05$). These improvements were greater than most changes seen in the reference patients at both 6 and 12 months, particularly for daily activities, which achieved statistical significance at 6 months

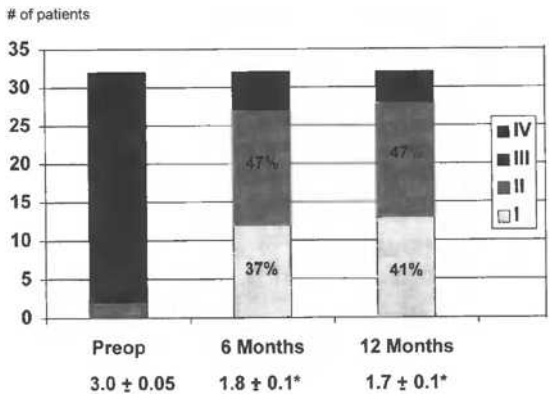

Figure 4 Changes in NYHA class in DCMP patients. The percentage of survivors in the various NYHA classes is shown at each follow-up point. Preoperatively, 94% of patients with 12-month follow-up had been in NYHA class III or IV; at 12 months 88% were in NYHA class I or II, with the mean NYHA class improving from 3.0 to 1.7 ($p = 0.0001$)

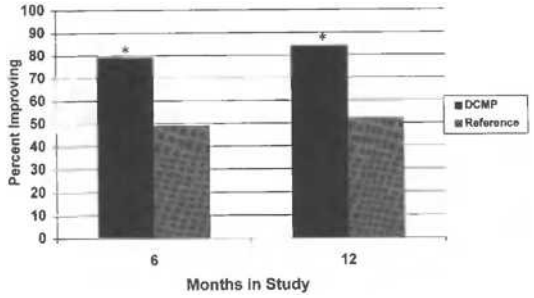

Figure 5 Percentage of patients showing improvement in NYHA class. Data shown represent percentages of surviving patients in DCMP and reference groups showing increased NYHA class. Significantly more DCMP patients improved their NYHA class at 6 and 12 months when compared to reference patients (^{*79} vs 49%, $p = 0.003$, and 84 vs 52%, $p = 0.009$, respectively)

that would support the NYHA assessment. Patients responded to questions on daily activities, social activities, quality of interaction, and mental health. Scores range from 0 to 100; lower scores are associated with more compromised quality of life. Upon entering the study, both groups were quite compromised in their daily activities and social activities, but were normal or near-normal in their quality of interactions and mental health. At 6 months postoperatively the DCMP patients showed statistically significant improvements in all four areas, and their average scores moved into the test-defined normal zones for daily activities and social activities (Figure 3). Results were similar at 12 months but, with fewer patients, only the change in daily activities was statistically significant. While not statistically significant, most of the reference group's mean scores showed modest increases at 6 and 12 months. However, the treatment group's improvements were greater in most areas, particularly for daily activities, which achieved statistical significance at 6 months.

Morbidity as reflected in hospitalizations for cardiac therapy was also evaluated. Both groups had approximately the same percentage of patients hospitalized for cardiac therapy (excluding initial cardiomyoplasty hospitalization) during the first year of

follow-up. The frequency of hospitalizations was also similar. While the mean number of hospital-days per visit was less for treatment patients, this did not reach statistical significance. However, the treatment patients entered the ICU significantly less frequently, and if admitted, stayed in the ICU for a significantly shorter time. Therefore, patients in both groups made the same number of trips to the hospital, but it appears that the treatment patients' visits were less serious. If confirmed with a larger sample size this may not only translate into a better quality of life for patients, but possibly less cost to society.

MECHANISMS OF DYNAMIC CARDIOMYOPLASTY

The results of the phase II trial appear promising. DCMP alleviates the symptoms of moderate to severe heart failure and improves the quality of life in most treated patients. Patient selection criteria were also further advanced in order to make the procedure safer and outcome more consistent. However, evidence that DCMP augments systolic cardiac function is modest. This is not entirely a new finding. There has always been a lack of consistent measurable objective data confirming that DCMP could increase the failed contractile force of the diseased myocardium and deliver systolic augmentation. In spite of this, almost all series report significant symptomatic improvement in their patients[17–19,22,24,26,27,29,30,34–40]. These seemingly paradoxical findings have led investigators to wonder if there are other possible mechanisms that may help explain why these patients respond so well clinically.

One possible mechanism is that DCMP reduces the myocardial wall stress, which is a major determinant of myocardial oxygen consumption. Experimentally it was shown that, in an acute heart failure mode, DCMP decreased the myocardial wall stress while at the same time increasing peak LV systolic pressure[41]. A reduction in myocardial wall stress was thought to occur through enhanced systolic contractility, decreased end-systolic dimensions, and increased ventricular wall thickness. An increase in wall thickness, according to LaPlace's law, would cause a reduction in the stress per unit of muscle mass. Long-term reduction in wall stress, therefore, may act to improve patient outcome and perhaps survival. This reduction in the myocardial stress by DCMP has been called 'myocardial sparing effect'.

Clinical evidence to support this concept has been reported by Moreira *et al.*[27]. Pressure–volume loops obtained by simultaneous echocardiographic–hemodynamic studies with the cardiomyostimulator turned on compared to off showed a significant reduction of left ventricular end-systolic and end-diastolic stress with the device on. This was associated with a significant decrease in left ventricular chamber stiffness and an increase in left ventricular maximal elastance.

DCMP may also provide a more passive benefit to the failing hearts in the treated patients. Having skeletal muscle wrapped around the heart may prevent the progressive ventricular dilatation and deterioration of ventricular function seen with worsening heart failure. This in turn may delay or slow down the further advance of the underlying disease process. Support for the concept of preventing progressive ventricular dilatation in heart failure patients can be found in Cohn's publication entitled 'Structural basis for heart failure'[42]. Here Cohn discusses a recent hypothesis that systolic dysfunction, which has been thought to be related to contractile failure, could be a *consequence* rather than a cause of a structural increase in ventricular chamber volume. If this is so, perhaps remodeling, rather than contractile dysfunction, is the key to understanding heart failure, and the regression of remodeling or prevention of progression of remodeling is a therapeutic goal in heart failure management.

Experimental animal and clinical DCMP data from several sources are relevant here. Experimental studies of this 'girdling effect' have shown that even a non-stimulated wrap can significantly reduce the progression of LV dilatation and decrease in ejection fraction seen with worsening heart failure[43,44]. In phase II clinical trial data from the American Cardiomyoplasty Group the mean left ventricular end-diastolic diameter via echocardiography lessened over time, but did not reach statistical significance[19]. Carpentier *et al.*[35] reported a stable cardiothoracic ratio to 3 years following cardiomyoplasty in the largest single-center series to date. Most compelling, however, are the results of pressure–volume studies reported by Kass *et al.*[45]. These studies demonstrated that substantial benefit was derived from reverse chamber remodeling, most probably from the cardiomyoplasty acting 'like an elastic girdle around the heart'. If DCMP can affect the remodeling process of failing hearts, then this may also contribute to the improved clinical outcome seen in the DCMP patients.

Whatever the mechanism of action, ongoing research in two other areas should lead to improved results in future patients. First, several reports have recently been published on the ability of optimal stimulation timing to significantly improve outcome in cardiomyoplasty patients[36,46–51]. Also, compelling research is being conducted to improve LD muscle viability long-term[52–57].

TRANSPLANTATION VERSUS CARDIOMYOPLASTY: A FUTURE PERSPECTIVE

Dynamic cardiomyoplasty has been in clinical use since 1985 in many parts of the world, but is not yet currently approved by the FDA in the USA. Many cardiologists are convinced that patients in NYHA class III and less can be well managed medically. Those patients who have progressed to end-stage heart failure have a high perioperative mortality and are therefore not selected. Thus patients who might benefit from DCMP are often not referred. This question of patient selection continues to be one of the most controversial issues facing DCMP today.

Currently, it is generally accepted that heart transplant is the treatment of choice for end-stage heart failure patients[58], since 5-year survival in most centers is over 60%. However, this procedure is not without its disadvantages, as mentioned earlier. An estimated 40 000 Americans below age 65 are transplant candidates, but only about 2000 heart transplants are done each year, due to the limited supply of donors[59]. With the aging population, more patients are going to be rejected for transplant and more patients will be waiting longer for their hearts, suffering and even dying as the lists get longer. The population of patients who will most likely be considered for DCMP will probably prove to comprise those patients clinically deteriorating despite being on maximum medical therapy, and who either are rejected for heart transplant or appear to have little chance of finding a timely donor.

COMMENT

A randomized prospective phase III DCMP clinical trial organized by Medtronic, Inc. is currently under way in North America using Medtronic's second-generation Transform™ cardiomyostimulator system. Patients who meet the selection criteria will be randomized to either DCMP or medical therapy. In order to achieve statistical power, 200 patients will be assigned to each arm of the study. Although such a study is the gold standard in clinical trials, a prospective randomized trial for DCMP will face a number of methodological, ethical and logistic challenges which need to be overcome for its successful completion[60]. Conclusive confirmation of its clinical efficacy will establish DCMP as a useful modality of therapy for selected patients with chronic heart failure.

Acknowledgment

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Medtronic cardiomyoplasty clinical centers contributing data to this report
(listed in order of cumulative months of implant)

Institution	*City, country*
Hôpital Broussais	Paris, France
Instituto Do Coração*	São Paulo, Brazil
Hôpital Cardiologique	Lyon, France
Allegheny General Hospital*	Pittsburgh, PA, USA
Hôpital La Pitié	Paris, France
St Vincent Hospital*	Portland, OR, USA
Spedali Civili	Brescia, Italy
Ruprecht-Karls-Universität	Heidelberg, Germany
Cleveland Clinic*	Cleveland, OH, USA
McGill University (Montreal General Hospital)*	Montreal, Quebec, Canada
Academic Hospital	Maastricht, Netherlands
Presbyterian Medical Center*	Philadelphia, PA, USA
Institut Arnault Tzanck	Nice, France
C.H.U. de la Timone	Marseille, France
Karolinska Hospital	Stockholm, Sweden
Policlinico S. Matteo	Pavia, Italy
Policlinica De Guipuzcoa	San Sebastian, Spain
Johns Hopkins Hospital*	Baltimore, MD, USA
Türkiye Yüksek Ihtisas Hospital	Ankara, Turkey
Wythenshawe Hospital	Manchester, UK
University of Milan	Milan, Italy
Edinburgh Royal Infirmary	Edinburgh, UK
Hospital Juan Canelejo Insalud	La Coruña, Spain
King Faisal Hospital	Riyadh, Saudi Arabia
University Western Ontario*	London, Ontario, Canada
Royal Northshore Hospital	Sydney, Australia
University Hospital	Istanbul, Turkey
Stättisches Klinikum	Ludwigshafen, Germany
Hospital Centro Medico Siglo XXI	Mexico City, Mexico

* American Cardiomyoplasty Group.
Note: List does not include seven centers with 24 patients contributing data to Medtronic between December 1982 and June 1992.

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Cultured Cardiomyocytes

F.W. SMART, W. CLAYCOMB, J. DELCARPIO AND C. VAN METER

INTRODUCTION

The shortage of donor organs for cardiac transplantation continues to fuel the search for alternatives to the transplantation of human heart allografts[1,2]. To alleviate this profound cardiac donor shortage, attention has recently focused on the use of biological and mechanical assist devices for permanent implantation[3–5] and xenogeneic whole organ transplants. Therapies to repair damaged hearts are also gaining acceptance, such as high-risk coronary bypass grafting and transmyocardial laser revascularization[6,7]. Cell transplantation is one method of augmenting these repair processes.

Adult heart muscle has no reserve capacity to regenerate following injury to the myocardium[8,9]. As a result, myocardial infarction or progressive heart disease, such as cardiomyopathy, leads to the loss of myocytes and impairment of ventricular function. Once a significant amount of cardiac mass is damaged the patient develops intractable heart failure and is responsive only to cardiac transplantation.

The use of biologic agents to repair heart muscle has gained considerable attention in recent years. Cardiomyoplasty is a procedure in which the latissimus dorsi muscle is mobilized, still attached to its neurovascular pedicle, and is wrapped around the failing heart[10,11] (Chapter 4). When paced in conjunction with the recipient heart beat, this muscle wrap may augment the ventricular function. Similar skeletal muscle wraps have also been used around the aorta to form a counterpulsation device or, in other efforts, to create a skeletal muscle ventricle that would work in series with the patient's heart[12]. Another type of biologic augmentation is the use of skeletal muscle myoblasts. Experiments have been conducted into the ability of skeletal muscle to regenerate following injury. The presence of satellite cells capable of dividing and growing allows this muscle to repair itself. These satellite cells are harvested and injected into a recipient whose muscle may have been damaged by muscular dystrophy[13]. Cell transplants have been shown to improve function in the skeletal muscle of recipients with muscular dystrophy. Satellite cells have also been injected into the hearts of both mice and swine[14]. The cells grow in this living matrix, but studies thus far do not show appropriately formed gap junctions. This raises the question as to whether these cells will ever electrically couple to the cardiac muscle cell.

A potentially more promising area of investigation now focuses on the use of fetal muscle cells that still have the ability to divide. Such cells are harvested and placed into the recipient adult myocardium. Early experiments in this area have focused on the use of syngeneic mouse cardiomyocytes, placed by direct injection into the free wall of the left ventricle of an adult mouse[15,16]. Subsequent genetic modification of cells so that they are more apt to grow after injection has also been reported[17–19]. The following will explain the use of such a genetically modified cell line. The results of experiments thus far undertaken in *in-vitro* culture and research ongoing in the adult swine will be reviewed.

IN-VITRO AND RODENT STUDIES

Co-culture experiments

Experiments utilizing 1-day-old or neonatal rat ventricular cardiomyocytes were conducted. Heart muscle cells were isolated by the protocols of Claycomb and Palazzo[20], and modified after Claycomb and Lanson[21]. Rats were anesthetized and their hearts were removed and placed into cold Joklik's medium. The hearts were then attached to a short Frederick's condenser by the use of a three-way stop-cock and retrogradely perfused through the aorta with enzyme-free Joklik's medium. This was followed immediately by retrograde perfusion with Joklik's medium containing 1 mg/ml of type II collagenase. All solutions were maintained at 37°C, and gassed with a 0.8 ml filtered mixture of 95% O_2 and 5% CO_2. Once softened, the hearts were removed, minced and subjected to gentle digestion in fresh collagenase by stirring in a heated rotating shaker bath. The cells were collected and washed in serum-free Joklik's medium and then pooled. Cells were then plated and grown to confluence.

A lineage of AT-1 tumor cells was obtained from transgenic mice produced by targeting the expression of SV40 large T antigen to the cardiac atria. These mice exhibited unilateral right atrial tumor genesis. Transgenic atrial cells were isolated from

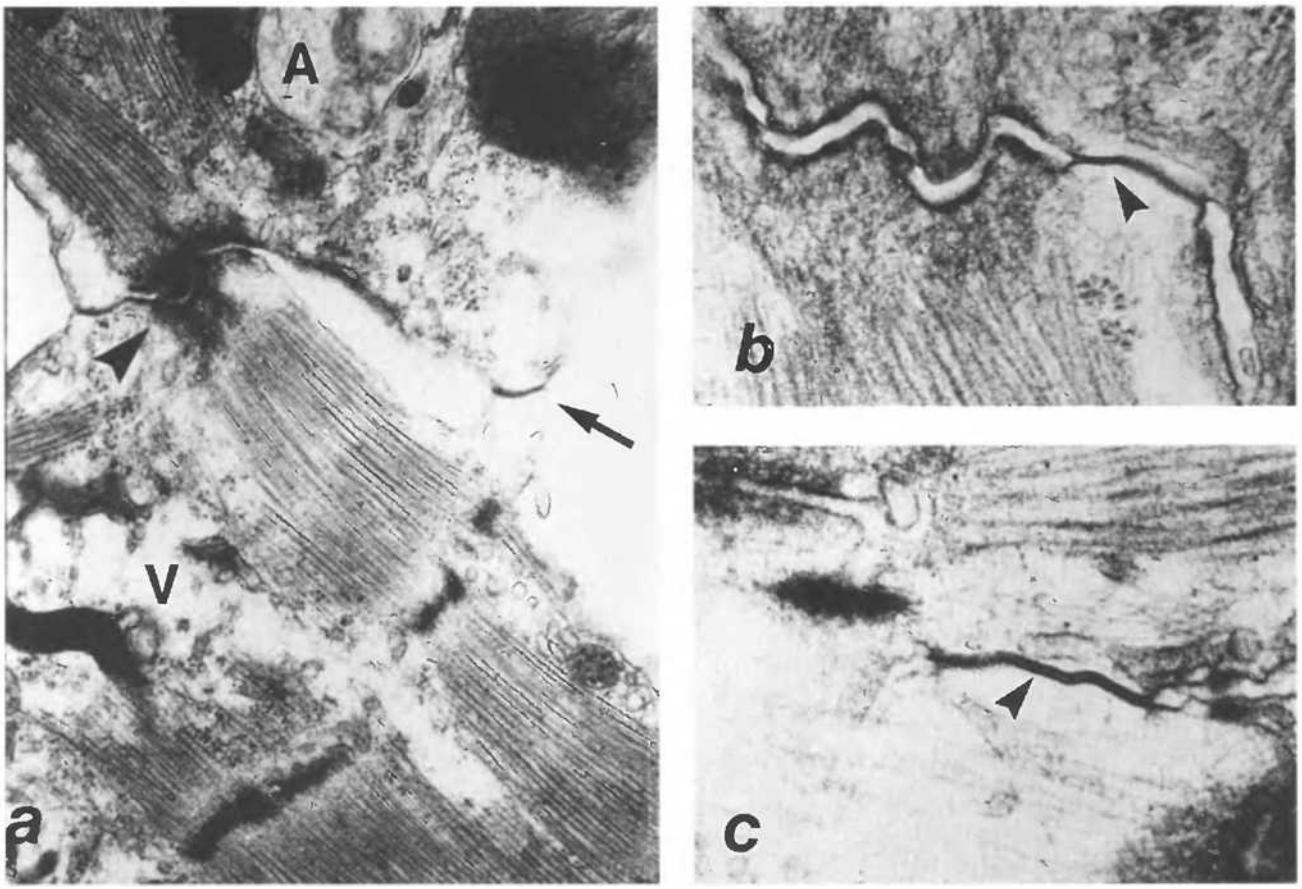


Figure 1 Panel (**a**) depicts the co-culture of two cell lineages. (A) is the AT-1 tumour cell and (V) is the adult rat ventricular myocyte. Panel (**b**) shows a gap junction between these two cells (arrow). Panel (**c**) is a higher magnification of this same gap junction

these atria, and injected subcutaneously into syngeneic host mice. Cardiomyocytes derived from this transplanted tumor lineage retained the capacity to proliferate in culture and express cardiac specific genes. These cardiomyocytes exhibit all the morphologic characteristics typically associated with adult atrial cardiac myocytes. This isolate of cells in *in-vitro* culture with adult rat ventricular cardiomyocytes was noted to form gap junctions (Figure 1). This was confirmed by immunohistochemical staining for the connexin protein pan-cadherin (Figure 2). Under real-time microscopy they were also observed to beat in synchrony with the rat ventricular myocyte. This *in-vitro* work confirmed the assumption that, by using either genetically altered or undifferentiated cells, xenogeneic cell transplantation was feasible.

Syngeneic and xenogeneic injections in rodents

Subsequent experiments focused on the AT-1 cell being transplanted by direct injection into the adult rat ventricle. AT-1 cells were prepared by the method previously outlined. The cells were injected into syngeneic mice and immunosuppressed rats. Rats were immunosuppressed with cyclosporin A (given through gavage feeding) as the sole immunosuppressant agent. Again, the cells were transplanted by direct injection into the left ventricular wall and allowed to grow for several weeks. Histological analysis at the time of harvesting indicated that, in both the syngeneic mouse and the immunosuppressed rat, AT-1 cells formed nascent intercalated disks with each other, and morphologically rudimentary junctions with host myocytes. Contractile proteins and atrial granules were also present (Figure 3). Having demonstrated that these genetically altered AT-1 tumor cells would grow and form what appeared to be gap junctions with host myocytes, the stage was set for using myocytes with a potential to differentiate into an adult myocyte.

Fetal and neonatal myocyte injections

Isolation of fetal mouse and neonatal rat cardiomyocytes followed previously published protocols[21]. Briefly, the hearts of 2-day-old neonatal rats were dissected out, minced and rinsed in sterile phosphate-buffered saline (PBS). Tissue pieces were shaken overnight in 0.125% trypsin in PBS at 4°C. After 16 hours they were rinsed in PBS and subjected to four or five sequential digestions in 0.1% collagenase in PBS at 37°C. Isolated cells from each digestion were rinsed in PBS and pooled. These cells were then injected into the adult rat, and again were found to grow within the host myocardium and to form rudimentary intercalated disks. Interestingly, there was also an increase in angiogenesis. The microscopy of these cells indicated that the morphology is

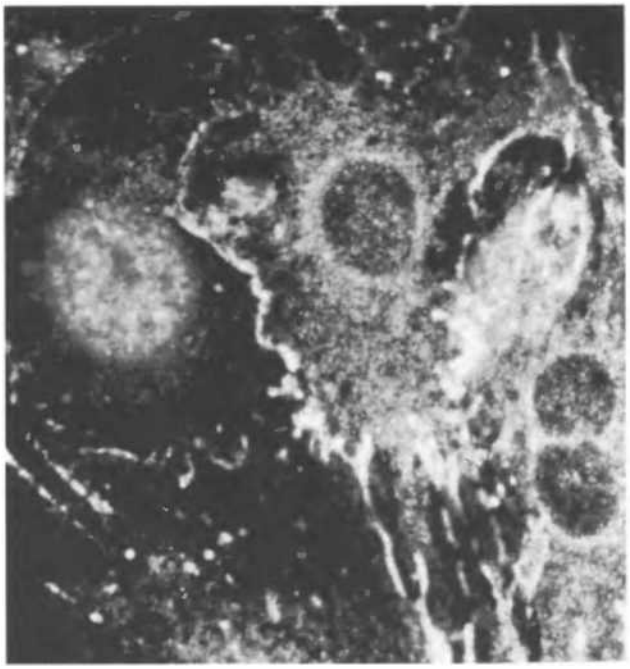


Figure 2 Immunohistochemical stain using two antibodies. The first is an antibody directed against the large T antigen of the AT-1 cell. This stains the nucleus of the AT-1 cell bright orange. The second, an antibody directed against Pan-Cadherin (a connexin protein), stains with light green to white and can be seen just to the right of the AT-1 cell nucleus. This is the site of the gap junction between the AT-1 cell and the rat ventricular myocyte

still in a more neonatal form (Figure 4). However, these experiments suggested that it would be possible to use a neonatal allogeneic or even xenogeneic cell to potentially repair a damaged host myocardium. Additionally, the presence of neovasculature in the area of the cell transplant indicated that this repair process would potentially augment the vascular supply not only to the transplanted cell lineage, but also to other areas of potentially ischemic host myocytes following myocardial infarction.

LARGE-ANIMAL STUDIES

Xenogeneic transplants

This protocol was then taken to the large animal for testing, to determine if the adult swine would accept these cells as readily as rodents. The swine model was chosen because the coronary anatomy is similar to the human, and creating a percutaneous infarction in these animals is possible.

Seven adult Yorkshire swine were anesthetized with ketamine and pentobarbital, intubated and ventilated with isofluorane by a pressure-controlled respirator. Venous access was accomplished by placement of a subcutaneous infusion catheter, and subsequently beryllium (5 mg/kg) and diltiazem (2.5 mg/kg) were given intravenously by constant infusion to prevent ventricular dysrhythmias. Using aseptic technique a median sternotomy was performed and a pericardial well was created. Isolated cardiomyocytes in Joklik's medium were directly injected into the myocardium through a 26-gauge needle using a tuberculin syringe.

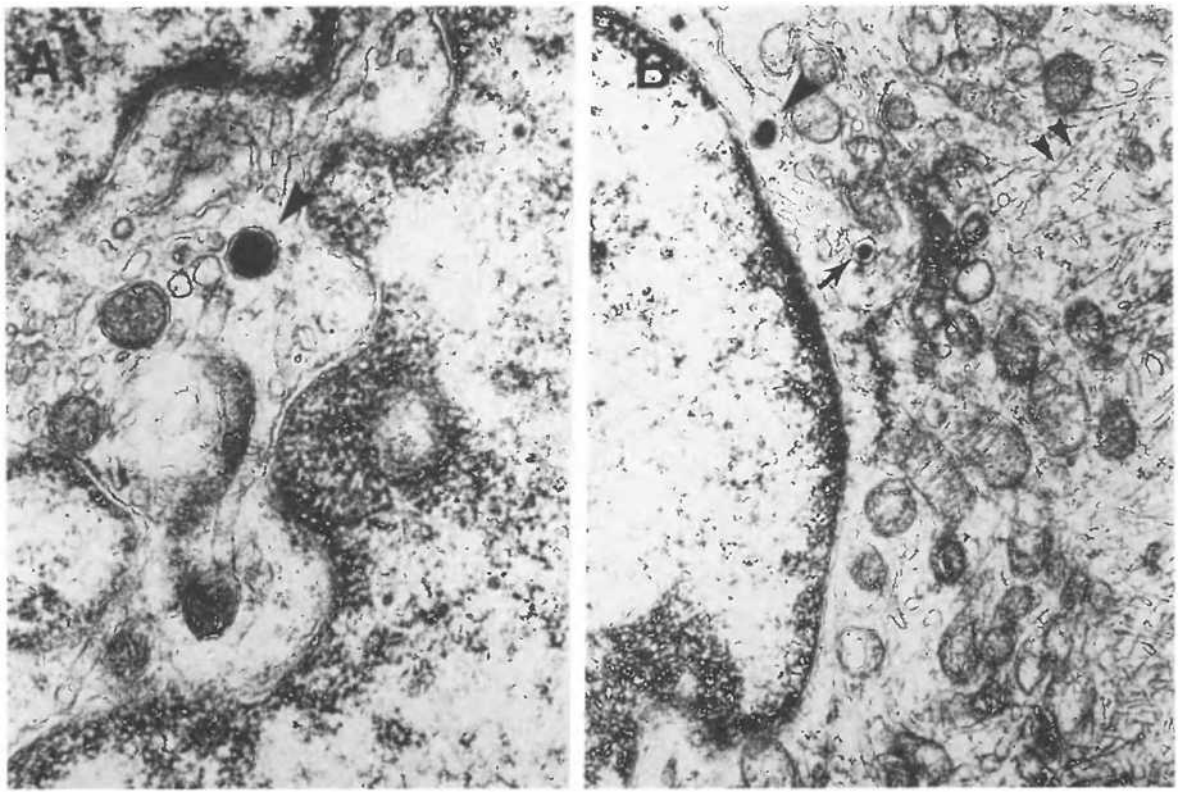


Figure 3 A: The arrow indicates an atrial granule present in the AT-1 transplant itself. B: The double arrow highlights the immature contractile protein present in the AT-1 cell after having been transplanted into an immunosuppressed rat

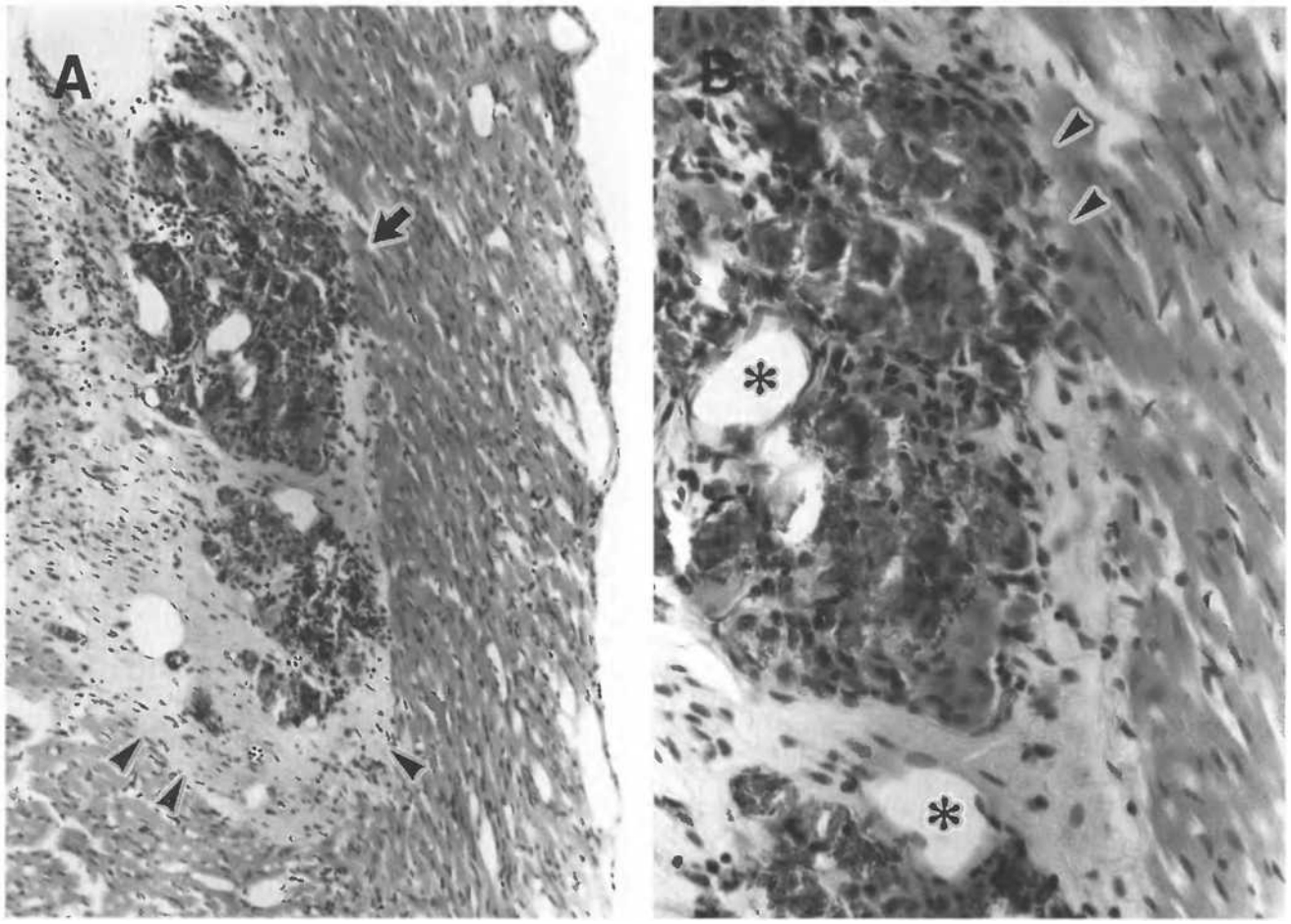
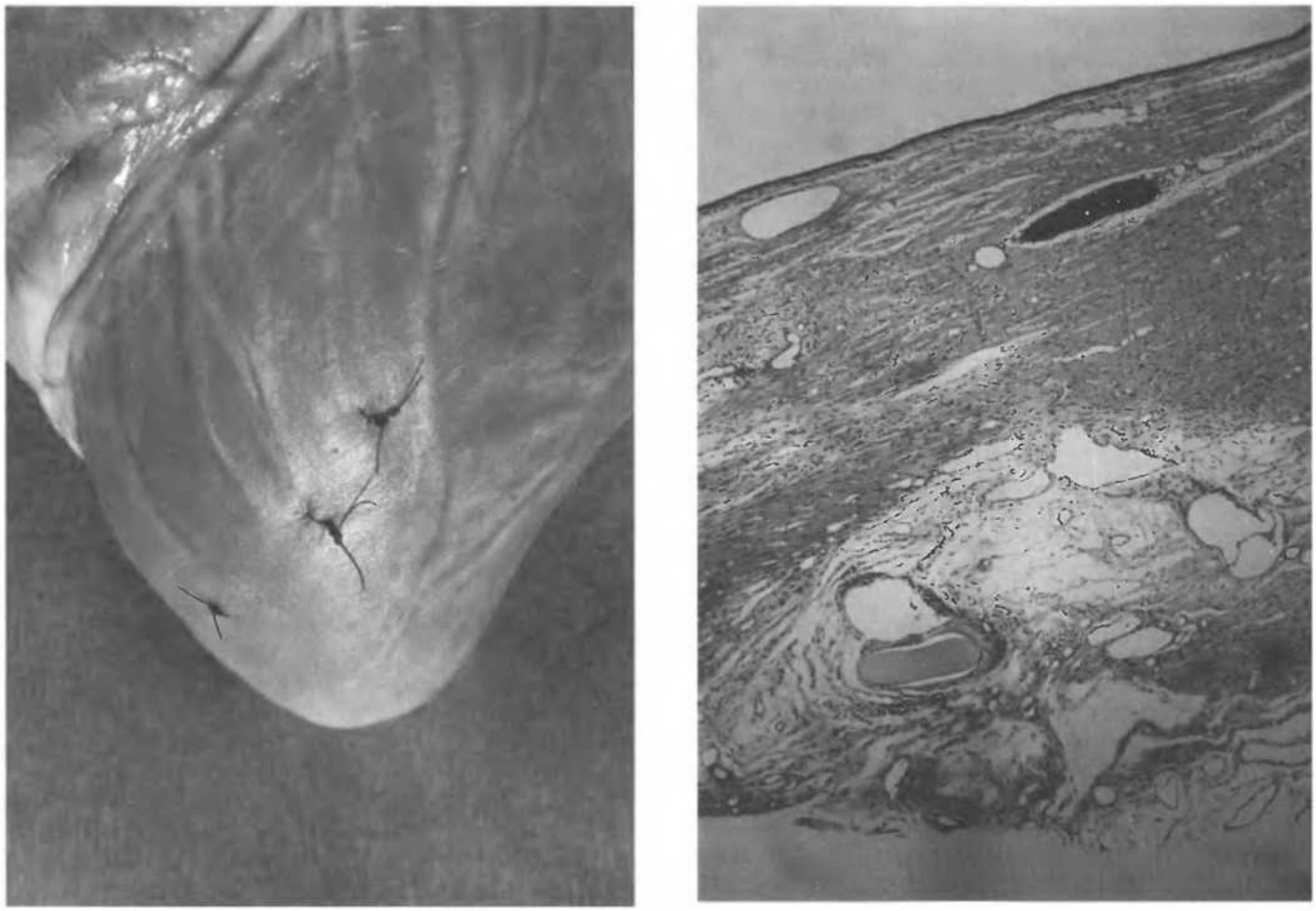


Figure 4 Four weeks post-transplantation of freshly isolated fetal mouse ventricular cardiomyocytes. **A**: Medium magnification of transplanted ventricular cardiomyocytes from a 16-day mouse fetus. While the transplant region shows some encapsulation (arrowheads), there is nevertheless close contact between host cardiomyocytes and the darkly stained transplanted cells (arrow) (× 135). **B**: Enlargement of the region surrounding the arrow in **A**, indicating the close contact of host and transplant cells (arrowheads); the asterisk indicates lumen of blood vessels in the transplant region (× 320, H&E)

Approximately 1×10^4 cardiomyoblasts were transplanted with each injection in a volume of approximately 100 μl. Control injections were performed utilizing 100 μl of Joklik's medium alone. The injection sites were identified by placing a 7/0 proline suture into the myocardium in the area of the injection (Figure 5). The midline sternotomy was closed, and the pericardial and thoracic spaces were evacuated of residual air.

Animals were then started on an immunosuppressant regimen consisting of oral cyclosporin (15 mg/kg per day) to maintain whole blood cyclosporin levels of 150–300 ng/dl by TDX (Abbot Labs) assay. Additionally, animals were given prednisone (0.3 mg/kg per day) and were allowed to recover for 1 month following the transplant of these cardiomyocytes. All animals transplanted tolerated the procedure very well, and in no instance did an animal die prior to the scheduled harvesting time. CK (creatinine kinase) and CKMB isoenzyme fractions were obtained within 24 hours of injection of the cells. There was no significant increase in MB fraction, indicating no myocardial death from the cellular implantation. No animals developed any significant illness. Two animals had a postoperative fever that was attributable to local inflammation of the skin incision in the area of the Porta Cath. They were treated with amoxicillin, and quickly improved.

At the end of 1 month the animals were again sedated with ketamine and pentobarbital, and placed on a pressure-cycle ventilator with isoflurane. The chest was again opened through a median sternotomy and the heart freed of any adhesions. The animals were then heparinized to prevent clotting in the microvasculature and the organ explanted with subsequent exsanguination of the animal. The hearts were then perfusion-fixed by selectively cannulating the left and right coronary arteries, flushing with saline, and then continually flushing under 100 mmHg pressure with phosphate-buffered 4% paraformaldehyde/1% glutaraldehyde fixative for 40 minutes. The excised whole hearts were then fixed an additional 7 days in 4% glutaraldehyde/0.1 molar sodium cacodylate. Following adequate fixation, injection sites were identified by locating the proline sutures, and the 1 cm^2 areas around these injection sites were excised and rinsed for an additional week in 0.1 molar sodium cacodylate buffer, with frequent changes of buffer. The blocks were further sectioned for both



A B

Figure 5 **A**: A whole heart specimen in which one can see three prolene sutures in the epicardial surface, marking the area of the transplanted cells. **B**: Low-power micrograph (hematoxylin–eosin stain) of myocardium. Towards the lower portion of the micrograph a prolene suture can be identified. Above it is a nest of injected cells in the epicardial surface

light and electron microscopy. For the purposes of electron microscopy, tissue samples were post-fixed in 1.0 osmium tetroxide/0.1 mol/l sodium cacodylate, in block. Staining used 0.5% aqueous uranyl acetate, dehydrated in acetone and infiltrated and embedded in Polybed 812 (Polysciences). Thin sections were examined in a JEOL 12/10 transmission electron microscope. Images were obtained on a Kodak 4489 EM film. Tissue blocks were prepared for light histology by embedding in paraffin, sectioning, and staining with hematoxolyn and eosin.

Neonatal rat myocytes, AT-1 mouse cells, mouse ventricular cells, and neonatal porcine myocytes were implanted into the adult pig ventricle. The preparation of the porcine cells was similar to the technique used for rat myocytes previously described. Figures 6–11 illustrate the results of these experiments. In all of these experiments, cells were identified in the transplanted region. There seemed to be no gross encapsulation or significant T cell or other inflammatory cell response, indicating no significant rejection of the allogeneic or xenogeneic transplanted cells. In addition, all injection sites demonstrated a marked increase in the neovascularization, which was felt to be a result of these transplanted cells. The sham injections of Joklik's

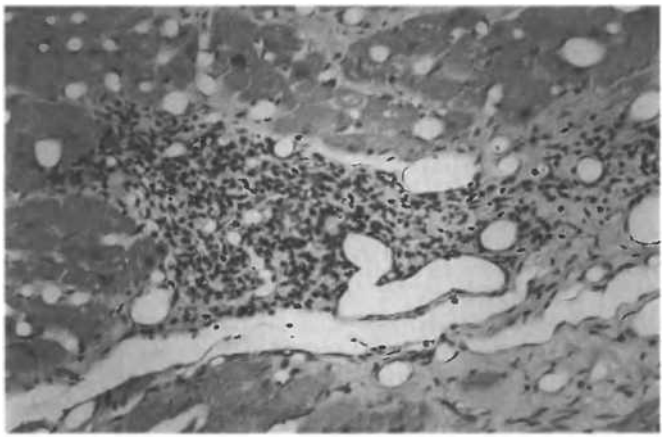


Figure 6 This demonstrates injection of mouse ventricular cells into the adult pig myocardium. Note that the mouse ventricular transplanted cells are easily identifiable as small basophilic cells. They can be seen moving in between the myocardial fibres of the host myocardium. Of note is the striking amount of neovascularization, as represented by multiple capillaries dispersed among the transplanted cell line and the host myocardium

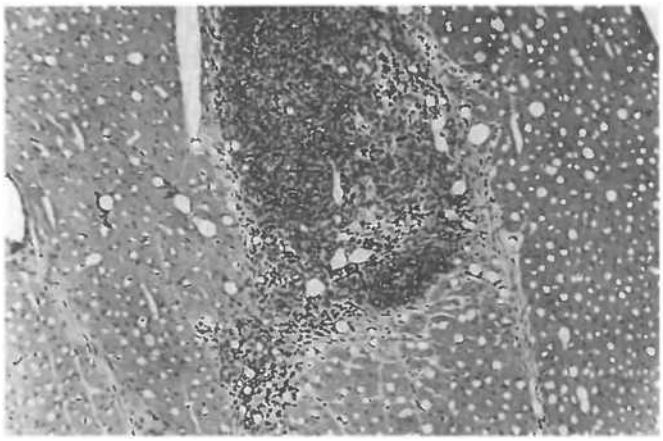


Figure 7 Fetal pig cell line injected into an adult pig ventricle seen at low power (× 90) and stained with hematoxylin–eosin. The transplanted cell line is represented by the small basophilic cells in the center of the panel. This experiment does show slightly more fibrosis around the transplanted cell line than had previously been seen. However, the increase in neovascularization is striking, with the entire field being taken up by the presence of new capillaries throughout the myocardium

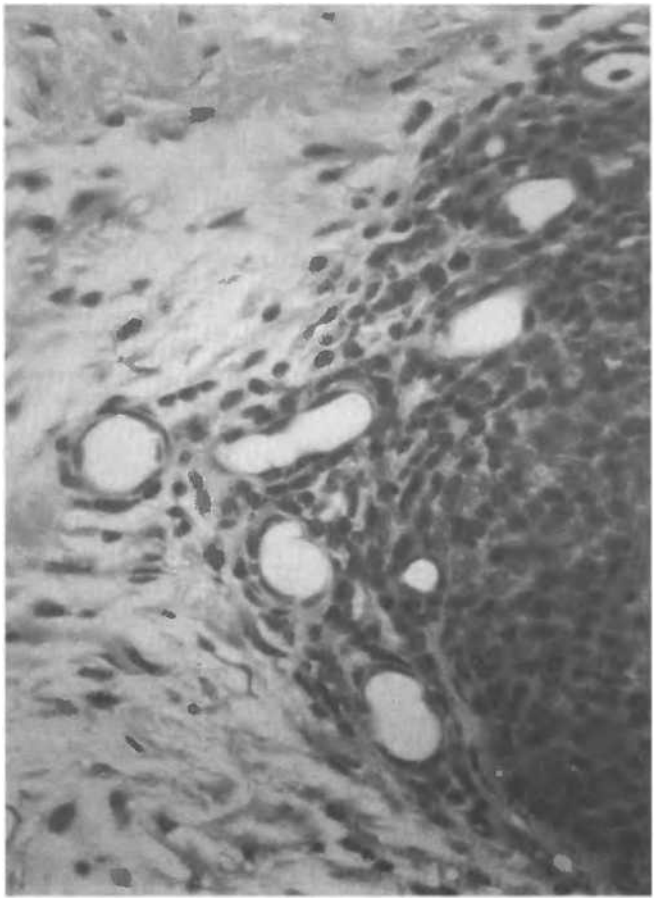


Figure 8 Higher magnification (× 330) H&E stain of fetal pig cells transplanted into the ventricle of an adult pig. The fibroid encapsulation that was noted in Figure 7 is present surrounding the transplanted cell line. Multiple small capillaries, lined with what histologically appear to be endothelial cells, are present at the border zone of the transplanted cell line

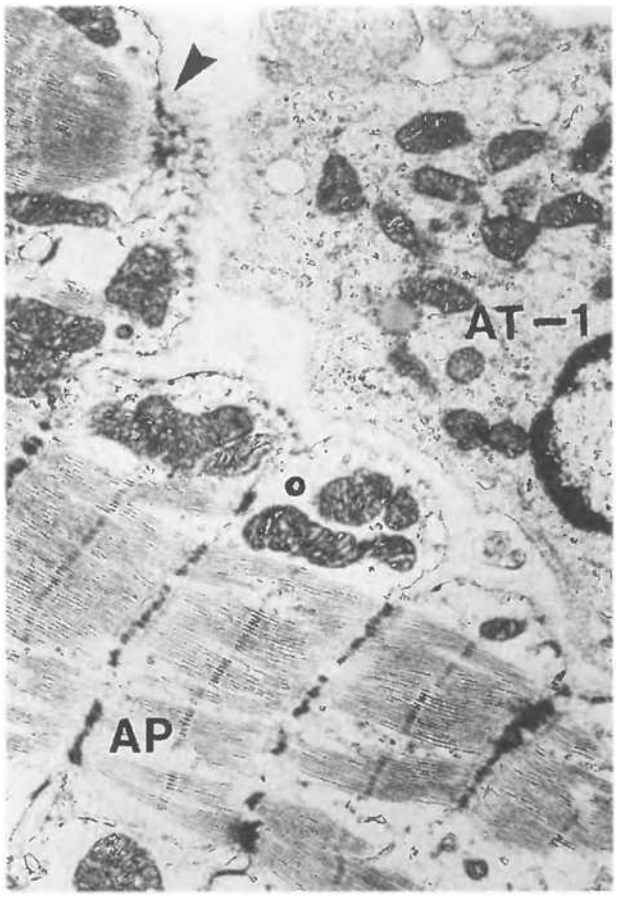


Figure 9 Electron micrograph of AT-1 cells transplanted into an adult pig heart. AT-1 labelling denotes the transplanted cell line, and AP labels the adult pig host cell. The arrow depicts a gap junction between the AT-1 cell and an adult pig myocyte

medium containing no cells did not show this degree of neovascularization.

Using electron microscopy we have been able to identify nascent junctional contacts between host ventricular cardiomyocytes and transplanted AT-1 cells, again suggesting that these transplanted cells may, in the large animal, significantly add to the overall contractile mass of a damaged host myocardium.

Armed with the anatomic information described, physiologic studies have now been undertaken. Similar transplant experiments utilizing strictly mouse ventricular cells and fetal pig cells have been undertaken in a myocardial infarct model.

Myocardial infarct preparation

Adult Yorkshire swine are anesthetized with ketamine and phenobarbitol and placed under isofluorane anesthesia delivered by pressure ventilation. Through a 2 cm incision in the right groin, the femoral artery is isolated and cannulated with a sterile 8 French introducer sheath (Cordis Co.). Through this, a 100 cm Cordis multipurpose catheter is placed into the aortic root, using direct fluoroscopic guidance. Intracoronary angiograms are per-

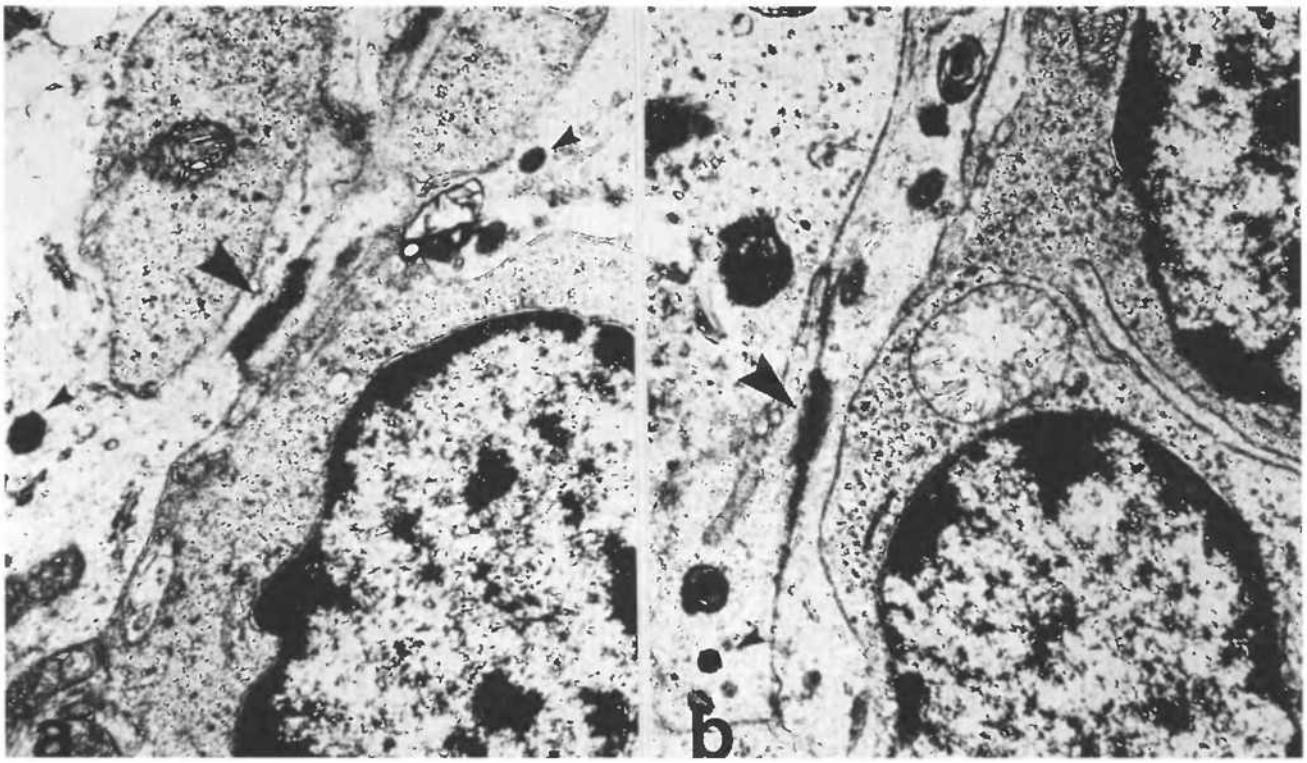


Figure 10 Double panel figure of an AT-1 cell transplanted into an adult pig heart. Both panels depict adherence junctions between transplanted AT-1 cells. These results indicate that transplanted AT-1 cells make gap junctions with themselves and, as previously noted, with the adult pig myocyte

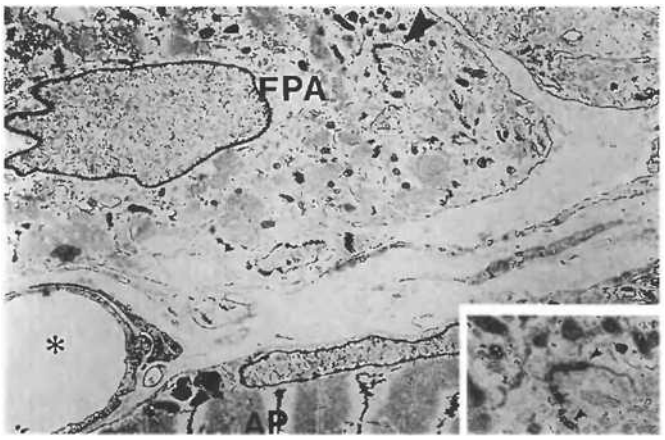


Figure 11 Electron micrograph depicting a transplanted fetal pig atrial cell in an adult pig heart. The host adult pig cell (AP) is noted at the bottom of the panel. The arrow depicts an adherence junction between two transplanted fetal atrial cells. The asterisk indicates a capillary that was induced in the area. The box in the lower right of the panel depicts at higher power the adherence junction noted above

formed with the injection of Renografin (E.R. Squibb Inc.) contrast material, and the coronary anatomy is diagrammed.

Through this 8 French guide catheter, a 5 French H1 embolization catheter is placed. This is maneuvered down into the left anterior descending coronary artery or one of the obtuse marginal branches of the circumflex coronary artery, depending on the individual coronary anatomy. Again, cine angiography is used to confirm the placement of this catheter. Through the catheter, a Cooper embolization coil (Cook Co.) is then placed using a 0.035 guidewire for deployment. The embolization coil is deployed in the mid portion of the left anterior descending coronary artery or at one of the proximal sites of one of the larger obtuse marginal branches of the circumflex coronary artery.

Electrocardiographic confirmation of myocardial infarction is seen by the presence of marked ST segment elevation, and occlusion is confirmed by a repeat coronary angiogram. The animals are given high doses of bretylium and diltiazem to prevent ventricular fibrillation, and standby DC cardioversion is present if ventricular tachycardia/fibrillation were to occur. The catheter apparatus is then removed from the animal. The swine is allowed to recover for 4 hours under anesthesia with the arterial line in place. During this time, bretylium and diltiazem are titrated to relative bradycardia and the presence of significant ventricular dysrhythmias. With this model approximately 60–70% of the animals survive the myocardial infarction and go on to recover for approximately 1 month.

Cell transplant into an infarct zone

At the end of 1 month the animals are opened by median sternotomy, and the zone of myocardial infarction is identified by direct visualization. The infarct zone is then confirmed through

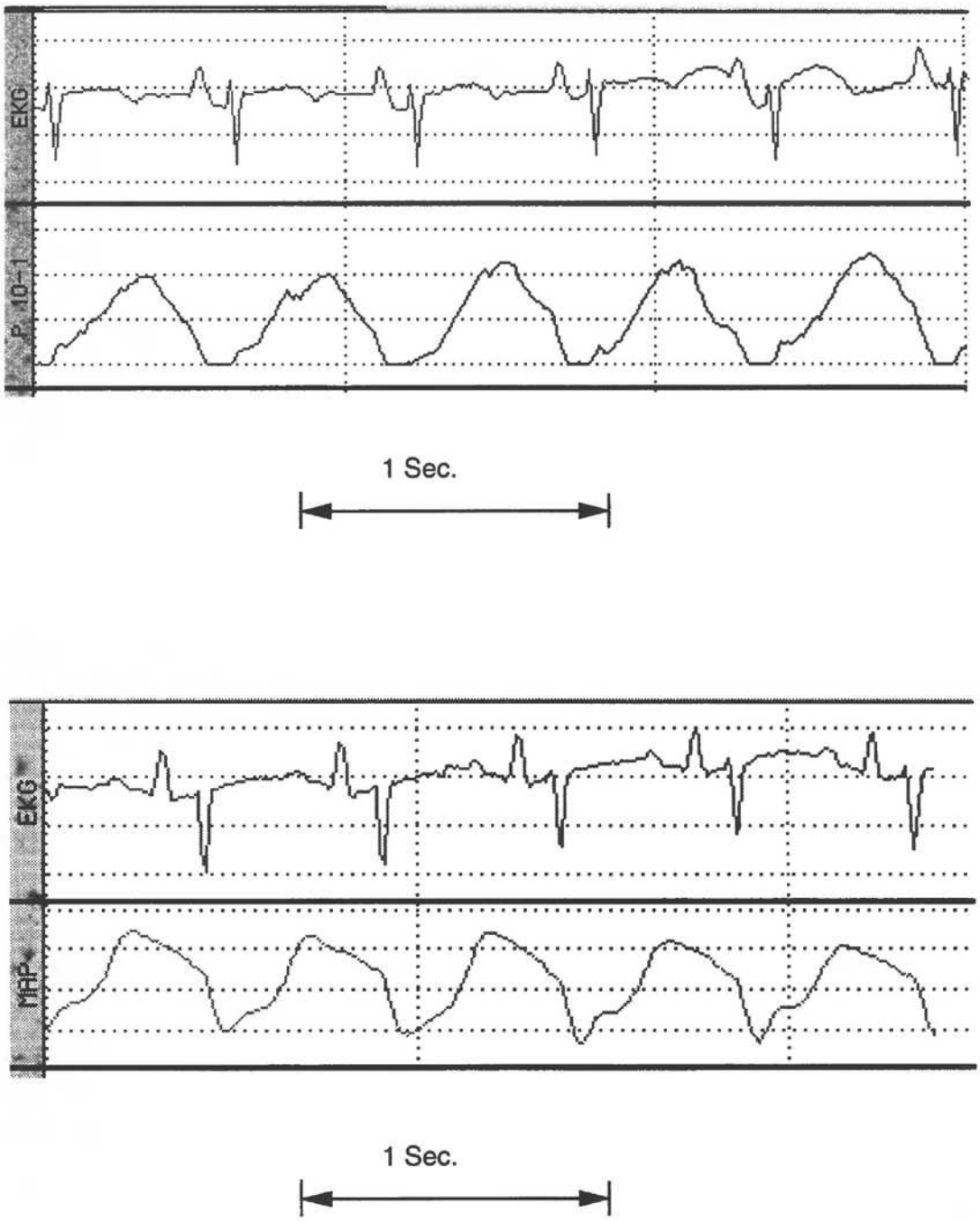


Figure 12 A representative Doppler thickening probe tracing. The upper panel shows an upward deflection from baseline following electrical systole (QRS) on the ECG. In the lower panel a negative deflection from baseline is seen following the QRS complex on the ECG, representing thinning in the infarct region

use of a Doppler thickening probe placed on the surface of the myocardium, which measures myocardial thickening in the region of interest (Figure 12). This probe generates the curve shown in normal myocardium, and shows myocardial thinning in the region of infarction. These probes are then left in place, sutured to the cardiac surface with 7/0 proline sutures. mouse ventricular cells or neonatal pig myocytes are then implanted in the area of infarction and also at the border zone between infarcted tissue and normal myocardium. Again, the median sternotomy is closed, and the animals are allowed to recover for an additional month

while being immunosuppressed with cyclosporin (15 mg/kg per day) and prednisone (0.3 mg/kg per day).

At the end of a month the animals are again anesthetized and sacrificed with perfusion fixation of the hearts as previously described. This time, however, prior to sacrifice, repeat readings are taken using a Doppler thickening probe in the area of interest, to determine if there is a physiologic improvement in myocardial function. Very preliminary data indicate that there may be some improvement in the function of the myocardium in the area of interest, and studies are currently ongoing to delineate the overall thickening and the electrical activity of the cells in the region of interest by the measurement of monophasic action potentials.

Figure 13 depicts the mouse ventricular cells transplanted into the infarct zone. The section shows the transplanted cell line growing in the center of a zone of infarction with no normal myocardium adjacent. One can see that, even in an area of infarction, these cells are able to grow and induce neovascularization, a process that would be necessary for the overall improvement in function required in a patient with ischemic cardiomyopathy.

FUTURE STUDIES AND CLINICAL APPLICATIONS

While cardiomyocyte transplantation is still in the early stages, advances have been made that are certainly promising for the development of a procedure by which certain hearts can be repaired using either allogeneic or xenogeneic transplanted cells. Additionally, an understanding of the mechanisms that control the myocyte cell cycle may allow investigators to design manipulations which could initiate repair or regeneration of the adult myocardium.

Direct transplantation of genetically altered cardiomyocytes will likely result in three important considerations for the ultimate repair of a failing heart. First, an actual increase in viable myocytes can be accomplished. It remains to be seen whether these myocytes will differentiate and manifest adult contractile proteins. However, since no other impediments to cell differentiation have been induced, and the signal for terminal differentiation certainly exists in the adult animal, it is presumed that with time these cardiomyocytes will produce adequate amounts of adult contractile protein.

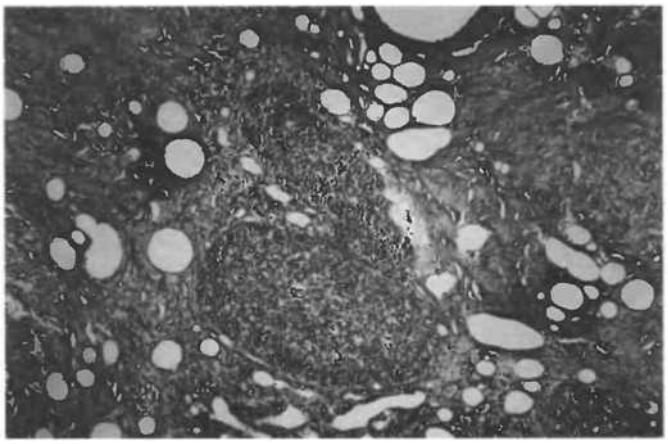


Figure 13 Low-power (× 100) trichrome slide depicts mouse ventricular cells transplanted into the center of a myocardial infarction after 1 month of healing of the infarct. The basophilic transplanted mouse ventricular cells can be seen in the center of the slide, with the acellular scar tissue completely surrounding the transplanted cell line. It is of extreme interest to note the amount of neovascularization present even in the scar tissue of the mature myocardial infarction

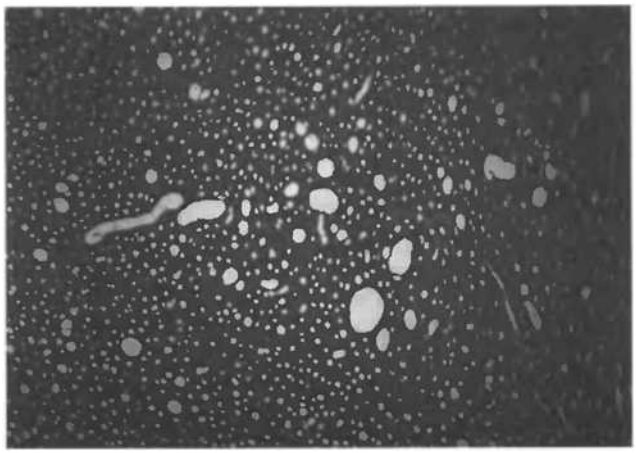


Figure 14 Center of a mature myocardial infarction. The swine had the infarct induced 1 month prior to cell implant by occlusion of the obtuse marginal coronary artery. At that time, mouse ventricular cells were transplanted into the infarct and allowed to grow for 1 month. This panel of hematoxylin–eosin-stained infarct shows the acellular scar present with a large amount of neovascularization. This section was taken at a site distant from the HL-1 transplanted cell line, so that no transplanted cells are seen in this panel

Secondly, these transplanted cells can be used to induce repair of the damaged myocardium. The profound angiogenesis noted at the areas of transplantation suggests that these cells have the ability to induce neovascularization. This occurs even in a zone of infarction (Figure 14). With this technique a number of cell lineages can be made and used to induce changes in the native myocardium. Angiogenic factors, such as VEGF, could potentially increase the vascular flow to an ischemic area of myocardium.

Finally, this area of study also provides valuable insight into the field of xenogeneic transplantation, since the amount of rejection present in these transplanted xenogeneic cells is substantially less than seen in whole organ xenografts. This is possibly because of a lack of cell surface markers in these isolated neonatal ventricular myocytes. Additionally, almost pure myocytes and no endothelial cells are being transplanted. This may explain why the recognition of the MHC antigens on these transplanted cells seems to be less than in the whole organ. Cardiomyocyte transplantation will clearly expand our knowledge of transplantation in general, and has the potential to act as a patch to repair a damaged heart, potentially decreasing the need for transplantation of entire hearts.

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Myocardial Regeneration with Skeletal Muscle Satellite Cells

G.J. MAGOVERN Sr

INTRODUCTION

Heart failure, arising from multiple etiologies, results in extensive myocardial damage as dying myocytes are replaced by hyperplasia of non-contractile connective tissue. Loss of contractile function leads to a reduction in pumping capacity of the heart, and initiates a cascade of systemic compensatory actions which ultimately exacerbate and extend myocardial destruction.

At Allegheny General, and other centers, an important new focus for cardiac assist research is the use of autologous skeletal muscle to augment the failing myocardium. Obviously, normal skeletal muscle would fatigue rapidly if stimulated to perform at the level of the myocardium, but skeletal muscle does retain the genetic capability for transformation to a fatigue-resistant metabolic state. Transformation techniques (most often by electrical stimulation) are now well established, and the biochemical correlates of this transformation have been described by ourselves[1] and others[2]. Although much remains to be done to elucidate the mechanisms of muscle transformation, we have expanded our studies to a corollary investigation into myocardial regeneration via skeletal muscle satellite cells, myogenic stem cells which can differentiate and form contractile tissue.

In vertebrates, muscle cells lose the ability to undergo mitosis following the neonatal period. Skeletal muscle, unlike cardiac muscle, retains the ability to regenerate, and the source for cellular repletion is the skeletal muscle satellite cell. Satellite cells divide to produce cells resembling myoblasts. In a process similar to the embryonic development of muscle tissue, these new myoblasts fuse to form a primitive structure known as a myotube, which is capable of maturing into a contractile myofiber.

First described by Mauro in 1961[3], satellite cells are found under or imbedded in the basal lamina of skeletal muscle. Autoradiographic studies completed by Snow in 1977[4] provided strong presumptive evidence that the satellite cell was the precursor for new myofibers in denervated or injured muscle. The myogenic potential of satellite cells was confirmed by Lipton and Schultz [5] in 1979 in a definitive study using pure clonal cultures of satellite cells. Pioneering work by Morgan *et al.*[6] established the ability of these cells to initiate muscle regeneration in congenital myopathies or injuries in the skeletal muscle following culture and translocation. This work was recently extended by Alameddine *et al.*[7], who demonstrated functional improvement in damaged muscles grafted with autologous cultured satellite cells. We hypothesized that skeletal muscle satellite cells could serve a similar function if cultured and translocated into the damaged myocardium of a syngeneic host.

Using animal models we have successfully isolated skeletal muscle satellite cells, cultured them *in vitro*, and implanted them into injured myocardium. The implanted cells have been demonstrated to multiply and differentiate into muscle cells with some characteristics of cardiac myocytes[8]. Studies recapitulating these findings have recently been described by Zibaitis *et al.*[9]. Using these techniques it should be possible to develop an entirely new approach to therapy for heart failure using the patient's own 'home-grown' repair cells as the therapeutic agent, and directly targeting the injury which has initiated the disease process.

SUMMARY OF SATELLITE CELL INVESTIGATIONS AT ALLEGHENY GENERAL HOSPITAL

Satellite cell culture yield

Initial studies focused on optimization of satellite cell isolation techniques. Cell yield and purity are critical for long-term implantation studies. For the technique to be widely usable for clinical myocardial transplantation the amount of muscle resected needed to be limited to the 0.02–0.04 g available from a standard muscle biopsy. The minimum requirement for satellite cell yield from a biopsy sample is approximately 10^7 cells.

The first challenge was to improve the yield of satellite cells for a given muscle sample without compromising the mitotic capability of the cells. A set of experiments was performed to determine the optimal concentration of fibroblast growth factor (FGF) for culture of animal satellite cells. It was determined that 40 μl FGF/plate resulted in the greatest increase in cell numbers; this concentration was therefore used for all subsequent cultures. Cell

yield was further enhanced by maintaining high optimal nutrient and FGF concentrations via multiple feedings, and by keeping the plates on a rocker during incubation to prevent local depletion of FGF.

A second set of experiments was designed to improve satellite cell purity by decreasing fibroblast contamination. Because fibroblasts have a greater plating efficiency than myoblasts, using uncoated plates to preplate cell suspensions will preferentially remove fibroblasts from culture during transfer. The optimal collagenase treatment time for detaching satellite cells, but not fibroblasts, from the plates was also determined. The resultant subcultures contained at least 98% satellite cells and, in many cases, were greater than 99% pure.

Using our improved techniques a 0.025–0.035 g sample of mouse hindlimb muscle was shown to produce approximately 7.8×10^4 myosatellite cells with a doubling time of 1.7 ± 0.1 times/day at day 3 of culture. Based on these estimates it was calculated to take less than 7 days and 12 doublings to achieve a yield of 10^7 cells. Myosatellite cells are capable of doubling approximately 30–35 times before becoming senescent. Thus, a satisfactory yield of satellite cells was obtained from a small muscle sample without exhausting the mitotic capability of the cells.

Cryoinjury model

Another focus of our studies was to develop an animal model with a reproducible myocardial defect similar to the injury patterns seen in humans. The ideal lesion had to demonstrate destruction of the myocytes in the area of injury with reversible damage to the peripheral blood vessels and connective tissue cells. Retained vascularity was necessary for cultured cells to survive in this area, and connective tissue was needed to provide a matrix for attachment of the transplanted cells. A reproducible injury model was achieved via cryoinjury using a 5 cm diameter cryoprobe cooled to –160°C by internally circulating liquid nitrogen. An obvious contrast between injured and normal tissue was documented on gross examination, and uniformly destroyed cardiac myocytes and fibrous scar formation were documented histologically.

Cell marking

A reliable method of labeling the satellite cells prior to implantation was needed to document survival and adaptation of the cells in the myocardial injury site. Successful labeling was attained with the radioisotope [^{3}H]thymidine. This method, however, was limited by the loss of labeling intensity over time as the cell divided.

Our work then concentrated on marking the cells with fluorescent beads. Heavily labeled dilutions resulted in cellular death. A 1:10 dilution appeared to be the best concentration, since it caused little cell disruption and produced a high percentage of labeling (>90%), with a significant number of beads per cell.

Transplantation of satellite cells

Cultured satellite cells were transplanted into canine myocardium. Four-day cultures were selected to allow more of the differentiation process to take place within the myocardial injury site. Viable muscle was identified both grossly and by Mason trichrome stains in the implant sites. As judged by the appearance of the mitochondria, glycogen, and intercalated discs, regenerated muscle cells displayed morphological characteristics similar to those of cardiac myocytes. In some cases the newly formed muscle cells appeared to have developed specialized cell junctions either with adjacent cardiac cells or with each other, and to have taken on the appearance of cardiomyocytes.

In another group of animals satellite cells labeled with beads or [^{3}H]thymidine were implanted in the myocardium. At 6 weeks, implanted sites demonstrated clearly viable labeled satellite cells. Presence of the label was definitive evidence that these cells arose from transplanted cells and not the native heart. Only scar tissue, with no evidence of muscle cells, was observed in injury areas which had not been injected with the cultured cells.

COMMENT

In animal studies, techniques have been refined to reliably produce cultures of satellite cells with clinically applicable levels of cell yield and purity. An animal model of myocardial injury has been developed and used to evaluate the short-term response of cultured cells to myocardial implantation. The results of these studies have confirmed that cultured satellite cells survive translocation and begin a process of differentiation which closely resembles that seen during skeletal muscle repair. In future studies techniques for animal cell culture will be adapted and refined for use with human satellite cells. Development of genetic markers for canine and human satellite cells will allow us to document the fate of these cells through multiple mitotic cycles. Animal studies will then examine the long-term effects of satellite cell implantation for functional improvement of the heart.

The ability of myofibers regenerated from skeletal muscle precursors to function within the myocardium will depend on the adaptive capabilities which these cells retain through culture and translocation. One recent study examined the potential of satellite cells for diversification[10]. In fused cultures of human satellite cells all clones examined revealed a mix of fast and slow myosin heavy-chain isoforms. Studies by Wehrle[11] confirmed that isoform transformation can be induced by chronic electrical stimulation in myotube cultures derived from the satellite cells of rat soleus (slow-twitch) muscle.

The future of myocardial regeneration via skeletal muscle satellite cells will most probably be realized through an approach which may be called 'cellular cardiomyoplasty'. Combining elements of transplantation and cardiomyoplasty, the goal of this technique will be to transplant autologous satellite cells into damaged myocardium for differentiation and growth. Using methods refined from skeletal muscle cardiomyoplasty these grafts can be trained for fatigue-resistance and stimulated to contract with the heart.

Currently our work has focused on methods for culture and translocation of syngeneic cell lines. Other investigators[12] have reported successful reimplantation of allogeneic or xenogeneic neonatal myoblasts into porcine myocardium. The translocated cells were shown to proliferate within the implant sites. These studies are promising; however, ethical and political difficulties

involved with the use of neonatal tissue may limit the usefulness of these techniques in the clinic.

The work described here presents a fascinating but nascent area of cardiac assist research. Many questions remain to be answered, and it is hoped that this brief review will stimulate the growth of research in the field. The theoretical basis for myocardial regeneration with skeletal muscle satellite cells is sound and the initial studies are promising. We look forward to collaborating in the development of a novel, yet ideal, approach to the treatment of heart failure.

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Lung Volume Reduction Surgery in Patients with Emphysema

W. KLEPETKO, E. TSCHERNKO, W. WISSER AND T. WANKE

HISTORY

Surgical interest has long been directed to the treatment of patients with emphysema. This has resulted in a remarkable number of different approaches in surgical history.

The earliest interventions focused on the marked hyperinflation of the lungs, and were intended to restore the normal size of the thoracic cavity by procedures such as costochondrectomy, thoracoplasty, phrenic nerve paralysis, or creation of pneumoperitoneum. Other procedures were directed towards treatment of expiratory airway collapse. These included suturing of a bone graft to the posterior wall of the trachea and major bronchi, or reinforcement of the tracheal wall with a polyethylene prosthesis. In a third concept it was intended to overcome the bronchospastic component of the disease. Resections of various parts of the autonomic nervous system, such as the vagus, cervical and stellate ganglia or the posterior pulmonary plexus, were performed with the same frustrating results that had been achieved with the approaches mentioned before.

The list of different concepts and postulated procedures can be continued with parietal pleurectomy and poudrage, with the intention of increasing the blood supply to the emphysematous parenchyma, and with radiotherapy, to achieve shrinking of the enlarged lung. However, all of these approaches only partially understood (or even completely misunderstood) the underlying pathophysiology of the disease. Therefore, only marginal improvement or, in the majority of patients, deterioration of lung function was observed.

As a consequence of these failures, surgical interest in the treatment of patients with emphysema focused on the bullous form of the disease for more than two decades[2]. In these patients it was demonstrated very impressively that resection of large bullae resulted in relief of compressed lung parenchyma and improvement in lung function. On the contrary, 'diffuse' or 'homogeneous' emphysema was believed to be a clear contraindication for surgical intervention.

It is therefore interesting that the original idea for the procedure that has recently been introduced under the name 'lung volume reduction surgery' had been formulated by O.C. Brantigan as early as the late 1950s. By that time he had performed multiple peripheral segmental lung resections on patients with chronic obstructive pulmonary disease, with the intention of reducing overall lung volume and restoring circumferential traction upon both small airways and blood vessels. However, his ingenious work never achieved widespread acceptance, and the method survived only in the literature[3].

Only recently, this old concept was revised and modified by J.D. Cooper, who came across Brantigan's ideas in his search for alternatives to lung transplantation in patients with end-stage emphysema[4]. Lung volume reduction surgery (LVRS), although still in its infancy, has the potential to become a standard procedure in thoracic surgery.

PATHOPHYSIOLOGIC BACKGROUND

Chronic obstructive lung disease at an advanced stage can result in severe hyperinflation. This leads to severe disturbances of the musculoskeletal component of the respiratory system. The diaphragm is pressed downwards and becomes flattened or even inverted. As a consequence it contracts from a shorter than normal initial fiber length, and is placed on an unfavorable part of its force–length curve. Flattening further increases the radius of its curvature and, according to Laplace's law, whatever tension is developed in the contracting diaphragm is poorly converted to transdiaphragmatic pressure[5].

A second major pathologic component of advanced emphysema is functional airway collapse on expiration[6], which is due to loss of alveolar relaxation pressure and of parenchymal networks, and leads to intrinsic positive end-expiratory pressure (PEEPi). The more advanced the disease becomes, the more the pathophysiologic causes and consequences can influence and intensify each other.

Finally, diffuse emphysema is not completely uniform. With modern imaging techniques regional variations can be distinguished, and patients with sometimes marked heterogeneity of their disease can be identified. In these patients, ventilation and perfusion is unequally distributed. Due to their mechanical

interaction, regions with poor function and severe loss of parenchymal structure indirectly influence alveolar gas exchange in regions with preserved parenchyma.

By removal of the most diseased parts of the lung, LVRS is aimed at: (a) reducing residual and total lung volumes, (b) bringing the diaphragm back to a normal position, and (c) restoring transdiaphragmatic pressure generation. Functional collapse during expiration should be diminished by an increase of effective elastic recoil and reduction of PEEPi. This would result in a reduction in the increased work of breathing, and would decrease the sensation of dyspnea. The more heterogeneous the disease, the more benefit to the remaining normal (or less-diseased) lung tissue can be expected.

It must be emphasized that the goal of LVRS can only be palliation, and the process can never lead to cure of the underlying disease. Ideally, improvement in the patient's condition with relief from disabling symptoms is achieved, which represents a reversal in the evolution of the emphysema.

PATIENT SELECTION

The selection process of patients for the procedure is based primarily on chest radiography and lung function (Table 1). Candidates for LVRS must have the typical signs of hyperinflation on chest radiography. These are: (a) distended intercostal spaces, (b) flattened or inverted diaphragm, and (c) a large retrosternal space (Figure 1). Due to its inability to move, the diaphragm shows no or only limited excursion between inspiration and expiration.

Identification of the heterogeneity of emphysematous change throughout the lungs is by CT scan. Patients with COPD usually have their maximum diseased lung parenchyma in the upper regions of the lung (Figures 2A and B), whereas patients with α_1-antitrypsin deficiency frequently have the most prominent loss of parenchymal substance in the basal regions (Figures 3A and B). A third group of patients can be identified who present with a truly homogeneous distribution of emphysematous change. Visualization of the extent of parenchyma with preserved structure helps to estimate the severity of the disease. Further information gained from the CT scan includes the identification of areas of bronchiectasis and the presence of small nodules or tumors. All of these represent contraindications for LVRS.

An additional ventilation/perfusion scan allows further screening for differences in distribution of emphysema between the two lungs, although this can be estimated from the CT scan alone.

Further selection of patients is performed using standard lung function tests and body plethysmography, which allow an assessment of the degree of hyperinflation. Possible candidates for LVRS must have a residual volume (RV) greater than 250–300% predicted. Under these conditions total lung capacity (TLC) is usually measured as 130–160% of predicted. FEV_1 is significantly reduced, and indicates the severity of the disease.

Table 1 Indications for lung volume reduction surgery (LVRS)

Radiographic signs of hyperinflation
Residual lung volume > 250–300% predicted
Total lung capacity > 130–150% predicted
Heterogeneity of disease

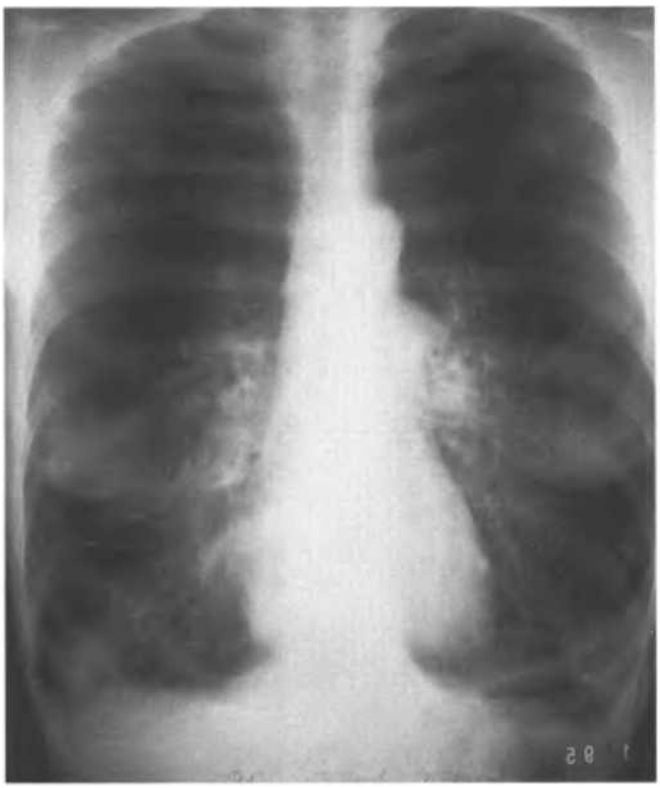
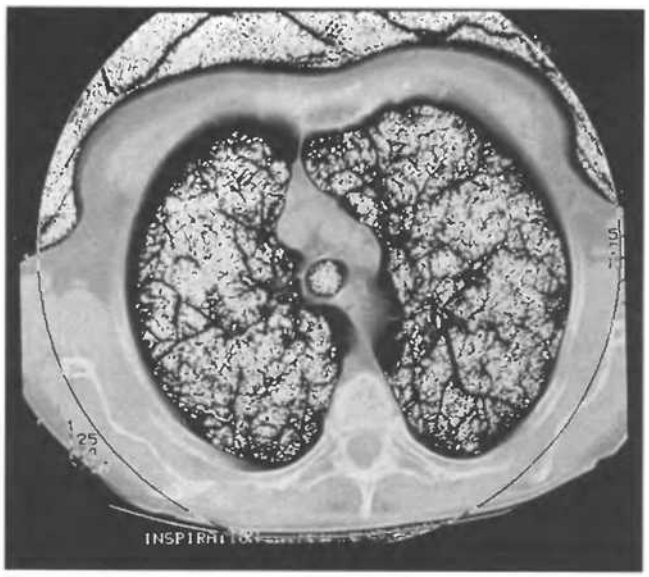


Figure 1 Typical chest radiograph of a patient with severe hyperinflation. Maximum parenchymal destruction is seen in the upper lobe

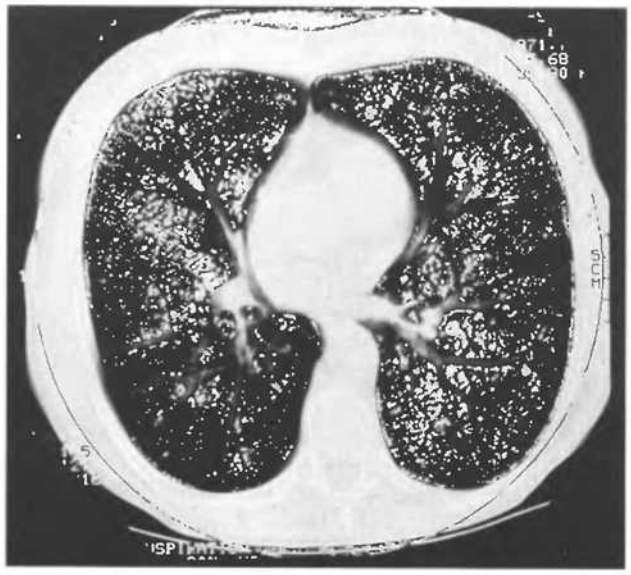
The functional limitation of patients who should be referred for the procedure remains inadequately determined. Before a surgical intervention is considered the patient must be substantially disabled from the disease despite optimal conventional anti-inflammatory and anti-obstructive medication. In terms of lung function, LVRS seems to be indicated when the FEV_1 has fallen to about 35% of predicted.

The lower limit of FEV_1, where the procedure can still safely be applied, is far more difficult to determine, and depends on the morphological changes of the particular patient and on his/her overall condition. Patients who have marked heterogeneity of their disease, with areas of severe destruction interspersed with relatively normal lung tissue, will benefit from the procedure to a greater extent than patients with homogeneous disease. For heterogeneous disease LVRS can therefore safely be applied even at FEV_1 values of <15% of predicted. Under these circumstances, hypercarbia and global insufficiency are common findings. Although these do not represent absolute contraindications for the procedure, the overall risk is clearly increased. Most patients have the need for supplemental oxygen at rest, or at least on exertion.

Further patient assessment should include studies of diaphragmatic function as well as measurements of PEEPi and the work of breathing. Exercise tests, such as the 6-minute walk test, and standard spiroergometry should be performed to document the patient's functional status, and to allow for subsequent comparison of pre- and postoperative values.



A

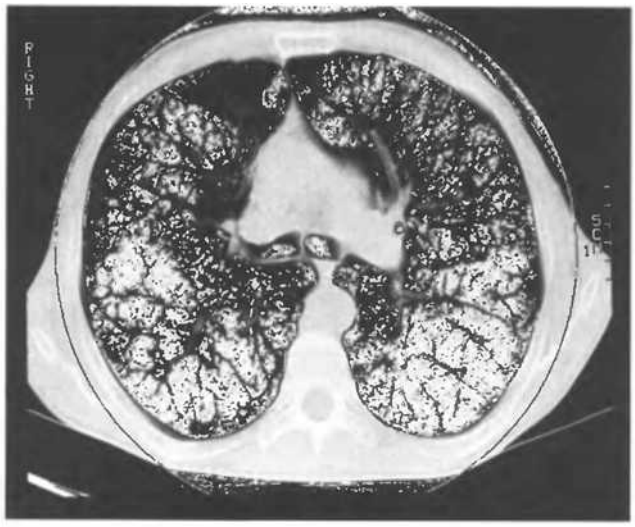


B

Figure 2 **A**: In patients with COPD, a cross-section CT scan of the chest with density mask reveals the most prominent loss of parenchymal structure to be in the upper region. **B**: A lower cross-section of better-preserved lung parenchyma

Established contraindications for LVRS are: (a) recurrent pulmonary infection, (b) daily corticosteroid medication of >25 mg, and (c) a mean pulmonary artery pressure >35 mmHg (Table 2). Patient workup is completed by echocardiography, right heart catheterization if indicated, and coronary angiography in patients in whom coronary artery disease is suspected.

Once a patient has been accepted for the procedure, he or she should enter a standardized rehabilitation program to optimize overall condition and exercise endurance.



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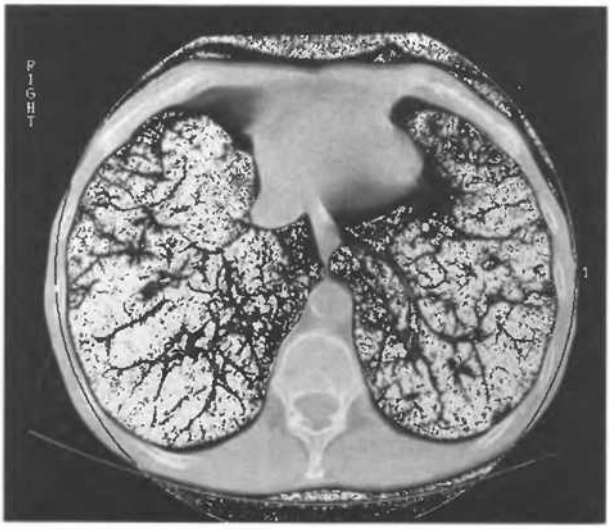


B

Figure 3 **A**: In patients with α_1-antitrypsin deficiency, the upper lobes show a fairly well-preserved parenchymal structure. **B**: In contrast, a greater degree of diseased parenchyma is visualized in the lower lobes

Table 2 **Contraindications for lung volume reduction surgery (LVRS)**

Pulmonary hypertension (mPAP > 35 mmHg)
Bronchiectasis
Purulent sputum and frequent bronchitic episodes
Homogeneity of disease
Previous major thoracic surgery
Lack of rehabilitation potential
Concomitant diseases

SURGICAL TECHNIQUE

The standard technique of LVRS requires intubation with a double-lumen endotracheal tube. The operative approach is via a median sternotomy. Both pleural spaces are opened in a longitudinal way. One-lung ventilation is initiated and the other lung is

deflated. This may take a considerable length of time depending on the extent of air trapping. The most diseased areas will usually remain inflated for the longest time, and therefore can easily be identified. Mobilization of the pulmonary ligament, together with any adhesions, is carried out, and moist packs are placed behind the hilum to elevate the lung.

Excision of peripheral segments is performed with stapling devices, buttressed with bovine pericardium to prevent post-resectional air leakage (Figure 4). Resection is performed of the most diseased areas of lung, which are usually located at the apex in patients with COPD, and in the lower parts in patients with α_1-antitrypsin deficiency. Patients with a uniform distribution of emphysematous change demand resection distributed over all lobes.

The usual objective is to resect about 30% of the lung tissue (Figure 5). Intermittent reinflation of the lung allows for repeated assessment of the extent of resection. Care must be taken to direct the resection lines in a way that allows the remaining lung to keep its anatomical shape, to continue to fill the thoracic cavity, and to avoid large extrapulmonary air spaces.

In cases of severe air trapping we have found it helpful to cross-clamp the lung parenchyma with a long rubber-mounted clamp, parallel to the proposed staple line, before the stapling device itself is applied. This takes traction from the stapling line and avoids shear stresses during the stapling procedure. In addition, it may be necessary to incise the area which undergoes resection, to allow the trapped air to evacuate.

At the end of the procedure the remaining inflated lung should be somewhat smaller than the thoracic cavity. All stapling lines are checked for air leaks and, if necessary, fibrin glue is applied to seal small insignificant leaks. Every effort must be undertaken to avoid larger air leaks. Rarely, it is necessary to place single 4/0 PDS sutures mounted with small pledgets of pericardium to close a persistent air leak.

After partial resection of the first lung is completed, the same procedure is applied on the opposite side. At the end of the procedure two pleural drains are placed in each thoracic cavity, and the pleura is closed with running sutures to separate the two pleural cavities. Suction of 10 cmH_2O pressure is applied to the water-seal bottles.

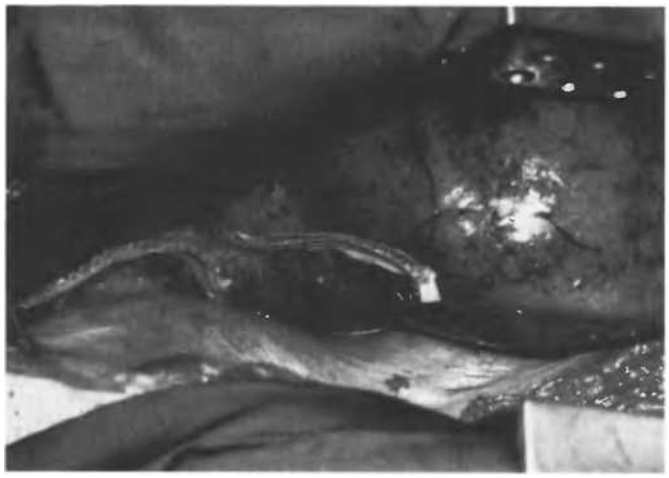


Figure 4 Operative view of LVRS. Multiple resections of peripheral segments of the lung are performed with stapling devices buttressed with bovine pericardium

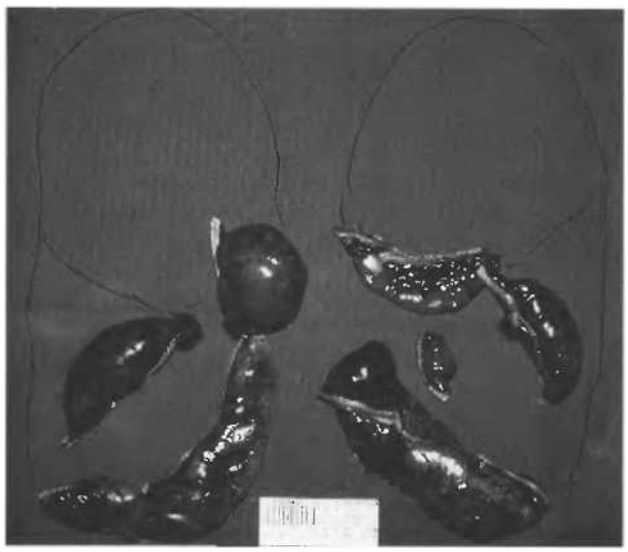


Figure 5 Operative specimens following LVRS

VIDEO-ENDOSCOPIC TECHNIQUES

The above-described technique has gained the most widespread acceptance, but several video-endoscopic approaches towards the reduction of emphysematous lung volume have been introduced.

Laser resection of small bullae, together with 'laser painting' of the lung surface, has been favored by one group[7], but they have not yet been able to prove significant functional benefit from the procedure. A unilateral thoracoscopic approach with laser ablation, together with stapler resection, has been reported by another group[8].

Recently, a bilateral endoscopic technique of LVRS with endostaplers has been reported from a Swiss center[9]. Basically this represents the minimal invasive variant of the conventional technique via a sternotomy. The patient is placed in the supine position and, under endotracheal double-lumen intubation, both sides are operated on sequentially. Resection is performed with regular 45 mm endostaplers without pericardial reinforcement.

ANESTHESIA, MONITORING AND INTENSIVE CARE

The success of the procedure depends as much on good anesthetic management as on surgery. Optimal preoperative preparation of the patient is essential. This consists of dilatation of the airways with β_1-agonists, theophylline and cromolyn sodium. Loosening of secretions is achieved with humidifiers, systemic hydration, and mucolytic drugs. Finally, extensive respiratory therapy assists removal of secretions.

Monitoring of the patient at operation is standard, with radial artery and central venous cannulae. An epidural catheter for intra- and postoperative analgesia is inserted at the level of T4–5. The correct position of this catheter is particularly important and must be checked with a test dose of 2% lidocaine plus epinephrine

1:200 000. Prevention of bronchospasm, together with optimal analgesia, and extubation of the patient on the operating table are the main goals of the anesthesiologist. Isoflurane is the drug of choice for maintenance of anesthesia, since it is a potent bronchodilator. Muscle relaxation should be achieved with pancuronium and vecuronium, as neither release histamine and both have a very short half-life. Postoperative analgesia is provided via the epidural catheter using bupivacaine 0.25%, together with sufentanil.

Postoperative management in the intensive-care unit is based on extensive respiratory therapy, early mobilization, and effective analgesia. Frequent therapeutic sessions of chest vibration and percussion are followed by periods of rest. In order to enable early mobilization, pain therapy has to be efficient. The drains should be removed as soon as there is no air leak with only moderate serous drainage.

Since almost all patients are prone to postoperative episodes of bronchospasm, bronchodilator therapy with intravenous theophylline and β_1-agonists administered via a humidifier six times per day are essential. If severe bronchospasm cannot be reversed successfully by these drugs, epinephrine should be administered via a humidifier.

Pain management is performed via the epidural catheter. The local anesthetic, bupivacaine 0.25%, is continuously infused, together with two or three bolus dosages of sufentanil during the day. Antibiotic therapy is determined by the results of sputum cultures monitored daily.

FUNCTIONAL RESULTS

The initial description of LVRS by J.D. Cooper and his colleagues reported on a series of 20 patients with COPD. There was no early or late mortality. Mean preoperative FEV_1 of 0.77 l (25% predicted) increased to 1.4 l (44% predicted) postoperatively. Preoperative residual volume of 5.9 l (288%) diminished to 3.5 l (177%). Highly significant improvement was reported for measurements of different dyspnea indices.

Data on a much larger group of patients were presented from the same center 1 year later[10]. Operative mortality was 5/84 (5.9%). The increase in FEV_1 over a 1-year observation period was 0.48 (± 0.33) l/min or 40 (± 37)% compared to preoperative values. The need for supplemental oxygen at rest was significantly reduced from 55% of all patients preoperatively to only 3% after 1 year.

Our own experience to date consists of a series of 15 patients who have undergone LVRS. Preoperatively, 12 patients required supplemental oxygen at rest, and five patients were 'globally insufficient' with a PCO_2 > 40 mmHg and a PO_2 < 55 mmHg. Mean FEV_1 for the entire group was 0.65 (± 0.21) l/s (21% predicted) and mean PAP was 27 (± 7) mmHg.

Perioperative mortality was 2/15 (13%). Both patients died from septic complications after 15 and 11 days, respectively. Two other patients suffered from postoperative pneumonia. Three months after the procedure, FEV_1 had increased 0.45 l/s (69%) to 1.1 (± 0.23) l/s (36% predicted) (Figure 6A). RV was reduced from a preoperative value of 325 (± 57) % to 212 (± 35) % (Figure 6B). Only one patient still requires supplemental oxygen at rest. Comparison of pre- and postoperative chest radiographs has demonstrated significant reductions in hyperinflation, together

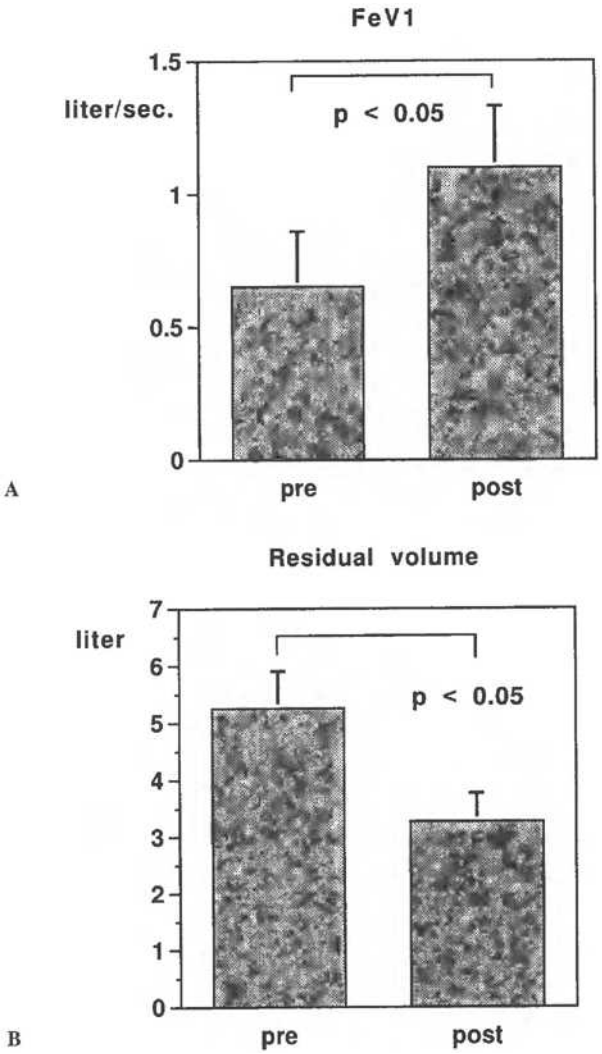
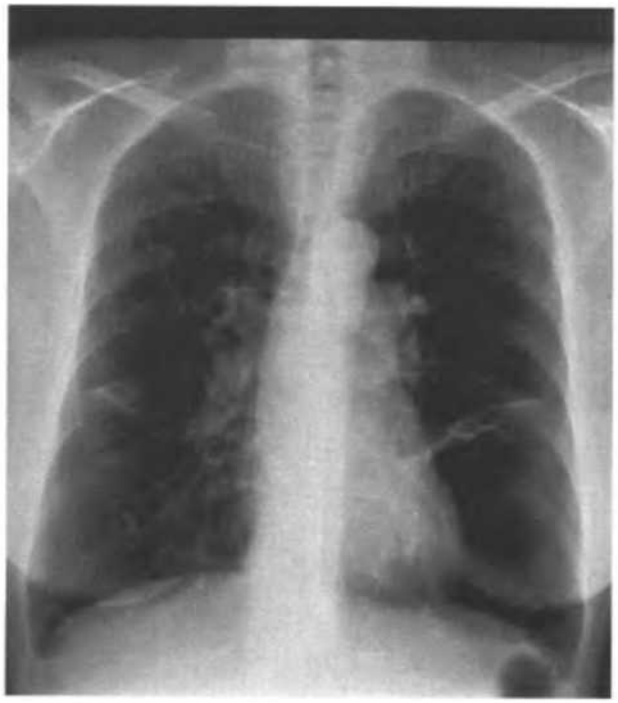


Figure 6 Measures of lung function before (pre) and 1–6 months after (post) LVRS. **A**: FEV_1 = forced expiratory volume during the first second; **B**: RV = residual volume

with significant improvements in diaphragmatic motility (Figures 7A and B).

In addition to lung function parameters we measured the work of breathing, both preoperatively and immediately after the operation, in all patients. A highly significant reduction was demonstrated as early as the first postoperative day, which was followed by a continuous further decrease to normal values by the 7th postoperative day (Figure 8).

Data currently available regarding the results of endoscopic LVRS are limited. For unilateral procedures a 7% mortality (3/42) has been reported[8]. FEV_1 increased >20% in more than 60% of patients within the first month. However, no significant changes in blood gases were reported. Interesting results have been described for the bilateral approach, where no mortality was observed in a series of 18 patients[9]. However, the reported improvement of FEV_1 of 36% (range 0–63%), together with a reduction in RV of only 43%, seems to be less successful than those reported after open sternotomy LVRS.



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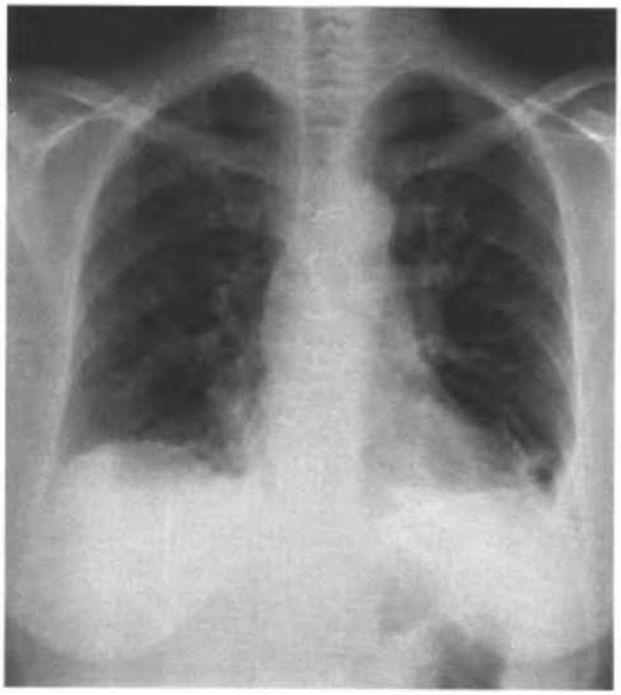

B

Figure 7 Chest radiograph in the same patient **(A)** pre- and **(B)** post- (3 weeks) LVRS. The preoperative hyperinflation of the thorax has disappeared. The diaphragm has moved upwards and has regained its normal shape. In the post-LVRS radiograph 3 weeks after the operation, small pleural effusions still can be seen

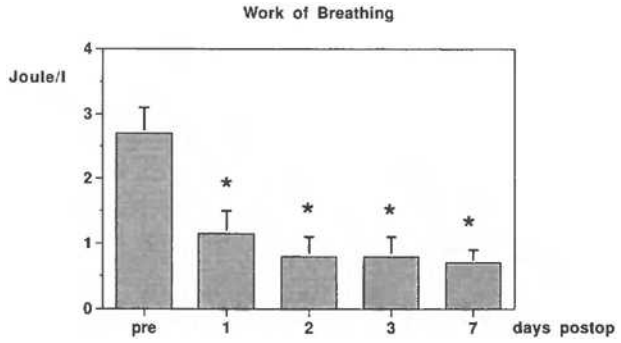

Figure 8 Work of breathing (WOB) after LVRS. Preoperative WOB is increased three times over the upper limit of normal (0.5–0.7 Joules/l). LVRS results in a significant and immediate reduction in WOB (p <0.01). Even on the first postoperative day it reaches a value close to normal, and within the first postoperative week it has returned to within the normal range

COMMENT

LVRS is still in its infancy, but would seem to have enormous potential in the treatment of patients with end-stage emphysema with severe hyperinflation. Many questions, however, currently remain unanswered.

It is unclear, and cannot be predicted from the data available, how long the significant improvement in lung function will persist after the procedure. LVRS takes the patient a functional step back in the developing process of his emphysema. The significant improvement in functional performance and quality of life that has already been demonstrated in an increasing number of patients is so impressive that one has reason to believe that LVRS will attain a definite place in the therapy of patients with end-stage emphysema. Its potential benefit for survival, however, has not yet been proven, and could be ascertained only by a randomized trial comparing the best conservative care with LVRS.

To better define its therapeutic role in the future, the group of patients that will benefit from the procedure has to be determined more fully. It is already fairly well established that patients with heterogeneous disease, having their major structural deficits in the upper lobes, are ideal candidates. More uncertain is the functional benefit that is derived in patients with lower lobe disease, i.e. α_1-antitrypsin deficiency and, in particular, those with completely homogeneous disease.

Furthermore, the functional limits within which LVRS can be safely carried out remain uncertain. The safe upper limit of pulmonary artery pressure and the degree of impairment of right ventricular function have not yet been determined. There is reason to believe that the major factor in the selection of patients will be related to the extent of parenchymal destruction. Patients with heterogeneous disease and extensive but patchy destruction of the parenchyma of the lung should benefit from LVRS even in the presence of higher PA pressures when compared with patients with homogeneous disease (even with lower PA pressures). In terms of lung function, LVRS should be performed only in clearly symptomatic patients when conservative treatment has failed. When considering the lower limits of lung function it is important to estimate the amount and functional potential of lung parenchyma that will remain after LVRS. Global insufficiency and poor diffusion capacity indicate that the lung parenchyma is severely damaged. The operative risk under these conditions is significantly increased. LVRS should therefore be offered only to patients with marked heterogeneity and/or an FEV_1 of >15% predicted.

Finally, it has not yet been clarified which operative procedure – median sternotomy or unilateral or the bilateral endoscopic approach – represents the ideal method for LVRS. Operative mortality and morbidity, as well as the extent of functional improvement, must all be considered. Although the minimal invasive approaches would seem to be less traumatic, it remains unclear whether the patients operated on using these approaches have been comparable in terms of the severity of their disease. How much functional benefit can be obtained with each method remains uncertain. Those advocating the endoscopic methods will have to prove that the amount of functional improvement obtained is comparable to that after open sternotomy LVRS. Most likely, these questions will be answered satisfactorily only by randomized, prospective trials.

In summary, LVRS is currently the most promising alternative to lung transplantation for patients with emphysema and hyperinflation. In the near future its exact value and place will be better defined. There is already strong evidence that a large number of patients have already benefited from the procedure to an impressive extent.

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Pulmonary Endarterectomy – Treatment of Choice for Patients with Pulmonary Hypertension due to Emboli

S.W. JAMIESON

INTRODUCTION

Chronic thromboembolic pulmonary hypertension is a relatively common entity, but underdiagnosed. Those who practice pulmonary transplantation probably encounter patients with chronic pulmonary embolism often, as it is one of the more common causes of pulmonary hypertension. However, its exact frequency is hard to determine, because most patients presenting with thromboembolic pulmonary hypertension have not experienced clinically evident deep venous thrombosis or symptomatic episodes of pulmonary thromboembolism.

Endarterectomy is the treatment of choice for this condition, as the wait for a donor is eliminated and the risk of operation (about 7%) compares very favorably to the 1-year survival figures for either single or double lung transplantation. The operation appears to be permanently curative, and immunosuppression is not required.

Every effort should be made to eliminate chronic thrombi as a cause for pulmonary hypertension since medical therapy is ineffective, but surgical treatment is curative and can be performed at relatively low risk. More than 650 patients with chronic pulmonary thromboembolism and severe pulmonary hypertension have now been operated upon by endarterectomy, rather than transplantation, at the University of California at San Diego.

INCIDENCE

Acute pulmonary embolism is the third most common cause of death in the United States today. Dalen and Alpert[1] calculated that pulmonary embolism resulted in 630 000 symptomatic episodes in the United States yearly, making it at the time (1975) about half as common as acute myocardial infarction, and three times as common as cerebral vascular accidents. A recent autopsy study with an analysis of 13 216 patients[2] showed pulmonary thromboembolism in 5.5% of autopsies, being up to 31.3% in the elderly. Acute pulmonary embolism, then, is common and surprisingly underdiagnosed.

The true incidence of pulmonary hypertension due to *chronic* thromboembolic disease is more difficult to determine. Of the over 600 000 cases of massive acute thromboembolism in the United States annually there are more than 500 000 survivors. In addition, there are probably 3–4-fold more patients who have acute thromboembolic episodes which remain undiagnosed, for a total of perhaps 2 million patients yearly in the United States who survive episodes of pulmonary embolism. Although the majority of these patients resolve their emboli substantially, and perhaps completely, some patients fail to do so, and present with progressive pulmonary hypertension.

The majority of these patients can give no history of a deep vein thrombosis or pulmonary embolism, and the clinical picture is non-specific until right heart failure becomes evident. The diagnosis is therefore often missed, and it is likely that pulmonary disease due to chronic pulmonary embolism is very much more common than is generally appreciated. Presti and colleagues[3] found chronic massive thrombosis of *major* pulmonary arteries in 0.9% of 7753 autopsies. Since many of the patients we operate on successfully have disease in *minor* pulmonary arteries (the lobar, segmental or subsegmental branches) the incidence of significant disease that could be alleviated by surgery could be in the region of 100 000 patients a year in the United States alone.

ETIOLOGY

Most patients resolve acute pulmonary emboli with spontaneous lysis of the clot. The pulmonary circulation, and the pulmonary artery pressures, then return to normal. Whether the failure to resolve embolic material is a result of abnormalities of clotting and lytic mechanisms, or repetitive emboli, or both, remains unclear. Studies of the pulmonary vascular endothelium in affected patients have failed to demonstrate any consistent abnormality. In some patients the disease is related to autoimmune disease and antibody production. Abnormalities such as lupus anticoagulant, protein C deficiency, or antithrombin III deficiency are found in approximately 10% of patients. In addition, some patients with a paradoxical response to heparin have been identified (in these cases special precautions must be taken during the perioperative period with the use of prostacyclin during cardiopulmonary bypass).

PROGNOSIS

It is now well established that the prognosis for patients with pulmonary hypertension is limited, and is worse for those who do not have intracardiac shunts, as in Eisenmenger's syndrome. Thus patients with primary pulmonary hypertension, and pulmonary hypertension due to pulmonary emboli, fall into a higher-risk category. In our experience the mortality of patients with primary pulmonary hypertension is considerable. Between January 1990 and October 1994, 98 patients were referred to our transplant program with primary pulmonary hypertension. Thirty-seven (38%) were considered suitable transplant candidates, 13/37 (35%) underwent transplantation, 14/37 (37%) died awaiting transplantation and 10/37 (10%) remain listed. Because of the very significant loss of patients awaiting donors, a true comparison with the efficacy of a non-transplantation treatment should also take into account the total mortality once the patient has been accepted on a waiting list. In most series this is approximately 40% for those with pulmonary hypertension. A predictor of poor prognosis among our patients with primary pulmonary hypertension has been found to be a cardiac index < 2.8, and mean pulmonary artery pressures above 50.

We have had some success with the performance of balloon atrial septostomy in patients who continue to deteriorate with primary pulmonary hypertension, and in whom the wait for a donor lung has been prolonged. Though difficult to document, it has seemed to us that this procedure has considerably lengthened survival in selected patients, and allowed subsequent transplantation. Once the right to left shunt is absent after transplantation the atrial septal defect closes. We are currently conducting a randomized trial to attempt to document the efficacy of this procedure.

The prognosis for patients with chronic pulmonary hypertension due to thromboembolic disease is also poor, and is also proportional to the degree of hypertension. Riedel *et al.*[4] followed 147 patients with serial right heart studies and pulmonary arteriograms, and found that those with mean pulmonary artery pressures over 30 mmHg had a 30% 5-year survival rate, and only 10% of those with mean pulmonary pressures over 50 mmHg were alive at 5 years.

Medical therapy for embolic pulmonary hypertension, using anticoagulants, vasodilators, or thrombolytic agents, is ineffective[5,6]. An important aspect of the pulmonary circulation, however, is that the bronchial circulation maintains blood supply to the lung parenchyma after pulmonary artery embolization, and thus pulmonary embolization uncommonly results in tissue necrosis. Removal of organized thrombotic material thus allows the lung to regain its function. Since there is no restriction in the timing of the operation, as with awaiting a donor in primary pulmonary hypertension, pulmonary endarterectomy may be performed electively, and allow the patient's condition to be optimized pre-operatively.

DIAGNOSIS

The clinical presentation of chronic pulmonary embolism is often insidious, since most episodes are asymptomatic. Further, it is not until over 50% of the pulmonary vasculature has been occluded, and the right heart begins to fail, that the patient first notices any manifestations of this illness. In addition, the two major symptoms, effort dyspnea and fatigue, are very non-specific. Other symptoms that may occur, usually in the later stages of the disease, include exertional chest pain, cough and hemoptysis and, of course, eventually classic right heart failure.

The origin of the thromboembolic material is from a deep venous thrombosis in the legs or pelvis in over 90% of cases. However, many of these episodes are silent, and less than half the patients with chronic thromboembolic pulmonary hypertension in our series can relate a history of deep venous thrombosis. The clinical history may therefore not be helpful, but predisposing causes for deep venous thrombosis should be sought, as should a history of leg swelling or anything to indicate episodes of pulmonary embolism.

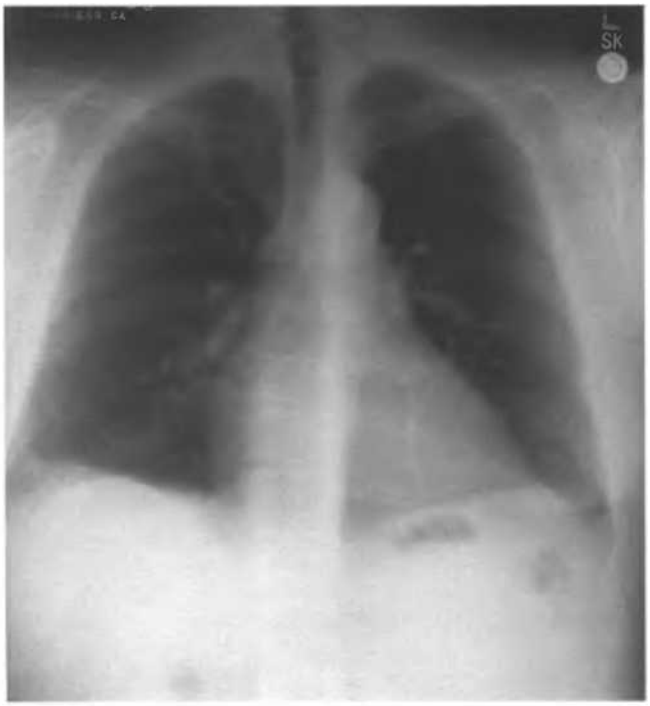
Clinical examination is usually non-productive if right heart failure has not occurred. Flow murmurs may be heard[7], especially over the back. It is thought that these murmurs are due either to flow through stenotic pulmonary arteries, or aggressive bronchial flow.

Chest X-ray, electrocardiogram, and pulmonary function tests are of little value in the differentiation of thromboembolic pulmonary hypertension from other forms of pulmonary hypertension. However, these investigations often give the initial clues that pulmonary hypertension exists when the physical findings are less conclusive.

The radiographic signs of pulmonary hypertension on chest X-ray may be difficult to determine, and are variable according to the degree of pulmonary hypertension and right heart failure (Figures 1 and 4). Enlargement of the pulmonary artery, either main (Figure 1) or right and left (Figure 4) and paucity of flow to the pulmonary vascular bed may indicate occlusion of major vessels. The lateral view of the chest X-ray may show right ventricular hypertrophy (Figure 4). However, the chest X-ray appearances may be interpreted as normal, even with severe disease.

Echocardiography demonstrates enlarged right-sided heart chambers and varying degrees of tricuspid regurgitation. Standard two-dimensional echocardiography is also helpful in defining the presence and severity of pulmonary hypertension and excluding certain other causes such as Eisenmenger's syndrome[8]. Continuous wave Doppler of the tricuspid regurgitant jet is helpful in estimating the pulmonary artery systolic pressure. Sometimes it is possible to visualize proximal, chronic, organized thrombus in the main pulmonary artery or main right and left pulmonary arteries with transthoracic echocardiography, but this technique lacks sensitivity and is inadequate for visualization of the lobar vessels, where the embolic material may be localized. Transesophageal echocardiography has proven to be more promising, especially with the newer multiplane probes that allow angulation of the imaging plane so that the origin of most of the lobar vessels can be identified. Early attempts are being carried out at visualizing the pulmonary arteries with transbronchial echocardiography, but it is unlikely that either of these techniques will ever provide data of sufficient precision to replace angiography.

A perfusion scan may be helpful, particularly to differentiate between primary pulmonary hypertension, in which the scan is usually normal, or has a patchy and mottled appearance. In embolic pulmonary hypertension there are usually multiple punched-out lobar or segmental defects. However, the perfusion scan is not always diagnostic, and in particular it tends to underestimate the degree of occlusion of the pulmonary vessels.



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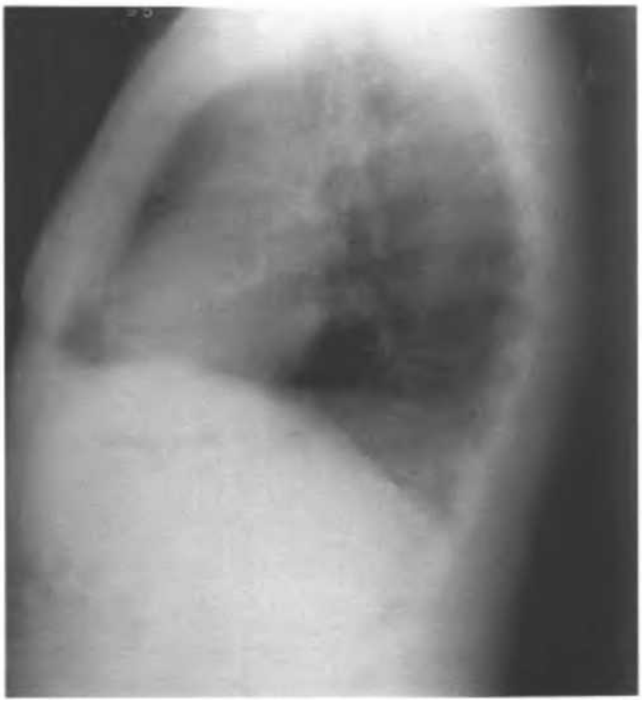
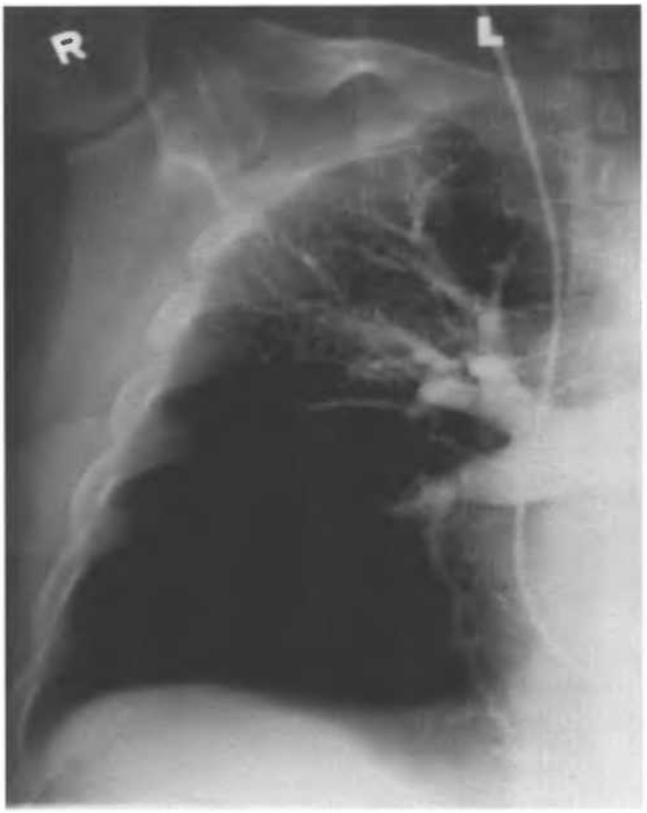


Figure 1 (A) PA chest X-ray of a patient with thromboembolic pulmonary hypertension. Note enlarged pulmonary artery shadow, possible hypoperfusion of left lower lobe. (B) Lateral chest X-ray of the same patient

A CT scan may be useful[9], and recent work has been performed using computer-enhanced images of CT scanning, in both the acute and the chronic forms of this condition. Spiral CT scans help to define the major pulmonary vessels. These images are



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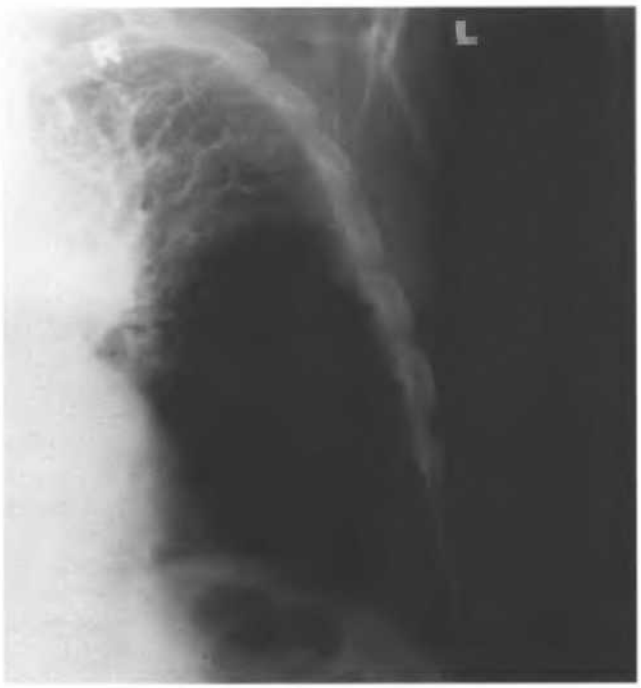


B

Figure 2 (A) Right pulmonary arteriogram of patient shown in Figure 1. There is right middle and lower lobe occlusion. Note the web in the main lower lobe branch. (B) Left pulmonary arteriogram of patient shown in Figure 1. Upper lobe normal, but absent filling of lingula and lower lobe branches

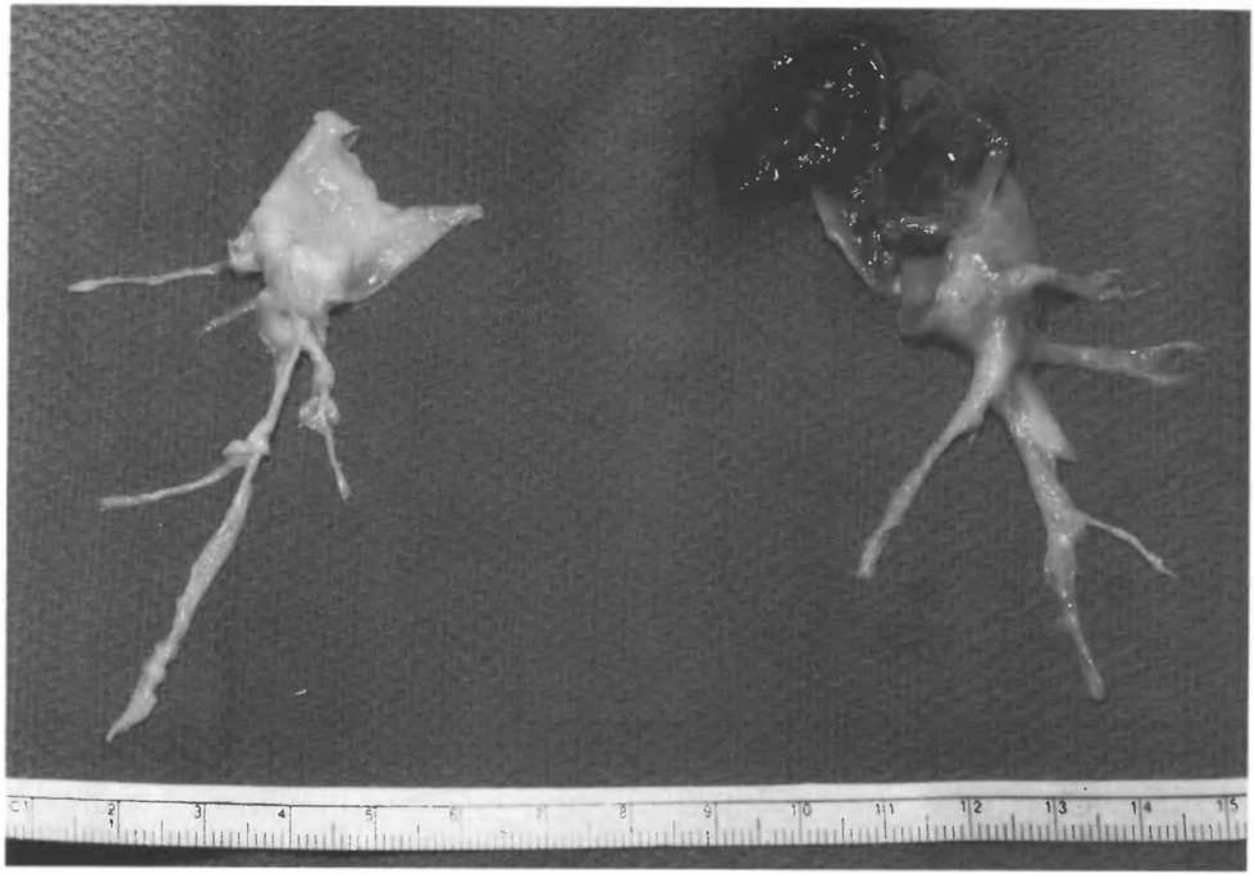


Figure 3 Specimen obtained at operation in patient shown in Figures 1 and 2. This patient had a pulmonary vascular resistance of 800 dyne/s per cm^5, which returned to normal postoperatively

capable of confirming occlusion in at least the main and lobar pulmonary arteries. Further, a mosaic pattern of lung attenuation at CT is a sign of variable regional perfusion, and may suggest chronic pulmonary thromboembolism as a cause for pulmonary hypertension[10].

Electrocardiogram-gated spin-echo magnetic resonance images (MRI) of the chest have been studied in patients with pulmonary disease, including some who had pulmonary thromboembolism[11]. Signal intensity could be correlated with pulmonary arteriolar resistance and cardiac index. This technique is being studied further.

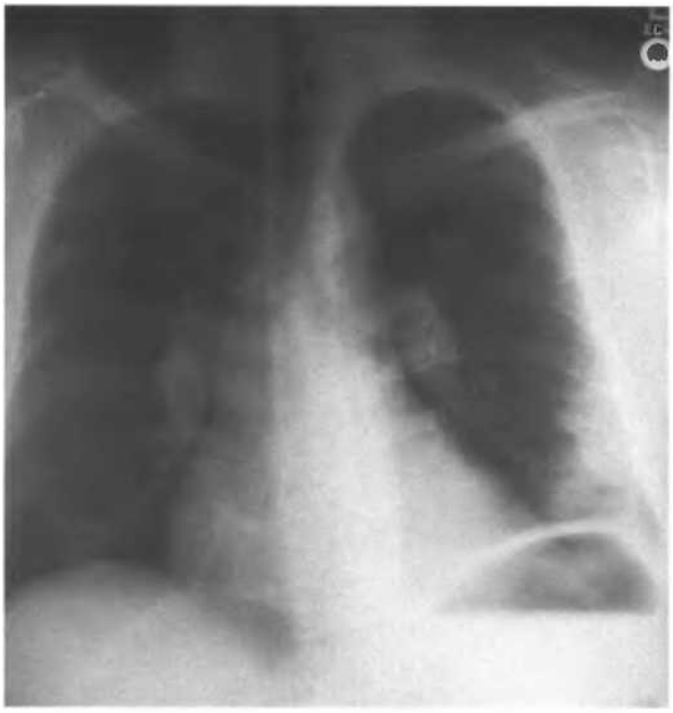
Once pulmonary hypertension as a result of chronic thromboembolic disease is suspected, the specific evaluation of the patient prior to planning surgical intervention depends on right heart catheterization and pulmonary angiography. Pulmonary angiography remains the 'gold standard' for assessing the operative risk and surgical accessibility[12]. The classical signs of disease on pulmonary angiography include an irregular lumen, indicating thrombus attached to the vessel wall, the appearances of bands or webs across the lumen of vessels (Figures 2 and 5), sometimes with post-stenotic dilatation, and occlusion of branches with lack of filling out to the periphery, often with an abrupt termination of pulmonary vessels with a pouch-like appearance (Figure 5a).

Concern is often voiced that pulmonary angiography is a high-risk procedure in patients with pulmonary hypertension. However, we have not found this to be the case. Selective power injections of the right and left pulmonary trunks using non-ionic contrast agents to prevent the cough response are well tolerated[12].

In addition to pulmonary angiography, patients over 35 undergo coronary arteriography, and other cardiac investigation as necessary. If significant disease is found, valve replacement or repair, or coronary artery surgery, is performed at the time of pulmonary thromboendarterectomy.

In approximately 20% of cases the differential diagnosis between primary pulmonary hypertension and distal and small vessel pulmonary thromboembolic disease remains unclear. In these patients pulmonary angioscopy has been found to be helpful[13]. The pulmonary angioscope is a fiberoptic telescope that is placed through a central venous line into the pulmonary artery. The tip contains a balloon that is then filled with saline and pushed against the vessel wall. In this way a bloodless field can be obtained for visualization of the pulmonary artery wall.

The classical appearances of chronic pulmonary thromboembolic disease at angioscopy are those of intimal thickening, with intimal irregularity and scarring, and webs across small vessels. These webs are thought to be the residua of resolved occluding thrombi of small vessels, but are diagnostic of the presence of



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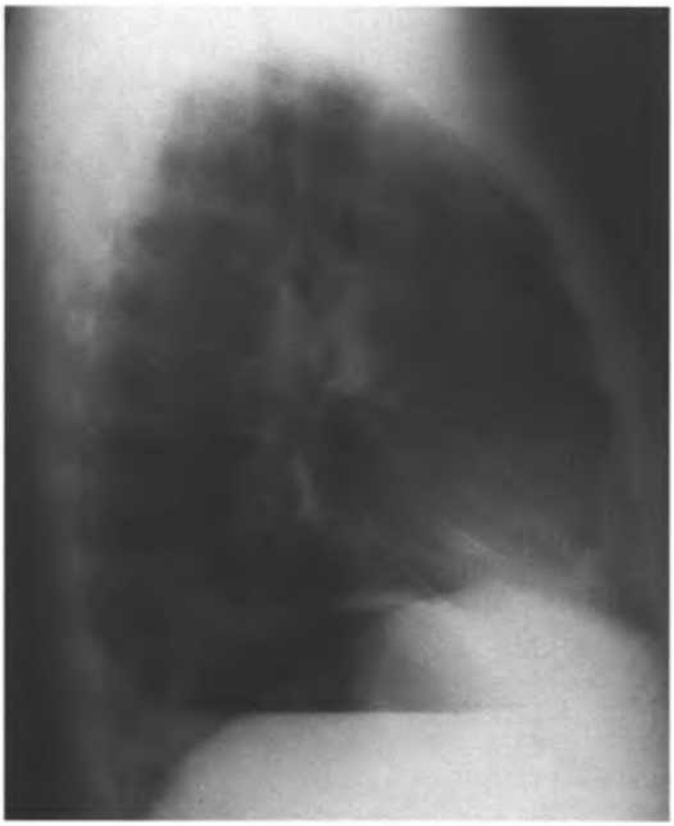

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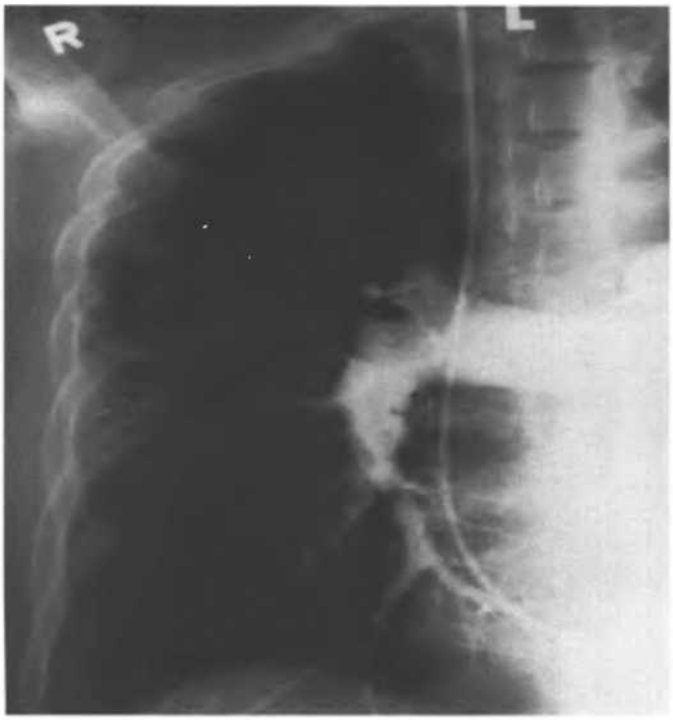
Figure 4 (**A**) PA chest X-ray of a patient with thromboembolic pulmonary hypertension. Compare with Figure 1. Right and left main pulmonary artery shadows are enlarged. (**B**) Lateral view in the same patient. Right ventricular enlargement



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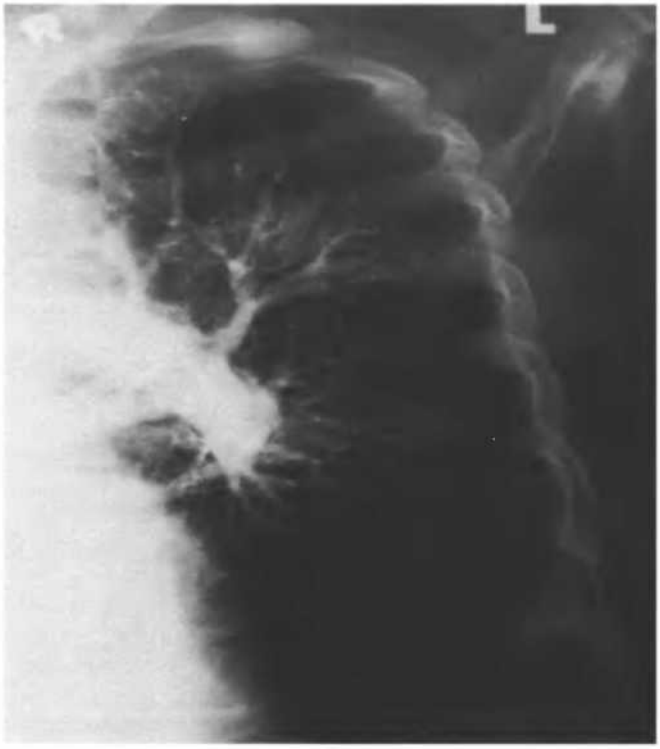

B

Figure 5 (**A**) Right pulmonary arteriogram in this patient. The angiography catheter is placed through the jugular vein. Note occlusion of the right upper lobe with a pouch, loss of middle lobe, and diffuse disease of lower lobe. Note partial web in lower lobe branch. (**B**) Left pulmonary arteriogram in this patient. Flow to the left upper lobe is largely preserved, but there is absent flow to much of the lower lobe. Though this disease is distal, it will be accessible at operation

embolic disease. Occlusion of vessels or the presence of thrombotic material is diagnostic. Pulmonary angioscopy should be reserved for those cases where real doubt exists in the differentiation from primary pulmonary hypertension, after other investigative tests have been completed.

SURGERY

Surgery for the chronic form of pulmonary embolism was first performed in 1951[14], when a patient suspected of a pulmonary aneurysm was treated with a pneumonectomy. A planned approach of pulmonary thromboendarterectomy for pulmonary hypertension due to chronic pulmonary thromboembolism was suggested by Hollister and Cull[15] and then carried out by Hurwitt *et al.* in 1957[16]. This patient was operated upon using inflow occlusion and systemic hypothermia, without success. In 1958 Allison *et al.* performed the first successful endarterectomy, again using inflow occlusion[17]. A right thoracotomy approach was used by Snyder *et al.*[18] in 1963 in a 71-year-old man initially operated on for a suspected tumor. In the same year Houk and his associates[19] also reported a thromboendarterectomy through a thoracotomy approach.

Cardiopulmonary bypass for this operation was used in 1964 by Castleman *et al.*[20], and several other cases using either thoracotomy or median sternotomy with bypass were later reported over the next 20 years. A review of the world's literature in 1984 showed 85 cases who had had surgery, with a mortality of 22%[21].

There have been occasional other case reports, particularly from the groups at Duke University[21], La Pitié Hospital in Paris[22], and Chiba University[23]. However, most of the surgical experience in pulmonary thromboendarterectomy has been reported from our group at the University of California, San Diego (UCSD) medical center[24,25], with a current total of more than 650 cases.

The specific preoperative evaluation of patients at our center includes right heart catheterization and pulmonary angiography. Pulmonary artery pressures are confirmed, and angiography performed. In cases where residual doubt exists, pulmonary angioscopy may be done. Aside from the establishment of the diagnosis, the decision for operation is then made on the general condition of the patient, and the severity of symptoms. Patients accepted for surgery typically include those who have chronic thrombi judged to be surgically accessible, the absence of significant co-morbid disease, and a pulmonary vascular resistance over 300 dyne/s per cm^5.

As the surgical experience has continued to grow, patients have been accepted for surgery with more distal thromboembolic disease, and with advanced (though presumed reversible) hepatic and renal dysfunction due to right-sided cardiac failure. Ascites may be present, and some patients have suprasystemic pulmonary artery pressures with a pulmonary vascular resistance above 1200 dyne/s per cm^5. Occasional patients have had a pulmonary vascular resistance less than 300 dyne/s per cm^5. These have been young patients with total unilateral pulmonary artery occlusion and unacceptable exertional dyspnea. The ages of the patients have ranged from 15 to 81 years.

As our experience with this condition has grown we have come to realize that the vasculature of the remaining open vessels can become affected with an Eisenmenger-like change, in which the increased flow and pressure in these initially unaffected vessels can lead to small-vessel changes that are irreversible. We have thus become more inclined to operate earlier in the younger patient whose vascular capacitance in the remaining open vessels may, at least initially, allow for the maintenance of low pulmonary artery pressures.

An inferior vena cava filter is always placed prior to surgery unless it is obvious that the legs or pelvis are not the source of embolic material (e.g. intraventricular pacing wires, or ventriculoatrial shunt). If necessary, any leads or wires are removed and, when possible, placed in extravascular locations. All patients are treated with warfarin until the time of surgery, and this is continued for life postoperatively.

As outlined above, the pulmonary thromboendarterectomy operation has gradually evolved from a unilateral approach via a thoracotomy to a bilateral approach through a median sternotomy and using cardiopulmonary bypass. Other changes have been made to improve operative exposure and to minimize ischemic, bypass and circulatory arrest times[25]. A bilateral approach is essential. Both pulmonary arteries must be substantially involved if the patient has pulmonary hypertension, since this does not occur even with complete unilateral occlusion, as with a pneumonectomy. In chronic pulmonary embolism the right ventricle is hypertrophied, and pulmonary hypertension, even to suprasystemic levels, is possible. A unilateral approach without bypass is therefore also more likely to result in an unstable intraoperative course, particularly after clamping one pulmonary artery.

The only practical approach to both pulmonary arteries is through a median sternotomy incision. Further, in order to define an adequate endarterectomy plane, and to then follow the pulmonary endarterectomy specimen all the way out into the subsegmental vessels, very good visibility is required, in a bloodless field. Therefore, cardiopulmonary bypass is required, with the subsequent institution of profound hypothermia and circulatory arrest.

The patient is prepared as for any open-heart procedure, with arterial and pulmonary artery pressure, and EEG monitoring. However, a femoral artery line is also placed because the profound vasoconstriction that tends to occur with hypothermic circulatory arrest makes readings from the radial artery catheter unreliable during the immediate postoperative course.

A median sternotomy incision is made and the sternum divided. Bypass is instituted with high ascending aortic cannulation and two caval cannulae. Standard flow for cardiopulmonary perfusion is used, and the patient cooled, maintaining a 10°C gradient between arterial blood and bladder or rectal temperature[26].

The patient's head is surrounded by ice and the cooling blanket turned on. During perfusion the venous saturations increase; saturations of 80% at 25°C and 90% at 20°C are typical. Hemodilution is carried out to decrease the blood viscosity during hypothermia and to optimize capillary blood flow; the hematocrit is maintained in the range of 18–25 during profound hypothermia. Phenytoin is administered intravenously during cooling at 15 mg/kg, to a maximum dose of 1 g.

A temporary pulmonary artery vent is inserted. When the heart fibrillates, a further vent is placed in the left atrium through the right upper pulmonary vein. A large amount of bronchial arterial blood flow is common with these patients, since viability of the

pulmonary parenchyma has been maintained by the bronchial vessels after pulmonary artery occlusion. During the cooling period, preliminary dissection can be carried out, with full mobilization of the ascending aorta and the superior vena cava. The superior vena cava is mobilized all the way to the innominate vein, and dissected free of the right pulmonary artery. The reflection of the right pulmonary artery to the left atrium is also divided. Most of this dissection is performed with electrocautery since, with advanced right heart failure and hepatic congestion, coagulation is usually abnormal. However, care must be taken to preserve the integrity of the right phrenic nerve lying lateral to the superior vena cava. All dissection of the pulmonary arteries occurs intrapericardially, and it is not necessary to enter either pleural cavity.

The right pulmonary artery is now exposed so that the take-off of upper and middle lobes can be seen. The upper pulmonary vein is usually not visualized, but reflected upwards from the plane of the pulmonary artery wall. An incision is made in the right pulmonary artery from beneath the ascending aorta out under the superior vena cava and entering the lower lobe branch of the pulmonary artery just after the take-off of the middle lobe. It is important that the incision stays in the center of the vessel. Only one incision is needed, and it is easier to endarterectomize the right upper lobe from a central incision than through a separate incision in the upper lobe artery. The distal limit of the lower lobe pulmonary artery incision is dictated by the accessibility required in order to repair this subsequently.

Any loose thrombus is now removed, and if the bronchial circulation is not excessive, the endarterectomy plane can be found. However, although a small amount of dissection can be carried out prior to the initiation of circulatory arrest, it is unwise to proceed further unless perfect visibility is obtained.

Surgical therapy for chronic thromboembolic pulmonary hypertension involves not only an embolectomy of chronic laminated thrombus where this is present, but a true endarterectomy of the pulmonary arterial bed. It is most important to recognize that first, embolectomy without endarterectomy is quite ineffective, and second, that in 90% of patients with chronic thromboembolic hypertension, direct examination of the pulmonary vascular bed at operation shows no obvious embolic material. Thus, to the inexperienced or cursory glance, the pulmonary vascular bed may appear normal prior to endarterectomy. The thrombus that might be seen within the vessel (Figures 3 and 6) is often secondary thrombus superimposed upon fibrotic intimal disease, and bears no relationship to the underlying pathology.

When the patient's temperature reaches 20°C the aorta is crossclamped and a single dose of cold cardioplegic solution (1 liter) administered. Additional myocardial protection is obtained by the use of a cooling jacket. The entire procedure is carried out with a single aortic crossclamp period with no further administration of

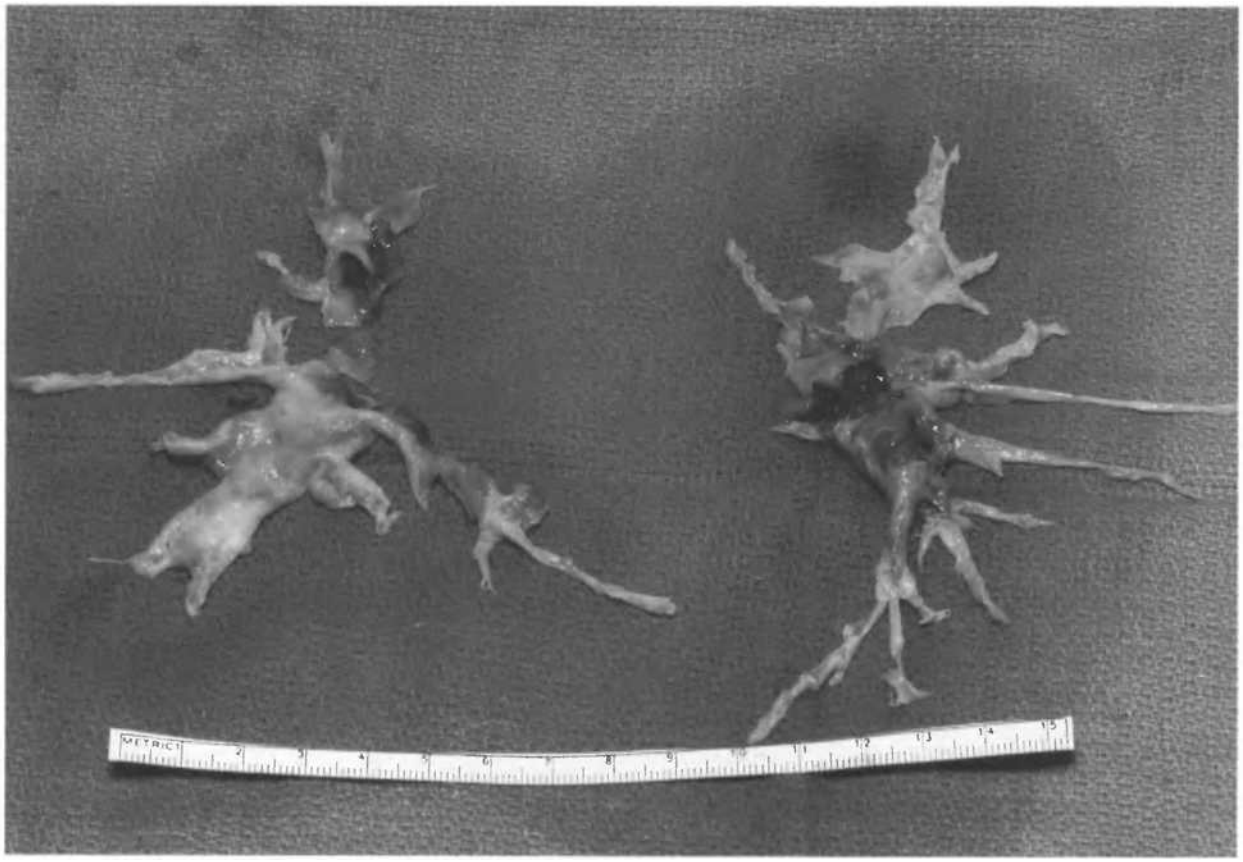

Figure 6 Specimen removed at operation in this patient. Some secondary thrombus is seen in the left lower lobe. This patient had a pulmonary vascular resistance of 1420 dyne/s per cm^5 preoperatively; this returned to normal after surgery

cardioplegic solution. After crossclamping of the aorta thiopental is administered (500 mg to 1 g) until the EEG becomes isoelectric. When circulatory arrest is initiated, all monitoring lines to the patient are turned off, and the patient exsanguinated. The endarterectomy plane is then developed, and the endarterectomy specimen is progressively followed all the way to the subsegmental vessels. Each lobe is endarterectomized, and then each segmental and subsegmental artery pursued distally. Although many of these vessels cannot be seen initially, progressive dissection and traction allow a complete endarterectomy of the entire pulmonary vascular bed. It is important that each subsegmental branch is followed and freed individually until it ends in a 'tail', beyond which there is no further obstruction.

Circulatory arrest periods are limited to 20 min, followed, if necessary, by a reperfusion period. Reperfusion is carried out at 18°C until the venous saturations reach 90%, or for a minimum of 10 min. However, with experience the entire endarterectomy on one side can be performed within a 20-min circulatory arrest period.

When the endarterectomy on the right side has been completed, reperfusion is established, and the pulmonary arteriotomy closed using a running suture of polypropylene. Hemostasis of the suture line is absolutely necessary, as visualization of the distal incision can only be obtained later with the reinstitution of bypass.

Attention is now turned to the left side, and an incision made from the main pulmonary artery down, again intrapericardially, to the take-off of the left upper lobe. Any loose thrombus is removed and an endarterectomy under profound hypothermia with circulatory arrest again carried out. The most difficult part of this operation on the left side is that of the left lower lobe which proceeds posterior to the left bronchus, thus making visibility more difficult. Progressive traction and freeing of each segmental branch make this possible.

After the completion of the endarterectomy, cardiopulmonary bypass is re-instituted and warming commenced. 500 mg methylprednisolone is administered intravenously, and during warming a 10°C temperature gradient is maintained between the perfusate and body temperature. If the systemic vascular resistance is high, nitroprusside is administered to promote vasodilatation and warming. The rewarming period generally takes about 90 min, but this period varies according to the body mass of the patient.

The left pulmonary arteriotomy is repaired. The right atrium is then examined to remove any incidental thrombus and to close an atrial–septal defect or persistent foramen ovale if this is present. This is important, since if pulmonary pressures do not immediately return to normal, right-to-left shunting may contribute to postoperative hypoxemia. Although tricuspid valve regurgitation is invariable in these patients, and is often severe, tricuspid valve repair is not performed. Right ventricular remodeling occurs within a few days, with return of tricuspid competence.

If other cardiac procedures are required, such as coronary artery or mitral or aortic valve surgery, these are conveniently performed during the systemic rewarming period.

Wound closure is routine, though both atrial and ventricular pacing wires are left *in situ*.

POSTOPERATIVE MANAGEMENT

Careful postoperative management is essential to a successful outcome. Although the pulmonary artery pressures in most patients come down immediately to normal levels, some may have residual high resistance of the pulmonary vascular bed (usually patients with long-standing chronic thromboembolic disease) which resolves after about 24 h. The pulmonary artery systolic pressure may remain transiently high; however, if a good endarterectomy has been performed the diastolic pressure is low, indicating rapid run-off in residually stiff vessels. With time the pulmonary artery regains its capacitance and the systolic pressure falls.

Some patients develop a degree of reperfusion pulmonary edema. This is now seen in only about 10% of patients, probably as a result of the more complete and expeditious performance of the endarterectomy that has come with a large experience over the past few years, with subsequently reduced ischemic times of the lung. The cause of reperfusion edema is uncertain. It is possibly related to the absolute ischemia of the lung (the pulmonary parenchyma, of course, is absolutely ischemic during the arrest period – it receives blood from neither the bronchial arteries nor the pulmonary arteries), to reperfusion of chronically underperfused areas, and to the endarterectomy itself, as well as other factors. In any event, reperfusion edema occurs only in the areas of the lung from which obstruction has been removed. The phenomenon varies in severity from a mild form of edema, seen more commonly, to an acute and fatal complication seen in 1–2% of cases. However, some degree of postoperative hypoxemia is common from this complication. The areas of alveolar edema involve the segments of lung that are endarterectomized, and these now become preferentially perfused with blood that is diverted away from previously normal areas ('steal'). The resulting hypoxemia results in further pulmonary vasoconstriction, thus worsening the situation. Meticulous ventilatory management is thus required, together with very careful management of fluid balance. An aggressive diuresis should be instituted, and the hematocrit kept high (32–36) in order to minimize the alveolar capillary leak. The patient's ventilatory status may be dramatically position-sensitive. Because this complication resolves with time, management hinges on adequate support until this resolution occurs.

RESULTS

More than 650 patients have now been operated upon at the University of California Medical Center, and approximately 500 since 1990. The overall mortality (30 days or in-hospital if the hospital course is prolonged) was 9% for the entire patient group. This encompasses a time span of 20 years, and during the early experience with this procedure mortality was related to many causes, including myocardial infarction, bilateral phrenic nerve paralysis, pulmonary hemorrhage, and sepsis. Of 196 patients operated upon from July 1970 to December 1989 the mortality was 15%, with no appreciable change over the years. A change in the operative method, as described above, was instituted in 1990[25]. Since this time the mortality rate has been in the range of 5–6%.

Residual causes of mortality are operation upon patients in whom thromboembolic disease was then found not to be the cause of the pulmonary hypertension, and the rare case of reperfusion pulmonary edema which progresses to a respiratory distress syndrome of long standing, which is not reversible.

These results obviously compare very favorably with those for transplantation, in both the short term and the long term. The data

from the registry of the International Society for Heart and Lung Transplantation for the years 1987–1992 show a 1-year survival of 52% for single lung transplantation and 52% for double lung transplantation. Results have been somewhat better in recent years, and are also somewhat better at individual centers, but still do not achieve figures comparable to the current results for endarterectomy at UCSD.

Long-term results with endarterectomy show persistent hemodynamic and respiratory improvement[27]. The New York Heart Association (NYHA) functional classification improves markedly, with the majority of patients changing from NYHA III or IV to NYHA I functional status. Long-term results with transplantation, of course, are not as well sustained, since there is a progressive morbidity and mortality due to chronic rejection and the side-effects of immunosuppression, including infection.

COMMENT

It is increasingly apparent that pulmonary hypertension due to chronic pulmonary embolism is a condition which is under-recognized. Medical therapy for this condition is ineffective. The operation of pulmonary endarterectomy is technically demanding, and requires careful dissection of the pulmonary artery planes and the use of circulatory arrest. There is a distinct learning curve for the procedure. However, surgical therapy is curative, with currently excellent short- and long-term results. There are distinct advantages to this procedure as compared to transplantation. The wait for a donor, with its attendant mortality, is eliminated. Short- and long-term problems with rejection and the side-effects of immunosuppression are avoided. There seems little doubt, with the increasing recognition of patients presenting with pulmonary hypertension due to emboli, and the realization that pulmonary endarterectomy can be a relatively safe and effective procedure in experienced hands, that this will be an expanding area of surgical therapy in the future.

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