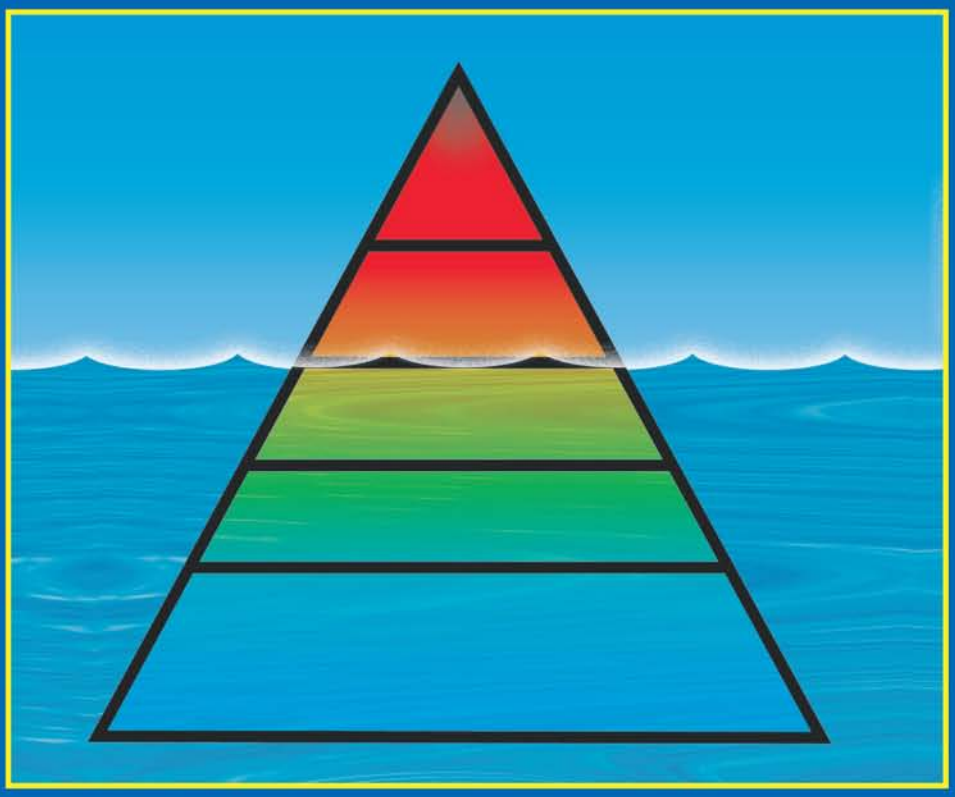
Fundamental and Clinical Cardiology Series / 62



HEPARIN-INDUCED THROMBOCYTOPENIA

Fourth Edition

Edited by
Theodore E. Warkentin
Andreas Greinacher

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Edited by

Theodore E. Warkentin
McMaster University
Hamilton, Ontario, Canada

Andreas Greinacher
Ernst-Moritz-Arndt-University-Greifswald
Greifswald, Germany

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healthcare

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To the late Professor Michael F. X. Glynn,
for initiating my hemostasis interests;
to Dr. John G. Kelton,
for amplifying these through boundless opportunities;
and to Erica, Andrew, Erin, and Nathan,
for downregulating my passion, as a caring family must.

T.E.W.

To my co-workers and students for their contributions and efforts;
to Sabine, Sebastian, Anja, and Jan.

A.G.

Introduction

Informa Healthcare has developed various series of beautifully produced books in different branches of medicine. These series have facilitated the integration of rapidly advancing information for both the clinical specialist and the researcher.

My goal as editor-in-chief of the Fundamental and Clinical Cardiology Series is to assemble the talents of world-renowned authorities to discuss virtually every area of cardiovascular medicine. I feel we have achieved this objective with *Heparin-Induced Thrombocytopenia, Fourth Edition*.

Theodore Warkentin, MD, PhD, and Andreas Greinacher, MD, have written and edited a much-needed practical and timely book. These world-renowned investigators have assembled a multidisciplinary and global team of key opinion leaders to assist them in creating one of the best written and most highly successful efforts in the history of our Fundamental and Clinical Cardiology Series. In the current edition, the editors hone in on the hot topics and controversies. These include assessment of the clinical likelihood of heparin-induced thrombocytopenia (HIT), the increasing problem of excessive diagnosis of HIT, and new strategies to manage suspected and proven HIT.

HIT with thrombosis can lead to gangrene, amputation, and death. It is one of the most feared catastrophes in cardiovascular medicine. The optimal approach requires a multidisciplinary team. Drs. Warkentin and Greinacher have chosen experts from many continents and academic backgrounds to provide comprehensive data and guidelines in this informative and carefully edited book.

HIT is a rapidly changing field. I will take this book with me on the wards when I am confronted with new cases of this deadly disease. I especially appreciate the clear explanations of pathophysiology and therapy that the authors provide. Their coverage of direct thrombin inhibitors (lepirudin, argatroban, bivalirudin), danaparoid, pentasaccharides, and other therapeutic alternatives is superior to any resource available on the Internet or elsewhere.

Future contributions to the Fundamental and Clinical Cardiology Series will include books on molecular biology, interventional cardiology, and clinical management of such problems as coronary artery disease, venous thromboembolism, peripheral vascular disease, and cardiac arrhythmias.

Samuel Z. Goldhaber, MD
Professor of Medicine
Harvard Medical School
Senior Staff Cardiologist
Brigham and Women's Hospital
Boston, Massachusetts, U.S.A.

Editor-in-Chief
Fundamental and Clinical Cardiology Series

Preface to the Fourth Edition

This fourth edition of *Heparin-Induced Thrombocytopenia* continues to expound on the themes enunciated in the Preface to the First Edition (2000): heparin-induced thrombocytopenia (HIT) is a *clinicopathologic syndrome*; HIT pathogenesis melds both platelet and coagulation activation—a key to comprehending its prothrombotic nature; and HIT exhibits a strikingly variable frequency in different clinical situations.

WHAT IS NEW IN THIS FOURTH EDITION?

We have a better understanding of the diagnostic approach to HIT. The combination of a clinical assessment of pretest probability, together with the results of laboratory testing for the pathogenic HIT antibodies, enables the clinician to stratify patients into risk categories for HIT-associated complications. Clinical scoring systems, such as the *4 T's,* are now available and evaluated, which help to standardize this approach. A major achievement is the high negative predictive value of this scoring system, i.e., a low score effectively "rules out" HIT. Further, the diagnostic value of the magnitude of a positive test result for "HIT antibodies" is increasingly appreciated. As well, the high sensitivity of the platelet factor 4 (PF4)-dependent enzyme-immunoassays (EIAs) and washed platelet activation assays (when performed by experienced laboratories) is increasingly accepted. New test concepts, such as the rapid assays, and novel methods for detecting antibodies against several heparin-binding proteins simultaneously by EIA are being introduced. But caution is required: there is growing recognition that HIT may be *over*diagnosed, at least when low probability clinical situations and weak-positive EIAs are misinterpreted as indicating clinical HIT. Our selection of the "iceberg model" of HIT for the book's cover is meant to highlight the reality that only a minority of heparin-dependent antibodies have the potential to cause HIT, and that the various laboratory assays differ regarding their usefulness in detecting these pathologic "HIT antibodies."

The pathogenesis of HIT is now better understood, especially the biophysical nature of the PF4/heparin immune complexes. Ultrastructural studies have helped to clarify the basis of the greater immunogenic potential of unfractionated heparin [vis-à-vis low molecular weight heparin (LMWH) and fondaparinux], and have also provided a glimpse into the basis of the paradox of immunization induced by the sulfated pentasaccharide, fondaparinux, while at the same time its inability to form well the conformational changes leading to autoepitope formation on PF4. Still, the future of HIT research remains the unraveling of its unusual immunobiology.

Important new concepts in HIT treatment have evolved. There is emerging consensus that the "package insert" dosing for the direct thrombin inhibitors—particularly lepirudin—is too high. That the dangers of coumarin (warfarin, phenprocoumon, acenocoumarol) use during the acute phase of HIT include not only its potential to induce protein C depletion (thereby predisposing to

microthrombosis syndromes such as coumarin-induced venous limb gangrene), but also to predispose to *under*dosing of direct thrombin inhibitor therapy (through prolongation of the partial thromboplastin time by coumarin). Also new to the fourth edition is the approval of bivalirudin for anticoagulation in percutaneous coronary intervention in patients with acute or previous HIT, an indication previously held only by argatroban. New data on the efficacies of the standard HIT therapies—danaparoid, lepirudin, and argatroban—are also discussed.

The growing use of LMWH results in a shift of patient populations affected by HIT, from post–major surgery to the intensive care setting, where unfractionated heparin (UFH) use still predominates. But, among critically-ill patients, HIT explains only a minority of platelet count declines, reflecting the high frequency of numerous thrombocytopenia-inducing comorbidities. How should such thrombocytopenic patients be managed in whom the diagnosis of HIT is raised, but where the probability is judged to be only low or intermediate? What anticoagulant options are available—and in what doses? An evolving concept in countries in which danaparoid is available is to use this agent in *prophylactic* doses in this setting of low (or even moderate) probability of HIT (when thrombosis is not present), pending clarification of the diagnosis. This approach could reduce the risk of bleeding associated with therapeutic-dose regimens. And, this edition includes a new chapter describing novel anticoagulants, such as fondaparinux, that could have future roles either for prevention or management of HIT.

HIT comprises a myriad of complexities and counter-intuitions. The new chapter on "paradoxes, myths, and realities" of HIT highlights some of the potential sources of error that can lead to catastrophic outcomes in affected patients and is a fitting conclusion to a topic with paradoxes aplenty.

SPECIAL THANKS

A multi-author book depends on many contributors. For us, as editors, it was a great pleasure to produce this 4th edition together with our contributing colleagues throughout the world, many of whom became our friends, all dedicated to research and resulting improved clinical management of HIT. We would also like to acknowledge the help of many individuals in this project. Paula Garber was superb in managing this edition on behalf of the publisher. In Hamilton, we wish to thank in particular Maria Adamek, Jo-Ann Sheppard, Jim Smith, Jane Moore, Carol Smith, Di Moffatt, Junior Santos, Rumi Clare, and Dr. Greg Lo; in Greifswald, gratitude is owed to Uta Alpen, Norbert Lubenow, Petra Eichler, Kathleen Selleng, David Juhl, Birgitt Fürll, Ulrike Strobel, Ricarda Raschke, and Carmen Blumentritt, for their invaluable technical and administrative support, for ideas and discussions, and especially for being part of the team dedicated to research in HIT.

Theodore E. Warkentin
Andreas Greinacher

Preface to the Third Edition

Since the appearance of the second edition of *Heparin-Induced Thrombocytopenia* over three years ago, new and important advances in the understanding and treatment of this paradoxical adverse reaction to heparin have continued to emerge.

For example, mapping of the target epitopes recognized by HIT antibodies to platelet factor 4 (a "self protein" found within platelets) rather than on heparin itself helps to explain some of the "autoimmune" features of HIT, such as its potential to present as thrombocytopenia and thrombosis several days after stopping heparin ("delayed-onset HIT"), as well as the increased risk of thrombosis that can persist for several days or weeks even after HIT is recognized and the inciting agent, heparin, stopped. A new double-transgenic animal model of HIT (with mice engineered to produce both human platelet factor 4 and human platelet Fcγ receptors provides direct evidence that platelet Fc receptor-mediated platelet activation by HIT antibodies indeed helps to explain thrombocytopenia and the associated risk of thrombosis in HIT.

There is increasing recognition of the danger of microvascular thrombosis in HIT, particularly when warfarin or other coumarin anticoagulants are given when HIT remains active. This can lead to a variant of coumarin-induced necrosis, with a predisposition to involve the extremities, i.e., warfarin-induced venous limb gangrene complicating HIT-associated deep-vein thrombosis. The recognition of this syndrome underscores the importance of continuing therapy with a direct thrombin inhibitor or danaparoid (where available), and postponing warfarin therapy until substantial resolution of the thrombocytopenia of HIT has occurred.

In 2002, danaparoid was withdrawn from the United States, leaving the direct thrombin inhibitors, lepirudin and argatroban, as the two main agents in that market for managing the difficult clinical situation of HIT complicated by thrombosis. (Danaparoid remains available in Canada, the European Union and elsewhere.) Another recent clinical development is the recognition that lepirudin bolus infusion can (rarely) trigger life threatening anaphylaxis, perhaps because of antibodies that have been formed against this foreign protein (although lepirudin is manufactured using recombinant biotechnology, its blueprint is drawn from the medicinal leech).

Perhaps most important, there is increasing agreement that patients suspected as having HIT should not merely have their heparin stopped, but should additionally have an alternative anticoagulant given, so as to reduce the risk of subsequent thrombosis. Indeed, argatroban has FDA approval for this novel indication of prophylaxis against thrombosis in HIT. Post-marketing studies indicate that lepirudin is also effective in this situation. Thus, physicians need to consider carefully the possibility of HIT in many hospitalized patients who develop thrombocytopenia during heparin therapy, or even in patients who return to the emergency room with thrombosis and thrombocytopenia following a recent hospitalization in which heparin was given (delayed-onset HIT). If HIT is strongly suspected, alternative anticoagulation is indicated.

All 20 chapters from the second edition have been revised, and two new chapters added. One reviews pediatric HIT, and the other discusses use of a new direct thrombin inhibitor, bivalirudin, in the context of preventing and, possibly, treating HIT. Of course, for their generosity of time in updating and adding to this book, we thank the contributors. And, for their invaluable efforts, we thank (in Canada) Jo-Ann I. Sheppard, Aurelio Santos, Jr., James W. Smith, and Maria Adamek, and (in Germany) Uta Alpen, Petra Eichler, Norbert Lubenow, Lena Carlsson, and Theresia Lietz.

Despite the lower risk of HIT with low molecular weight heparin and the novel factor Xa-inhibiting pentasaccharide (fondaparinux), compared with unfractionated heparin, HIT does not seem to be going away. It remains an issue particularly in cardiac surgery patients, where unfractionated heparin remains the prevailing drug for providing anticoagulation during the cardiac surgery itself, as well as in the postoperative period in many centers. Further, the increasing awareness of HIT, and the increasing availability of laboratory testing for HIT antibodies, means that more cases of HIT continue to be recognized, even if, perhaps, the overall frequency of this reaction is in decline (due to increasing use of low molecular weight heparin). Further, the common concurrence of thrombocytopenia and heparin therapy in hospitalized patients, and the medicolegal consequences of a missed diagnosis of HIT, mean that this diagnosis continues to be considered and discussed daily around the world. We hope that this compilation of information and practical guidelines on HIT diagnosis and treatment will assist the health care professional in managing this challenging and fascinating disorder.

Theodore E. Warkentin
Andreas Greinacher

Preface to the Second Edition

Since the first edition of *Heparin-Induced Thrombocytopenia* appeared, there is much new on this topic. In particular, there is growing awareness of the intense "thrombin storm" characteristic of heparin-induced thrombocytopenia (HIT), especially after heparin has been stopped. Recently, another therapeutic option became available to manage this situation, namely, argatroban, a synthetic, small-molecule, direct thrombin inhibitor. Significantly, the indication approved by the Food and Drug Administration for this novel anticoagulant was "for prophylaxis or treatment of thrombosis in patients with HIT."

This approval of argatroban *for prevention of thrombosis* in HIT parallels the growing recognition that even "isolated HIT" (i.e., HIT recognized on the basis of thrombocytopenia alone, rather than because of a new thrombotic complication) is associated with an unacceptably high risk of life- and limb-threatening thrombosis, even when heparin has been promptly discontinued because of a falling platelet count. Indeed, this view that isolated HIT itself is an indication for prescribing an alternative anticoagulant in most patients has been accepted by the 2000 Consensus Conference on Antithrombotic Therapy of the American College of Chest Physicians.

Thus, there now exist three anticoagulant agents for which there is consensus regarding efficacy treatment for HIT: danaparoid sodium, recombinant hirudin (lepirudin), and argatroban (listed in order of availability). Approval status of the three drugs with respect to HIT varies in different countries, but even if unapproved they may be available for "off-label" use, or on a "compassionate-use" basis. Important differences in pharmacokinetics, particularly in half-life, metabolism, and monitoring, mean that each of these three agents will be appropriate in some of the complex clinical settings in which HIT occurs.

Other developments in HIT include new understanding of the molecular structure of the target antigen, a proposed role for activation of monocytes in thrombin generation, and new animal models for studying this fascinating syndrome. Even the clinical syndrome itself is now better understood: The influence of previous heparin exposure on the timing of onset of HIT has been clarified, and the peculiar transience of HIT antibodies has been shown. These clinical and laboratory insights provide a firmer basis for estimating pretest probabilities of HIT in various clinical settings, and also give a scientific rationale for considering re-exposure to heparin in a patient with previous HIT but whose antibodies are no longer detectable.

Ironically, improvements in a laboratory testing also mean that not all detectable HIT antibodies are truly pathogenic. Thus, physicians need to interpret results of diagnostic assays in the clinical context, so as to estimate accurately the posttest probability of HIT. A major aim of this book is to provide the relevant information for understanding such a "clinipathologic" framework of HIT.

More and more, it seems, HIT is an issue in cases of alleged medical malpractice. This is because HIT often occurs in patients who received heparin for prophylaxis of thrombosis. So it can seem evident even to a nonmedical person

that something fundamentally went wrong if the patient ended up with severe pulmonary embolism or even limb loss.

To address these new developments, and others, all chapters of the first edition have been updated. In addition, two new chapters have been added, one discussing the use of argatroban for management of HIT and the other dealing with U.S. perspectives on medicolegal aspects of HIT.

Theodore E. Warkentin
Andreas Greinacher

Preface to the First Edition

An anticoagulant turns procoagulant; an antithrombotic causes thrombosis. This is the fundamental paradox of heparin-induced thrombocytopenia (HIT), an antibody-mediated prothrombotic drug reaction without parallel in clinical medicine.

Heparin justifiably is listed as an "essential" drug by the World Health Organization (1997): with a rapid onset of action, simple laboratory monitoring, and a low cost, heparin has benefited countless patients. And yet, beginning some 40 years ago, a few physicians asserted that heparin caused unusual and sometimes catastrophic thrombi in some of their patients who received the drug for a week or more. Subsequently, two landmark studies led by a vascular surgeon, Donald Silver, identified the key elements of the HIT syndrome: thrombocytopenia, thrombosis, and heparin-dependent antibodies in the patient's blood (Rhodes et al., 1973, 1977; see Chap. 1). But key questions remained: how can heparin cause thrombosis? What is the frequency of this event? How should these patients be treated? This book summarizes a quarter-century of observation and study that has begun to provide answers to these questions.

The reader will observe several "themes" in this book. One is that HIT should be considered a clinicopathologic syndrome. This means that HIT should be diagnosed only when 1) one or more unexpected clinical events occur during heparin treatment (most commonly, thrombocytopenia with or without thrombosis), and 2) heparin-dependent antibodies can be demonstrated in the laboratory. A corollary is that the inability to demonstrate the antibodies using reliable assays means that an alternative diagnosis must be considered. Both editors view HIT through this "filter" of confirmatory laboratory testing. For us, the laboratory has been crucial to defining the HIT syndrome, by making it possible to distinguish patients who really have HIT from those affected by the numerous other causes of thrombocytopenia encountered in clinical medicine. Indeed, our own first studies on HIT, presented at the XIIIth Congress of the International Society on Thrombosis and Hemostasis in Amsterdam, focused on improvements and innovations in diagnostic testing using platelet activation assays (Greinacher et al., 1991; Warkentin et al., 1991). Coincidentally, this was the same time scientific meeting at which Jean Amiral and colleagues (1991) announced the identity of the protein coantigen of HIT (platelet factor 4; PF4), providing another diagnostic avenue (enzyme immunoassay). Thus, when we stepped onto the patient wards, we increasingly relied on the laboratory to confirm or refute the diagnosis of HIT. Through mutually reinforcing experiences of clinic and laboratory, the nature of the HIT syndrome was unfolded. And, over time, the wide spectrum of complications of HIT, and its high frequency in certain clinical settings, became apparent.

Our focus on HIT as a clinicopathologic syndrome has implications for the terminology we have used in this book. Because the causative role of heparin can generally be established—in the appropriate clinical context—by the demonstration of pathogenic, heparin-induced thrombocytopenia to describe this syndrome (i.e., heparin can be shown convincingly to have "induced" the platelet count fall in a particular patient). In contrast, we use the term nonimmune

heparin-associated thrombocytopenia (nonimmune HAT) to describe patients who have developed thrombocytopenia during heparin treatment and in whom a pathogenic role for HIT antibodies cannot be shown. In our view, this term unambiguously denotes that HIT antibodies are not responsible for the thrombocytopenia, while leaving open the possibility that heparin may have played a role in the causation of the platelet count fall by nonimmune mechanisms (although coinciding thrombocytopenia from another cause is probably the most frequent explanation for this event). We have also introduced the term pseudo-HIT to indicate those patients with nonimmune HAT that, by virtue of associated thrombosis or the timing of onset of thrombocytopenia, closely mimics HIT (see Chap. 11).

A second theme of this book is the importance of in vivo thrombin generation in the pathogenesis of HIT. By virtue of antibody-mediated activation of platelets and endothelium, and the neutralization of heparin by PF4 released from activated platelets, the HIT syndrome can be understood as a prothrombotic disorder characterized by activation of the coagulation system. This concept of HIT helps explain its association with venous as well as arterial thrombosis (by analogy with other hypercoagulable states, such as congenital deficiency of natural anticoagulant factors), and also the occasional HIT patient with decompensated, disseminated intravascular coagulation (DIC).

Marked thrombin generation in HIT also helps explain its association with coumarin-induced venous limb gangrene, an unusual syndrome now recognized as a potential complication of coumarin treatment of HIT-associated deep venous thrombosis (see Chap. 2). This iatrogenic disorder represents perhaps the most striking of all the HIT treatment paradoxes (see Chap. 12): two antithrombotic agents with distinct adverse event profiles that interact to produce a profound disturbance in procoagulant-anticoagulant balance, i.e., increased thrombin generation (secondary to HIT) together with acquired, severe protein C deficiency (secondary to coumarin treatment). Finally, the concept of HIT as a hypercoagulable state with in vivo thrombin generation provides a rationale for understanding the efficacy of new therapies that either reduce thrombin generation by inhibition of factor Xa (e.g., danaparoid) or directly inactive thrombin (e.g., lepirudin).

A third theme of this book is the peculiarly inconstant nature of HIT, in particular, its variable frequency and clinical presentation among different patient populations treated with heparin. Figure 1 depicts HIT as an iceberg within which a variety of clinical and laboratory factors interact to influence antibody formation, development of thrombocytopenia, and, finally, resulting clinical complications, such as thrombosis. A recent, novel concept is that the size and buoyancy of the HIT icebergs can vary among different patient populations who receive heparin. This concept of multiple icebergs of HIT is shown in Chapter 3, Figs. 3 and 5. Unraveling the clinical and laboratory determinants for these differences in the icebergs among patient populations is a major challenge of current and future investigation.

Why should HIT be the subject of a book? First and foremost, HIT is common, and nonimmune HAT is very common. According to the Council for International Organization of Medical Sciences (CIOMS III), adverse drug reactions can be classified as common if they occur in 1—10% of patients, and very common if they occur in 10% or more of patients. There is convincing evidence that HIT occurs in as many as 5% of certain patient populations, such as postoperative orthopedic patients receiving unfractionated heparin. The clinical

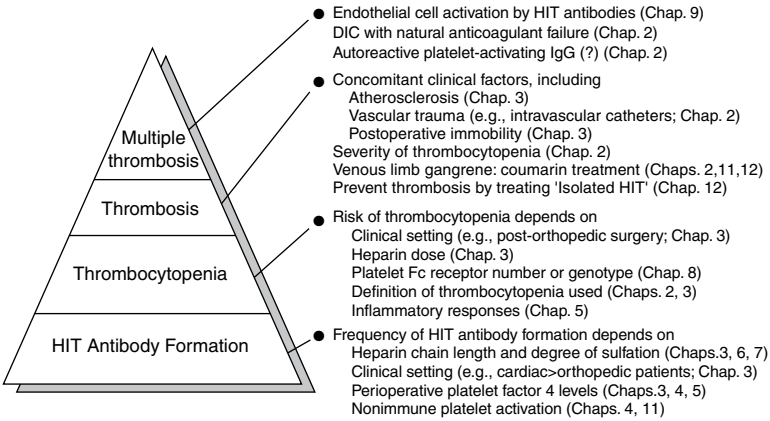

FIGURE 1 The iceberg model of heparin-induced thrombocytopenia as an index for this book.

influence of HIT is substantial: about half of these patients develop HIT-associated thrombosis. Nonimmune HAT occurs in as many as 30% of certain patient populations. Thus, physicians need to be able to reliably distinguish among the various thrombocytopenic disorders that occur during heparin treatment. This will minimize the risk of inappropriate treatments, such as failing to stop heparin administration in a patient with probable HIT, or deciding to stop heparin in a patient with nonimmune HAT or pseudo-HIT. Because HIT is a life- and limb-threatening iatrogenic illness with many diagnostic and treatment pitfalls, medicolegal consequences of caregiver's action or inaction can be significant (see Chap. 21).

A second reason for the compilation of this book is that most of the pieces of the HIT puzzle are now firmly in place. Consensus has emerged on several key aspects of the syndrome, including the nature of its target antigen, the participation of platelet and endothelial cell activation in the pathogenesis of thrombosis, the frequency of HIT, and optimal laboratory testing. The publication of this book reflects this coherence in our understanding of the HIT syndrome. Yet there remain important unresolved issues: for example, what is the fundamental nature of the "autoimmune" response to the PF4-heparin neoepitope? Why is the immune response to the HIT antigen so transient? Why do only a subset of patients who form HIT antibodies develop clinical HIT?

Heparin has been, and will continue to be, one of the most important agents for the prophylaxis and treatment of venous and arterial thromboembolism. Consequently, HIT will continue to be an important management problem for some time to come. Both of us have spent a decade of our scientific careers providing, in the context of other investigators' work, a rational management approach aimed at minimizing morbidity and mortality among the many patients who develop the most common immune-mediated adverse drug reaction in clinical medicine. The importance of controlling thrombin generation in HIT is now widely appreciated. The book should help guide clinicians through the often paradoxical clinical and management problems posed by patients with HIT (see Chaps. 12–19).

The book is a tribute to our scientific mentors, John G. Kelton and Christian Mueller-Eckhardt, and the close cooperation of many of our scientific colleagues and personal friends whose efforts made this project possible.

We would also like to acknowledge the help of many individuals in this project. In North America, we thank Jo-Ann Sheppard for technical support over the years, and also for preparing many of the figures in this book; Aurelio Santos, Jr., for preparing the key Fig. 12.1; James W. Smith, for help with vexing computer problems; Katherine Bean and Maria Adamek, for able secretarial assistance; and Erica Warkentin, for checking references and manuscripts. In Germany, we thank Uta Alpen for excellent secretarial assistance and Petra Eichler, Norbert Lubenow, Lena Carlsson, and Oliver Ranze for valuable discussion and review of manuscripts.

Theodore E. Warkentin
Andreas Greinacher

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Contents

Contributors

Susanne Alban Christian-Albrechts-University of Kiel, Kiel, Germany

Jean Amiral Hyphen Biomed Research, Zac Neuville-Université, Neuville-Sur-Oise, France

Gowthami M. Arepally Duke University Medical Center, Durham, North Carolina, U.S.A.

Richard H. Aster Medical College of Wisconsin and Blood Research Institute, The Blood Center of Wisconsin, Milwaukee, Wisconsin, U.S.A.

John R. Bartholomew Cleveland Clinic, Cleveland, Ohio, U.S.A.

James E. Bradner Dana-Farber Cancer Institute, Boston, Massachusetts, U.S.A.

Beng Hock Chong St. George Hospital and University of New South Wales, Kogarah, New South Wales, Australia

Douglas B. Cines University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Gregory A. Denomme University of Toronto, Canadian Blood Services and Mount Sinai Hospital, Toronto, Ontario, Canada

John W. Eikelboom Michael G. DeGroote School of Medicine, McMaster University, Hamilton, Ontario, Canada

Karl-Georg Fischer University Hospital Freiburg, Freiburg, Germany

Andreas Greinacher Ernst-Moritz-Arndt-University-Greifswald, Greifswald, Germany

McDonald K. Horne III Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland, U.S.A.

Marcie J. Hursting Clinical Science Consulting, Austin, Texas, U.S.A.

Anne F. Klenner Ernst-Moritz-Arndt-University-Greifswald, Greifswald, Germany

Andreas Koster Deutsches Herzzentrum Berlin, Berlin, Germany

David H. Lee Queen's University, Kingston, Ontario, Canada

Bruce E. Lewis Loyola University Medical Center, Maywood, and Catholic Health Partners, Chicago, Illinois, U.S.A.

Chao Yan Liu University at Buffalo, The State University of New York, Buffalo, New York, U.S.A.

Katharina Madlener Kerckhoff-Klinik, Bad Nauheim, Germany

Harry N. Magnani Medical Consultant, Oss, The Netherlands

Mortimer Poncz University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, U.S.A.

Bernd Pötzsch Rheinische Friedrich-Wilhelms-University Bonn, Bonn, Germany

Lawrence Rice The Methodist Hospital, Weill Cornell Medical College, Houston, Texas, U.S.A.

Gian Paolo Visentin University at Buffalo, The State University of New York, Buffalo, New York, U.S.A.

Anne Marie Vissac Hyphen Biomed Research, Zac Neuville-Université, Neuville-Sur-Oise, France

Theodore E. Warkentin Michael G. DeGroote School of Medicine, McMaster University, and Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada

1 History of Heparin-Induced Thrombocytopenia

Theodore E. Warkentin
Michael G. DeGroote School of Medicine, McMaster University, and Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada

I. THE DISCOVERY OF HEPARIN AND ITS FIRST CLINICAL USE

The following account of the discovery and first clinical development of heparin was recorded by the physiologist Best (1959), a codiscoverer of insulin as well as a pioneer in studies of heparin. Incidentally, in 1916, while working at Johns Hopkins University to characterize procoagulant substances, McLean (1916) identified a natural anticoagulant substance. Further studies of this material were performed by his supervisor, Dr. Howell, who coined the term, "heparin" to indicate its first extraction from animal hepatic tissues (Gr. ἡ παρ *[hepar]*, liver) (Howell and Holt, 1918). Despite its in vitro anticoagulant action, the inability of heparin to prevent platelet-mediated thrombosis (Shionoya, 1927) made it uncertain whether it had antithrombotic potential. However, animal (Mason, 1924) and human studies (Crafoord, 1937) showed that heparin could prevent thrombosis. By the 1950s, heparin was established as an important therapeutic agent in the treatment of venous and arterial thrombosis.

II. THE PARADOX OF HEPARIN AS A POSSIBLE CAUSE OF THROMBOSIS

A. Weismann and Tobin

On June 1, 1957, at the Fifth Scientific Meeting of the International Society of Angiology (North American Chapter) in New York, two physicians suggested that heparin might *cause* arterial embolism in some patients. Rodger E. Weismann, a 43-yr-old Assistant Professor of Clinical Surgery at the Dartmouth Medical School (Fig. 1), and his Resident in Surgery, Dr. Richard W. Tobin, presented their 3-yr experience with 10 patients who developed unexpected peripheral arterial embolism during systemic heparin therapy at the Mary Hitchcock Memorial Hospital, in Hanover, New Hampshire. Their first patient with this complication was reported in detail, and to this day represents a classic description of the syndrome:

> This 62-yr-old white woman was admitted to the Hitchcock Hospital Feb. 8, 1955, with left retinal detachment, complicating longstanding myopia . . . Left scleral buckling was carried out on Feb. 10, and strict bed rest was required during the ensuing 3 wk. On her beginning ambulation, on March 6, signs and symptoms of left iliofemoral thrombophlebitis were noted, for which systemic heparinization was begun (. . . heparin sodium in divided subcutaneous doses, totaling 150–300 mg per day . . .). On March 16, after 10 days of anticoagulation therapy, sudden signs of right common femoral arterial occlusion led to the diagnosis of common femoral arterial embolism. Successful femoral embolectomy was carried out. She was kept on adequate heparinization and made a satisfactory initial recovery until March 19, . . . when signs of sudden occlusion of the distal aorta appeared.

FIGURE 1 Photograph of Dr. Rodger Elmer Weismann, taken circa 1958.

> ... [P]rompt transperitoneal distal aortic and bilateral iliac embolectomies were performed. In the ensuing 24 h, because unsatisfactory distal circulation persisted, the patient underwent left femoral exploration, with negative findings, and right popliteal exploration, revealing an embolus. She subsequently pursued a favorable course, ... never showing more serious ischemic changes than a small area of superficial gangrene of the right great toe and several small areas of skin infarction of the right leg (Weismann and Tobin, 1958).

The report included a photograph of the emboli removed from the distal aorta and both iliac arteries, with the authors noting their "unusual length and cylindrical shape, suggesting origin in [the] proximal aorta," as well as a corresponding photomicrograph of the embolus. The thromboemboli were described by the authors as "pale, soft, salmon-colored clots" that "histologically ... were comprised mostly of fibrin, platelets and leukocytes; red cells were rare." This appearance was distinguishable from the typical appearance of thrombi originating in the heart (i.e., mulberry-colored thrombi tending to contain cellular elements of the blood in approximately normal proportions), leading the authors to propose "the source for the emboli ... to be aortic mural platelet-fibrin thrombi."

A summary of the 10 reported patients noted that the onset of arterial embolism began between 7 and 15 days, inclusive, of commencing heparin treatment (mean, day 10). Multiple thromboemboli occurred in nine patients; six of the patients died as a direct result of these complications; two survived with extensive amputations, and two were discharged with their extremities intact. The temporal time frame was consistent with the later realization by others that this syndrome represented an immune-mediated reaction initiated by the heparin.

The authors noted that further embolization stopped when the heparin was discontinued, leading to their recommendation that "heparin should be promptly reduced in dosage, and, if possible, discontinued if the presence of fibrin-platelet thrombi adherent to the intima of the aorta is suspected." Aggressive surgical management of emboli was also recommended, as some limbs were salvageable in this way. The authors summarized well the clinical dilemma: "In each instance there was a feeling of futility in the management of the problem, due to anticipation of further emboli from the same or similar sources. Heparin was

badly needed to retard distal thrombosis; yet the agent was probably seriously altering the integrity and attachment of the thrombotic source" (Weismann and Tobin, 1958).

B. Roberts and Colleagues

The communication of Weismann and Tobin was met with considerable skepticism. When a show of hands was elicited to indicate those surgeons who had also observed similar events, none were raised (Weismann, personal communication, July 1998). However, a few years later, Roberts and colleagues from the University of Pennsylvania in Philadelphia described a series of patients who were remarkably similar to those reported by Weismann and Tobin (Roberts et al., 1964; Barker et al., 1966; Kaupp and Roberts, 1972). The key features were summarized as follows:

> To witness a series of apparently paradoxical events is disconcerting as well as challenging. When such paradoxes involve totally unexpected results following the use of a major therapeutic agent, it is at first difficult to know whether the relationship is causal or merely coincidental. When, however, the same series of events has been seen repeatedly it is difficult to escape the conclusion that there is some causal relationship, even though the mechanism by which it is accomplished may be unknown . . . . During the last 9 yr at the Hospital of the University of Pennsylvania, we have seen a group of 11 patients who suffered unexplained arterial embolization for the first time while being treated with heparin for some condition that could not of itself reasonably be expected to cause arterial emboli . . . . All patients had been receiving heparin for 10 days or more when the initial embolus occurred . . . . All emboli removed were of a light color, seemingly made up primarily of fibrin and platelets, and microscopically appeared to be relatively free of red cells. All patients in this group had multiple emboli . . . . Of the four deaths, three were attributed to cerebral vascular accidents presumably embolic in origin and one was thought to have resulted from a perforation of the small bowel 2 wk after the removal of a mesenteric embolus (Roberts et al., 1964).

Roberts' group also viewed the likely pathogenesis as that of embolization of platelet-fibrin-rich material originating within the aorta, rather than the heart. Furthermore, they believed that the thrombi were initially formed on aortic ulcerations that acted as a nidus for thrombus formation. This pathogenesis was suggested by the observation that such adherent thrombi could be removed from the proximal aorta in a few of the patients (Roberts et al., 1964; Kaupp and Roberts, 1972).

C. An Immune Basis for Heparin-Induced Thrombosis?

The delay between initiation of heparin therapy and onset of embolization caused Roberts and colleagues (1964) to speculate that the etiology could represent an "antiheparin factor," resulting perhaps from "an antigen-antibody mechanism." Furthermore, the observation that the first 21 patients reported with this syndrome from both Hanover and Philadelphia had received heparin exclusively by subcutaneous or intramuscular, rather than intravenous, injection also was offered by Roberts' group as support for immune sensitization. Apparent heparin-induced thrombosis did not seem rare to these investigators: at least 13 of 110 (12%) patients with peripheral arterial emboli managed by the Philadelphia group over a decade were believed to have been caused by preceding heparin treatment (Barker et al., 1966).

III. HEPARIN-INDUCED THROMBOCYTOPENIA AND PARADOXICAL THROMBOSIS

A. Heparin-Induced Thrombocytopenia

Routine platelet count measurements were not a feature of hospital laboratory practice until the 1970s, and neither the Dartmouth nor Philadelphia surgeons reported thrombocytopenia in their patients with heparin-induced arterial thrombosis. Ironically, the first report of severe heparin-induced thrombocytopenia (HIT) involved a patient who did not develop paradoxical thrombosis. Natelson and coworkers (1969) reported on a 78-yr-old man with prostate carcinoma and pulmonary embolism, who on day 10 of treatment with therapeutic-dose heparin developed severe thrombocytopenia. Three days after discontinuing the heparin therapy, the patient's fibrinogen fell to 1 g/L, attributed to carcinoma-associated disseminated intravascular coagulation (DIC). Heparin treatment was restarted and, although fibrinogen levels normalized, the platelet count fell to 5×10^9/L, rising to 115×10^9/L 6 days after stopping heparin administration. Simultaneously, however, the fibrinogen value fell to less than 0.5 g/L. When heparin was given for the third time, the platelet count fell over 2 days to 10×10^9/L, although the fibrinogen values again normalized. In vitro studies showed that heparin added to the patient's citrated platelet-rich plasma produced platelet count reductions. This early report of severe HIT is interesting, as it illustrates the dichotomy of heparin reproducibly producing severe thrombocytopenia while at the same time maintaining anticoagulant activity (correction of DIC). However, it remained for later workers to link thrombocytopenia and thrombosis to heparin therapy.

B. Rhodes, Dixon, and Silver: "HIT with Thrombotic and Hemorrhagic Manifestations"

Laboratory evidence implicating an immune basis for HIT was first provided by studies performed by a vascular surgeon (Donald Silver; Fig. 2), with two medical housestaff (Glen R. Rhodes and R. H. Dixon). The first two patients described by Silver's group (Rhodes et al., 1973) developed severe thrombocytopenia (platelet count nadirs, 8 and 10×10^9/L), myocardial infarction, petechiae, and heparin resistance, with complete platelet count recovery on discontinuing heparin treatment.

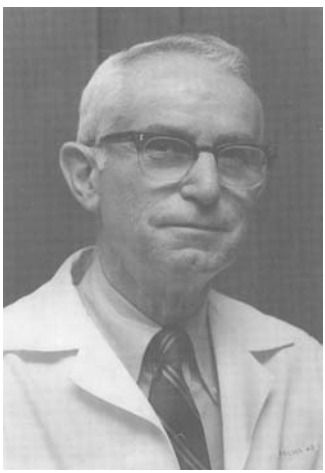

FIGURE 2 Photograph of Dr. Donald Silver, taken circa 1975.

Both patients developed rapid recurrence of thrombocytopenia when heparin rechallenges were given within 1 wk of platelet count recovery.

The immune basis of this syndrome was suggested by several laboratory observations. First, increased platelet consumption was suggested by increased numbers of marrow megakaryocytes, as well as immediate recurrence of thrombocytopenia on reexposure to heparin. Second, a circulating platelet-activating substance was found in both patients' blood: patient, but not control, serum resulted in aggregation of normal donor platelets in the presence of heparin. Third, the possible identity of the aggregating agent as an immunoglobulin G (IgG) was shown by fractionation of one patient's serum to show the presence of heparin-dependent, complement-fixing activities within the IgG fraction.

A second report from this group (Rhodes et al., 1977) represented the landmark study in establishing HIT as a distinct syndrome. Eight patients were reported with thrombocytopenia that occurred during intravenous therapeutic-dose or subcutaneous prophylactic-dose heparin. The mean platelet count nadir was 25 (range, 5–54 $\times 10^9$/L). The predominance of thrombotic, rather than hemorrhagic, complications was demonstrated: seven patients had new or recurrent thromboembolic events, and the remaining patient had a stroke leading to evacuation of a temporal lobe hematoma. Complement-fixing, heparin-dependent antibodies were identified in five of the patients. The authors also cited the previous work by Weismann and Tobin (1958) and Roberts and colleagues (1964) as likely representing the identical syndrome. Thus, for the first time, the concept of an immune-mediated hypercoagulable state, with a predisposition to arterial thromboembolism that occurred in association with thrombocytopenia, was proposed.

C. Platelet-Activating Antibodies in the Pathogenesis of HIT

Although some limited studies of heparin-dependent platelet aggregation by patient serum were performed in the classic study by Rhodes and colleagues (1973), the next few years saw increasing emphasis on this characteristic feature of HIT antibodies. In 1975, National Institutes of Health investigators Fratantoni et al. described a patient who developed severe thrombocytopenia (4×10^9/L) and pulmonary embolism while receiving therapeutic-dose unfractionated heparin (UFH) to treat deep venous thrombosis. Recurrent thrombocytopenia resulted following heparin rechallenge. The patient's serum produced both aggregation and serotonin release from normal platelets in the presence of heparin. The platelet-activating factor was presumed, but not proved, to be caused by an antibody.

During the next 5 yr, at least eight groups of investigators reported similar patients, confirming the presence of heparin-dependent, platelet-activating antibodies (Babcock et al., 1976; Green et al., 1978; Nelson et al., 1978; Trowbridge et al., 1978; Wahl et al., 1978; Cimo et al., 1979; Hussey et al., 1979; Cines et al., 1980). Babcock and colleagues (1976) described five patients who developed thrombocytopenia (mean platelet count nadir, 28×10^9/L) during heparin treatment; heparin-dependent antibodies were detected that produced platelet factor 3 activity (i.e., patient globulin fractions incubated with heparin, platelet-rich plasma, and celite-activated contact product shortened the clotting time following recalcification). Three patients developed thrombotic complications, and none developed hemorrhage. The five patients were observed within a 6 wk time span, leading the authors to suggest that "this syndrome may occur more often than has previously been suspected."

A consistent theme was evident from these various reports. Patients developed arterial or venous thrombotic complications, in association with thrombocytopenia that generally began after 5 or more days of heparin treatment. A platelet-activating antibody that aggregated platelets suspended in citrated plasma was usually detected. The platelet count nadirs seen in some of the larger series (e.g., 33 and 48 $\times$ 10^9/L, respectively) observed by Cimo et al. (1979) and Hussey et al. (1979), were higher than in previous reports, indicating that as recognition of the syndrome grew, less severely thrombocytopenic patients were recognized.

D. The "White Clot Syndrome"

Jonathan Towne, a vascular surgeon in Milwaukee, reported with his colleagues (1979) that the pale thrombi characteristic of this syndrome consisted of fibrin-platelet aggregates (electron microscropy). These workers coined the term "white clot syndrome" to describe the characteristic appearance of these arterial thromboemboli. Ironically, their report is also the first to note the occurrence of phlegmasia cerulea dolens (severe venous limb ischemia) that progressed to venous limb gangrene in two of their patients (i.e., a syndrome of limb loss due to extensive venous thrombosis without arterial white clots). Nonetheless, the designation of white clot syndrome has become virtually synonymous with HIT in both North America and Europe (Benhamou et al., 1985; Stanton et al., 1988), despite the lack of specificity of these thrombi for HIT (see Chapter 11).

IV. NONIMMUNE HEPARIN-ASSOCIATED THROMBOCYTOPENIA

A. Nonimmune Mechanisms in Heparin-Associated Thrombocytopenia

Klein and Bell (1974) reported on two patients who developed severe thrombocytopenia, thrombotic complications, and DIC, with hypofibrinogenemia and microangiopathic red cell abnormalities; i.e., these patients likely had severe HIT. This experience prompted Bell to perform the first prospective study investigating the frequency of thrombocytopenia complicating therapeutic-dose UFH (Bell et al., 1976). Sixteen of 52 patients (31%) developed a platelet count fall to less than 100 $\times$ 10^9/L, and some of these patients developed hypofibrinogenemia and elevated fibrin(ogen) degradation products. The authors speculated that a "thromboplastic contaminant" extracted along with heparin from beef lung could explain the thrombocytopenia. A subsequent randomized controlled trial by Bell and Royall (1980) found the frequency of thrombocytopenia to be higher in patients who received bovine heparin (26%) compared with heparin of porcine intestinal origin (8%).

These investigators found no platelet-activating antibodies in plasma from the patients who developed thrombocytopenia (Alving et al., 1977), leading Bell (1988) to challenge the view that an immune pathogenesis explained HIT. However, as the Johns Hopkins group did not report thrombotic complications in any of their 37 patients who developed thrombocytopenia in their prospective studies, and given the apparent early onset of thrombocytopenia in many of their patients, it is likely that most of their patients did not have immune-mediated HIT.

B. Nonimmune (Type I) Versus Immune (Type II) HIT

A confusing situation arose. The terms "heparin-induced thrombocytopenia" or "heparin-associated thrombocytopenia" were often applied to any patient who

developed thrombocytopenia during heparin therapy, whether presumed or proved to be caused by heparin-dependent antibodies or otherwise. Investigators in Australia, led by Chong (1981), also observed patients with thrombocytopenia in whom heparin-dependent, platelet-activating IgG antibodies could be identified. In a subsequent report that appeared in the *Lancet*, two distinct syndromes of "HIT" were described by Chong and colleagues (1982). The first, called "group 1," developed severe, delayed-onset thrombocytopenia with thrombotic complications in association with IgG antibodies that caused platelet activation. In contrast, "group 2" patients had mild asymptomatic thrombocytopenia of early onset.

In 1989, at a Platelet Immunobiology Workshop in Milwaukee, it was suggested to Chong that terminology describing these two types of HIT be formalized. Accordingly, Chong recommended the terms in a review article that appeared in *Blut* (Chong and Berndt, 1989), although (in reverse of the *Lancet* article nomenclature) the early, nonimmune disorder was named "HIT type I" and the later-onset, immune disorder referred to as "HIT type II." These terms subsequently became popular.

V. LABORATORY TESTING TO CHARACTERIZE THE HIT SYNDROME

A. A Sensitive and Specific Platelet Activation Assay for HIT

Many clinical laboratories began to use platelet aggregation assays (Fratantoni et al., 1975; Babcock et al., 1976) to diagnose HIT. Problems with this type of assay, however, included low sensitivity (Kelton et al., 1984) as well as technical limitations in simultaneous evaluation of multiple patient and control samples. In 1983–1984, while working as a research fellow in the McMaster University laboratory of John Kelton, Dave Sheridan overcame problems of low test sensitivity by showing that washed platelets, resuspended in a buffer containing physiological concentrations of divalent cations, were very sensitive to platelet activation by HIT sera (Sheridan et al., 1986). The assay, known as the "platelet serotonin release assay (SRA)," was adapted from a method of platelet washing developed at McMaster University by the laboratory of Dr. Fraser Mustard. In particular, the emphasis on using physiological calcium concentrations was based on observations that "artifacts" of agonist-induced platelet activation were caused by the use of citrate anticoagulation resulting in low plasma calcium concentrations. One example of an artifact induced by citrate is that of two-phase aggregation triggered by adenosine diphosphate (ADP). At physiological calcium concentrations, only weak single-phase aggregation without thromboxane generation is triggered by ADP (Kinlough-Rathbone et al., 1983). Fortuitously, the washed platelet technique previously developed at McMaster University by Mustard and colleagues that Sheridan evaluated for its HIT serum-sparing properties rendered platelets far more sensitive to the platelet-activating properties of HIT antibodies than assays based on citrated platelet-rich plasma. Modified washed platelet assays have subsequently been developed by other investigators (see Chapter 10).

Sheridan and colleagues also made the observation that heparin concentrations strongly influenced platelet activation by HIT sera: therapeutic (0.05–1 U/mL), but not high (10–100 U/mL), heparin concentrations resulted in platelet activation, i.e., the characteristic "two-point" serotonin release activation profile of HIT. Later, Greinacher and colleagues (1994) showed that high heparin concentrations in solution release platelet factor 4 (PF4) from PF4-heparin complexes bound

covalently to a solid phase, with a corresponding decrease in binding of HIT antibodies to the surface. Thus, the inhibition of platelet activation by high heparin concentrations probably results from a similar disruption of the multimolecular antigen complex on the platelet surface.

The high sensitivity of washed platelets to activation by HIT antibodies led to new insights into the pathogenesis of platelet activation. For example, 2 yr after describing their washed platelet assay for HIT, Kelton and coworkers (1988) reported that the platelet activation process was critically dependent on the platelet Fc receptor. This represented a fundamental new pathobiological mechanism in a drug-induced thrombocytopenic disorder.

B. Prospective Studies of Serologically Defined HIT

Although several prospective studies of the frequency of HIT were performed (see Chapter 3), until the 1990s, none had systematically evaluated serum or plasma from study participants for HIT antibodies. Often the distinction between "early" and "late" thrombocytopenia was blurred. Thus, the relative frequency and clinical importance of immune versus nonimmune HIT were unclear. This is illustrated by a prospective study reported by Powers and colleagues (1979) that found HIT to be "uncommon" during treatment with porcine mucosal heparin, as "only" 4 of 120 (3%) patients developed thrombocytopenia, in contrast with the 26–31% frequency of thrombocytopenia reported for bovine lung heparin. However, 2 of these 120 patients probably died as a result of HIT-associated thrombosis (Warkentin and Kelton, 1990), underscoring the need for a specific laboratory marker for this immune-mediated syndrome.

In a prospective study of HIT that performed systematic testing for HIT antibodies (Warkentin et al., 1995), the authors showed the dramatic clinical effects of HIT. Of 665 patients participating in a clinical trial of UFH versus low molecular weight heparin (LMWH) after orthopedic surgery, nine patients developed "late" thrombocytopenia serologically confirmed to represent HIT. These patients had a thrombotic event rate far greater than controls. Moreover, the spectrum of thrombosis in HIT patients included venous thromboembolism, rather than only the classic problem of arterial thrombosis. This study also showed that early postoperative thrombocytopenia occurred frequently, but was not explained by HIT antibodies (see Chapter 3).

However, even this study did not initially capture the complete clinical profile of HIT. This is because it defined the platelet count fall indicating possible HIT using the "standard" definition of thrombocytopenia, i.e., a platelet count fall to less than 150×10^9/L (Warkentin et al., 1995). Subsequent review of the database, together with correlative analysis of the results of systematic serological testing for HIT antibodies (performed in most study subjects), showed that this standard definition underestimated the number of patients who had HIT (Warkentin et al., 2003). Rather, a proportional fall in platelet count (50% or greater)—in relation to the postoperative peak platelet count—provided a more accurate definition of thrombocytopenia (applicable at least to this postoperative patient population). This improved definition identified twice as many patients as having had HIT in this clinical trial, without compromising diagnostic specificity. Indeed, the study suggested that the risk of immune HIT is about 5% ($16/332 = 4.8\%$) in postoperative orthopedic surgery patients receiving UFH for a week or more (see Chapter 3).

VI. THE TARGET ANTIGEN OF HIT: PF4–HEPARIN

In 1992, Jean Amiral, working in the laboratory of Dominique Meyer, reported that the antigen recognized by HIT antibodies was a complex between heparin and PF4, an endogenous platelet α-granule protein (Amiral et al., 1992). This important discovery led to an explosion of basic studies in numerous laboratories that led to further characterization of the basic pathogenesis of HIT (see Chapters 4–9). Amiral's discovery also led to the development of new assays for HIT antibodies based on enzyme immunoassay techniques (see Chapter 10).

The antigen site(s) recognized by HIT antibodies were identified as being on PF4, rather than on heparin itself or a compound antigen (Li et al., 2002) (see Chapters 5–7). This observation highlights intriguing parallels between HIT and the antiphospholipid syndrome. This latter disorder is also characterized by pathogenic antibodies directed against one or more proteins that express neoepitopes when bound to certain negatively charged phospholipid surfaces (see Chapter 11). The presence of neoepitopes on the "self" protein, PF4, suggests that HIT can be conceptualized as a transient, drug-induced, platelet- and coagulation-activating autoimmune disorder. Indeed, high-titer HIT antibodies that are able to activate platelets in vitro even in the absence of pharmacologic heparin have been associated with the onset of thrombocytopenia and thrombosis beginning several days after heparin has been discontinued, so-called delayed-onset HIT (Warkentin and Kelton, 2001) (see Chapter 2).

VII. TREATMENT OF THROMBOSIS COMPLICATING HIT

The treatment of HIT is discussed in Chapters 12–21. Here we will discuss only a few vignettes relating to the initial use of selected treatments for HIT.

A. Danaparoid Sodium

In 1982, a 48-yr-old vacationing American developed deep venous thrombosis and pulmonary embolism following a transatlantic flight to Germany. Heparin treatment was complicated by thrombocytopenia and progression of venous thrombosis. Professor Job Harenberg of Heidelberg University, who had performed phase I evaluations of the experimental glycosaminoglycan anticoagulant danaparoid, requested this agent from the manufacturer (NV Organon, The Netherlands). The platelet count recovered and the venous thrombosis resolved (Harenberg et al., 1983, 1997). Over the next 6 yr, this patient developed recurrent thromboembolic events, and was successfully treated each time with danaparoid. This favorable experience led to a named-patient, compassionate-release program ending in March 1997, during which time, over 750 patients were treated with this agent. Additionally, Chong and colleagues (2001) performed the first randomized, controlled clinical trial evaluating danaparoid (see Chapter 13).

B. Recombinant Hirudin (Lepirudin)

The medicinal leech, *Hirudo medicinalis*, has been used for medical purposes for many centuries. Given the observation that the medicinal leech can prevent clotting of blood it has ingested, crude preparations derived from this animal were given experimentally at the beginning of the twentieth century. However, because this treatment's daily cost (75 Reichsmark) in 1908 was equivalent to the monthly salary of a factory worker, it was judged to be infeasible. After World War I, Haas,

at Justus-Liebig University in Giessen, began his experiments using crude extracts of leech heads for hemodialysis. The major complication in these animal experiments was severe bleeding. The first human hemodialysis patients were treated by him with hirudin during dialysis when a more purified, but still crude protein extract of leech heads became available (Haas, 1925).

In 1956, Dr. F. Markwardt began his work to extract the active component of the leech at the Ernst-Moritz-Arndt University, in Greifswald. Still today, elderly peasants in the small villages around Greifswald tell stories of how they earned their pocket money by collecting leeches for the researchers at the nearby medical school.

The production of large amounts of hirudin by recombinant technology allowed assessment of this direct thrombin inhibitor in clinical trials. Dr. Andreas Greinacher, at that time working at the Justus-Liebig University in Giessen, first used a recombinant hirudin (lepirudin) to anticoagulate a patient who developed acute HIT following heart transplantation. After Greinacher's move to Greifswald, he further assessed the use of hirudin in patients with HIT in two clinical studies that led to the first approval of a drug for parenteral anticoagulation of patients with HIT in both the European Community (March 1997) and the United States (March 1998) (Greinacher et al., 1999) (Table 1).

C. Warfarin-Induced Venous Limb Gangrene

A theme of this book is the central importance of increased thrombin generation in the pathogenesis of thrombosis complicating HIT. The recognition that warfarin therapy can be deleterious in some patients with HIT illustrates the importance of uncontrolled thrombin generation in HIT.

TABLE 1 U.S. Approvals for Three Direct Thrombin Inhibitors

	Date of U.S. approval		
Use	Lepirudin	Argatroban	Bivalirudin
HIT indications			
For patients with HIT and associated thromboembolic disease in order to prevent further thromboembolic complications	March 6, 1998		
For prophylaxis or treatment of thrombosis in patients with HIT		June 30, 2000	
Anticoagulation in patients with or at risk for HIT undergoing PCI		April 3, 2002	
For patients with or at risk of HIT/HITTS undergoing PCI			November 30, 2005
Non-HIT indications			
Use as an anticoagulant in patients with unstable angina undergoing PTCA			December 15, 2000
Use (with provisional use of GP IIb/IIIa inhibitor) as an anticoagulant in patients undergoing PCI			June 13, 2005

Abbreviations: GP, glycoprotein; HIT, heparin-induced thrombocytopenia; HITTS, heparin-induced thrombocytopenia/thrombosis syndrome; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty.

In December 1992, in Hamilton, Canada, while receiving ancrod and warfarin treatment for deep vein thrombosis complicating HIT, a 35-yr-old woman developed progressive venous ischemia, culminating in venous limb gangrene. This occurred despite a supratherapeutic international normalized ratio (INR). The following day, Kelton observed an area of skin necrosis on the abdomen of this patient, suggesting the diagnosis of warfarin-induced skin necrosis. The author questioned whether the warfarin had also contributed to the pathogenesis of the venous limb gangrene. This hypothesis was directly tested just 2 mo later when a second young woman developed severe phlegmasia cerulea dolens of an upper limb during treatment of deep vein thrombosis complicating HIT with ancrod and warfarin. Treatment with vitamin K and plasma given by pheresis reversed the phlegmasia. Further laboratory studies supported this hypothesis of a disturbance in procoagulant-anticoagulant balance during treatment of HIT with warfarin (Warkentin et al., 1997) (see Chapters 2, 11, and 12).

Increasingly, HIT became viewed as a syndrome characterized by multiple prothrombotic events, including not only platelet and endothelial cell activation, but also profound activation of coagulation pathways. This conceptual framework provides a rationale for antithrombotic therapy that reduces thrombin generation in patients with HIT (Warkentin et al., 1998).

VIII. TREATMENT OF ISOLATED HIT

Isolated HIT refers to HIT diagnosed on the basis of thrombocytopenia alone, rather than because of HIT-associated thrombosis. Often, the initial reason for administering heparin includes routine postoperative prophylaxis or a medical indication such as acute stroke or myocardial infarction. Until the early 2000s, the standard approach upon suspecting HIT in such patients was discontinuation of heparin, sometimes with substitution of oral anticoagulants.

A. Natural History of Isolated HIT

During the mid-1990s, new data indicated a high risk for venous thrombosis in postoperative orthopedic patients who developed HIT, particularly for pulmonary embolism (Warkentin et al., 1995) (see Chapter 2). Thus, HIT came to be viewed as a dramatic, albeit transient, prothrombotic state, even when the original indication for heparin was routine antithrombotic prophylaxis.

In July 1992, the author became aware of a 68-yr-old patient whose platelet count fell from 151 to 51×10^9/L between days 5 and 8 following coronary artery bypass surgery, during routine postoperative heparin antithrombotic prophylaxis. The heparin was stopped, and laboratory testing confirmed HIT. The platelet count recovered, and the patient was discharged to home on postoperative day 12. Three days later, the patient complained of dyspnea, and then died suddenly. Postmortem examination showed massive pulmonary embolism. This tragic outcome prompted the question: Is mere cessation of heparin sufficient for a patient with isolated HIT?

To address this problem, the author performed a study of the natural history of HIT (Warkentin and Kelton, 1996). From a database of patients with serologically proven HIT, a 62-patient cohort with isolated HIT was identified: the cumulative 30-day thrombotic event rate was 52.8% (see Fig. 2 in Chapter 3). The rate of thrombosis was similarly high whether heparin was simply stopped or substituted with warfarin.

Similar findings were reported later by Wallis and colleagues (1999) from Loyola University. These investigators also found a high frequency of subsequent thrombosis (43 of 113, or 38%) in patients with isolated HIT managed by discontinuation of heparin. Surprisingly, a trend was observed for the highest risk of thrombosis in those patients in whom heparin was stopped most promptly (see Table 7 in Chapter 3).

Further evidence supporting an unfavorable natural history of untreated HIT was provided by a prospective cohort study (Greinacher et al., 2000). These investigators found that the thrombotic event rate was 6.1% per day during the mean 1.7-day interval between diagnosis of HIT (and cessation of heparin) and initiation of lepirudin therapy. This event rate corresponded closely to the 10% rate of thrombosis observed in the Hamilton study in the first 48 h following diagnosis of isolated HIT (Warkentin and Kelton, 1996).

B. Argatroban

A synthetic small-molecule thrombin inhibitor derived from L-arginine, now known as argatroban, was used in Japan during the 1980s as a treatment for chronic arterial occlusion (Tanabe, 1986). During this time, argatroban also underwent investigation as treatment for HIT in Japan, particularly in the setting of hemodialysis (Matsuo et al., 1988). In 1993, exclusive rights to the compound for the United States and Canada were acquired from Mitsubishi-Tokyo Pharmaceuticals, Inc. by Texas Biotechnology Corporation (TBC) of Houston. In 1995, clinical evaluation of this agent for HIT began in the United States, using a prospective, multicenter, open-label design with historical controls, the ARG-911 study (Lewis et al., 2001) (see Chapter 15). Two groups of patients were studied: HIT without thrombosis (i.e., isolated HIT) and HIT complicated by thrombosis (heparin-induced thrombocytopenia/thrombosis syndrome [HITTS]). Eligibility was based on clinical suspicion of HIT, and serological confirmation of the diagnosis, therefore, was not required. Both patient groups received the identical therapeutic-dose regimen of argatroban (initially, 2 μg/kg/min, then adjusted by activated partial thromboplastin time [aPTT]). The favorable results of the ARG-911 and subsequent studies (ARG-915, ARG-915X) led to the approval of argatroban on June 30, 2000, by the U.S. Food and Drug Administration (FDA) as "anticoagulant for prophylaxis or treatment of thrombosis in patients with HIT" (Table 1). Thus, for the first time in the United States, a drug was approved for the novel indication of prevention of thrombosis in isolated HIT. A marketing partnership between TBC (now, Encysive) and SmithKline Beecham (now, GlaxoSmithKline) commenced in August 1997. Marketing of argatroban began on November 13, 2000. More recently (April, 2002) argatroban received approval for anticoagulation in patients with or at risk for HIT undergoing percutaneous coronary intervention (PCI).

C. Therapeutic-Dose Anticoagulation for Isolated HIT

The approval by the FDA of identical therapeutic-dose regimens of argatroban for both prophylaxis and treatment of HIT highlighted the emerging view that HIT is a high-risk prothrombotic state. This contrasted with the earlier concept that HIT was generally benign, provided that thrombocytopenia was promptly recognized and heparin discontinued. Further support for the new view included studies showing HIT to be a profound hypercoagulable state (markedly elevated

molecular markers of in vivo thrombin generation) (Warkentin et al., 1997; Greinacher et al., 2000) and recognition that many patients already have subclinical deep vein thrombosis at the time that isolated HIT is first recognized (Tardy et al., 1999).

Indeed, therapeutic doses of an alternative anticoagulant might be generally applicable for treatment of most patients with isolated HIT (Farner et al., 2001) (see Chapters 12–15). For example, although the prophylactic-dose regimen of lepirudin for HIT is initially lower than the therapeutic-dose regimen (0.10 mg/kg/h, rather than 0.15 mg/kg/h, and without an initial lepirudin bolus), subsequent dose adjustments are made using the aPTT; thus, the eventual infusion rate approaches the one given using the therapeutic regimen (see Chapters 12 and 14 for current recommendations regarding dosing of lepirudin). A high success rate (91.4%) was observed using such "prophylactic" doses of lepirudin for isolated HIT (Farner et al., 2001).

In contrast, the prophylactic-dose regimen using danaparoid (750 U bid or tid) may be somewhat less effective than therapeutic-dose danaparoid (usually, 150–200 U/h after an initial bolus) for preventing new thromboembolic complications in acute HIT: 81.4% versus 91.6% (Farner et al., 2001) (see Chapter 13). If this difference is real, it could be explained by greater efficacy of the therapeutic-dose regimen, in which at least twice as much danaparoid is usually given (3600–4800 vs. 1500–2250 U/24 h). The implication of Farner's study is that the approved prophylactic-dose regimen of danaparoid may not be optimal, either when used for its approved indication in Europe (i.e., prevention of HIT-associated thrombosis) or for the corresponding "off-label" use for HIT elsewhere (Warkentin, 2001) (see Chapter 12).

D. Bivalirudin

The 20-amino acid hirudin analog, bivalirudin (Angiomax, formerly, Hirulog), was first used over 10 yr ago in the United States on a compassionate use basis for the treatment of four patients with HIT (Nand, 1993; Reid and Alving, l994; Chamberlin et al., 1995). Since then, it has undergone limited off-label use for the treatment of HIT (Francis et al., 2003), often in patients with both renal and hepatic compromise (see Chapter 16). In contrast to its limited use in managing HIT, bivalirudin is widely used for anticoagulation in the setting of percutaneous transluminal coronary angioplasty (PTCA) as well as other types of PCI (Warkentin and Koster, 2005). Indeed, bivalirudin is the only direct thrombin inhibitor that is approved for an indication beyond that involving HIT (Table 1). In November 2005, approval was also granted for use of bivalirudin for anticoagulation of patients with (or at risk of) HIT (or HIT-associated thrombosis) undergoing PCI (Table 1).

IX. REDUCING THE RISK OF HIT

A. Low Molecular Weight Heparin

For over 50 yr, UFH has been used in numerous clinical situations. However, UFH has several limitations, and efforts to develop potentially superior LMWH preparations began during the 1980s. Advantages of LMWH included better pharmacokinetics (e.g., improved bioavailability, predictable and stable dose response obviating the need for monitoring, lower risk of resistance to anticoagulation, longer plasma half-life) and favorable benefit-risk ratios in experimental animals (Hirsh, 1994; Hirsh et al., 2001). Advantages of UFH include its low cost, widely available

TABLE 2 U.S. Approvals for Enoxaparin and Fondaparinux

Use[a]	Date of U.S. approval	
	Enoxaparin[b]	Fondaparinux
Prophylaxis after hip replacement surgery	March 29, 1993	December 7, 2001
Prophylaxis after knee replacement surgery	March 9, 1995	December 7, 2001
Prophylaxis after hip fracture surgery	–	December 7, 2001
Extended prophylaxis after hip replacement surgery	January 30, 1998	–
Extended prophylaxis after hip fracture surgery	–	June 17, 2003
Prophylaxis after general (abdominal) surgery	May 6, 1997	May 26, 2005
Prophylaxis for unstable angina and non-Q wave myocardial infarction (given together with aspirin)	March 27, 1998	Under regulatory review[c]
Acute deep-vein thrombosis, with or without pulmonary embolism,[d,e] together with warfarin	December 31, 1998	May 28, 2004
Prophylaxis in medical patients at risk for deep-vein thrombosis or pulmonary embolism	November 17, 2000	–

[a]Use described may not necessarily conform precisely to the wording of the approved indications.
[b]Other LMWH preparations (dalteparin, tinzaparin) have been approved (at later times) for various indications (not shown).
[c]Fondaparinux has been recently studied extensively for treatment of patients with acute coronary syndrome and will undergo FDA review for use in that setting.
[d]Wording of approved indication for enoxaparin Includes "inpatient" treatment of acute deep vein thrombosis with or without pulmonary embolism and "outpatient" treatment of acute deep vein thrombosis without pulmonary embolism.
[e]Wording of approved indication for fondaparinux: "for the treatment of acute deep vein thrombosis when administered in conjunction with warfarin sodium; and the treatment of acute pulmonary embolism when administered in conjunction with warfarin sodium when initial therapy is administered in the hospital".

laboratory monitoring, and potential for neutralization using protamine. But the question remained: Was the risk of HIT lower with LMWH? This was an important and relevant question, particularly as differences in risk of HIT exist even among UFH preparations derived from different animal sources (see Chapter 3). As discussed earlier (Sec. V.B), there is indeed evidence that LMWH has both a lower risk of HIT antibody formation and (more importantly) a lower risk of HIT and HIT-associated thrombosis. Table 2 provides a historical timeline of the introduction of the LMWH enoxaparin in the United States in various clinical situations.

B. Fondaparinux

Fondaparinux (Arixtra®) is a synthetic pentasaccharide modeled after the antithrombin-binding site of heparin. It selectively binds to antithrombin, causing rapid and specific inhibition of factor Xa. In contrast to LMWH, HIT antibodies fail to recognize PF4 mixed with fondaparinux, both in platelet activation and PF4-dependent antigen assays (Warkentin et al., 2005).

Interestingly, evidence suggests that although HIT antibody formation occasionally occurs in association with fondaparinux use, such antibodies fail to react in HIT assays in which fondaparinux replaces UFH or LMWH in vitro (Warkentin et al., 2005; Pouplard et al., 2005). Thus, this pentasaccharide anticoagulant seems unlikely to cause an adverse effect resembling HIT. Although no patients developed HIT with either LMWH (enoxaparin) or fondaparinux in the two orthopedic surgery trials reported (Warkentin et al., 2005), the duration of anticoagulant

therapy may have been too brief to reveal a true difference in risk of immune thrombocytopenia between LMWH (frequency 0.1–1.0%) and fondaparinux (anticipated negligible frequency). Fondaparinux is approved in the United States, Canada, and the European Union for antithrombotic prophylaxis in orthopedic surgery as well as other clinical situations (Table 2).

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2 Clinical Picture of Heparin-Induced Thrombocytopenia

Theodore E. Warkentin
Michael G. DeGroote School of Medicine, McMaster University, and Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada

I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a distinct clinicopathologic syndrome caused by platelet-activating antibodies that recognize complexes of platelet factor 4-heparin (PF4/H). Its strong association with venous and arterial thrombosis represents a striking paradox. However, thrombocytopenia itself is common in clinical medicine. Furthermore, heparin is usually given to patients who either have thrombosis, or who are judged to be at high risk for thrombosis. Thus, thrombocytopenia with or without thrombosis during heparin treatment does not necessarily indicate a diagnosis of HIT. Indeed, several disorders can closely resemble HIT (see Chapter 11).

On the other hand, HIT is associated with a wide spectrum of unusual thrombotic and other complications (Table 1). Unrecognized HIT may have been an important contributing factor in otherwise bizarre clinical events that have occurred in certain heparin-treated patients (Anderson et al., 1981; Solomon et al., 1988; Pfueller et al., 1990; Muntean et al., 1992). Laboratory documentation of HIT antibodies has been crucial in determining the clinical scope of the HIT syndrome. Accordingly, this chapter emphasizes clinical data obtained from large prospective and retrospective studies that have used diagnostic testing for HIT antibodies.

Estimated frequencies of the various complications of HIT are taken from reports with serological confirmation of the diagnosis (Warkentin et al., 1995, 1997; Warkentin and Kelton, 1996). "Rare" indicates an estimated frequency <3% of HIT patients.

II. THROMBOCYTOPENIA

Thrombocytopenia, using the standard definition of a platelet count of less than 150×10^9/L, is the most common clinical effect of HIT, occurring in 85–90% of patients (Warkentin, 1998a). An even higher proportion develops "thrombocytopenia" if a definition appropriate for the clinical situation is used.

A. Timing

The characteristic delay of 5 or more days between initiation of heparin and onset of thrombocytopenia was the major clue that led early investigators to recognize the immune pathogenesis of HIT (Roberts et al., 1964; Rhodes et al., 1973). King and Kelton (1984) noted that thrombocytopenia occurred between days 6 and 15 for more than 90% of patients in whom HIT occurred during their first exposure to heparin. In contrast, for patients who developed HIT during a repeat course of heparin, the onset of thrombocytopenia was often more rapid, occurring within 2 days. These data have been interpreted as indicating an "anamnestic"

TABLE 1 Thrombotic and Other Sequelae of HIT

Venous thrombosis	Arterial thrombosis	Miscellaneous
DVT (50%): new, progressive, recurrent; lower limb (often bilateral); upper limb (at site of venous catheter); phlegmasia cerulea dolens	Aortic or iliofemoral thrombosis resulting in acute limb ischemia/infarction (5–10%) or spinal cord infarction (rare)	Heparin-induced skin lesions at heparin injection sites (10–20%): Erythematous plaques Skin necrosis
Coumarin-induced venous limb gangrene (~5–10% of DVT treated with warfarin)	Acute thrombotic stroke (3–5%)	Coumarin-induced skin necrosis complicating HIT involving "central" sites (breast, abdomen, thigh, leg, etc.) (rare)
Pulmonary embolism (25%): with or without right-sided cardiac intra-atrial or intraventricular thrombi	Myocardial infarction (3–5%)	Acute systemic reactions postintravenous heparin bolus (~25% of sensitized patients who receive an intravenous heparin bolus): Inflammatory: e.g., fever, chills, flushing
Cerebral dural sinus thrombosis (rare)	Cardiac intraventricular or intra-atrial thrombosis, in situ or via embolization of DVT (rare)	Cardiorespiratory: e.g., tachycardia, hypertension, dyspnea; cardiopulmonary arrest (rare)
Adrenal hemorrhagic infarction (rare): bilateral (acute or chronic adrenal failure) or unilateral	Thrombosis involving miscellaneous arteries (rare): upper limb, renal, mesenteric, spinal, and other arteries	Gastrointestinal: nausea, vomiting, diarrhea
DIC, with hypofibrinogenemia and acquired natural anticoagulant deficiency, causing multiple venous and arterial thromboses (rare)	Embolization of thrombus from heart or proximal aorta can also contribute to microvascular ischemic syndromes	Neurological: transient global amnesia, headache

Abbreviations: DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis.

(Gr., memory) or "secondary" immune response in HIT; i.e., the immune system produces HIT antibodies more quickly on reencountering an antigen "remembered" within its memory cell repertoire. Recent data, however, suggest another explanation for these two temporal profiles of HIT, typical and rapid (discussed subsequently).

Typical Onset of HIT

A prospective study of serologically confirmed HIT showed that the platelet count typically begins to fall between days 5 and 10 (inclusive) of postoperative subcutaneous heparin prophylaxis (Warkentin et al., 1995, 2003) (Fig. 1). Note that the data refer to the day the platelet count begins to fall, and not the later day on which an arbitrary threshold defining thrombocytopenia is crossed. This study also showed that most patients who developed thrombocytopenia beginning after day 5 had HIT rather than another explanation for the thrombocytopenia. The data suggest the following clinical rule:

Rule 1

A thrombocytopenic patient whose platelet count fall began between days 5 and 10 of heparin treatment (inclusive) should be considered to have HIT unless proved otherwise (first day of heparin use is considered "day 0").

HIT-IgG antibodies generally are not detectable before day 5 of heparin treatment, but are readily detectable using sensitive assays when the platelet count first begins to fall due to HIT.

A recent study (Warkentin and Kelton, 2001a) that analyzed temporal aspects of the platelet count fall in 243 patients with serological confirmed HIT in

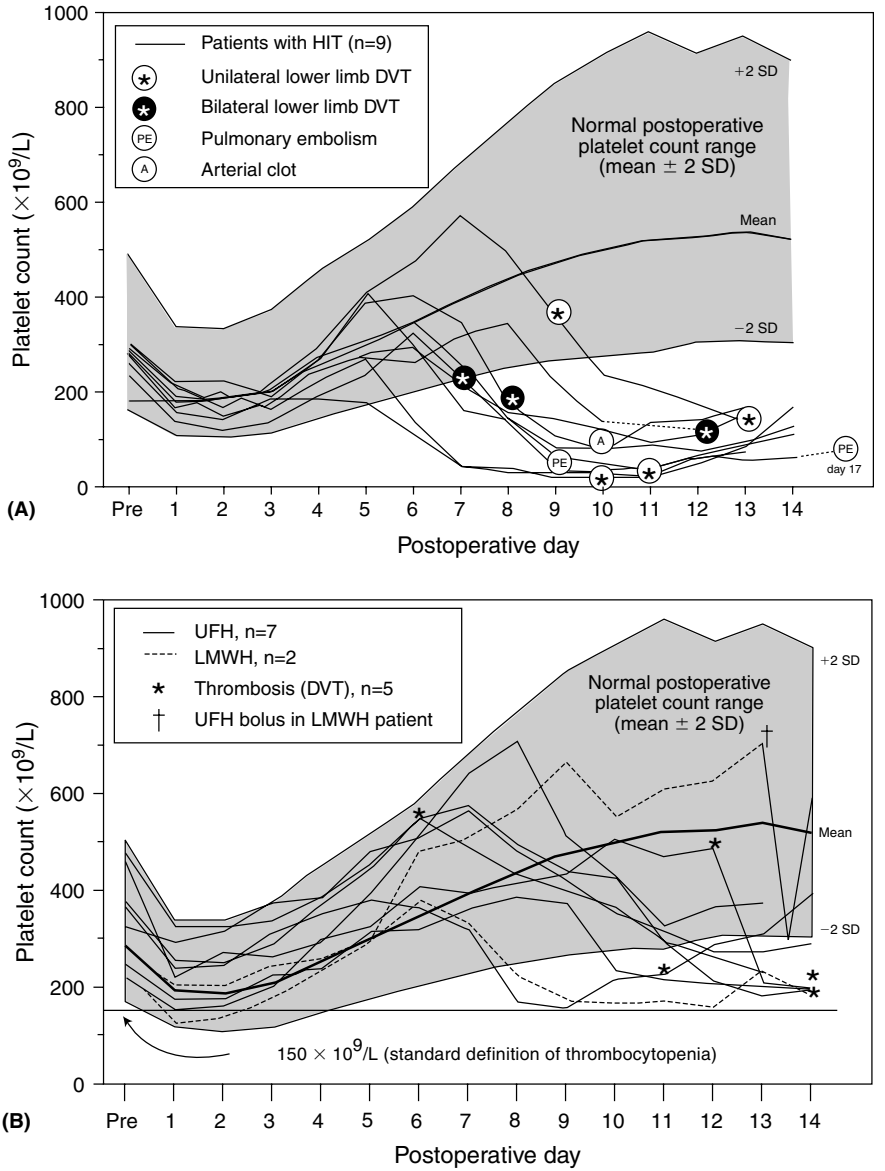
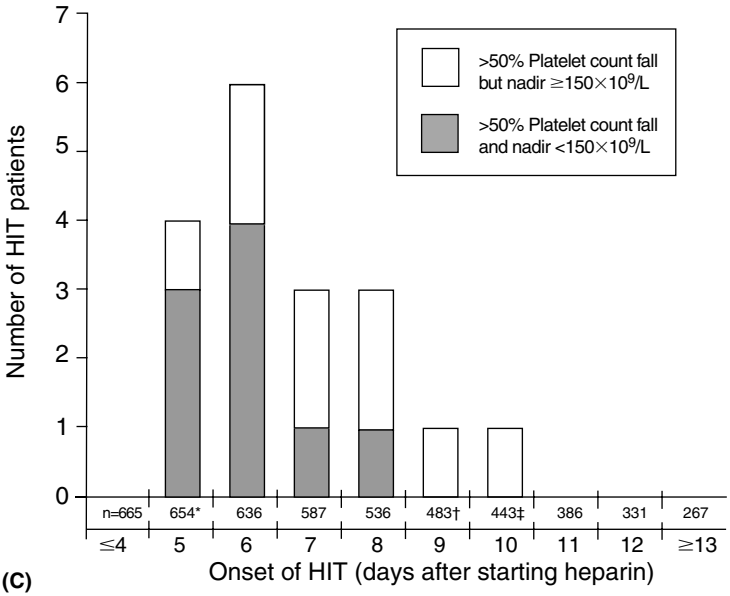


FIGURE 1 (*Continued on next page.*)

relation to heparin use (both past and present) also found that the onset of the platelet count fall typically occurs between days 5 and 10 (Fig. 2). Interestingly, among these patients with typical onset of HIT, there was no significant difference in the time to onset of HIT, irrespective of whether or not the patients had been



(C)

FIGURE 1 (*Continued from previous page*.) HIT in a clinical trial of postoperative orthopedic patients. (**A**) Serial platelet counts of nine patients with HIT (platelet count nadir $<150\times10^9$/L). The bold line and shaded area indicate the mean (±2 SD) platelet count in the reference population (367 patients who tested negative for HIT antibodies). The reference population indicates the occurrence of postoperative thrombocytopenia (days 1–3), followed by postoperative thrombocytosis (maximal, days 11–14). Nine patients developed serologically confirmed HIT, with a platelet count fall to $<150\times10^9$/L; eight of the nine patients developed HIT-associated thrombosis (see insert for description of the types of thrombi observed; all thrombi were venous, except for a mesenteric artery thrombosis). (**B**) Serial platelet counts of nine patients with HIT (platelet nadir $>150\times10^9$/L, but platelet count fall >50%). Five patients developed DVT. (For one of the patients, the platelet count began to fall on day 5 after UFH "flushes" were received through an intra-arterial catheter placed at the time of surgery.) HIT developed in seven patients receiving UFH and two receiving LMWH (The platelet count fell abruptly on day 12 (postoperative day 13), together with symptoms and signs of an acute systemic reaction, following administration of a 5000 U intravenous UFH bolus. However, the first clinical manifestation of HIT was on day 9 (erythematous skin lesions at heparin injection sites). The platelet count fell abruptly on postoperative day 13 when 5000 U of intravenous UFH was given. (**C**) Day of onset of HIT for 18 patients observed in a clinical trial. HIT began between days 5 and 10, inclusive, in all 18 patients. Length of heparin treatment was variable; thus, the remaining number of patients at risk for HIT for each day of follow-up is shown (*n*). The platelet count fell abruptly on day 10 after administration of a 5000 U intravenous UFH bolus, followed by therapeutic-dose UFH infusion. However, positive HIT antibodies were first detected by platelet factor 4-heparin-enzyme-immunoassay on day 6 of treatment with subcutaneous UFH, 7500 U twice daily. *Abbreviations*: DVT, deep vein thrombosis; HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; UFH, unfractionated heparin. *Source*: (**A**, **C**) from Warkentin et al., 1995, and Warkentin, 2000; (**B**) from Warkentin et al., 1995, 2003.

exposed to heparin in the past. For most patients with typical onset of HIT, previous heparin exposure had occurred in the "remote" past, arbitrarily defined as more than 100 days earlier (Fig. 2).

Gruel and colleagues (2003) have reported that the onset of the platelet count fall may occur on average several days later in patients who develop HIT during low molecular weight heparin (LMWH) therapy. More time may be required to generate clinically important levels of HIT-IgG so as to activate platelets in the presence of PF4/LMWH, rather than PF4/H, complexes.

Diminishing Risk of HIT After Day 10

The risk of HIT decreases after the day 5–10 "window" passes (Fig. 1c). In my experience, a platelet count fall after day 10 is usually caused by another pathological process, such as septicemia. In a notable exception, sometimes an invasive procedure "resets the clock"; that is, a platelet count fall that begins on day 12 of a course of heparin that consists of two 6-day treatments with heparin (before and after intervening surgery) is likely HIT. Perhaps the surgery causes circumstances that favor seroconversion (e.g., release of PF4) (see Chapter 5). Tholl and colleagues (1997) reported on a patient who for 9 yr uneventfully received unfractionated heparin (UFH) for hemodialysis; nevertheless, HIT complicating hemodialysis began shortly after the patient underwent parathyroidectomy.

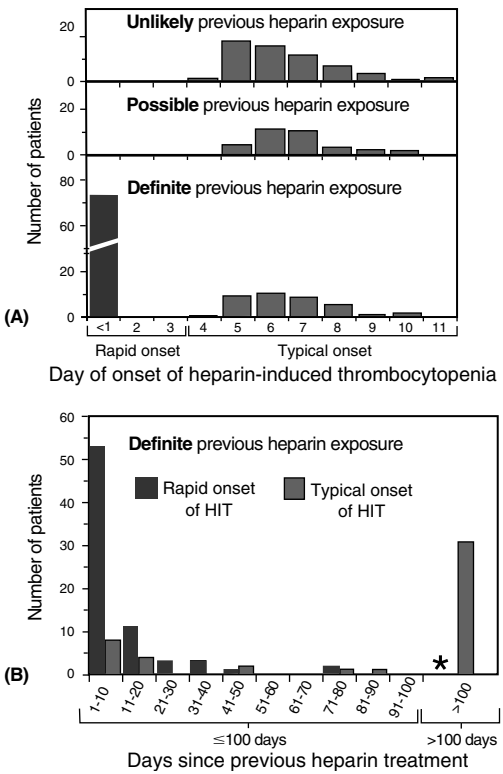


FIGURE 2 Temporal patterns of HIT in 243 patients in relation to previous treatment with heparin. (**A**) Data are shown for the patients in whom the day of onset of HIT could be determined to within a 3-day period. Among 170 patients with typical onset of HIT, there was no significant difference in onset of HIT (median day), irrespective of whether previous heparin exposure had been definite (6.5, $n = 47$), possible (7.0, $n = 49$), or unlikely (6.0, $n = 74$) ($p = 0.88$, definite vs. unlikely). Among 120 patients who had definite previous exposure to heparin, 73 had rapid onset of HIT. (**B**) For the subgroup of patients with definite previous exposure to heparin, the 73 patients with rapid onset of HIT invariably had been exposed to heparin within the past 100 days (i.e., no patients shown at the asterisk [*]); in contrast, only 16/47 patients with typical onset of HIT had been exposed to heparin within the past 100 days ($p < 0.001$). *Abbreviation*: HIT, heparin-induced thrombocytopenia. *Source*: From Warkentin and Kelton, 2001a.

Rapid Onset of HIT

Sometimes patients develop *rapid-onset HIT*. This is defined as an unexpected fall in the platelet count that begins soon after heparin is started. Indeed, it is generally evident on the first postheparin platelet count, whether obtained minutes, hours, or a day later. Patients who develop such a rapid fall in the platelet count and who are confirmed serologically to have HIT antibodies invariably have received heparin in the past (Warkentin and Kelton, 2001a; Lubenow et al., 2002). A characteristic feature of this prior heparin exposure has been recently identified: it generally includes a *recent* exposure to heparin, usually within the past 2–3 wk, and almost always within the past 100 days (Figs. 2 and 3).

This temporal profile of onset of HIT can be explained as follows: the rapid fall in platelet count represents abrupt onset of platelet activation caused by residual circulating HIT antibodies that resulted from the recent heparin treatment, rather than antibodies newly generated by the subsequent course of heparin.

This explanation is supported by other observations. First, for patients with typical onset of HIT, there was no difference in its median day of onset, irrespective of whether or not patients had previously been exposed to heparin. Second, patients did not generally develop thrombocytopenia that began between days 2 and 4. Had

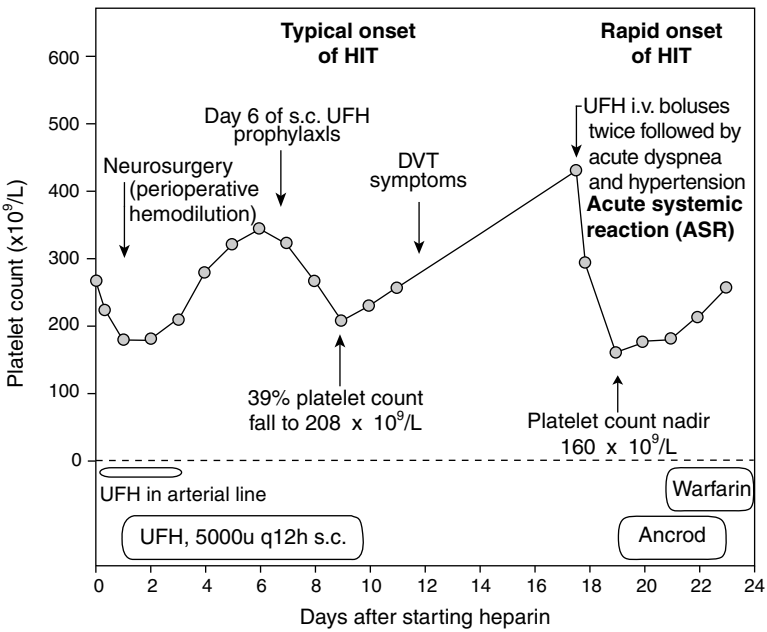


FIGURE 3 A 49-yr-old patient exhibiting both typical- and rapid-onset HIT: The platelet count began to fall on day 6 of sc UFH injections given for antithrombotic prophylaxis following neurosurgery (typical HIT). An abrupt fall in platelet count occurred twice on day 18, each after a 5000 U iv UFH bolus (rapid HIT). Symptoms and signs of acute systemic reaction occurred 10 mins after each bolus (dyspnea, tachypnea, hypertension, chest tightness, restlessness). Note that the patient's platelet count never fell below 150×10^9/L, even though her serum tested strongly positive for HIT antibodies by serotonin release assay. She developed proximal DVT shortly after developing HIT. *Abbreviations*: ASR, acute systemic reaction; DVT, deep venous thrombosis; HIT, heparin-induced thrombocytopenia; iv, intravenous; sc, subcutaneous; UFH, unfractionated heparin.

there truly been an anamnestic immune response more rapid than the usual 5- to 10-day period, one might have expected to identify such a group of patients. Third, patients reexposed to heparin following disappearance of HIT antibodies do not necessarily form HIT antibodies again; those who do appear to form antibodies after day 5 (Gruel et al., 1990; Warkentin and Kelton, 2001a). Indeed, several patients with well-documented previous HIT have received full treatment courses of heparin several months or years later without incident (Warkentin and Kelton, 2001a; Lindhoff-Last et al., 2002).

HIT Antibodies Are Transient

There is a plausible biological basis to explain why patients who develop rapid-onset HIT have received heparin in the recent, rather than in the remote, past: HIT antibodies are transient and become undetectable at a median of 50 days (95% CI, 32–64 days) after first testing positive, using the platelet serotonin release assay. The median time to a negative test is somewhat longer (85 days; 95% CI, 64–124 days) using a commercial antigen assay (Fig. 4). At 100-day follow-up, the probability of the activation and antigen assays being negative is approximately 90% and 60%, respectively (Warkentin and Kelton, 2001a).

Rule 2

A rapid fall in the platelet count that began soon after starting heparin therapy is unlikely to represent HIT unless the patient has received heparin in the recent past, usually within the past 30, and latest, 100 days.

To summarize, the rapid fall in platelet count appears to be caused by the repeat administration of heparin to a patient with residual circulating HIT antibodies, rather than resulting from a rapid regeneration of HIT antibodies.

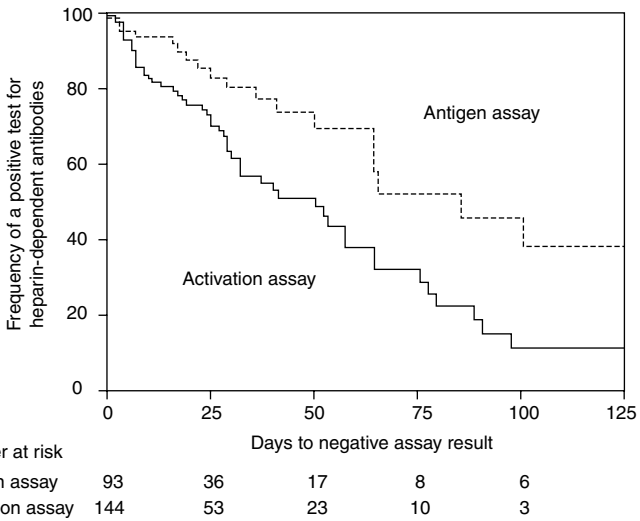


FIGURE 4 Proportion of patients with HIT antibodies after an episode of HIT. The time (in days) to a negative test by the activation assay ($n = 144$) or the antigen assay ($n = 93$) is shown. The antigen test tended to become negative more slowly than did the activation assay ($p = 0.007$). *Abbreviation*: HIT, heparin-induced thrombocytopenia. *Source*: From Warkentin and Kelton, 2001a.

A Hypothesis to Explain the Timing of HIT

There is a possible explanation for these unusual temporal features of HIT: because the HIT antigen(s) is a "cryptic" autoantigen (or neoantigen) comprised of two autologous substances (PF4 and heparin), HIT can be regarded as an *autoimmune* disorder. Indeed, the target of the immune response appears to be one of at least three dominant conformation-dependent neoepitopes formed on PF4 when it binds to heparin (see Chapters 5–7). There is evidence that transient IgG-mediated autoimmune responses can occur, particularly when the responsible antibodies have a relatively low affinity for the neoepitope (thus having avoided prior clonal deletion as occurs with lymphocytes that have high-affinity binding to autoantigens). In this situation, the antibodies are generated only as long as the autoantigen is present, thus explaining why there is a rapid fall in anti-PF4/H antibodies soon after discontinuation of heparin. The affinity of the HIT antibodies may be substantially enhanced when both Fab "arms" of the IgG molecule can bind to linked epitopes, i.e., two PF4 molecules bound to a single heparin molecule (Newman and Chong, 1999).

This hypothesis could explain several unusual aspects of the timing of HIT, such as: (1) why HIT tends to occur fairly rapidly, beginning as soon as 5 days after starting heparin even in a patient who has never been exposed previously to heparin (autoreactive T-cell or B-cell clones might already be present in small numbers prior to starting heparin); (2) why HIT occurs more often in certain patient populations, such as postoperative patients (cytokine-driven immune responses); and (3) why HIT does not necessarily recur in patients with a previous history of HIT who are subsequently treated with heparin (there is a rapid loss of HIT antibodies following resolution of HIT, and the specific circumstances that favored immune stimulation the first time—e.g., large, stoichiometric concentrations of PF4 and heparin, occurring in an inflammatory milieu—may not be recapitulated during the subsequent heparin exposure).

Implications for Repeat Use of Heparin in a Patient with a History of HIT

The (1) transient nature of the HIT antibody, the (2) apparent minimum of 5 days to regenerate clinically significant HIT antibodies even in a patient who once had HIT, and (3) the observation that HIT antibodies do not necessarily recur, despite heparin rechallenge in a patient with definite prior HIT, all suggest that it may be safe to readminister heparin to such patients. Fortunately, this potentially risky situation is not frequently necessary, as there are several alternative anticoagulants that can be substituted for heparin (see Chapters 12–19).

However, UFH is the unparalleled drug of choice in certain therapeutic settings, particularly heart surgery when using cardiopulmonary bypass, or vascular surgery. Furthermore, there are important disadvantages of newer anticoagulants for these procedures (see Chapter 19). In my opinion, therefore, for patients with a remote history of HIT (>100 days) who require cardiac or vascular surgery, a rational approach is to prove serologically that HIT antibodies are no longer present, and then to give heparin for a brief time to permit the surgery (Olinger et al., 1984; Pötzsch et al., 2000; Warkentin and Kelton, 2001a) (see Chapter 19). We have even used this approach successfully in a patient who required heparin for major vascular surgery 1 mo following an episode of HIT, when the HIT antibodies had just become undetectable. After surgery, it seems prudent to avoid postoperative heparin completely and to administer an alternative anticoagulant, such as danaparoid, fondaparinux, lepirudin, or argatroban, as indicated. The actual risk of recurrent HIT beginning 5–10 days later, either following a transient

intraoperative heparin exposure or even during prolonged postoperative heparin use, is unknown, but may be low.

For planning a brief reexposure to heparin in a patient who had HIT in the past few weeks or months, a dilemma would arise if the follow-up patient serum now tested negative using a sensitive activation assay (e.g., platelet serotonin release assay), but positive by antigen assay. There is evidence that activation assays are better at detecting clinically significant levels of HIT antibodies (Warkentin et al., 2000, 2005a) (see Chapter 10). Thus, use of heparin in this situation might be a reasonable option, provided that one had confidence in the activation assay performed, the antigen assay result was "weak" (e.g., 0.400–0.750 OD units), there was a strong indication for surgery requiring heparin, and there was limited experience with an alternative anticoagulant. Continued watchful waiting is another option, given the transience of HIT antibodies.

Sensitization by Incidental Heparin Exposure

Sensitizing exposures to heparin can be relatively obscure. For example, incidental use of intraoperative line "flushes" that were not even documented in the medical records has led to HIT antibody formation or acute onset of HIT, with tragic consequences (Brushwood, 1992; Ling and Warkentin, 1998). Greinacher and colleagues (1992) reported a patient who developed recurrent HIT when reexposed to heparin present in prothrombin complex concentrates. Physicians should suspect possible heparin exposure in a patient whose clinical course suggests HIT, especially if the patient was recently hospitalized or has undergone procedures in which heparin exposure may have occurred.

Delayed Onset of HIT

Rarely, HIT begins several days after discontinuing heparin therapy or persists for several weeks even though heparin administration has been stopped (Castaman et al., 1992; Tahata et al., 1992; Warkentin and Kelton, 2001b; Rice et al., 2002; Warkentin and Bernstein, 2003; Shah and Spencer, 2003; Levine et al., 2004; Smythe et al., 2005; Jackson et al., 2006; Arepally and Ortel, 2006) (Fig. 5). A dramatic case encountered by the author was a female outpatient who presented with transient global amnesia and a platelet count of 40×10^9/L 7 days after receiving two doses of UFH; despite the diagnosis and serologic confirmation of HIT and avoidance of all heparin, this patient's platelet count fell over the next 4 days to 14×10^9/L, along with laboratory evidence for disseminated intravascular coagulation (DIC) (low fibrinogen and elevated fibrin *D*-dimer levels). This patient's platelet counts gradually recovered to normal over several months, during which time recurrent thrombotic events were managed successfully with an alternative anticoagulant.

The unusual clinical course of these patients could be related to very high titers of platelet-activating IgG antibodies (Warkentin and Kelton, 2001b). Moreover, substantial platelet activation in vitro can be caused by some of these patients' sera even in the absence of added heparin. This finding of substantial heparin-independent platelet activation resembles that described in other patients with drug-induced immune thrombocytopenia, in which prolonged thrombocytopenia has been reported in association with drug-independent binding of IgG to platelets (Kelton et al., 1981). Given the apparent rarity of these cases, it is perhaps surprising that this syndrome does not occur more frequently, given that HIT—once initiated—resembles somewhat an autoimmune disorder, with IgG

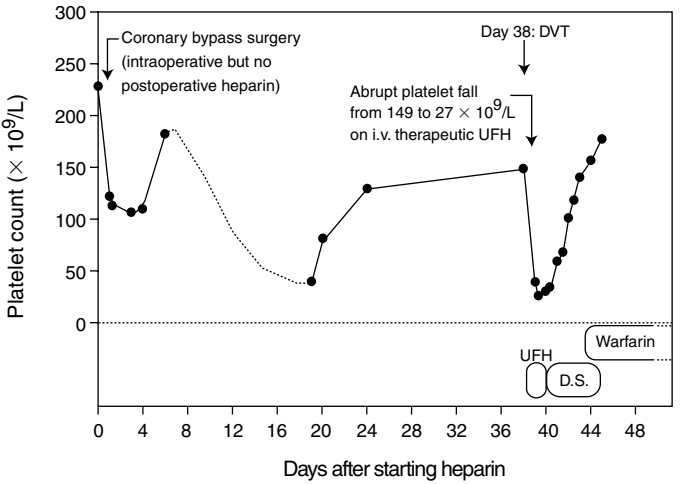


FIGURE 5 Delayed onset of HIT: a 68-yr-old woman who received UFH for heart surgery was noted to have a platelet count of 40×10^9/L on postoperative day 19 and a "rash" of her lower extremities. She presented on day 38 with symptomatic DVT and developed rapid-onset recurrent thrombocytopenia after receiving iv UFH. The patient was successfully treated with D.S. and warfarin. In retrospect, the thrombocytopenia first observed on postoperative day 19 almost certainly was caused by delayed onset of HIT. *Abbreviations*: D.S., danaparoid sodium; DVT, deep venous thrombosis; iv, intravenous; UFH, unfractionated heparin.

recognizing an autologous protein, PF4. On the other hand, earlier discharge from the hospital and a higher index of suspicion for this syndrome might mean that delayed onset of HIT will become a relatively more common presentation of HIT in the future.

Delayed onset of HIT, however, should not be confused with delayed clinical manifestation of HIT-associated thrombosis. For example, Figure 3 shows a patient who developed typical onset of HIT while receiving postoperative heparin prophylaxis. However, isolated HIT was not clinically recognized, and the patient presented subsequently with a deep vein thrombosis (DVT) and a normal platelet count; when heparin boluses were given, rapid onset of thrombocytopenia occurred. Presumably, subclinical HIT-associated DVT that began during the episode of isolated HIT progressed to symptomatic thrombosis in the absence of anticoagulation. In contrast, patients with delayed onset of HIT develop thrombocytopenia several days after the use of heparin and are thrombocytopenic when they present with thrombosis. Exacerbation of thrombocytopenia occurs if further heparin is given.

The existence of delayed onset of HIT presents a diagnostic dilemma in patients who are no longer receiving heparin but who develop thrombocytopenia 5 or more days after placement of a heparin-coated device, e.g., certain intravascular grafts or stents (Cruz et al., 2003). Such a puzzling situation of delayed onset of thrombocytopenia postvascular surgery prompted investigators to postulate heparin contamination of a graft (the manufacturer insisted otherwise) (Burger et al., 2001). In my view, either delayed onset or a protracted course of thrombocytopenia could reflect the generation and persistence of unusual "autoimmune" HIT antibodies without the need to invoke continuing exposure to heparin.

B. Severity of Thrombocytopenia

Figure 6 shows the platelet count nadirs of patients with laboratory-proved HIT: the median platelet count nadir was approximately 60×10^9/L (Warkentin, 1998a, 2007). This contrasts with "typical" drug-induced immune thrombocytopenic purpura (DITP; e.g., caused by quinine/quinidine, sulfa antibiotics, or vancomycin), for which the median platelet count nadir is 20×10^9/L or less, and patients usually develop bleeding (Pedersen-Bjergaard et al., 1997). The platelet count is 20×10^9/L or fewer in only about 5–10% of patients with HIT (Warkentin, 2003, 2007). But even in this minority of HIT patients with very severe thrombocytopenia, thrombosis, rather than bleeding, predominates. Patients with atypical drug-induced thrombocytopenic purpura caused by anti-GPIIb/IIIa-blocking drug (e.g., abciximab [ReoPro]) appear to develop severity of thrombocytopenia resembling that of typical DITP (Fig. 6).

Definition of Thrombocytopenia

Figure 6 illustrates that HIT is associated with thrombosis even when the platelet count nadir is more than 150×10^9/L. This suggests that the standard definition

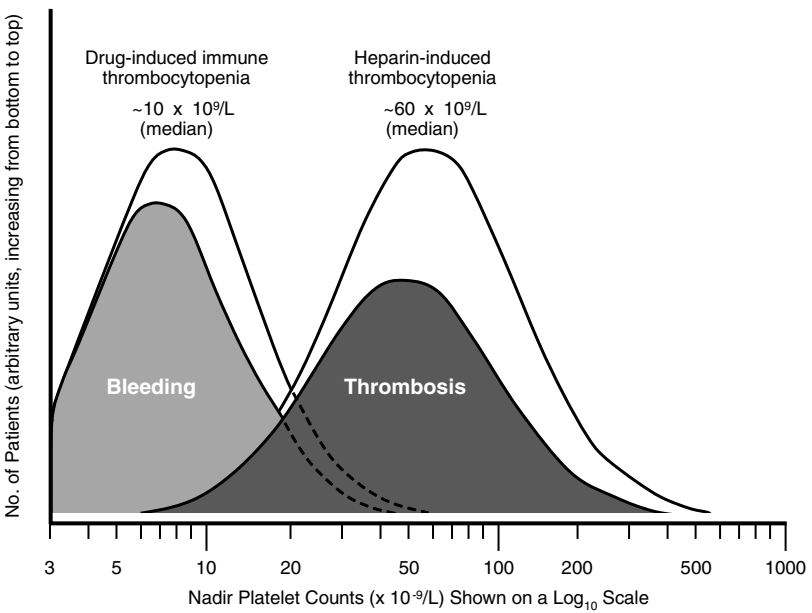


FIGURE 6 Platelet count nadirs and clinical profile of classic drug-induced immune-mediated thrombocytopenia versus serologically confirmed HIT. "Classic" drug-induced immune-mediated thrombocytopenia (e.g., caused by quinine, vancomycin, or glycoprotein IIb/IIIa antagonists, among other drugs) typically produces severe thrombocytopenia (median platelet count nadir, approximately 10 x 10^9/L) and associated mucocutaneous bleeding. In contrast, HIT typically results in mild-to-moderate thrombocytopenia (median platelet count nadir, about 60 x 10^9/L) and associated venous or arterial thrombosis. Note that the relative heights of the two peaks are not drawn to scale, as HIT is much more common than all other causes of drug-induced immune-mediated thrombocytopenia combined. *Source*: From Warkentin, 2007.

of thrombocytopenia (<150 $\times$ 10^9/L) may be inadequate for many patients with HIT. Particularly in postoperative patients, a major fall in the platelet count can occur without the nadir falling to less than 150 $\times$ 10^9/L (Figs. 1b and 3). Indeed, studies indicate that a 50% or greater fall in the platelet count from the postoperative peak is strongly associated with HIT antibodies, even when the platelet count nadir remains higher than 150 $\times$ 10^9/L (Ganzer et al., 1997; Warkentin et al., 2003). Moreover, this patient subgroup is at increased risk for thrombosis.

Rule 3

A platelet count fall of more than 50% from the postoperative peak between days 5 and 14 after surgery associated with heparin treatment can indicate HIT even if the platelet count remains higher than 150 $\times$ 10^9/L.

It is possible that a greater than 50% platelet count fall definition is also appropriate for medical patients (Girolami et al., 2003). Regardless of the patient population, a clinician should have a high index of suspicion when unexpected large-percentage declines in the platelet count occur during heparin treatment, irrespective of whether an arbitrary absolute threshold for "thrombocytopenia" is crossed. Indeed, some investigators have used other thresholds to define thrombocytopenia, such as platelet count declines of 40% (Pouplard et al., 2005) or even 30% (Greinacher et al., 2005a).

Platelet Count Monitoring in Patients Receiving Heparin

In postoperative patients, the onset of HIT coincides with rising platelet counts (postoperative thrombocytosis); thus, the platelet count profile of HIT resembles an "inverted V" (Λ Fig. 1a,b). The postoperative peak platelet count preceding HIT is often higher than the preoperative platelet count. Therefore, the postoperative peak platelet count is the appropriate baseline for calculating the magnitude of a subsequent platelet count fall (Warkentin et al., 2003; Pouplard et al., 2005) (Table 2).

HIT-Associated Thrombosis Without Thrombocytopenia

Anecdotal reports indicate that HIT-associated thrombosis can occur in the absence of thrombocytopenia, as conventionally defined (Phelan, 1983; Hach-Wunderle et al., 1994; Warkentin, 1996a, 1997; Houston, 2000). However, most of these patients do have an associated fall in the platelet count, although the nadir remains higher than 150 $\times$ 10^9/L. Perhaps the most dramatic example of this phenomenon was a patient with essential thrombocythemia who developed serologically confirmed HIT: the platelet count fell by 49% from 1235 to 633, i.e., concomitant "thrombocytopenia" and thrombocytosis (Risch et al., 2000).

A study suggested that HIT antibody formation without thrombocytopenia is not associated with a thrombosis rate greater than control patients (Warkentin et al., 1995, 2003). However, the subset of patients who formed HIT antibodies and whose platelet count fell by 50% or more—but remained above 150 $\times$ 10^9/L—did have an increased risk for thrombosis (odds ratio, 6.0). Figure 7 illustrates this concept of the central importance of thrombocytopenia (defined broadly as a large relative fall in the platelet count) in determining risk for thrombosis. These observations provide indirect evidence suggesting that in vivo platelet activation by HIT antibodies probably contributes to the pathogenesis of HIT-associated thrombosis.

As shown by Figure 6, thrombosis commonly complicates HIT irrespective of the severity of the thrombocytopenia. Nevertheless, there is evidence that both

TABLE 2 Determining the Day of Onset of Thrombocytopenia: A 35-Year-Old Woman Who Developed HIT After Heart Surgery

	Postoperative day												
	-1	0 (surgery)	1	2	3	4	5	6	7	8	9	10	11
Heparin used		UFH during CPB	Line flushes	Nil	Nil	UFH 5000 b.i.d. sc	UFH 5000 b.i.d. sc	UFH 5000 b.i.d. sc	UFH 5000 b.i.d. sc	D.S	D.S	D.S	D.S
Platelet count	227	98	137	209	255	300	374	378	310	224 (PE[a])	166	171	161 (nadir)
Percent platelet count fall	Platelet fall during day 0–4 is unlikely to be HIT unless there was recent heparin use (past 100 days) and the magnitude of the platelet fall is greater than expected.						Rising platelet count	Peak platelet count	18% (378→ 310)	41% (378→ 224)	56% (378→ 166)	No further fall	57% (378→ 161)

[a]Pulmonary embolism (PE) occurred on postoperative day 8, in association with a platelet count fall of 41%, from 378 (postoperative peak) to 224×10^9/L. The platelet count began to fall on day 7. The case illustrates why it is wrong to use the preoperative platelet count value as the "baseline," as the fall in platelet count from 227 (preoperative [day -1]) to 224 (day 7) would be considered trivial, even though HIT-associated pulmonary embolism occurred. The preoperative (day -1) and first three postoperative days should be censored in the interpretation of platelet counts in HIT. In this patient, the abrupt fall in platelet count from 227 to 98 (day 0) is expected (heart surgery). This patient was treated successfully with danaparoid sodium (D.S.), with longer-term anticoagulation with warfarin. The patient's clinical course is also shown in Fig. 4B in Chapter 11.

Abbreviations: D.S., danaparoid sodium; HIT, heparin-induced thrombocytopenia; PE, pulmonary embolism; UFH, unfractionated heparin.

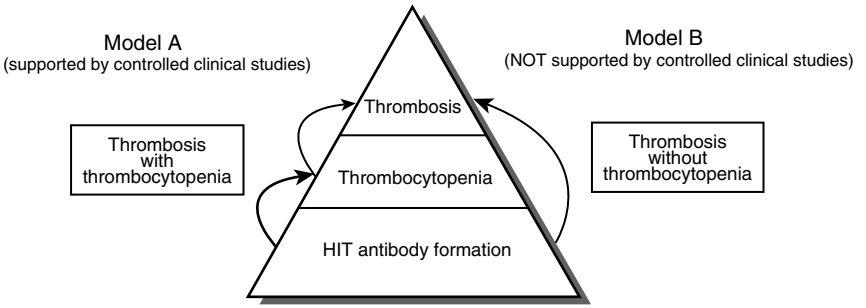


FIGURE 7 "Iceberg" model of HIT: Model A indicates that thrombosis occurs in patients who develop HIT antibody formation and thrombocytopenia. This model is supported by clinical data. In contrast, model B indicates the possibility of HIT antibody formation contributing to thrombosis without the intermediary process of thrombocytopenia. Although anecdotal experience suggests occasional patients consistent with model B, controlled studies indicate that HIT antibody formation without thrombocytopenia does not have an increased frequency of thrombosis, compared with controls (Warkentin et al., 1995, 2003). Note that thrombocytopenia is broadly defined and includes patients with large relative falls in the platelet count, even if the platelet nadir is $>150\times10^9$/L. *Abbreviation*: HIT, heparin-induced thrombocytopenia. *Source*: From Warkentin, 1999.

the frequency and the severity of thrombotic complications increase somewhat in relation to the magnitude of the platelet count decline, whether quantitated in relative or absolute terms (Warkentin et al., 2003; Greinacher et al., 2005b; Lewis et al., 2006).

Platelet Count Recovery Following Discontinuation of Heparin

The median time to platelet count recovery to more than 150×10^9/L after stopping heparin administration is about 4 days, although several more days may be required for the platelet count to reach a stable plateau. In patients with very severe HIT, the platelet count may take 2 wk or more to recover (Warkentin, 1998a). Unlike nonimmune heparin-associated thrombocytopenia, the platelet count will generally not recover in patients with HIT unless the heparin is discontinued.

III. THROMBOSIS

A. The HIT Paradox: Thrombosis but not Hemorrhage

Table 1 summarizes the clinical spectrum and approximate frequency of clinical sequelae associated with HIT. Spontaneous hemorrhage is not characteristic of HIT, and petechiae are not typically observed, even in those occasional patients whose platelet count is less than 10×10^9/L. Bleeding complications were not increased over controls in two prospective studies of HIT (Cipolle et al., 1983; Warkentin et al., 1995).

Rule 4

Petechiae and other signs of spontaneous bleeding are not clinical features of HIT, even in patients with very severe thrombocytopenia.

The explanation for this clinical feature is unknown, but could be related to unique pathophysiological aspects of HIT, such as in vivo platelet activation,

generation of procoagulant, platelet-derived microparticles, and procoagulant alterations of endothelium and monocytes (see Chapters 8 and 9).

B. HIT Is a Hypercoagulable State

A large controlled study (Warkentin et al., 1995, 2003) concluded that HIT is independently associated with thrombosis, even in a patient population at a high baseline risk for thrombosis (postoperative orthopedic patients). Moreover, both venous and arterial thrombosis was seen. Thus, HIT can be considered a *hypercoagulable state* (Table 3), a designation consistent with increased in vivo thrombin generation seen in almost all patients with HIT (Warkentin et al., 1997; Greinacher et al., 2000).

C. Timing of Thrombotic Complications

Thrombosis occurs in association with HIT in at least four ways. Only the last three situations are conventionally considered as HIT-associated thrombosis. First, thrombosis can precede heparin treatment, for which it usually represents the initial indication for heparin therapy. Second, HIT can be the presenting clinical manifestation of HIT, sometimes even occurring prior to the platelet count fall (Greinacher et al., 2005b) (Fig. 8). Indeed, new thrombosis is the initial clinical manifestation in about 40% to 50% of all HIT patients (Warkentin and Kelton, 1996; Greinacher et al., 1999, 2005b).

Third, thrombosis can occur during the period of thrombocytopenia or early platelet count recovery despite discontinuation of the heparin (discussed subsequently). Finally, thrombosis can occur following platelet count recovery (Gallus et al., 1987; Warkentin and Kelton, 1996). In these patients, it is possible that subclinical thrombosis occurred during the thrombocytopenia, but became clinically evident only later. The term *heparin-induced thrombocytopenia-thrombosis (syndrome),* also known as HITT or HITTS, is sometimes used to describe patients with HIT-associated thrombosis.

Natural History of "Isolated HIT"

There is a high probability of subsequent thrombosis even when heparin administration is stopped because of thrombocytopenia caused by HIT. A retrospective cohort study (Warkentin and Kelton, 1996) identified 62 patients with serologically confirmed HIT in whom the diagnosis was clinically suspected because of thrombocytopenia alone, and not because of signs and symptoms indicative of possible new thrombosis. Thus, this cohort was identified without an apparent recognition

TABLE 3 The Prothrombotic Nature of HIT: Comparison with Other Hypercoagulable States

Hypercoagulable state	Odds ratio for thrombosis
Heparin-induced thrombocytopenia:	–
Platelet count $<150\times10^9$/L	36.9
Platelet fall >50% beginning $\geq$5 days of heparin	12.4
Platelet fall >50%, but platelet count remains $>150\times10^9$/L	6.0
Factor V Leiden	6.6
Congenital protein C deficiency	14.4
Congenital protein S deficiency	10.9
Congenital antithrombin deficiency	24.1
Dysfibrinogenemia	11.3
Lupus anticoagulant	5.4

Source: Warkentin, 1995; Warkentin et al., 1995, 2003.

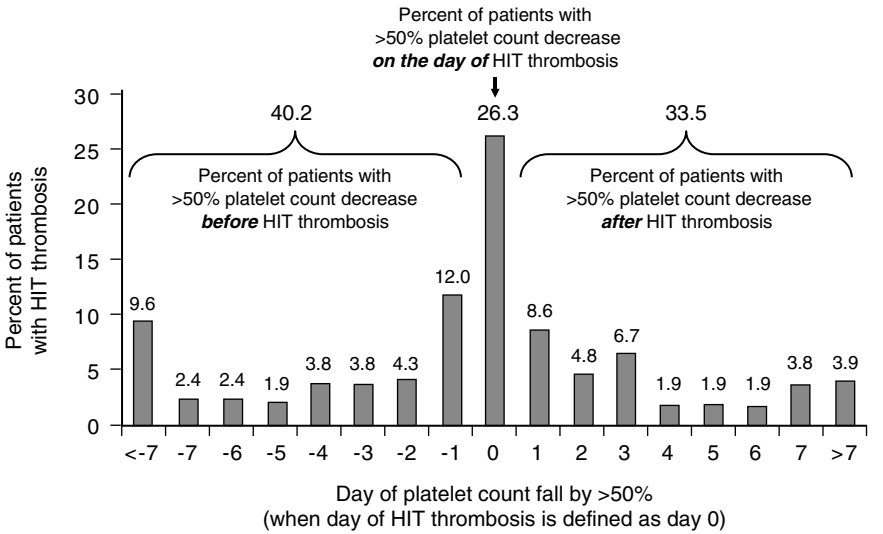


FIGURE 8 Relation of onset of platelet count decrease and onset of HIT-associated thrombosis. The data summarize 209 patients with HIT-associated thrombosis. About one quarter (26.3%) of patients develop thrombosis on the same day that the thrombocytopenia occurs (defined arbitrarily as the day the platelet count has fallen by more than 50%), and in 33.5% the platelet count reached thrombocytopenia levels only *after* the occurrence of thrombosis. *Abbreviation*: HIT, heparin-induced thrombocytopenia. *Source*: From Greinacher et al., 2005b.

bias caused by symptomatic thrombosis. Nevertheless, the 30-day thrombosis event rate was about 50% (Fig. 2 in Chapter 3). This high frequency of thrombosis occurred whether the heparin administration was simply stopped or substituted by warfarin.

In 1999, Wallis and colleagues provided further support for this concept that isolated HIT had an unfavorable natural history. In their retrospective cohort study of 113 patients with serologically confirmed HIT, these workers also found a relatively high risk of thrombosis (23–38% at 30-day follow-up, depending on whether patients who developed thrombosis at the time heparin was stopped are included) in patients with isolated HIT managed by cessation of heparin. Further, early cessation of heparin (within 48 h after a 50% or greater fall in platelet count) did not appear to reduce risk of thrombosis, compared with patients in whom heparin was discontinued later.

More recently, Zwicker and coworkers (2004) performed a retrospective study that evaluated the risk of symptomatic thrombosis among patients with isolated HIT, based upon the magnitude of a positive enzyme-immunoassay (EIA) for anti-PF4/H antibodies. Thrombosis was seen in five (36%) of 14 patients with a strong positive EIA (>1.0 optical density units), but only three (9%) of 34 patients with a weak positive test ($p = 0.07$). This is consistent with data indicating that the greater the magnitude of a positive EIA, the greater the likelihood that the patient has heparin-dependent platelet-activating antibodies and, hence, clinical HIT (Warkentin et al., 2005a).

Meta-analyses of two prospective cohort studies also found a high initial thrombotic event rate (6.1% per day after stopping heparin therapy and before

beginning alternative anticoagulant therapy with lepirudin) (Greinacher et al., 1999, 2000) (Fig. 4 in Chapter 14). Taken together, these large retrospective and prospective cohort studies suggest the following rule:

Rule 5

HIT is associated with a high frequency of thrombosis despite discontinuation of heparin therapy with or without substitution by coumarin: the initial rate of thrombosis is about 5–10% per day over the first 1–2 days; the 30-day cumulative risk is about 50%.

About 5% of patients (3 of 62) in the largest study died suddenly, two with proved or probable pulmonary embolism (Warkentin and Kelton, 1996). This experience supports the recommendation that further anticoagulation be considered for patients in whom isolated HIT has been diagnosed (Hirsh et al., 1998, 2001; Warkentin and Greinacher, 2004; Warkentin et al., 1998) (see Chapters 1 and 12–15).

D. Clinical Factors in the Pathogenesis of HIT-Associated Thrombosis

Clinical factors help determine the location of thrombosis in HIT. For example, Makhoul and colleagues (1986) observed prior vessel injury (e.g., recent angiography) in 19 of 25 patients with lower limb HIT-associated thrombosis. Similarly, central venous catheters are crucial for the occurrence of an upper limb DVT in patients with HIT (Hong et al., 2003).

Prospective studies of HIT in medical patients show that venous and arterial thrombotic events occur in approximately equal numbers; in contrast, there is a marked predominance of venous thrombosis when HIT occurs in surgical patients (Table 6 in Chapter 3). In a retrospective study, Boshkov and colleagues (1993) found that HIT patients with cardiovascular disease were more likely to develop arterial thrombosis, whereas venous thrombosis was strongly associated with the postoperative state.

Rule 6

Localization of thrombosis in patients with HIT is strongly influenced by independent acute and chronic clinical factors, such as the postoperative state, arteriosclerosis, or the location of intravascular catheters in central veins or arteries.

E. Venous Thrombosis

Large case series suggest that venous thrombotic complications predominate in HIT (Warkentin and Kelton, 1996; Nand et al., 1997; Greinacher et al., 2005b) (Tables 6 and 7 in Chapter 3). Indeed, pulmonary embolism occurs more often than all arterial thrombotic events combined. Furthermore, the strength of association between HIT and venous thromboembolism increases in relation to the severity of thrombosis (Table 4). Other unusual venous thrombotic events complicating HIT include cerebral vein (dural sinus) thrombosis (v.i.), adrenal vein thrombosis (v.i.), hepatic vein thrombosis (Theuerkauf et al., 2000), and perhaps retinal vein thrombosis (Nguyen et al., 2003). Thus:

Rule 7

In patients receiving heparin, the more unusual or severe a subsequent thrombotic event, the more likely the thrombosis is caused by HIT.

TABLE 4 Association of HIT and Thrombosis

Patient population (Ref.)	Thrombosis	Thrombosis rate		OR (95% CI)	*p*-Value
		HIT	Controls		
Postorthopedic surgery[a] (Warkentin et al., 1995, 2003)	Proximal DVT	8/18 (44.4%)	26/647 (4.0%)	19.1 (5.9–58.3)	<0.001
	Bilateral proximal DVT	2/18 (11.1%)	4/647 (0.6%)	20.1 (1.7–150)	0.01
	Pulmonary embolism	2/18 (11.1%)	2/647 (0.3%)	40.3 (2.7–572)	0.004
	Any thrombosis	13/18 (72.2%)	112/647 (17.3%)	12.4 (4.0–45.2)	<0.001
Patients with central line[b] (Hong et al., 2003)	Upper-limb DVT	14/145 (9.7%)	3/484 (0.6%)	17.1 (4.9–60.5)	<0.001
Medical[a] (Girolami et al., 2003)	Any thrombosis	3/5 (60%)	21/593 (3.5%)	40.8 (5.2–163)	<0.001

[a]HIT defined as >50% platelet count fall.
[b]HIT defined as any abnormal platelet count fall with positive HIT serology (platelet fall was >50% in 93% of study patients).
Abbreviations: DVT, deep vein thrombosis; HIT, heparin-induced thrombocytopenia; OR, odds ratio.

Regardless of the severity of thrombosis, in any patient who develops a symptomatic venous or arterial thrombosis while receiving heparin, the platelet count should be measured to evaluate whether HIT could be present.

Recently, Levine and colleagues (2006) estimated from published data that HIT could be present in about one in eight patients who develop venous thromboembolism subsequent to UFH treatment or prophylaxis. However, the risk could be substantially higher (~45–75%) among patients who develop *symptomatic* venous thromboembolism following postoperative UFH thromboprophylaxis (Warkentin, 2006a; Greinacher et al., 2005a).

Lower Limb DVT

Lower limb DVT is the most frequent thrombotic manifestation of HIT. Many venous thrombi are extensive and are often bilateral (Table 4).

Sometimes the DVT is sufficiently severe on clinical grounds as to merit use of the term "phlegmasia cerulea dolens" (i.e., an inflamed, blue, painful limb). However, progression of phlegmasia to venous limb gangrene is rare in the absence of coumarin anticoagulation (discussed subsequently).

There is slight left-sided predominance involving lower limb DVT: we found that 76/137 (56%) of lower limb DVT complicating HIT involved the left lower limb (Hong et al., 2003), a similar proportion as in control patients (57%). A slight left-sided predominance (~55 vs. ~45%) for lower limb DVT has also been noted in non-HIT populations (Kerr et al., 1990; Markel et al., 1992). This is attributed to the left iliac vein crossing the left iliac artery, causing an increase in left-sided lower limb venous pressures. Pregnancy amplifies further this phenomenon, thus explaining the marked predominance (>95%) of left lower limb DVT in pregnancy (Ginsberg et al., 1992; Breinachet et al., 2005a).

Upper Limb DVT

Upper limb DVT is relatively common in HIT, occurring in about 5% of patients with HIT (Hong et al., 2003). Notably, in these patients the upper limb DVT occurred at the site of a current or recent central venous catheter. Most (86%) of the patients therefore had right upper limb DVT complicating HIT, reflecting strong physician preference to using the right neck veins for insertion of central lines. This study suggests that a systemic hypercoagulable state (HIT) interacts with a local factor (location of central lines) to result in clinical events (upper limb DVT).

Recurrence of Venous Thromboembolism

Gallus and colleagues (1987) identified HIT as a significant risk factor for recurrence of venous thromboembolism in a prospective treatment study: three of the nine patients with HIT developed recurrent venous thromboembolism, compared with 12 of the 223 patients in whom HIT was not diagnosed (odds ratio, 8.8; $p < 0.01$).

Warfarin-Induced Venous Limb Gangrene

Venous limb gangrene is one of two clinical syndromes associated with HIT in which coumarin anticoagulation paradoxically plays an important pathogenic role (Fig. 9). Venous limb gangrene is defined as acral (extremity) necrosis that occurs in a limb affected by DVT. Additional features include (1) absence of large artery occlusion (i.e., there are palpable or doppler identifiable pulses); (2) extensive thrombotic occlusion of large and small veins, as well as venules; and (3) the characteristic hallmark of a *supra*therapeutic international normalized ratio (INR), generally >4.0 (Fig. 10).

Anticoagulation with warfarin, phenprocoumon, or other coumarins is a crucial factor to explain the progression of DVT to venous limb gangrene (Warkentin, 1996b; Warkentin et al., 1997). A case-control study of eight patients with HIT-associated venous limb gangrene found a higher median INR, compared with 58 control HIT patients treated with warfarin for DVT who did not develop venous gangrene (5.8 vs. 3.1; $p < 0.001$). Laboratory studies showed a characteristic hemostatic profile for patients with venous gangrene: persisting in vivo thrombin generation (elevated thrombin-antithrombin complex levels), together with reduced protein C activity (Fig. 11). The high INR is a surrogate marker for severely reduced protein C (through parallel coumarin-induced reduction in factor VII). Thus, venous limb gangrene appears to result from a profound disturbance in procoagulant-anticoagulant balance.

The association between venous limb gangrene and HIT was first reported by Towne and colleagues (1979). They noted a prodrome of phlegmasia cerulea dolens before progression to distal gangrene (information on possible coumarin treatment was not given). Other reports of venous limb gangrene complicating HIT, however, do suggest that warfarin had been used during the evolution to necrosis (Thomas and Block, 1992; Hunter et al., 1993; Kaufman et al., 1998).

Patients have also developed venous limb gangrene during combined treatment with both ancrod and warfarin (Warkentin et al., 1997; Gupta et al., 1998); because thrombin generation *increases* during treatment of HIT with ancrod (Warkentin, 1998b; Fig. 2 in Chapter 12), ancrod could predispose to a greater risk for venous gangrene during warfarin treatment.

Recently, several patients have been reported who developed venous limb gangrene during the transition to coumarin from parenteral anticoagulation with a direct thrombin inhibitor (lepirudin or argatroban) (Smythe et al., 2002; Srinivasan

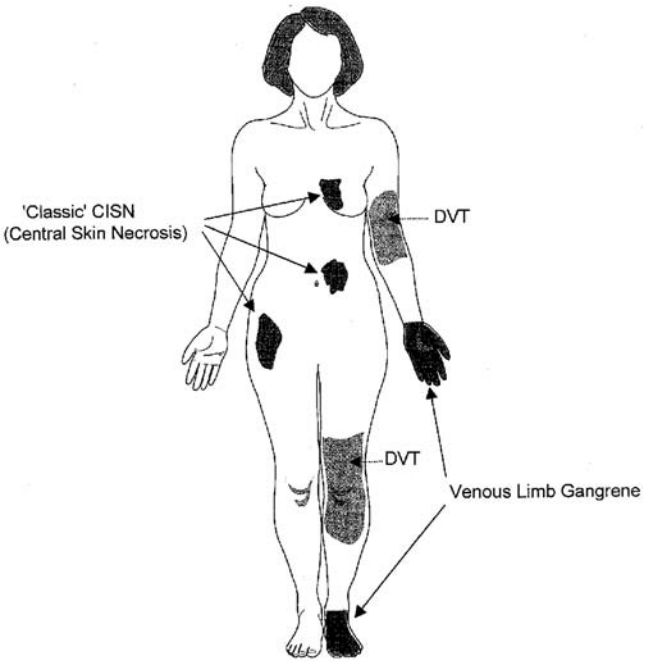


FIGURE 9 Coumarin-induced necrosis: HIT is associated with two forms of necrosis: (1) venous limb gangrene, affecting extremities with active deep vein thrombosis, and (2) "classic" CISN, which involves central (nonacral) tissues, such as breast, abdomen, thigh, flank, and leg, among other tissue sites. Coumarin-induced necrosis complicating HIT typically manifests as venous limb gangrene (~90%) (Warkentin et al., 1997, 1999), whereas necrosis in other clinical settings most commonly affects central tissues (~90%) (Cole et al., 1988). *Abbreviations*: DVT, deep vein thrombosis; CISN, coumarin-induced skin necrosis; HIT, heparin-induced thrombocytopenia. *Source*: From Warkentin, 1996b.

et al., 2003; Warkentin, 2006b). Typically, patients had symptomatic DVT in the affected limb and had their direct thrombin inhibitor started and stopped while they remained thrombocytopenic. Additionally, the INR was supratherapeutic at the time that limb ischemia or gangrene occurred *after stopping* the direct thrombin

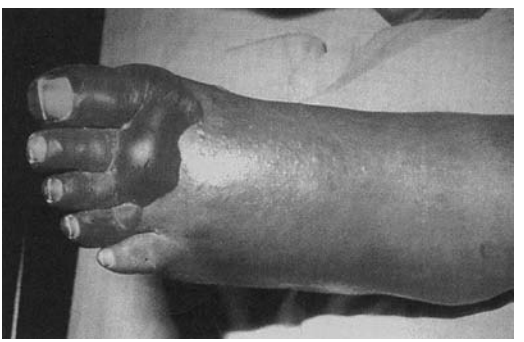


FIGURE 10 (*See color insert*) Warfarin-associated venous limb gangrene. Progression of deep vein thrombosis to acral necrosis (leading to below-the-knee amputation) occurred despite the presence of palpable arterial foot pulses in this 49-yr-old woman with HIT treated with warfarin (international normalized ratio = 7.2 at the onset of limb gangrene). *Source*: From Warkentin et al., 1997.

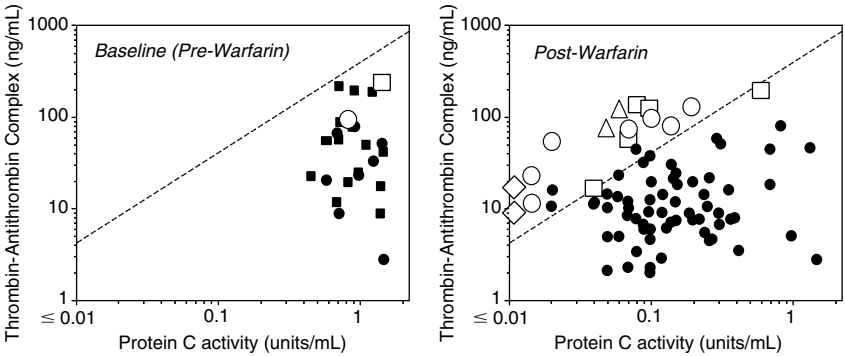


FIGURE 11 TAT complexes compared with protein C activity in patients with HIT: Each data point represents TAT complexes and protein C activity per single treatment day per patient. In both panels the open symbols represent three patients with warfarin-induced venous limb gangrene and one patient with phlegmasia cerulea dolens (*open squares*). The diagonal line represents an arbitrary ratio of TAT complex to protein C of 400. (*Left*) Results when HIT was first diagnosed and before warfarin therapy. Control samples included eight patients (*closed circles*) who subsequently received warfarin for DVT without developing venous limb gangrene and 14 patients without DVT who did not later receive warfarin. (*Right*) Results in 16 patients who were receiving warfarin for HIT, including four patients (*open symbols*) who developed venous limb gangrene/phlegmasia and 12 patients (*closed circles*) who received warfarin without developing venous limb gangrene. The data suggest that patients who develop venous limb gangrene or phlegmasia have a higher ratio of TAT to protein C, consistent with a disturbance in procoagulant-anticoagulant balance during warfarin treatment of HIT. *Abbreviations*: DVT, deep vein thrombosis; HIT, heparin-induced thrombocytopenia; TAT, thrombin-antithrombin. *Source*: From Warkentin et al., 1997.

inhibitor. This experience indicates that the transition from parenteral anticoagulation to coumarin therapy should proceed cautiously, as suggested by the following "rule" (see Chapter 12):

Rule 8

Venous limb gangrene is characterized by (1) in vivo thrombin generation associated with acute HIT; (2) active DVT in the limb(s) affected by venous gangrene; and (3) a supratherapeutic INR during coumarin anticoagulation. This syndrome can be prevented by (1) delaying initiation of coumarin anticoagulation during acute HIT until there has been substantial recovery of the platelet count (to at least 150×10^9/L) while receiving an alternative parenteral anticoagulant (e.g., lepirudin, argatroban, danaparoid), and only if the thrombosis has clinically improved; (2) initiating coumarin in low, maintenance doses (e.g., 2–5 mg warfarin); (3) ensuring that both parenteral and oral anticoagulant overlap for at least 5 days, with at least the last 2 days in the target therapeutic range; and (4) if applicable, physicians should reverse coumarin anticoagulation with intravenous vitamin K in a patient recognized with acute HIT after coumarin therapy has been commenced.

The frequency of venous limb gangrene in HIT patients with DVT who receive warfarin is unknown. This complication happened in 8 of 66 (12.1%; 95% CI 5.4–22.5%) patients with HIT-associated DVT treated with warfarin (with or without ancrod) in Hamilton; venous limb gangrene was a more frequent cause of limb loss in HIT patients than was arterial occlusion in this medical community. Venous gangrene also occurred in 1 of 21 (4.8%; 95% CI 0.12–23.8%) patients

treated with phenprocoumon in Germany (Greinacher et al., 2000). In contrast, this complication was not observed by Wallis and colleagues in any of 51 patients who received warfarin with a diagnosis of HIT, although only 16 patients received warfarin to manage HIT-associated thrombosis (95% CI for 0/16, 0–20.6%). Besides cotherapy with ancrod, factors that could influence the risk for venous gangrene include the dosing of coumarin, the rate of coagulation factor turnover/consumption related to HIT severity and/or DIC, and vitamin K deficiency.

Rarely, coumarin therapy contributes to microvascular thrombosis and acral limb ischemia in the absence of DVT. Figure 12 shows multiple digital necrosis of the right hand complicating the initiation of warfarin therapy (maximal INR = 4.3) in a patient with Raynaud's phenomenon who developed HIT following aortic valve replacement for adenocarcinoma-associated noninfective thrombotic endocarditis (Warkentin et al., 2004). Although digital necrosis occurred in all four limbs in this patient, only the right foot (which exhibited the greatest amount of ischemic necrosis) was found to have DVT by duplex ultrasonography. It was hypothesized that microcirculatory disturbances secondary to paraneoplastic Raynaud's phenomenon interacted with altered procoagulant-anticoagulant balance (secondary to HIT and warfarin therapy) to cause this dramatic clinical syndrome.

Cerebral Venous (Dural Sinus) Thrombosis

Thrombosis of the dural venous sinuses is an unusual cause of stroke in HIT patients that was first reported by Stevenson (1976). Often, there is a second hypercoagulable state, such as pregnancy (Van der Weyden et al., 1983; Calhoun and Hesser, 1987) or myeloproliferative disease (Kyritsis et al., 1990), that may have interacted with HIT to cause this complication. Platelet-rich "white clots" were identified in the superior sagittal venous sinus in one necropsy study (Meyer-Lindenberg et al., 1997). Clinicians should have a high index of suspicion for dural sinus thrombosis when a patient develops progressive focal neurological signs, decreased level of consciousness, seizures, or headache during or soon after stopping heparin treatment (Beland et al., 1997; Pohl et al., 1999, 2000; Warkentin and Bernstein, 2003). Treatment includes immediate discontinuation of heparin, use of an alternative anticoagulant, and possibly, intravenous gammaglobulin (see Chapter 12).

Adrenal Hemorrhagic Infarction (Adrenal Vein Thrombosis)

Clinicians should suspect bilateral adrenal hemorrhagic infarction when thrombocytopenic patients develop abdominal pain and/or hypotension in association

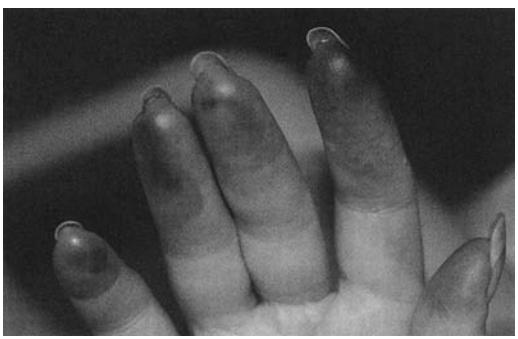


FIGURE 12 (*See color insert*) Warfarin-associated multiple digital necrosis of the right hand in a 61-yr-old woman with paraneoplastic Raynaud's phenomenon and adenocarcinoma-associated thrombotic endocarditis who developed HIT following aortic valve replacement surgery (see text for additional clinical details). *Source*: From Warkentin et al., 2004.

with heparin treatment (Arthur et al., 1985; Dahlberg et al., 1990; Ernest and Fisher, 1991; Delhumeau and Granry, 1992; Bleasel et al., 1992; Kovacs et al., 2001; Warkentin, 2002a, 2006c). Fever and hyponatremia occur in some patients. These patients require corticosteroid replacement to prevent death from acute or chronic adrenal failure (Rowland et al., 1999). Unilateral adrenal hemorrhagic infarction typically presents with ipsilateral flank pain without signs of adrenal failure (Warkentin, 1996a). HIT explained at least 5% of patients with adrenal hemorrhage at one institution (Vella et al., 2001).

This hemorrhagic manifestation of HIT is caused by thrombosis of adrenal veins leading to hemorrhagic necrosis of the glands (Warkentin 2002a, 2006c). Other hypercoagulable states associated with adrenal necrosis include DIC complicating meningococcemia (Waterhouse-Friderichsen syndrome) and the antiphospholipid antibody syndrome (McKay, 1965; Carette and Jobin, 1989).

DIC and Acquired Anticoagulant Deficiency

Although increased thrombin generation occurs in virtually all patients with HIT, overt *decompensated DIC,* defined as reduced fibrinogen levels or an otherwise unexplained increase in the INR, is relatively uncommon, occurring in about 5–10% of patients (Natelson et al., 1969; Klein and Bell, 1974; Zalcberg et al., 1983; Castaman et al., 1992; Betrosian et al., 2003). Protein C consumption is also well compensated, as protein C levels are usually within the normal range when HIT is diagnosed (Warkentin et al., 1997).

Nevertheless, acquired natural anticoagulant failure from DIC could contribute to thrombosis in some patients with HIT. Markedly reduced antithrombin levels were found in a young woman with three-limb DVT and bilateral adrenal infarction complicating HIT; following recovery, antithrombin levels were normal (unpublished observations of the author). This hypothesis implies that plasmapheresis could benefit patients by correcting acquired anticoagulant deficiency; if so, the replacement fluid must be plasma, rather than albumin, to correct antithrombin and other natural anticoagulant deficiencies.

Other patients with HIT-associated DIC evince clinical signs of microvascular thrombosis. For example, Figure 13 shows livedo reticularis and patchy foot necrosis (despite palpable foot pulses) in a postoperative cardiac surgery patient with HIT (platelet count nadir, 39×10^9/L) complicated by hypofibrinogenemic DIC. Evidence for acquired natural anticoagulant failure included mildly reduced antithrombin levels (0.76 U/mL; normal, 0.77–1.30 U/mL) and moderately reduced protein C activity (0.50 U/mL; normal, 0.70–1.80 U/mL) that subsequently resolved. Free protein S levels were normal (1.12 U/mL; normal, 0.62–1.38 U/mL). Evidence for DIC included a fibrinogen of 1.2 g/L (normal, 1.5–4.0 g/L) that rose to 4.7 g/L 1 wk later during therapeutic-dose danaparoid therapy, a strongly positive protamine sulfate paracoagulation assay (4+ reactivity at 15 min; normal, no reactivity), a fibrin *D*-dimer level that was greater than 2000 μg/L (normal, <500 μg/L), and the presence of red cell fragments. Additionally, the INR was elevated at 1.6 (normal, 0.9–1.2), even though coagulation factors VII, V, X, and II all measured between 0.73 to 0.83 U/mL (normal, 0.50–1.50 U/mL). The anticoagulant treatment was successful in avoiding limb amputation. In my experience, limb ischemia and necrosis associated with DIC that occurs in the absence of large artery thrombotic occlusion or warfarin therapy is the least common explanation for limb loss in HIT.

Livedo reticularis is also discussed on p. 48.

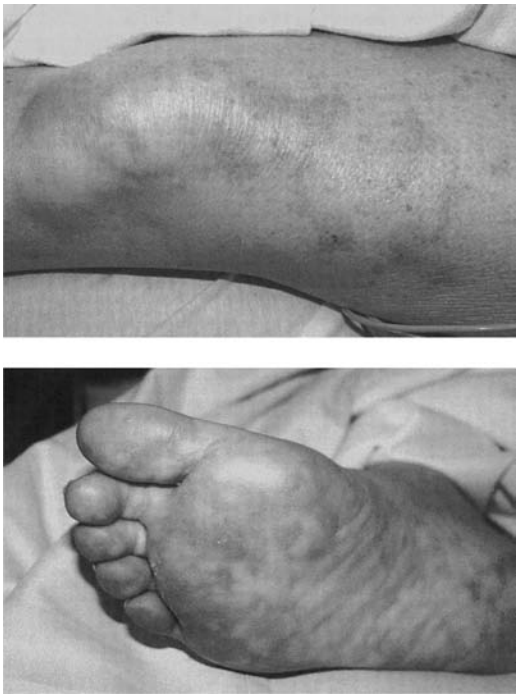


FIGURE 13 (*See color insert*) Clinical manifestations of DIC. (**A**) Livedo reticularis. (**B**) Patchy ischemic necrosis of right foot. This 70-yr-old woman developed HIT-associated DIC with hypofibrinogenemia, elevated INR, and reduced antithrombin and protein C activity levels 9 days after emergency cardiac surgery for cardiac catheterization-associated dissection of the left main coronary artery (see text for additional clinical information).

Congenital Hypercoagulability and HIT-Associated Thrombosis

Gardyn and associates (1995) reported a patient with fatal HIT and widespread microvascular thrombosis. The investigators identified heterozygous factor V Leiden (G1691A mutation) in this patient, and they speculated that this contributed to the severe clinical course. However, the complications may also have been related to the treatment with LMWH and warfarin.

The interaction between factor V Leiden and thrombotic sequelae of HIT was formally investigated in a study of 165 patients with HIT, 16 (9.7%) of whom had factor V Leiden (Lee et al., 1998). No increase in the number or severity of venous or arterial thrombosis was seen. This result is not surprising, as thrombosis occurs in about 50–75% of patients with HIT (Warkentin and Kelton, 1996). Thus, even if the most common congenital hypercoagulable disorders, factor V Leiden and the prothrombin G20210A mutation (each occurring in about 5% of the population), were strongly associated with increased risk for thrombosis in HIT, only a few HIT-associated thromboses could thereby be explained.

Carlsson and colleagues (2003) studied 142 patients with HIT (79 with thrombosis) to determine whether any of 10 established or putative platelet receptor or clotting factor polymorphisms (including factor V Leiden and prothrombin G20210A mutation) was associated with thrombosis. None was found.

Lindhoff-Last et al. (2002) also found no association between factor V Leiden or prothrombin G20210A mutation and thrombosis in a smaller study of 21 patients. However, they found that more HIT patients had elevated factor VIII levels (at mean 29 mo follow-up) than matched normal controls (16/21 vs. 4/21). The significance of this finding is unclear.

E. Arterial Thrombosis

Lower limb artery thrombosis was the first recognized complication of HIT (Weismann and Tobin, 1958; Roberts et al., 1964; Rhodes et al., 1973, 1977). Arterial thrombosis most commonly involves the distal aorta (e.g., saddle embolism) or the large arteries of the lower limbs, leading to acute limb ischemia with absent pulses. Sometimes, platelet-rich thromboemboli from the left heart or proximal aorta explain acute lower limb arterial ischemia (Vignon et al., 1996). Other arterial thrombotic complications that are relatively common in HIT include acute thrombotic stroke and myocardial infarction. The relative frequency of arterial thrombosis in HIT by location, namely, lower limb artery occlusion >> stroke syndrome > myocardial infarction (Benhamou et al., 1985; Kappa et al., 1987; Warkentin and Kelton, 1996; Nand et al., 1997), is reversed from that observed in the non-HIT population (myocardial infarction > stroke syndrome >> lower limb artery occlusion).

Uncommon but well-described arterial thrombotic events in HIT include mesenteric artery thrombosis (bowel infarction), brachial artery thrombosis (upper limb gangrene), and renal artery thrombosis (renal infarction). Multiple arterial thrombotic events are quite common, as are recurrences following surgical thromboembolectomy, especially if further heparin is given during or after surgery. Occasionally, microembolization of thrombus originating from the heart or aorta causes foot or toe necrosis with palpable arterial pulses.

Angiographic Appearance

Lindsey and colleagues (1979) reported a distinct angiographic appearance of heparin-induced thromboembolic lesions, described as "broad-based, isolated, gently lobulated excrescences which produced 30–95% narrowing of the arterial lumen. The abrupt appearance of such prominent luminal contour deformities in arterial segments that were otherwise smooth and undistorted was unexpected and impressive.... In each case, the lesions were located proximal to sites of arterial occlusion." The radiologic and surgical experience described suggests that distal embolization of "white" clots composed of "platelet-fibrin aggregates" accounted for the limb ischemia.

G. Graft, Prosthetic Device, and Extracorporeal Circuit Thrombosis

HIT predisposes to thrombosis of blood in contact with native or prosthetic grafts or vascular fistulae, valve or other intravascular prostheses, as well as extracorporeal circuits (Towne et al., 1979; Silver et al., 1983; Bernasconi et al., 1988; AbuRahma et al., 1991; Lipton and Gould, 1992; Hall et al., 1992). This presents serious management problems in certain situations, such as renal hemodialysis (see Chapter 18). Clinicians should check for unexpected platelet count declines, and test for HIT antibodies, in patients who develop thrombosis of grafts, prostheses, or other devices during heparin treatment.

IV. MISCELLANEOUS COMPLICATIONS OF HIT

A. Heparin-Induced Skin Lesions at Subcutaneous Injection Sites

Skin lesions that occur at the site(s) of subcutaneous heparin injection are a manifestation of the HIT syndrome. For unknown reasons, only 10–20% of patients who form HIT antibodies during subcutaneous UFH or LMWH treatment develop these lesions (Warkentin et al., 2005b). Furthermore, about 50% to 75% of patients who develop heparin-induced skin lesions do not develop thrombocytopenia, even

though heparin-dependent, platelet-activating HIT antibodies are readily detectable (Warkentin, 1996a, 1997; Handschin et al., 2005).

The skin abnormalities range in appearance from indurated, erythematous nodules or plaques (Fig. 14) to frank necrotizing lesions (Fig. 14) that start 5 or more days (median, day 8) after beginning heparin injections (Hasegawa, 1984; MacLean et al., 1990; Wütschert et al., 1999). The lesions can occur earlier if there was recent treatment with heparin given by another route that resulted in formation of HIT antibodies. Some erythematous plaques have an eczematous appearance. Necrotic lesions typically consist of a central black eschar surrounded by a cuff of induration and erythema (Fig. 14). Complex skin lesions can result—for example, several discrete areas of necrosis (each lesion corresponding to a different heparin injection site), each with a surrounding violaceous halo, with all circumscribed by a diffuse erythema. Even the least severe forms of heparin-induced skin lesions usually cause pain or pruritus.

Both UFH and LMWH can cause these reactions (Handschin et al., 2005). Patients who develop UFH-induced skin lesions generally will develop further lesions if LMWH is substituted for the UFH (Bircher et al., 1990). In contrast, it is uncommon for danaparoid to cause skin lesions in these patients.

Histopathology

Lymphocyte infiltration of the upper and middermis that can extend into the epidermis characterizes the erythematous plaque (Bircher et al., 1990). Dermal and epidermal edema (spongiosis) is observed in lesions that appear eczematous. The T lymphocytes of helper-suppressor (CD4+) phenotype predominate, together with

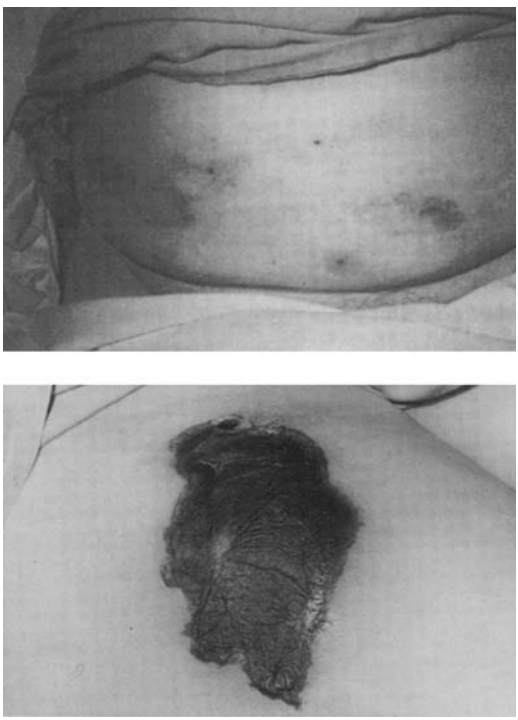


FIGURE 14 (*See color insert*) Heparin-induced skin lesions. (**A**) Heparin-induced erythematous plaques: UFH injections into the lower abdomen resulted in painful erythematous plaques beginning on day 7 of sc UFH treatment; at this time, the platelet count fell only by 9% from 340 to 311 $\times$ 10^9/L. HIT antibody seroconversion from a negative baseline was shown using the serotonin release assay (from 0% to 84% serotonin release). (**B**) Heparin-induced skin necrosis: UFH injections into the right anterior thigh led to skin necrosis: a large black eschar with irregular borders is surrounded by a narrow band of erythema. The platelet count fell to 32 $\times$ 10^9/L; despite stopping heparin, the patient developed symptomatic proximal deep vein thrombosis 10 days later. *Abbreviations*: HIT, heparin-induced thrombocytopenia; UFH, unfractionated heparin. *Source*: (**B**) From Warkentin 1996a.

CD1+/DR+ dendritic (Langerhans) cells, are consistent with a type IV delayed hypersensitivity immune response. Cytokine synthesis by activated CD4 cells could explain the peripheral blood eosinophilia that has been reported in a few patients (Bircher et al., 1994). In contrast, histopathology of lesions associated with cutaneous necrosis usually shows intravascular thrombosis of dermal vessels, with or without perivascular inflammation and red cell extravasation of variable degree (Hall et al., 1980; Kearsley et al., 1982; Cohen et al., 1988; MacLean et al., 1990; Balestra et al., 1994).

Management

Heparin-induced skin lesions should be considered a possible marker for the HIT syndrome (Warkentin et al., 2005b). Platelet count monitoring, if not already being performed, should be initiated and continued for several days, even after stopping heparin administration. The reason is that some patients develop a fall in platelet count, together with thrombosis (often affecting limb arteries), that begins several days after stopping the heparin (Warkentin, 1996a, 1997). An alternative anticoagulant, such as danaparoid, lepirudin, or argatroban, should be given, particularly in patients whose original indication for anticoagulation still exists or who develop progressive thrombocytopenia. The skin lesions themselves should be managed conservatively whenever possible, although some patients require debridement of necrotic tissues followed by skin grafting (Hall et al., 1980).

Rule 9

Erythematous or necrotizing skin lesions at heparin injection sites should be considered dermal manifestations of the HIT syndrome, irrespective of the platelet count, unless proved otherwise. Patients who develop thrombocytopenia in association with heparin-induced skin lesions are at increased risk for venous and, especially, arterial thrombosis.

B. Classic Coumarin-Induced Skin Necrosis

Classic coumarin-induced skin necrosis (CISN) is a very rare complication of oral anticoagulant therapy (Cole et al., 1988). In its classic form, it is characterized by dermal necrosis, usually in a central (nonacral) location, such as breast, abdomen, thigh, or leg, that begins 3–6 days after starting therapy with warfarin or other coumarin anticoagulants (Fig. 9). Initially, there is localized pain, induration, and erythema that progresses over hours to central purplish-black skin discoloration and blistering, ultimately evolving to well-demarcated, full-thickness necrosis involving skin and subdermal tissues. Some patients require surgical debridement. Case reports suggest that congenital deficiency of natural anticoagulant proteins, especially protein C, is sometimes a pathogenic factor (Broekmans et al., 1983; Comp, 1993).

There is evidence that HIT also predisposes to classic CISN (Celoria et al., 1988; Cohen et al., 1989; Warkentin et al., 1999; Srinivasan et al., 2003). Theoretically, this could result from increased consumption of anticoagulant factors, thereby leading to greater reduction in protein C in the setting of increased thrombin generation in HIT (Tans et al., 1991; Warkentin et al., 1997). However, central lesions of CISN seem less likely to complicate HIT than the related syndrome of coumarin-induced venous limb gangrene (Warkentin et al., 1997, 1999). Perhaps active DVT in HIT localizes the progressive microvascular thrombosis to acral tissues already affected by extensive venous thrombosis.

C. Other Heparin-Associated Skin Lesions

Skin Necrosis in the Absence of Coumarin Therapy

Other patients have developed skin lesions during *intravenous* heparin therapy, or at locations otherwise distant from subcutaneous injection sites, in the absence of coumarin therapy. Hartman and colleagues (1988) reported a man who received intravenous heparin for saphenous vein thrombosis: the platelet count fell from 864 to 44 $\times$ 10^9/L (day 10). On day 7, when the platelet count had fallen by 33% to 575 $\times$ 10^9/L, progressive necrosis of skin in the thigh at the region of the thrombosed vein occurred, necessitating surgical excision. Thrombosis of veins and capillaries, with arterial sparing, was noted. Balestra et al. (1994) reported a patient who developed thrombocytopenia (75 $\times$ 10^9/L) and skin necrosis of the thigh on day 9 of subcutaneous injections of LMWH given into the lower abdominal wall. A skin biopsy showed small vessel thrombosis with a mild inflammatory reaction.

Other clinicians have reported patients with HIT antibodies who developed skin lesions that occurred at locations distant from subcutaneous LMWH injection sites, even in the absence of thrombocytopenia (Tietge et al., 1998).

Other Skin Lesions Associated with Heparin Treatment

Livedo Reticularis. The bluish, reticulated (network-like), mottled appearance of livedo reticularis was reported in a patient with HIT complicating intravenous UFH given for atrial fibrillation after heart surgery (Gross et al., 1993). This patient also had DIC, microangiopathic peripheral blood abnormalities, and fibrin thrombi noted within small dermal vessels. The livedo appearance results from microvascular thrombosis, with slowing of blood flow and dilation of the horizontally oriented dermal venous drainage channels (Copeman, 1975). Figure 13a (see p. 44) shows livedo reticularis associated with HIT and DIC.

Urticaria and Other Miscellaneous Lesions. Other dermatological consequences of heparin treatment do not appear to be related to HIT. These range from common lesions (ecchymosis) to rare effects of intravenous heparin, such as vasculitis (Jones and Epstein, 1987) and cutaneous necrosis with hemorrhagic bullae (Kelly et al., 1981). Some patients have developed widespread urticarial lesions, sometimes accompanied by angioedema, during treatment with subcutaneous or intravenous heparin (Odeh and Oliven, 1992; Patriarca et al., 1994). In one patient skin testing suggested a generalized reaction against the preservative chlorbutol (Dux et al., 1981). Although LMWH injections were claimed to have caused distal extremity dermal lesions in a patient with HIT (Payne and Kovacs, 2003), it is possible these were related to concomitant warfarin therapy.

Cutaneous Type IV Hypersensitivity Reactions. Not all cutaneous lesions that develop at UFH or LMWH injection sites represent HIT. The so-called "type IV hypersensitivity reactions," which are characterized by pruritic infiltrations or blistering erythematous reactions of variable size at heparin injection sites, are often not associated with presence of anti-PF4/H antibodies. More than 90% of affected patients are females, and many are pregnant (Ludwig et al., 2006). The histopathology consists of epidermal spongiosis, dermal edema, and lymphocytic infiltrates accompanied by numerous eosinophils in the papillary dermis (Grasseger et al., 2001). Cutaneous allergy testing usually shows variable cross-reactivity with other heparin(oids), with frequency of cross-reactivity reportedly related to molecular weight, as follows (UFH > LMWH > danaparoid > fondaparinux) (Ludwig et al., 2005, 2006). However, some investigators have observed patients with cutaneous cross-reactivity against various LMWH preparations but not with UFH (Grasseger et al., 2001).

The distinction between non-HIT and HIT-associated skin lesions is not trivial: whereas intravenous heparin administration is appropriate for managing patients who cannot tolerate subcutaneous injections because of type IV hypersensitivity reactions (Koch et al., 1991; Gaigl et al., 2005; Ludwig et al., 2006), intravenous bolus heparin administration to a patient with HIT-associated skin lesions can lead to rapid-onset HIT and an associated acute systemic reaction (ASR) (Platell and Tan, 1986).

D. Acute Systemic Reactions Following Intravenous Bolus Heparin

ASR refers to a variety of symptoms and signs that characteristically begin 5–30 min after an intravenous heparin bolus is given to a patient with circulating HIT antibodies (Nelson et al., 1978; Warkentin et al., 1992, 1994; Popov et al., 1997; Ling and Warkentin, 1998; Warkentin, 2002b; Mims et al., 2004) (Table 5; Fig. 3). Only about one quarter of at-risk patients who receive a heparin bolus develop such a reaction. The most common signs and symptoms are fever and chills, hypertension, and tachycardia. Less common are flushing, headache, chest pain, dyspnea, tachypnea, and large-volume diarrhea. In some patients, severe dyspnea is the predominant sign, termed "pseudo-pulmonary embolism" (Popov et al., 1997; Hartman et al., 2006); multiple small perfusion defects on radionuclide lung scans can be shown (Nelson et al., 1978; Ling and Warkentin, 1998). Fatal cardiac and respiratory arrest has been reported (Ansell et al., 1986; Platell and Tan, 1986; Hewitt et al., 1998).

An abrupt fall in the platelet count invariably accompanies these reactions. However, the platelet count drop is often transient (Warkentin et al., 2005b). Thus, physicians should determine the platelet count immediately on suspecting the diagnosis and test for HIT antibodies. Heparin must be discontinued, as further use can lead to fatal complications (Ling and Warkentin, 1998).

Rule 10

Any inflammatory, cardiopulmonary, or other unexpected acute event that begins 5–30 min after an intravenous heparin bolus should be considered acute HIT unless proved otherwise. The postbolus platelet count should be measured promptly and compared with prebolus levels, because the platelet count fall is abrupt and often transient.

The clinical features of postheparin bolus ASR are *not* typical of IgE-mediated anaphylaxis (i.e., urticaria, angioedema, and hypotension are not seen). Rather, the syndrome resembles febrile transfusion reactions commonly observed after platelet transfusions, suggesting a common pathogenesis of proinflammatory cytokines associated with cellular activation (Heddle et al., 1994). Moreover, there are

TABLE 5 Clinical Features of Acute Systemic Reactions Following Intravenous Bolus Heparin

Timing: onset 5–30 min after intravenous heparin bolus
Clinical context: recent use of heparin (past 5–100 days)
Laboratory features: abrupt, reversible fall in the platelet count
Signs and symptoms
Inflammatory: chills, rigors, fever, flushing
Cardiorespiratory: tachycardia, hypertension, tachypnea, dyspnea, chest pain or tightness, cardiopulmonary arrest (rare)
Gastrointestinal: nausea, vomiting, diarrhea
Neurological: headache, transient global amnesia (rare)

similarities between ASR and the administration of ADP in humans, including acute dyspnea, tachycardia, and transient thrombocytopenia (Davey and Lander, 1964).

A few patients have developed acute, transient impairment of anterograde memory (i.e., the ability to form new memories) following an intravenous heparin bolus in association with acute HIT (Warkentin et al., 1994; Pohl et al., 2000). This syndrome resembles that of transient global amnesia, a well-characterized neurological syndrome of uncertain pathogenesis.

E. Heparin Resistance

Difficulty in maintaining therapeutic anticoagulation despite increasing heparin dosage, or heparin resistance, is a common finding in patients with HIT-associated thrombosis (Rhodes et al., 1977; Silver et al., 1983). Possible explanations include neutralization of heparin by PF4 released from activated platelets (Padilla et al., 1992) or pathophysiological consequences of platelet-derived microparticles (Bode et al., 1991). Heparin resistance is not specific for HIT, however, and occurs in many patients with extensive thrombosis of various etiologies (e.g., cancer).

V. RISK FACTORS FOR HIT

Besides duration of heparin administration, the three most important risk factors for HIT include: (1) type of heparin (UFH >> LMWH > fondaparinux); (2) type of patient (postsurgical > medical > obstetric/pediatric); and (3) patient gender (female > male) (Warkentin et al., 2005c, 2006) (see Chapter 3). Ironically, despite the greater risk of HIT in females (odds ratio, 1.5–2.0) (Warkentin et al., 2006), HIT is rare in pregnancy (Fausett et al., 2001), and has not been reported with LMWH administered during pregnancy (Greer and Nelson-Piercy, 2005).

VI. SPECIAL CLINICAL SITUATIONS

A. Cardiac and Neurological Complications of HIT

Although HIT can affect almost any organ system, some clinical specialties observe a wider spectrum of thrombotic and other sequelae of HIT. Table 6 lists complications encountered in cardiology and neurology.

B. HIT in Pregnancy

HIT has complicated UFH treatment given for venous thromboembolism complicating pregnancy (Van der Weyden et al., 1983; Meytes et al., 1986; Copplestone and Oscier, 1987; Greinacher et al., 1992) or the postpartum period (Calhoun and Hesser, 1987). HIT seems to be rare in this patient population; no pregnant patients have been diagnosed with HIT over a 25-yr period in Hamilton. Plasma glycosaminoglycans are increased during pregnancy (Andrew et al., 1992), which could contribute to lower frequency or pathogenicity of HIT antibodies. HIT antibodies cross the placenta (Greinacher et al., 1993), so it is at least theoretically possible that a heparin-treated newborn delivered from a mother with acute HIT could develop this drug reaction.

Pregnant patients with HIT have developed unusual events, such as cerebral dural sinus thrombosis (Van der Weyden et al., 1983; Calhoun and Hesser, 1987). Treatment options for pregnant patients with life-threatening thrombosis include danaparoid or fondaparinux as these drugs do not cross the placenta (see Chapters 12, 13 and 17). The more benign syndrome of heparin-induced skin

TABLE 6 Cardiological and Neurological Complications of HIT

Cardiological complications
Myocardial infarction (Rhodes et al., 1973; Van der Weyden et al., 1983)
Occlusion of saphenous vein grafts postcoronary artery bypass surgery[a]
Intra-atrial thrombus (left and right[b] heart chambers) (Scheffold et al., 1995; Olbricht et al., 1998)
Intraventricular thrombus (left and right[b] heart chambers) (Commeau et al., 1986; Dion et al., 1989; Vignon et al., 1996)
Prosthetic valve thrombosis (Bernasconi et al., 1988; Vazquez-Jimenez et al., 1999)
Right heart failure secondary to massive pulmonary embolism
Cardiac arrest postintravenous heparin bolus (Ansell et al., 1986; Platell and Tan, 1986; Hewitt et al., 1998)
Neurological complications
Stroke syndrome
In situ thrombosis
Progressive stroke in patients receiving heparin for treatment of stroke (Ramirez-Lassepas et al., 1984)
Cardiac embolization (Scheffold et al., 1995)
Cerebral vein (dural venous sinus) thrombosis (Van der Weyden et al., 1983; Kyritsis et al., 1990; Meyer-Lindenberg et al., 1997; Warkentin and Bernstein, 2003); complicating pregnancy (Calhoun and Hesser, 1987)
Amaurosis fugax (Theuerkauf et al., 2000)
Ischemic lumbosacral plexopathy (Jain, 1986)
Paraplegia, transient (Maurin et al., 1991) or permanent (Feng et al., 1993), associated with distal aortic thrombosis
Transient global amnesia (Warkentin et al., 1994)
Headache[c]

[a]Thrombosis preferentially affects saphenous vein grafts rather than internal mammary artery grafts (Liu et al., 2002; Ayala et al., 2002).

[b]Although adherent thrombi that likely developed in situ have been reported (Dion et al., 1989), emboli originating from limb veins can explain right-sided intra-atrial or intraventricular clots.

[c]Headache as a feature of HIT is suggested by (*i*) its occurrence in patients with acute systemic reactions postheparin bolus and (*ii*) its concurrence with onset of thrombocytopenia in several patients who developed HIT in a clinical trial (unpublished observations of the author).

Abbreviation: HIT, heparin-induced thrombocytopenia.

lesions without thrombocytopenia has also been reported in pregnant patients (Drouet et al., 1992). Danaparoid was reported to be effective in a patient who developed LMWH-induced skin lesions (de Saint-Blanquat et al., 2000).

C. HIT in Children and Neonates

There are anecdotal reports of HIT occurring in children, some as young as 3 mo of age (Laster et al., 1987; Oriot et al., 1990; Potter et al., 1992; Murdoch et al., 1993; Klement et al., 1996; Butler et al., 1997; Ranze et al., 1999, 2001; Klenner et al., 2004) (see Chapter 20). However, not all of these patients underwent confirmatory testing with specific diagnostic assays. HIT in children has a similar, often dramatic clinical course, as is seen in adults. The frequency of HIT in the pediatric population is unknown.

The frequency and clinical import of HIT in neonates receiving heparin in intensive care settings is controversial. Spadone and colleagues (1992) investigated 34 newborn infants (average gestational age, 29 wk) who developed thrombocytopenia or thrombosis, beginning an average of 22 days after starting heparin therapy. Platelet aggregation studies suggested the presence of HIT antibodies in 41% of these neonates. Aortic thrombosis complicating umbilical artery catheter use was the most common complication. Another group (Butler et al., 1997), also using

platelet aggregation studies, reported a neonate who may have developed fatal HIT shortly after birth. More specific activation or antigen assays were not performed in either study, however. A recent study of 108 neonates who received UFH flushes found no HIT antibodies using a sensitive antigen assay (Klenner et al., 2003).

D. HIT in Bone Marrow and Solid Organ Transplantation

Given the widespread use of heparin to maintain patency of indwelling catheters, it is surprising that there are few reports of HIT in patients undergoing intensive anticancer chemotherapy. Two reports describe patients with apparent HIT complicating allogeneic or autologous marrow or stem cell transplantation (Tezcan et al., 1994; Sauer et al., 1999). Subclavian vein thrombosis occurred in one patient. It is possible that the combination of intensive chemotherapy and treatment-induced thrombocytopenia reduces the likelihood of HIT antibody formation or clinical expression of HIT.

There is an intriguing report of a man recently recovered from HIT who was about to receive autologous marrow transplantation. When his marrow was collected into heparin anticoagulant, substantial ex vivo thrombus formation occurred, preventing adequate cell collection (Bowers and Jones, 2002).

Solid organ or tissue transplantation is rarely complicated by postoperative HIT (Hourigan et al., 2002; Anderegg et al., 2005; Rastellini et al., 2006). Whether postoperative immunosuppression reduces the risk of HIT compared with other postoperative patient populations is unknown.

VII. ESTIMATING THE PRETEST PROBABILITY OF HIT

A. Scoring Systems for HIT

Various scoring systems to estimate the probability of HIT based upon clinical information have been published, usually for the purpose of evaluating new laboratory tests for HIT (Greinacher et al., 1994; Pouplard et al., 1999; Alberio et al., 2003). These systems have included the platelet count recovery following heparin cessation, which limits their applicability for judging the clinical likelihood of HIT in "real time" when a thrombocytopenic patient receiving heparin is first evaluated. Further, these scoring systems were developed before various features of the timing and severity of platelet count fall in HIT were understood.

B. The "Four Ts"

A new scoring system, the "4 Ts," has been developed that takes advantage of new information regarding the clinical features of HIT (Warkentin, 2003; Warkentin and Heddle, 2003). Platelet count recovery is not a criterion, because this information often is not available at initial evaluation, or heparin may not have been stopped. For simplicity, four clinical features are assessed, given scores of 0, 1, or 2 (Table 7). Thus, the maximal total score is 8.

Estimated pretest probabilities of HIT thereby range from low (0–3) to high (6–8), with an intermediate score (4–5) indicating moderate risk.

Maximal scores for each category are given when the clinical features are highly consistent with HIT. Thus, a patient will score 8 if there is a substantial fall in the platelet count that begins 5–10 days after commencing heparin, together with thrombosis, and where no other plausible cause is apparent during clinical assessment. Even a patient with no clinical evidence of thrombosis can be assigned a high pretest probability (score 6 of 8) if the clinical features are otherwise consistent with HIT. Similarly, a patient can also score high (6 of 8) even in the absence of

TABLE 7 Estimating the Pretest Probability of HIT: "The Four Ts"

	Points (0, 1, or 2 for each of 4 categories: maximum possible score = 8)[a]		
	2	1	0
Thrombocytopenia (acute)	> 50% platelet fall (nadir $\geq 20 \times 10^9$/L)	30–50% platelet fall (or >50% fall due to surgery); or nadir 10–19 $\times 10^9$/L	<30% platelet fall; or nadir $\leq 10 \times 10^9$/L
Timing[b] of platelet count fall, thrombosis, or other sequelae (first day of heparin course = day 0)	Clear onset between days 5–10 or $\leq$1 day (if heparin exposure within past 30 days)	Consistent with day 5–10 fall, but not clear (e.g., missing platelet counts) or $\leq$1 day (heparin exposure within past 31–100 days) or platelet fall after day 10	Platelet count fall $\leq$4 days without recent heparin exposure
Thrombosis or other sequelae (e.g., skin lesions, ASR)	New thrombosis; skin necrosis; ASR after iv heparin bolus	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis (not yet proven); asymptomatic upper-limb DVT	None
o**T**her cause of thrombocytopenia not evident	No explanation (besides HIT) for platelet count fall is evident	Possible other cause is evident	Definite other cause is present

[a]Pretest probability score: 6–8 = high; 4–5 = intermediate; 0–3 = low.

[b]First day of immunizing heparin exposure considered day zero; the day the platelet count begins to fall is considered the day of onset of thrombocytopenia (it generally takes 1–3 more days until an arbitrary threshold that defines thrombocytopenia is passed). In general, giving heparin during or soon after surgery is most likely to induce immunization. The scoring system shown here has undergone minor modifications from previously published scoring systems (Warkentin, 2003; Warkentin and Heddle, 2003).

Abbreviations: ASR, acute systemic reaction (Table 5); DVT, deep venous thrombosis; HIT, heparin-induced thrombocytopenia.

thrombocytopenia; such clinical scenarios have been reported (Warkentin, 2005). Another feature of this system is that very low platelet count values (i.e., 10–19 and $<10 \times 10^9$/L) score only 1 and 0 points, respectively, thus reducing the chance that a patient with posttransfusion purpura (PTP) or DITP will be misclassified as HIT and inappropriately given anticoagulant therapy.

C. Clinical Use of a Scoring System

A practical use of the scoring system is to help make initial clinical decisions regarding therapy. Based on published data, we believe it is likely that a low score (0–3) is associated with a very low risk (<5%) of clinically significant HIT antibodies (defined arbitrarily as >50% serotonin release in a washed platelet activation assay) (Lo et al., 2006) (see Chapter 10). In contrast, a high score (6–8) appears to be associated with a high risk (>80%) of such strong HIT antibodies, at least in some centers. Further, 50–75% of patients evaluated for clinical HIT will have low or high scores. This leaves a smaller number of patients in whom the clinical suspicion of HIT is more uncertain (score 4–5) and in whom the results of diagnostic testing will be especially important for supporting (or refuting) the diagnosis of HIT (Warkentin, 2005).

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3 Frequency of Heparin-Induced Thrombocytopenia

David H. Lee
Queen's University, Kingston, Ontario, Canada

Theodore E. Warkentin
Michael G. DeGroote School of Medicine, McMaster University, and Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada

I. INTRODUCTION

Thrombocytopenia is a common problem encountered in hospitalized patients. For patients receiving heparin, there are three general explanations for thrombocytopenia: (1) heparin-induced thrombocytopenia (HIT), (2) non-idiosyncratic heparin-induced platelet activation (see Chapter 4), and—perhaps most often—(3) an unrelated clinical problem, either common (e.g., hemodilution, septicemia) or rare (e.g., posttransfusion purpura, drug-induced immune thrombocytopenic purpura) (see Chapter 11). The availability of sensitive and specific laboratory assays (e.g., enzyme immunoassay [EIA] and serotonin release assay [SRA]) for pathogenic HIT antibodies means that patients with HIT can usually be readily distinguished from those with the other conditions (see Chapter 10). However, the role of heparin in causing thrombocytopenia because of nonimmune platelet activation cannot readily be separated from other common medical problems encountered in hospitalized patients, on either clinical or laboratory criteria. Furthermore, these two conditions can coexist (Chong and Castaldi, 1986). Thus, the term "nonimmune heparin-associated thrombocytopenia" (nonimmune HAT) has been recommended to describe patients who develop thrombocytopenia during heparin treatment in which a role for HIT antibodies cannot be implicated (Warkentin et al., 1998a).

Unfortunately, many early studies of HIT frequency either did not perform laboratory testing or used relatively insensitive or nonspecific assays to diagnose HIT. In contrast, more recent studies have used one, or even two, sensitive and complementary assays. Perhaps for this reason, the understanding of the frequency and clinical impact of HIT has shifted over the years. Formerly, opinions on immune-mediated HIT were divergent: it was considered both nonexistent (Bell, 1988) and common (Kelton, 1986). Nevertheless, both viewpoints acknowledged that thrombosis resulting from HIT was very uncommon. Today's perspective on HIT is very different. The frequency of HIT is now shown to be *variable,* partly depending on patient population and type of heparin used. For example, the frequency ranges from negligible—e.g., a pregnant patient receiving low molecular weight heparin (LMWH)—to as high as 5% (orthopedic surgical patient receiving unfractionated heparin [UFH] for 2 wk). Anti-platelet factor 4 (PF4)/heparin antibody formation with use of UFH ranges from 2–5% (cardiac medical patients) to 15–30% (orthopedic surgical patients) to 30–70% (cardiac surgical patients). Most importantly, however, it is now becoming clear that the risk for

thrombosis in patients who develop HIT is at least 30–70%, a frequency that is far greater than in control patients who do not develop HIT (see Chapter 2).

The biological basis for this variability in frequency of HIT and antibody formation is now apparent. The HIT antigen is a cryptic autoantigen, or neoantigen, on PF4 that is formed when PF4 binds to heparin (see Chapters 5–7). Only stoichiometric concentrations of heparin and PF4 will form the antigen. Thus, it can be hypothesized that the frequency of anti-PF4/heparin antibody formation will be influenced not only by heparin dose and composition, but also by circulating PF4 levels. Conditions associated with fluctuating, but at times high, circulating PF4 and heparin levels (e.g., cardiac surgery) might be ideal for immunization. Thus, real differences in HIT frequency observed among prospective studies can be understood in a biologically plausible context.

The key role of the pathogenic HIT antibodies and the availability of sensitive and specific assays for their detection suggest that HIT should be considered a *clinicopathologic syndrome.* Consequently, this chapter will focus on studies that have used in vitro testing to evaluate HIT antibodies. However, other features known to be useful to diagnose HIT, such as the timing of the onset of thrombocytopenia in typical HIT, and the rapid platelet count fall on heparin rechallenge, will also be used (see Chapter 2). The importance of confirmatory laboratory testing should not be underestimated: prospective (Greinacher et al., 1994; Lee et al., 1996; Juhl et al., 2006) and retrospective (Look et al., 1997) studies suggest that only 15–55% of sera referred for evaluation test positive for antibodies. Furthermore, systematic analysis of a large clinical trial of heparin treatment (Warkentin et al., 1995, 2003) revealed several patients in whom unusual clinical events subsequently linked to HIT were initially attributed to other problems.

Table 1 lists various biological and technical explanations that underlie the reported variability in frequency of HIT among the prospective studies. We will begin our discussion by summarizing an important technical problem in many studies, namely, the failure to exclude patients with early, nonimmune HAT.

II. EARLY- VERSUS LATE-ONSET THROMBOCYTOPENIA

The distinction between thrombocytopenia that begins early (within 4 days) or late (5 or more days after beginning heparin treatment) is a simple clinical feature that is useful to distinguish nonimmune HAT, which begins early, from (immune) HIT, which begins late. For this assessment, the first day of heparin use is considered day 0. There is an important exception to this rule of timing for HIT: a rapid fall in platelet count on starting heparin therapy can represent acute HIT, but only if a patient already has circulating HIT antibodies, usually the result of a recent heparin exposure. HIT antibodies are transient, which could explain why the risk for rapid-onset HIT is restricted to a period of about 100 days following exposure to heparin (Warkentin and Kelton, 2001) (see Chapter 2).

Typically, nonimmune HAT begins 1–2 days after starting heparin administration and resolves during continued heparin therapy (Johnson et al., 1984; Chong and Berndt, 1989; Warkentin and Kelton, 1994; Warkentin et al., 1995; Greinacher, 1995). The platelet count fall is usually mild, with a nadir between 75 and 150 $\times$ 10^9/L. This early platelet count fall may be the result of a direct activating effect of heparin on platelets (Chong and Ismail, 1989; Chong and Castaldi, 1986) or of comorbid clinical factors.

TABLE 1 Explanations for Variable Frequency of HIT Among Prospective Studies

Biological explanations
Patient population studied (frequency of HIT antibody formation differs among patient populations, possibly because of differences in platelet activation and PF4 release)
Type of heparin used: immunogenicity (bovine UFH > porcine UFH > porcine LMWH ~ fondaparinux) and in vivo cross-reactivity (UFH > LMWH >> fondaparinux); also, possibility of lot-to-lot variability in immunogenicity/cross-reactivity among heparins
Variable duration of heparin treatment (HIT typically begins between days 5 and 10)
Gender: female > male (exception: HIT is rare during pregnancy)
Dose of heparin used (dose-dependent thrombocytopenia)
Technical explanations
Variable definition of thrombocytopenia used
Differing baseline platelet counts permitted for study entry
Requirement to repeat platelet count testing to confirm thrombocytopenia
Variable intensity of platelet count surveillance
Variable intensity of surveillance for thrombotic events
Failure to exclude nonimmune heparin-associated thrombocytopenia
Lack of use of in vitro test for HIT antibodies
Use of insensitive or nonspecific HIT antibody assays
Inclusion of patients with "early" thrombocytopenia
Failure to exclude patients whose platelet count recovered during continued heparin treatment
Failure to exclude patients with other explanations for thrombocytopenia

Abbreviations: HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; PF4, platelet factor 4; UFH, unfractionated heparin.

Early nonimmune HAT occurs in up to 30% of patients receiving heparin (Bell et al., 1976; Nelson et al., 1978; Warkentin et al., 1995). Systematic serological investigation of patients with early thrombocytopenia was performed in one study comparing UFH with LMWH for postoperative antithrombotic prophylaxis in patients who underwent hip replacement surgery (Warkentin et al., 1995). With 150×10^9/L as a platelet count threshold, early thrombocytopenia was observed in 189/665 (28%) patients; however, HIT antibodies were not detected in any of the 98 patients tested, and platelet count recovery to more than 150×10^9/L within 3 days occurred despite continuing the heparin (Warkentin et al., 1995). No difference in the frequency of early thrombocytopenia was observed between patients who received UFH (28%) and those who received LMWH (29%). This suggests that unrelated clinical factors, such as perioperative hemodilution with fluid and blood products, were primarily responsible. In contrast, the onset of late thrombocytopenia (i.e., between days 5 and 10 of heparin treatment) was strongly associated with the formation of heparin-dependent platelet-activating antibodies and occurred significantly more frequently in the patients who received UFH (discussed subsequently).

III. FREQUENCY OF IMMUNE HIT

Tables 2 and 3 list prospective studies of the frequency of HIT that employed in vitro testing for HIT antibodies or were studies in which the likelihood of HIT could be judged based on information provided, especially the timing of the onset of thrombocytopenia. Relevant variables influencing the frequency of HIT include the type of heparin used and the patient population.

(*Text continues on p. 74*)

TABLE 2 The Frequency of HIT: Prospective Studies of HIT in Medical Patients Using In Vitro Testing of Patient Serum/Plasma for HIT Antibodies or Indicating a High Likelihood of HIT Based on Timing of Platelet Count Fall

Study	Major indication for heparin	In vitro test	Route, dose	Frequency of (immune) HIT (%)			Timing of platelet count fall reported?	Definition of thrombocytopenia ($\times 10^9$/L)
				Bovine UFH	Porcine UFH	LMWH		
Comparisons between bovine UFH and porcine UFH (studies and data in **bold** *are RCTs)*								
Ansell et al., 1980 (RCT)	VTE	PRP (SR)	iv ther	**4/21 (19.0)**	**0/22 (0)**		Yes	<150
Green et al., 1984 (RCT)	VTE	HIPA	iv ther	**2/45 (4.4)**	**0/44 (0)**		Yes	<150
Powers et al., 1984 (RCT)	VTE	SRA[a]	iv ther	**2/65[a] (3.1)**	**0/66 (0)**		Yes	<150
Bailey et al., 1986 (RCT)	VTE, ATE	No test	iv ther	**1/21 (4.8)**	**0/22 (0)**		Yes	<100
Cipolle et al., 1983; Ramirez-Lassepas et al., 1984 [stroke subgroup]	VTE, ATE	PRP	iv ther	6/100[b] (6.0) [3/54] (5.6)	1/111 (0.9) [1/83] (1.2)		Yes	<100
Predominant treatment for VTE or ATE								
Bell et al., 1976; Alving et al., 1977	VTE, ATE	PRP/SRA[c]	iv ther	3/52[c] (5.8)			Yes	<100
Powers et al., 1979	VTE	No test	iv ther		2/120[d] (1.7)		Yes[d]	<150
Gallus et al., 1980	VTE	PRP	iv ther sc proph		3/166[e] (1.8) 0/5 (0)		Yes	<100
Holm et al., 1980	VTE	No test	iv ther		0/90[f] (0)		Yes	<100
Monreal et al., 1989	VTE	No test	iv ther sc proph		2/89 (2.2) 0/49 (0)	0/43 (0)	Low platelets day 8	<100
Kakkasseril et al., 1985	VTE, ATE	PRP	iv ther	4/142[g] (2.8)			No	<100
Malcolm et al., 1979	Multiple indications	PRP	iv ther sc proph		1/66[h] (1.5) 0/38 (0)		Yes	<100

Rao et al., 1989	Multiple indications	PRP (SR)	iv ther sc proph	0/94 (0) 0/99 (0)		NA	<100
Lindhoff-Last et al., 2002	VTE	EIA	iv ther; sc ther	1/356 (0.3)[i]	0/720[i] (0)	Yes	<100 or >50% fall
Prandoni et al., 2005	Multiple indications	EIA, HIPA	sc proph, ther		14/1754 (0.8)[j]	Yes	>50% fall
Predominant use of heparin for prophylaxis							
Bergmann and Neuhart, 1996	Prophylaxis	No test	sc proph	1/223 (0.4)	0/216 (0)	Yes	<100
Girolami et al., 2003	Prophylaxis, VTE, ATE	HIPA, EIA	sc proph sc ther	5/360 (1.4) 0/238 (0)		Yes	>50% fall
Harbrecht et al., 2004; Pohl et al., 2005	Prophylaxis, neurology	EIA, HIPA	sc proph, iv ther	5/200 (2.5)	0/111 (0)[k]	Yes	<120 or >50% fall
Predominant treatment for MI/ACS							
Kappers-Klunne et al., 1997	MI/ACS	HIPA, EIA	iv ther	1/358 (0.3) 2/358 (0.6)		Yes	<60 and >50% fall (<120)
Romeril et al., 1982	MI/ACS	PRP	sc proph	0/45 (0)		NA	<150
Weitberg et al., 1982	MI/ACS	No test	sc proph	0/50 (0)		NA	<150
Johnson et al., 1984	MI/ACS	No test	sc proph	0/66 (0)		Yes	<150
Foo et al., 2006	Cardiac catheterization	EIA	iv ther	0/357 (0)		NA	Not stated
Gluckman et al., 2005	PCI	EIA, SRA	iv ther	0/94 (0)		NA	Not stated
Matsuo et al., 2005	ACS	EIA	iv ther	4/252 (1.6)		No	<100 or >50% fall
Hemodialysis							
Yamamoto et al., 1996	New onset HD	EIA, PRP	iv ther	6/154 (3.9) 3/154 (1.9)		Yes	Clotting and >20% fall (>50% fall)
Peña de la Vega et al., 2005	Chronic HD	EIA, HIPA	iv ther	1/57 (1.8)		No	<150 or >50% fall

(*Continued*)

TABLE 2 The Frequency of HIT: Prospective Studies of HIT in Medical Patients Using In Vitro Testing of Patient Serum/Plasma for HIT Antibodies or Indicating a High Likelihood of HIT Based on Timing of Platelet Count Fall *(Continued)*

Study	Major indication for heparin	In vitro test	Route, dose	Frequency of (immune) HIT (%)			Timing of platelet count fall reported?	Definition of thrombocytopenia (10^9/L)
				Bovine UFH	Porcine UFH	LMWH		
Skouri et al., 2006	Pediatric chronic HD	EIA, HIPA	iv ther		0/38 (0)		No	Not stated
Healthy volunteers								
Saffle et al., 1980	NA	PRP	sc proph	0/25 (0)	0/14 (0)		No	<150
Schwartz et al., 1985	NA	No test	bolus iv ther	3/20 (15)	0/10 (0)		Yes	<150

Note: HIT was excluded if the platelet count rose during continued heparin after an early fall (e.g., Johnson et al., 1984). Also, where uncertainty existed as to the number of patients with probable HIT, the lower number was indicated in the table, to avoid overestimating the number of patients with HIT (contrast the analysis shown in Table 6). Some data relating to Cipolle et al. (1983) were obtained by personal communication, as reported (Warkentin and Kelton, 1991). The study by Gallus et al. (1980) was excluded because the source of heparin was not specified. Some reports (e.g., Nelson et al., 1978) were excluded because timing of thrombocytopenia was not reported. See Chapter 20 for data in children.

[a]Powers et al. (1984) described five patients who developed thrombocytopenia during bovine UFH use (none during porcine UFH use); 2 patients whose platelet counts fell beginning on day 7 (to nadir of 41×10^9/L) and on day 8 (to nadir of 53×10^9/L) had positive testing for HIT antibodies by SRA; test results are not available for 1 patient whose platelet count fell on day 5 (excluded from Table 2, but included in Table 6); 1 patient with proved HIT developed progression of deep venous thrombosis, as indicated in Table 6 (laboratory records of Dr. J. G. Kelton).

[b]Cipolle et al. (1983) described 10 patients who received bovine UFH who may have had HIT; only six are included here, all of whom developed a platelet count fall between days 7 and 10. Ramirez-Lassepas et al. (1984) examined the subgroup with underlying cerebrovascular disease and reported six patients who developed thrombocytopenia after receiving bovine heparin. Only the 3 who developed late thrombocytopenia are reported here.

[c]Only eight of 16 thrombocytopenia patients underwent PRP testing (all negative) and only five underwent SRA testing. The 3 who developed late thrombocytopenia (HIT) had negative PRP testing, but did not have SRA testing.

[d]Information on timing of platelet count fall to determine the likelihood of HIT was obtained by personal communication, as described (Warkentin and Kelton, 1991).

[e]Origin of heparin is uncertain and may have used mucosal heparin from sheep; at least 3, and as many as five, patients appeared to have HIT based on in vitro testing and timing of platelet count fall.

[f]One case of early thrombocytopenia due to DIC reported in original paper was excluded.

[g]At least four, and as many as nine, patients appeared to have HIT; the four that tested positive for HIT antibodies are included here.

[h]One patient appeared to have HIT based on positive heparin rechallenge, despite negative in vitro HIT test.

[i]Thrombocytopenic patients with negative EIA testing were excluded.

[j]Frequencies of HIT in patients with and without previous exposure to UFH or LMWH were 10/598 (1.7%) and 1/1156 (0.3%), respectively.

[k]These authors compared the frequency of HIT among a prospective cohort of LMWH-treated neurology patients with their previous UFH-treated patient cohort ($p = 0.17$).

Abbreviations: ATE, arterial thromboembolism; EIA, PF4-heparin enzyme-immunoassay; HD, hemodialysis; HIPA, heparin-induced platelet activation test; HIT, heparin-induced thrombocytopenia; iv ther, intravenous therapeutic-dose heparin; LMWH, low molecular weight heparin; MI/ACS, myocardial infarction/acute coronary syndromes; NA, not applicable; PCI, percutaneous coronary intervention; PRP, HIT assay using citrated platelet-rich plasma (PRP/SR, with serotonin release); sc proph, subcutaneous prophylactic-dose heparin; sc ther, subcutaneous therapeutic-dose heparin; RCT, randomized controlled trial; SRA, serotonin release assay using washed platelets; UFH, unfractionated heparin; VTE, venous thromboembolism.

TABLE 3 The Frequency of HIT: Prospective Studies of Surgical Patients Using Confirmatory In Vitro Laboratory Testing of Patient Serum or Plasma or Indicating a High Likelihood of HIT Based on Timing of Platelet Count Fall

Study	Major indication for heparin	In vitro test	Route, dose	Frequency of (immune) HIT (%)				Timing of platelet count fall reported?	Definition of thrombocytopenia ($\times 10^9$/L)
				Bovine UFH	Porcine UFH	LMWH	Fonda-parinux		
RCTs									
Leyvraz et al., 1991	Orthopedic	PRP	sc proph		2/204 (1.0)	0/205 (0)		Yes	<100, >40% fall
Warkentin et al., 1995	Orthopedic	SRA	sc proph		9/332 (2.7)	0/333 (0)		Yes	<150[a]
Warkentin et al., 2003, 2005a	Orthopedic	SRA/EIA	sc proph		16/332 (4.8)	2/333 (0.6)		Yes	>50% fall[a]
Francis et al., 2003	Cardiac	EIA	iv ther	0/99 (0)	0/108 (0)			NA	Not stated
Lubenow et al., 2006	Trauma	EIA, HIPA	sc proph		4/314 (1.3)	1/295 (0.3)		No	Not stated
Other studies									
Louridas, 1991	Vascular	PRP[b]	iv ther, sc proph		5/114[c] (4.4)			Yes	<100
Warkentin et al., 1998b	Orthopedic	SRA/EIA	sc proph			2/246 (0.8)		Yes	<150 or >50% fall
Ganzer et al., 1997, 1999[d]	Orthopedic	HIPA	sc proph		15/307 (4.9)	0/325 (0)		Yes	>50% fall[e]
Marx et al., 1999	Orthopedic	EIA, SRA	sc proph			0/586 (0)		NA	>50% fall
Mahlfeld et al., 2002	Orthopedic	HIPA	sc proph		5/252 (2.0)	1/252 (0.4)		Yes	>40% fall
Trossaert et al., 1998	Cardiac	PRP/EIA	sc proph		0/51 (0)			NA	Not stated
Warkentin et al., 2000	Cardiac	SRA	sc proph		1/100 (1.0)			Yes	>50% fall
Pouplard et al., 1999, 2002	Cardiac	SRA, EIA	sc proph		9/263 (3.4)	1/370 (0.3)		No	<100 or >40% fall
Greinacher et al., 2005a[d]	Orthopedic	HIPA, EIA	sc proph		12/231 (5.2)	0/271 (0)		Yes	>30% fall[e]
Kannan et al., 2005	Cardiac	EIA, PRP, SRA	iv ther		5/33 (15.2)			No	<100 or 35% baseline
Warkentin et al., 2005b	Orthopedic	EIA, SRA	sc proph			0/1349 (0)	0/1377 (0)	NA	>50% fall

[a]Results of same RCT reported using two different definitions of thrombocytopenia (<150 and >50% fall).
[b]Ineffective testing was used (platelet aggregation without heparin).
[c]Of seven patients with thrombocytopenia reported, two were excluded because of early onset of thrombocytopenia
[d]There is overlap in patients reported by Ganzer et al., 1997, 1999 and Greinacher et al., 2005a.
[e]Patients underwent laboratory testing for HIT antibodies if thrombosis developed even in the absence of thrombocytopenia. An additional 5 patients in Ganzer et al., 1997 developed thrombosis and positive HIPA test in absence of platelet count fall >50%, i.e., overall HIT frequency 20/307 = 6.5%.

Abbreviations: EIA, PF4-heparin enzyme-linked immunosorbent assay; HIPA, heparin-induced platelet activation test (aggregation of washed platelets); HIT, heparin-induced thrombocytopenia; iv ther, intravenous therapeutic-dose heparin; LMWH, low molecular weight heparin; NA; not applicable; PRP, HIT assay using citrated platelet-rich plasma; sc proph, subcutaneous prophylactic-dose heparin; SRA, serotonin release assay using washed platelets; UFH, unfractionated heparin.

A. Frequency of HIT in Medical Patients and Normal Volunteers: Comparison of UFH of Bovine Versus Porcine Origin

Five randomized controlled trials (RCTs) (Bell and Royall, 1980; Green et al., 1984; Powers et al., 1984; Ansell et al., 1985; Bailey et al., 1986) and one nonrandomized study (Cipolle et al., 1983; Ramirez-Lassepas et al., 1984) compared the frequency of HIT during treatment with UFH that was derived from either bovine lung or porcine intestinal mucosa. In addition, the frequency of HIT was evaluated in normal volunteers in one RCT (Schwartz et al., 1985) and one nonrandomized prospective study (Saffle et al., 1980) involving porcine and bovine heparins. The study of Bell and Royall (1980) has been excluded from primary analysis because neither laboratory testing for HIT antibodies nor data on the timing of onset of thrombocytopenia were provided.

Taken together, the four RCTs in medical patients strongly suggest that bovine UFH is more likely to cause HIT than porcine UFH, as all nine patients with HIT had received UFH of bovine origin ($p = 0.0059$ by Mantel-Haenszel) (Table 2). Similarly, an increased frequency of HIT in patients receiving bovine lung heparin was suggested in the nonrandomized comparison by Cipolle and colleagues (1983) (6/100, 6% vs. 1/111, 0.9%; $p = 0.055$), as well as in the study of Bell and Royall (1980) (26% vs. 8%; $p < 0.005$), although this latter study probably included patients with nonimmune HAT.

A significantly higher frequency of HIT antibody formation with bovine UFH was observed in a study of cardiac surgical patients randomized between bovine and porcine UFH (Francis et al., 2003). Although a smaller cardiac surgery study (Konkle et al., 2001) failed to detect a difference in antibody formation, blood samples were obtained only until postoperative day 5, which may have been too early to detect the majority of HIT antibodies (Warkentin, 2003).

A higher frequency of immune HIT with bovine lung heparin is biologically plausible. Bovine heparin has a higher sulfate:disaccharide ratio than does porcine heparin (Casu et al., 1983), and it is better able to activate platelets in vitro (Barradas et al., 1987). These properties could lead to greater platelet activation in vivo and, consequently, greater potential for PF4 release. Moreover, the bovine heparin chains would be expected to better form the large multimolecular complexes that compose the target antigen for HIT antibodies.

Lot-to-lot variability within heparin of a particular animal source could also contribute to variable frequency of HIT. Stead and coworkers (1984) reported a striking cluster of six patients with pulmonary embolism complicating HIT identified within a few weeks at one institution. A particular lot of bovine lung heparin in use in the operating room was linked to these events: patient serum-induced platelet aggregation occurred in the presence of this particular lot of heparin, but not when other lots of bovine lung heparin from the same manufacturer were used.

B. Frequency of HIT in Medical Patients Treated with Porcine Mucosal UFH

Table 2 also lists the frequency of HIT observed in several prospective studies that have evaluated medical patients receiving intravenous, therapeutic-dose porcine UFH, usually for venous thromboembolism (VTE), myocardial infarction and acute coronary syndromes (MI/ACS), or hemodialysis (HD). Excluding studies of HD, an overall frequency of HIT of slightly less than 1% is suggested. This is a relatively low number, particularly when one considers that, paradoxically, the frequency appears to be much higher in postoperative surgical patients who received lower (prophylactic) doses of porcine heparin (discussed subsequently).

In contrast, HIT may occur more often in prospective studies of acute HD patients receiving porcine UFH (Yamamoto et al., 1996). Whether this is a real difference that reflects increased platelet activation (and PF4 release) during HD or it reflects a more sensitive definition of thrombocytopenia (any platelet count fall associated with line clotting) is unknown. The frequency of clinical HIT in chronic HD patients appears to be less than 2% and may be considerably lower, but up to 18% develop a positive EIA for anti-PF4/heparin antibodies. Whether the incidence of elevated levels of anti-PF4/heparin antibodies and clinical HIT are dependent on the time since the initiation of HD is unclear. Some have suggested that the frequency of anti-PF4/heparin antibody increases with time (Palomo et al., 2005), while others have found no association (Peña de la Vega et al., 2005). Two studies suggest that the antibodies tend to develop early after initiation of HD, and may disappear after months, despite ongoing heparin exposure (Nakamoto et al., 2005; Skouri et al., 2006). Most studies have not found an association between vascular access thrombosis and elevated levels of anti-PF4/heparin antibodies (Greinacher et al., 1996; Sitter et al., 1998; O'Shea et al., 2002; Palomo et al., 2005; Peña de la Vega et al., 2005; Carrier et al., 2007).

The incidence of HIT in medical patients receiving prophylactic-dose UFH remains poorly defined but also appears to be lower than that observed in surgical patients receiving UFH prophylaxis. Girolami et al. (2003) prospectively evaluated 598 hospitalized medical patients who received prophylactic ($n = 360$) and therapeutic ($n = 238$) dose UFH. Overall, five patients developed HIT, all of whom were receiving UFH in prophylactic doses (0.8% of combined group, or 1.4% of patients receiving prophylactic-dose UFH). In an RCT comparing low-dose LMWH with UFH for VTE prevention, HIT was believed to have occurred in 1/223 (0.4%) of UFH-treated patients, based on the timing of the platelet count fall (serology was not performed) (Bergmann and Neuhart, 1996). In a retrospective analysis, Creekmore et al. (2006) reported a similar incidence of 0.5% (43/8420) in medical patients receiving UFH.

C. Frequency of HIT in Medical Patients Treated with LMWH

Although there have been several RCTs evaluating the efficacy of LMWH for prophylaxis in medical patients, published descriptions of secondary safety endpoints such as HIT are usually brief and often inadequate to judge whether the occurrences of thrombocytopenia were due to HIT or not (Samama et al., 1999; Turpie, 2000; Leizorovicz et al., 2004). In one RCT of prophylactic-dose LMWH (enoxaparin) versus UFH in medical patients, no cases of new onset of thrombocytopenia (platelet count $<100 \times 10^9$/L) were observed in 216 patients randomized to receive LMWH (Bergmann and Neuhart, 1996). In another RCT that compared deep-vein thrombosis treatment with LMWH (reviparin) versus UFH, none of 720 patients who received LMWH developed (antibody-positive) HIT, whereas one of 356 (0.3%) patients treated with UFH manifest this complication (Lindhoff-Last et al., 2002). Interestingly, if the definition of HIT in that study was expanded to include thrombosis and a positive test for anti-PF4/heparin antibodies (even without thrombocytopenia), then a greater event-rate was observed in the UFH-treated patients (2.2% vs. 0.1%; $p = 0.00087$) (Warkentin and Greinacher, 2005). This RCT also showed a greater frequency of antibody formation in the UFH-treated arm (21.1% vs. 6.2%; $p < 0.0001$).

In a prospective cohort study specifically designed to ascertain the incidence of HIT in medical patients receiving LMWH, 14/1754 (0.8%) developed HIT

(Prandoni et al., 2005), a frequency similar to that reported by the same investigators in medical patients receiving UFH (Girolami et al., 2003). In contrast, the retrospective study of VTE prophylaxis by Creekmore and colleagues (2006) observed a significantly lower frequency of HIT in medical patients receiving LMWH ($1/1189 = 0.08\%$) compared with the same patient population receiving UFH ($43/8420 = 0.5\%$; $p = 0.037$). Similarly, in "before-after" prospective cohort studies performed in neurologic patients, the frequency of HIT tended to be lower in patients treated with LMWH (nadroparin) compared with UFH (0% vs. 2.5%; $p = 0.17$), with a significantly lower frequency of heparin-dependent antibody formation among the patients receiving LMWH (1.8% vs. 10.5%; $p < 0.001$) (Harbrecht et al., 2004; Pohl et al., 2005).

D. Frequency of HIT in Surgical Patients Treated with Porcine Mucosal UFH

Two large prospective studies suggest that HIT is an important problem in orthopedic patients receiving UFH (Warkentin et al., 1995, 2003, 2005a; Greinacher et al., 2005a). When using a proportional fall in platelet count (e.g., 50% or greater) that began on or after day 5 of heparin treatment, and that was confirmed by serologic testing for HIT antibodies, both studies observed a frequency of HIT of about 5% (Table 3). Each study used porcine mucosal heparin, derived from a different manufacturer, given by the subcutaneous (sc) route at a dosage of 15,000 U/day. Other studies of postorthopedic UFH thromboprophylaxis (using confirmatory in vitro testing) have shown frequencies of HIT of about 2.0% (Leyvraz et al., 1991; Mahlfeld et al., 2002).

There is little prospective information of the frequency of HIT in other postoperative surgical populations treated with UFH (Table 3). Three studies have been performed on postoperative cardiac surgical patients who also received postoperative UFH in addition to high doses of UFH during preceding cardiopulmonary bypass (CPB) (Trossaert et al., 1998; Warkentin et al., 2000; Pouplard et al., 1999) (Table 3). Pooling the three studies, about 2.4% of the patients developed serologically confirmed HIT. Interestingly, the frequency of HIT in this population appears to be lower than in orthopedic patients receiving UFH, even though the cardiac surgical patients appear to have a higher frequency of formation of HIT antibodies (Warkentin et al., 2000).

Isolated limb perfusion (ILP) with melphalan employs extracorporeal circulation (and thus high-dose UFH) to treat melanoma or unresectable sarcoma limited to an extremity. In one study, HIT occurred in three of 108 patients (2.8%), who also received sc UFH prophylaxis following ILP (Masucci et al., 1999). The occurrence of arterial thrombosis and partial limb amputation in two of these patients with HIT led the investigators to discontinue routine UFH prophylaxis post-ILP. The hypothesis that ILP is a high-risk situation for HIT was supported by a follow-up prospective study by these same investigators showing that heparin-dependent antibodies were formed in all nine patients who underwent ILP (despite not receiving postoperative UFH prophylaxis), with eight having "strong" antibodies that effected serotonin release in vitro (Masucci et al., 1999).

E. HIT Is Less Frequent in Surgical Patients Receiving LMWH Compared with UFH Prophylaxis

Anecdotal reports indicate that HIT can occur during treatment with LMWH (Ball et al., 1989; Tardy et al., 1990; de Raucourt et al., 1996; Plath et al., 1997; Elalamy et al., 1996; Warkentin, 1998; Gruel et al., 2003; Ng and Lee, 2003). Clinical trial

data suggest that the frequency is low, however. Using a sensitive definition for HIT (>50% fall on or after day 5 and confirmed by positive HIT antibodies), two studies in Hamilton found an overall frequency of only 4/439 (0.9%; 95% CI 0.25–2.32%) for HIT complicating use of LMWH given for postoperative orthopedic patients (Warkentin et al., 1995, 1998b, 2003). In contrast, using the same definition of HIT, patients who received UFH had a much higher frequency of HIT, 16/332 (4.8%; 95% CI 2.78–7.71%) (Warkentin et al., 1995, 2003). A similar high frequency of UFH-induced HIT was observed in a German study, 15/307 (4.9%; 95% CI 2.76–7.93) (Ganzer et al., 1997).

The strongest evidence that LMWH is indeed associated with a lower frequency of HIT was provided by an RCT that directly compared the frequency of HIT between the two types of heparin (Warkentin et al., 1995, 2003, 2005a). The frequency of HIT in patients treated with the LMWH preparation, enoxaparin (itself derived from porcine mucosal heparin), was lower than that seen in patients treated with porcine UFH, irrespective of whether a standard definition (platelet fall to $<150 \times 10^9$/L on or after day 5 of heparin treatment) or a more sensitive definition (>50% platelet count fall from the postoperative peak) of thrombocytopenia was used. The frequency of HIT antibody formation also differed between the two patient groups, using either the SRA (Warkentin et al., 1995, 2005a) or a PF4/heparin (or PF4/polyanion) EIA (Warkentin et al., 2000, 2005a). Thrombocytopenia also appeared to be infrequent in other trials of LMWH (Leyvraz et al., 1991; Simonneau et al., 1997; ENOXACAN Study Group, 1997).

UFH appeared also to lead to greater frequency of HIT antibody formation than the LMWH reviparin in a randomized trial of post-hip and knee surgery patients (Ahmad et al., 2003a). HIT antibodies occurred somewhat more often in knee surgery patients. These same investigators also examined HIT antibody formation in orthopedic patients immobilized in a plaster cast who were randomized to receive reviparin or placebo (Ahmad et al., 2003b). A surprising finding was that the number of patients who apparently formed anti-PF4/polyanion antibodies (by EIA) was higher in the placebo group (10 cases vs. 6). No patient in either study developed clinical HIT.

A meta-analysis of five surgical thromboprophylaxis prospective cohort studies and RCTs (four postorthopedic, one postcardiac) that defined HIT using both clinical and serologic criteria found a substantial reduction in HIT frequency (odds ratio [OR], 0.10; $p < 0.001$) with LMWH compared with UFH (Martel et al., 2005) (Fig. 1). The authors found the absolute risk of HIT to be 0.2% with LMWH and 2.6% with UFH. In contrast, when they examined the OR for HIT using a non-serologically defined definition of thrombocytopenia, the difference in risk of apparent HIT was less marked.

Gruel and colleagues (2003) performed a systematic study that identified 11 patients with HIT (three with HIT-associated thrombosis) that had been exclusively treated with a LMWH preparation (dalteparin, nadroparin, or enoxaparin). Clinical and serologic features were similar to patients with HIT developing during UFH, except that there was evidence that thrombocytopenia may begin somewhat later during LMWH therapy. Based upon estimated relative use of UFH and LMWH in France, the authors estimated the frequency of HIT to be 40-fold less with LMWH, compared with UFH.

The data supporting a lower risk of HIT in postcardiac surgery patients receiving LMWH compared with UFH thromboprophylaxis is discussed later in the chapter (see Section V.B).

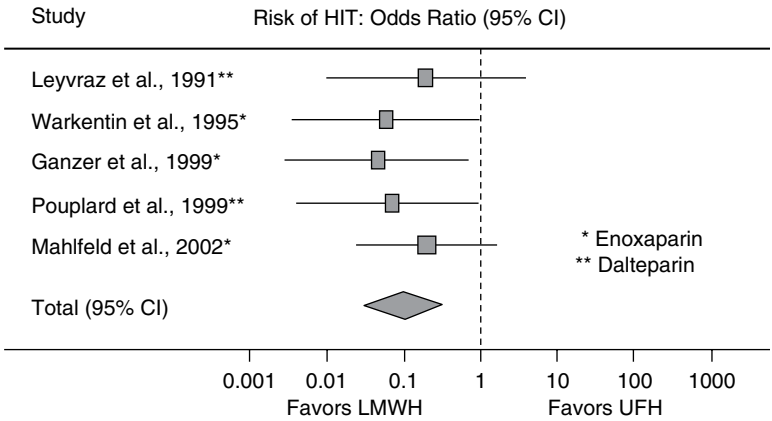


FIGURE 1 Frequency of HIT: UFH versus LMWH. Meta-analysis of five thromboprophylaxis studies comparing the frequency of serologically-confirmed HIT between UFH and LMWH (enoxaparin, 3 studies; dalteparin, 2 studies). *Abbreviations*: HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; UFH, unfractionated heparin. *Source*: From Martel et al., 1995.

F. Frequency of HIT in Critically Ill Patients

Thrombocytopenia is common in critically-ill patients, occurring in up to half of all patients in the intensive care unit (ICU). In this population, the presence of thrombocytopenia is associated with increased mortality, and depending on severity and etiology, is associated with increased hemorrhagic risk as well. ICU patients often have several potential causes of thrombocytopenia, making evaluation challenging. Heparin exposure (in the form of line flushes, prophylaxis, or therapeutic anticoagulation) is virtually ubiquitous in the ICU, making HIT a frequent diagnostic consideration.

There have been several prospective and retrospective studies that have evaluated the frequency of HIT in critically ill patients (Table 4). The two largest prospective studies (Verma et al., 2003; Crowther et al., 2005) found that although HIT was clinically suspected in 12 to 15% of ICU patients, compatible serology supporting the diagnosis (including the washed platelet activation assay, a relatively specific test for HIT antibodies) was found infrequently, with the overall frequency of HIT less than 1%. Indeed, if the frequency of HIT is only about 0.3–0.5% in ICU patients, yet the overall risk of thrombocytopenia is about 30–50%, then a useful clinical "rule" is that the true risk of HIT is only about one in 100 among all ICU patients who develop thrombocytopenia (Warkentin, 2006a; Napolitano et al., 2006; Selleng et al., 2007).

Other retrospective analyses utilizing anti-PF4/polyanion EIAs (which are much less specific for clinical HIT) raise the possibility that certain patient subgroups may be at higher risk of HIT. For example, Hoh et al. (2005) reported a much higher seropositivity rate in patients with subarachnoid hemorrhage admitted to a neurosurgical ICU who were suspected of having HIT on clinical grounds. Schmugge et al. (2002) claimed that 2.3% of critically ill pediatric patients exposed to heparin for at least 5 days developed HIT with thrombosis. Prospective studies utilizing platelet activation assays are required to ascertain the true frequencies of clinical HIT among these and other patient subgroups.

When HIT does occur in critically ill patients, it appears to be associated with a high risk of venous and arterial thrombotic events, similar to that observed in non–critically ill patients with HIT. Retrospective studies report a thrombosis frequency of 20–50%, or even greater (Wester et al., 2004; Hoh et al., 2005; Gettings et al., 2006). The HIT-associated mortality in these studies also appears to be high.

G. Frequency of HIT During Pregnancy

HIT appears to be uncommon during pregnancy even with UFH treatment. Fausett and colleagues (2001) reported that none of 244 pregnant women developed HIT during UFH use, although HIT occurred in 10 of 244 (4%) nonpregnant patients who received UFH ($p = 0.0014$). In a literature review, Sanson and coworkers (1999) identified no cases of HIT among 486 women who received LMWH during pregnancy. Ellison et al. (2000) studied 57 pregnancies in 50 patients and also found no episodes of HIT in pregnant women who received enoxaparin. More recently, Lepercq and colleagues (2001) found no cases of HIT in 624 pregnancies among 604 women treated with LMWH. A systematic review of studies published to the end of 2003 of the safety and efficacy of LMWH in pregnancy was reported by Greer and Nelson-Piercy (2005). From a total of 2777 pregnancies described in 61 reports, no cases of HIT were identified.

H. HIT and Gender

Ironically, in view of the rarity of HIT in pregnancy, it is now recognized that female gender is a modest risk factor for HIT (OR, 1.5–2.0) (Warkentin et al., 2006a). Whether this is related to gender differences in frequency of antibody formation, or in antibody levels, or in susceptibility of platelets to be activated by HIT antibodies, has not been reported.

I. Risk Factor Interactions

Table 5 summarizes the data supporting the role of three major risk factors for HIT: heparin type (UFH > LMWH), patient type (surgical > medical), and patient gender (female > male) (Warkentin et al., 2006a). (A fourth major risk factor, namely duration of heparin therapy, is not shown.) Note that the influence of heparin type is most striking in a female patient receiving postoperative thromboprophylaxis: HIT is much more likely to occur with UFH than with LMWH in such a patient (OR, 17.39 [95% CI, 4.22–71.70]; $p < 0.0001$); in contrast, the benefit of LMWH is significantly less in males receiving post-surgical thromboprophylaxis (Table 5).

J. Role of Incidental UFH Flushes in the Frequency of HIT

There are two ways that "incidental" exposure to heparin by "flushing" of intravascular catheters can affect the frequency or clinical effect of HIT. First, such minor heparin exposures can trigger formation of HIT antibodies (Ling and Warkentin, 1998; Warkentin et al., 1998b). And second, in patients who have already formed potent HIT antibodies for any reason, any ongoing or recurrent heparin exposure—including small-dose exposure—could lead to recurrence or exacerbation of thrombocytopenia or thrombosis (Rice and Jackson, 1981). Indeed, several patients have been reported in whom severe HIT occurred while only small amounts of heparin were being given as flushes to maintain the patency of

TABLE 4 Frequency of HIT in Critically Ill Patients

Study	Population	HIT clinical criteria	Frequency of thrombocytopenia n/N (%)	Suspected HIT n/N (%)	HIT cases: Positive HIT serology n/N (%)	HIT cases: HIT with thrombosis n/N (%)	In vitro test	Comments
Prospective studies								
Crowther et al., 2005	261 patients critically ill medical and surgical patients	Thrombocytopenia was defined as a platelet count $<150\times10^9$/L; other clinical criteria for HIT not stated	121/261 (46%) (62 patients were thrombocytopenic at admission; 59 developed thrombocytopenia during ICU admission)	33/261 (12.6)	0/261 (0)	NA	SRA, EIA	Post-op orthopedic and cardiac surgery patients not studied. Positive SRA needed to diagnose HIT (n = 33 tested)
Verma et al., 2003	748 UFH-treated patients admitted to a combined ICU and CCU over 2 yr. 267 had sufficient exposure to be considered at risk for HIT	>2 consecutive platelet counts $<150\times10^9$/L, or >33% drop, $\geqslant$5 days after UFH exposure, or sooner if previously exposed within preceding 8 wk	Not stated	40/267 (15.0)	1/259 (0.4)	0/1 (0)	EIA, SRA	Serology missing from 8/40 patients with clinically suspected HIT. Positive SRA needed to diagnose HIT (n = 32 tested)
Strauss et al., 2002	145 consecutive patients admitted to a medical ICU with a normal platelet count	Thrombocytopenia was defined as a platelet count $<150\times10^9$/L; other clinical criteria for HIT not stated	64/145 (44%)	Not stated	2/145 (1.4)	Not stated	Not stated	Clinical criteria for HIT not stated. Two HIT cases reported to be confirmed by laboratory testing, but type of assay not stated
Stéphan et al., 1999	147 consecutive patients admitted to a surgical ICU over 6 mo period	Thrombocytopenia was defined as a platelet count $<100\times10^9$/L; other criteria for HIT not stated	52/147 (35%)	Not stated	No cases described	No cases described	Not stated	UFH and LMWH were not found to be independent risk factors for the development of thrombocytopenia

Bouman et al., 2002	106 critically ill patients enrolled in a clinical trial of CRRT	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	5/106 (4.7%) of patients were diagnosed with HIT, but criteria for diagnosis were not stated
Retrospective studies								
Gettings et al., 2006	2046 critically ill post-op patients admitted to a surgical ICU over a 2 yr period	"4 T's" criteria (Lo et al., 2006) applied retrospectively	Not stated	210/2046 (10.3)	19/2046 (0.9)	4/19 (21.1)	EIA	6/19 (31.6%) patients with HIT died vs. 1/19 (5.3%) controls matched for age, sex, diagnosis, and severity of illness
Wester et al., 2004	20 consecutive ICU patients diagnosed with HIT over a 10 mo period	Criteria of Sheridan et al. (1986)	NA	NA	NA	10/20 (50.0%)	PAT	Total number of ICU patients at risk of HIT over study period not stated. 12/20 (60.0%) of patients with HIT died in ICU
Hoh et al., 2005	389 consecutive patients with subarachnoid hemorrhage admitted to a neurosurgical ICU over a 3.5 yr period	Platelet count $<100\times10^9$/L or >50% drop from baseline occurring days 4–14 of heparin exposure	Not stated	59/389 (15.2)	20/389 (5.1) (37.3% of 59 with suspected HIT had serology)	22/59 (37.3) (39/57 [66] developed new hypo-densities on head CT)	EIA	No serology on 22/59 (37.3%) patients with clinically suspected HIT. Incidence of thrombosis in serologically confirmed HIT not stated. Mortality was 29% in the 59 patients with suspected HIT
Schmugge et al., 2002; Risch et al., 2003	612 pediatric ICU patients who were exposed to heparin for $\geqslant$5 days, over a 2.5 yr period	HIT with thrombosis: radiographically confirmed thrombosis plus platelet count $<150\times10^9$/L, or <50% drop occurring $\geqslant$5 days after heparin exposure	Prevalence of thrombocytopenia in entire group at risk of HIT not stated. 28/38 patients with thrombosis had thrombocytopenia	Not stated	6/612 (1.0) had a positive EIA (adult cut-off) and 14/612 (2.3) with a lower cut-off	Overall, 14/38 (36.8) patients with thrombosis who were tested were considered to have HIT	EIA	57/612 (9.3%) developed thrombosis. Serology available on 38/57 (66.7%). Thrombocytopenia was present in 28/38 (73.7%). Authors argue for lower EIA cut-off in children

Abbreviations: CCRT, continuous renal replacement therapy; CCU, coronary care unit; CT, computed axial tomography; EIA, enzyme immunoassay; HIT, heparin-induced thrombocytopenia; ICU, intensive care unit; LMWH, low molecular weight heparin; NA, not applicable; PAT, platelet aggregation test; SRA, serotonin release assay; UFH, unfractionated heparin.

TABLE 5 Factors Influencing the Risk of HIT: Type of Heparin, Type of Patient Population, and Gender (Fixed-Effects Statistical Analysis)

Group (number of studies)	Common OR for HIT	95% CI Common OR		*p*-value
		Lower	Upper	
Overall effect of heparin type: UFH vs. LMWH (7)	5.29	2.84	9.86	<0.0001
Overall effect of patient type: surgical vs. medical (7)[a]	3.25	1.98	5.35	<0.0001
Overall effect of gender: female vs. male (7)	2.37	1.37	4.09	0.0015
Studies of *interactions* of gender (females, males), patient type (surgical, medical), or both (female/surgical; female/medical) on risk of HIT for UFH vs. LMWH[b]	Common OR for HIT: UFH vs. LMWH			
Female (7)	9.22	3.87	21.97	<0.0001
Male (7)[a]	1.83	0.64	5.23	0.2907
Comparison of the treatment effect of heparin type in females vs. the treatment effect in males, $p = 0.0199$				
Surgical (4)	13.93	4.33	44.76	<0.0001
Medical (3)	1.75	0.73	4.22	0.2327
Comparison of the treatment effect of heparin type in surgical vs. the treatment effect in medical patients, $p = 0.0054$				
Female/surgical (4)	17.39	4.22	71.70	<0.0001
Female/medical (3)	3.75	1.16	12.17	0.0252
Comparison of the treatment effect of heparin type in female surgical patients vs. the treatment effect in female medical patients, $p = 0.1028$				

[a]Studies were pooled across patient type to produce a simple 2×2 table; surgical (42/1999) and medical (25/3811), Fisher's exact test (2-sided) *p*-value.
[b]Male/surgical and male/medical not considered due to lack of events.
Abbreviations: HIT, heparin-induced thrombocytopenia; UFH, unfractionated heparin; LMWH, low molecular weight heparin.
Source: From Warkentin et al., 2006a.

intravascular catheters (Doty et al., 1986; Heeger and Backstrom, 1986; Kappa et al., 1987; Rama et al., 1991; Brushwood, 1992; Parney and Steinke, 2000).

In most of the reports of patients developing HIT during LMWH treatment, recent prior exposure to UFH was not excluded. Indeed, incidental exposure to UFH by intraoperative invasive catheters could lead to formation of HIT antibodies

that are inappropriately attributed to later postoperative LMWH prophylaxis (Shumate, 1995). However, if true, it would suggest that the apparent difference in immunogenicity between UFH and LMWH could be even greater than initially reported.

To address this issue, a randomized, double-blind clinical trial was performed to test the hypothesis that incidental exposure to UFH by intraoperative invasive lines, rather than postoperative LMWH antithrombotic prophylaxis, was the predominant explanation for postoperative HIT antibody formation (Warkentin et al., 1998b). Patients were randomized to receive either UFH or normal saline flushes during surgery. However, the data obtained essentially ruled out the hypothesis: the frequency of HIT antibodies was not higher in the patients who were randomized to receive UFH flushes (2.2% vs. 2.7%; $p = 0.73$). Rather, the results suggested that postoperative LMWH prophylaxis administered to both groups was the predominant factor in causing HIT antibody formation. However, HIT antibody formation occurred in two patients who received UFH flushes, but who subsequently were given warfarin anticoagulation. Because intraoperative UFH flushes occasionally result in formation of high levels of HIT antibodies that can lead to life-threatening, acute HIT if therapeutic-dose UFH is administered a few weeks later (Ling and Warkentin, 1998), and because there is no clinical benefit to flushing intravascular catheters with UFH (Warkentin et al., 1998b), it seems reasonable to recommend that normal saline flushes be considered for routine flushing of intravascular catheters used during surgery.

A double-blind placebo-controlled trial of UFH for maintaining peripheral vein catheter patency in neonates also evaluated the incidence of anti-PF4/heparin antibodies and clinical HIT. None of the 108 neonates who were allocated to receive heparin developed HIT or HIT antibodies (Klenner et al., 2003).

It is possible that heparin flushes for venous access devices in cancer patients can cause anti-PF4/heparin antibody formation. In a serosurveillance study, Mayo and colleagues (1999) found that about one-third of 49 such patients tested formed low levels of antibodies (detected by EIA) at least once. However, only one patient developed a positive SRA, and no patient developed thrombocytopenia. These data are in keeping with our own experience that HIT is very uncommon in this patient population.

In recent years, many centers have substituted saline for heparin to intermittently "flush" peripheral venous catheters. This is because saline flushing of such devices "locked" between use have similar patency rates as when heparin flushes are used (Randolph et al., 1998a). In contrast, heparin may help prolong the patency of intra-arterial, central venous, and pulmonary artery catheters (Randolph et al., 1998b), and consequently exposure to heparin by these routes remains common.

K. HIT and Heparin-Coated Devices

Heparin can be bonded to artificial surfaces (Larsson et al., 1987), either through ionic attachment, as used for pulmonary artery catheters (Eldh and Jacobsson, 1974), or by end-linked covalent bonding (e.g., Carmeda BioActive Surface [CBAS]) (Larm et al., 1983). CBAS has been used for CPB circuits and filters (Borowiec et al., 1992a,b, 1993), extracorporeal membrane oxygenation (ECMO) devices (Koul et al., 1992), and coronary stents (Serruys et al., 1996). During use in patients, ionically attached heparin is displaced by albumin from the catheter surface, where it could contribute to HIT (discussed subsequently). End-linked heparin is

an effective and longer-lasting anticoagulant, as the immobilized, but flexible, heparin chains are able to interact with fluid-phase antithrombin and thrombin (Elgue et al., 1993). Nevertheless, the end-linked, but relatively unconstrained, heparin is capable of interacting with PF4 (Suh et al., 1998). Therefore, it is theoretically possible that covalent heparin-bonded devices could result in formation of HIT antibodies or could cause HIT in a patient who has formed antibodies. Alternatively, even covalently bonded heparin might "leach" into blood by proteolytic mechanisms, thereby contributing in a more conventional way to the pathogenesis of HIT (Almeida et al., 1998a). Use of heparin-coated pulmonary catheters in contributing to HIT has been implicated by Laster and Silver (1988). These workers reported 10 patients with HIT whose platelet counts did not rise until the removal of their heparin-coated pulmonary catheters, despite discontinuing all other sources of heparin. Incubation of the heparin-coated catheters with platelets in the presence of patient sera resulted in catheter-induced platelet aggregation. Based on the identification of four such cases, during which time 1112 heparin-coated catheters had been used, they estimated the frequency of catheter-associated HIT to be 0.4%.

L. HIT and Ventricular Assist Devices

Ventricular assist devices (VADs) are surgically implanted mechanical pumps that have a large foreign surface area in direct contact with flowing blood, thereby creating an inherently prothrombotic environment. In a non-randomized study of patients who received heparin-coated and uncoated VADs, there was no difference in the development of anti-PF4/heparin antibodies and thromboembolism between the groups (Koster et al., 2001). In two more recent studies, 10/113 (8.8%) (Schenk et al., 2006, 2007) and 28/358 (7.8%) (Koster et al., 2007) of VAD patients developed apparent HIT. In both studies, the frequency of anti-PF4/heparin antibody formation (by EIA) was over 60%. While these apparent frequencies of clinical HIT (~8%) are among the highest reported in any patient population, it remains unclear how to distinguish true clinical HIT from a patient with cardiogenic shock or other non-HIT explanations for thrombocytopenia who coincidentally develop heparin-dependent platelet-activating antibodies (Warkentin and Crowther, 2007).

M. HIT Caused by Other Sulfated Polysaccharides

The cryptic HIT autoantigen is comprised of conformationally altered PF4 when it forms a multimolecular complex with heparin. Other negatively charged polysaccharides can interact with PF4 to produce the HIT antigen (Wolf et al., 1983; Greinacher et al., 1992a,b,c; Anderson, 1992) (see Chapter 7). These considerations explain why a number of high-sulfated polysaccharides, 10 or more subunits in length, have been reported to cause a syndrome of thrombocytopenia and thrombosis that essentially mimics HIT. These drugs include the semisynthetic five-carbon subunit-based "heparinoid" pentosan polysulfate (Gouault-Heilman et al., 1985; Vitoux et al., 1985; Follea et al., 1986; Goad et al., 1994; Tardy-Poncet et al., 1994; Rice et al., 1998), polysulfated chondroitin sulfate (Bouvier, 1980; Wolf et al., 1983; Greinacher et al., 1992a), and the anti-angiogenic agent, PI-88 (Rosenthal et al., 2002). The frequency of immune-mediated thrombocytopenia, with or without thrombosis, after exposure to these compounds is unknown, but may be high. On very rare occasions, a syndrome resembling HIT may be triggered by the pentasaccharide anticoagulant, fondaparinux (Warkentin et al., 2007).

Danaparoid, a mixture of anticoagulant glycosaminoglycans, has not been reported to cause HIT de novo. However, some HIT sera "cross-react" in vitro with danaparoid, and cases of apparent in vivo cross-reactivity have been reported (see Chapter 13) (Magnani and Gallus, 2006).

N. Spontaneous HIT

On exceptionally rare occasions, patients spontaneously develop an illness that clinically resembles HIT, i.e., thrombocytopenia, thrombosis, and presence of platelet-activating anti-PF4/heparin (HIT) antibodies (Warkentin et al., 2006b). Typically there is a preceding inflammatory or surgical event. Sometimes, patients with antiphospholipid syndrome develop thrombocytopenia rapidly upon receiving heparin treatment, consistent with preexisting HIT antibodies associated with their autoimmune diathesis (Martinuzzo et al., 1999; Bourhim et al., 2003).

O. Variable Duration of Heparin Treatment

As HIT typically begins 5–10 days after starting therapy with heparin, it follows that the length of heparin treatment can influence the risk for HIT, e.g., a 10–14 day course of UFH is far more likely to result in clinical HIT than a 1 day treatment period (>2% vs. 0.02%, i.e., an OR of ~100). Of note, there is evidence that the risk of HIT begins to decline after 10 days of uninterrupted heparin use (see Fig. 1C, Chapter 2). In a large study of postoperative orthopedic surgical patients receiving postoperative heparin prophylaxis, no patient developed HIT antibodies after day 10, even though many patients received heparin for up to 14 days (Warkentin et al., 1995). These data are consistent with a "point exposure" model for risk of HIT in this patient population, such as a brief time shortly after surgery, when high circulating PF4 levels coincide with the first few sc heparin injections. However, even if HIT antibody formation occurs during the day 5–10 window period, thrombocytopenia itself can occur somewhat later, particularly if a larger dose of heparin is given, or UFH is substituted for LMWH. The characteristic timing of HIT should assist clinicians in focusing their platelet count monitoring for HIT during the critical time period, so that the early diagnosis of HIT is improved (see Section VI).

P. Heparin Dose-Dependence in HIT

Analysis of individual patients with HIT often shows dose-dependence; that is, mild thrombocytopenia during sc heparin prophylaxis is followed by a marked drop in platelet count if the patient then receives therapeutic-dose heparin (Warkentin, 2006a).

However, dose-dependence of HIT is not readily apparent when reviewing prospective studies of HIT (Tables 2 and 3). However, this could be explained by differences in frequency of HIT among different patient populations that confounds the influence of heparin dose. For example, among medical patients, porcine UFH appears to be more likely to result in HIT when given in therapeutic, rather than prophylactic, doses. This difference, if real, could reflect dose-dependence of heparin in HIT. On the other hand, the relatively high frequency of HIT in surgical patients (up to 5%) receiving "only" prophylactic-dose porcine UFH more likely reflects differences in risk related to this surgical patient population.

Dose-dependence has also been reported to be associated with a higher incidence of anti-PF4/heparin antibody formation, but not a higher frequency of

HIT, following admission to hospital for ACS and percutaneous coronary intervention (PCI) (Gluckman et al., 2005; Matsuo et al., 2005).

IV. FREQUENCY OF THROMBOSIS COMPLICATING HIT

Ironically, although thrombosis was the first manifestation of the HIT syndrome, first recognized almost 50 yr ago (Weismann and Tobin, 1958), widespread recognition that thrombosis was a common complication of HIT did not occur until recently. Indeed, until 1995 no study of HIT had compared the frequency of thrombosis with a matched control population (Warkentin et al., 1995). This study quantitated the strength of the association between HIT and thrombosis and further noted that the more unusual the thrombotic event (e.g., bilateral deep venous thrombosis, pulmonary embolism), the stronger the association with HIT (see Chapter 2).

Table 6 summarizes the thrombotic events that have been observed during prospective studies of HIT. The major observation is that thrombosis is relatively common in HIT patients, occurring in approximately one-third of medical patients and about one half of postoperative surgical patients. The data also support findings from a prior retrospective study (Boshkov et al., 1993) that found the type of thrombotic event complicating HIT was influenced by the patient population. Table 6 suggests that the ratio of arterial to venous thrombosis is about 1 : 1 in medical patients, many of whom might have had arterial disease as their basis for hospitalization. Additionally, the therapeutic-dose heparin used in many of these studies may have partially protected against VTE, although it may not have prevented platelet-mediated arterial occlusion. In contrast, there appears to be a strong predisposition to VTE in postoperative orthopedic patients who have developed HIT (venous:arterial ratio at least 14:1) (Table 6).

The retrospective identification of patients with serologically confirmed HIT permits analysis of large groups of HIT patients (Table 7). This provides an alternative assessment of the spectrum of thrombotic complications in HIT. Three large studies (Warkentin and Kelton, 1996; Nand et al., 1997; Wallis et al., 1999) showed a predominance of venous thrombosis complicating HIT. Indeed, pulmonary embolism was even more frequent than all types of arterial thromboses combined.

In contrast, a different spectrum of thrombotic complications was reported by investigators at the University of Missouri-Columbia Health Sciences Center (Silver et al., 1983; Laster et al., 1987; Almeida et al., 1998b). Arterial, rather than venous, thromboembolism predominates in these patient series. Because this work is from the perspective of a vascular surgery service, it is possible that patients with arterial thrombosis are either more likely to be recognized as having HIT, or greater numbers of patients with preexisting arteriopathy are treated with heparin and thus at higher risk for developing arterial thrombosis if HIT develops.

Another pattern that emerges from the Missouri series is a progressively decreasing frequency of reported thrombotic or hemorrhagic complications, from 61% in 1983, to 23% in 1987, then to 7.4% in 1998. The authors believe this to be the result of earlier recognition of HIT. However, an alternative explanation could be greater awareness of HIT over time, and thus a higher likelihood of identifying patients with less severe HIT. Indeed, a study by Wallis and colleagues (1999) suggests that earlier recognition of HIT may *not* reduce the risk of thrombosis (Table 7).

A progressive reduction in HIT-associated mortality over time was also observed by the Missouri group (Table 7). However, early discontinuation of heparin was not associated with significantly lower mortality in another study (Wallis et al., 1999). This issue is complicated by the observation that deaths apparently unrelated to thrombosis are relatively common in patients with HIT (Warkentin and Kelton, 1996; Greinacher et al., 1999).

It is possible that nonthrombotic mortality may be higher than expected by chance in patients with HIT. This speculation is based on the observation that only a minority of patients who form anti-PF4/heparin antibodies develop HIT (discussed subsequently); a corollary to this statement is that comorbid factors that tend to result in increased pathogenicity of heparin-dependent antibodies may also independently contribute to increased patient morbidity and mortality (i.e., patients with septicemia or multisystem organ failure may be more likely to have platelet activation in the presence of HIT antibodies than "well" patients). Alternatively, because the patients develop thrombocytopenia they are tested for heparin-dependent antibodies, and non-pathogenic antibodies are detected.

A. Natural History of Isolated HIT

Isolated HIT is defined as the initial recognition of HIT because of thrombocytopenia alone, rather than because symptoms or signs of thrombosis draw attention to the possibility of underlying HIT. A large retrospective cohort study (Warkentin and Kelton, 1996) suggests that the subsequent frequency of new, progressive, or recurrent thrombosis is relatively high in such a patient population with serologically confirmed HIT (Fig. 2). Although these data are retrospective, the investigators attempted to minimize bias. First, the date that the HIT assay was ordered was used as an objective marker of first suspicion of the diagnosis of HIT. Second, patients were excluded from analysis if there was any evidence in the medical records to suggest the possibility of new signs or symptoms of thrombosis that may have caused the physician to suspect HIT. In other words, efforts were made to identify patients in whom HIT was suggested because of thrombocytopenia alone. Finally, only objectively documented new, progressive, or recurrent thrombotic events were analyzed.

The study identified 62 patients who met the definition of isolated HIT. The 30-day cumulative risk for thrombosis in this study was 52.8% (Fig. 2).

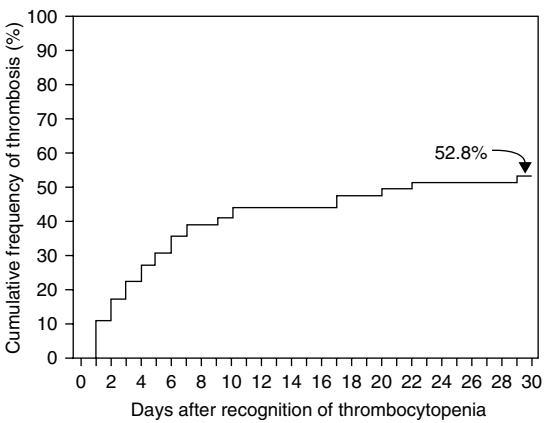


FIGURE 2 Cumulative frequency of thrombosis in patients presenting with isolated HIT ($n = 62$). Approximately 50% of HIT patients initially recognized with isolated HIT developed objective evidence for thrombosis during the subsequent 30-day period. The 1- and 2-day thrombotic event rates were approximately 10% and 18%, respectively. About 5% (3/62) patients developed sudden death as their presenting thrombotic event. *Abbreviation*: HIT, heparin-induced thrombocytopenia. *Source*: From Warkentin and Kelton, 1996.

TABLE 6 Proportion of Patients with HIT Developing HIT-Associated Thrombosis in Prospective Studies Employing In Vitro Laboratory Testing or in Which Data on Timing of Platelet Count Fall Were Reported

Study	Major indication for heparin	In vitro test	Number treated	Definition of thrombocytopenia ($\times 10^9$/L)	Patients with HIT (using more sensitive definition of HIT)	Thrombotic complication of HIT	
						Venous	Arterial
Medical patients (therapeutic dose heparin)							
Ansell et al., 1980	VTE	PRP (SR)	43	<150	4	0	0
Green et al., 1984; Green, 1986	VTE	HIPA	89	<150	2	1	1
Powers et al., 1984	VTE	SRA	65	<150	3	1	0
Bailey et al., 1986	VTE, ATE	No test	43	<100	1	0	1
Cipolle et al., 1983; Ramirez-Lassepas et al., 1984	Cerebrovascular ischemia	PRP	137[a]	<100 (>40% fall)	5 (16)	0 Not stated	1 (6)
Powers et al., 1979	VTE	No test	120	<150	2	1	1
Gallus et al., 1980	VTE	PRP	166	<100	5	1	0
Monreal et al., 1989	VTE	No test	89	<100	2	1	0
Kakkasseril et al., 1985	VTE, ATE	PRP	142	<100	9	2	2
Malcolm et al., 1979	Multiple	PRP	66	<100	1	1	0
Kappers-Klunne et al., 1997	Acute coronary syndromes	HIPA, EIA	358	<120, >30% fall (<60 or >50% fall)	2 (1)	1 (1)	0 (0)
Yamamoto et al., 1996	Hemodialysis	PRP, EIA	154	Clotting, platelet fall	5	0	1
Girolami et al., 2003	VTE, ATE	EIA, HIPA	238	>50% fall	0	0	0
Prandoni et al., 2005	Medical patients	EIA, HIPA	1754	>50% fall	14	2	2
Total medical: venous/arterial thrombosis ratio = 11/9 = 1.2			3464		56	11	9

Medical patients (prophylactic dose heparin)							
Girolami et al., 2003	Prophylaxis	EIA, HIPA	360	>50% fall	5	1	3
Harbrecht et al., 2004	Neurology patients	EIA, HIPA	200	<120 or >50% fall	5	5[b]	2[b]
Total medical: venous/arterial thrombosis ratio = 6/5 = 1.2			560		10	6	5
Orthopedic surgical patients (total joint arthroplasty)							
Warkentin et al., 1995, 2003	Hip	SRA	332	<150	9	7	1
				(50% fall)	(18)	(12)	(1)
Warkentin et al., 1998b	Hip, knee	SRA	246	<150, >50%	2	0	0
Ganzer et al., 1997	Hip, knee	HIPA	307	>50% fall	15	5[c]	0
Leyvraz et al., 1991	Hip	PRP	175	<100, >40% fall	2	2	0
Total orthopedic: venous/arterial thrombosis ratio = 14/1 = 14.0			1060		28	14	1
				(>50% fall)	(37)	(19)	(1)
Surgical, other							
Louridas, 1991	Vascular	PRP[d]	114	<100	5	0	0

Note: Where there was uncertainty over the numbers of patients with HIT, the higher estimated value was indicated in the table, to minimize the bias toward a high frequency of HIT-associated thrombosis (contrast analysis shown in Table 2).

[a]Detailed clinical data on thrombosis were available only on the subset of patients with cerebrovascular disease ($n = 137$).

[b]Thromboses diagnosed only at autopsy were not included; one stroke due to paradoxical embolism through patent foramen ovale classified as venous thrombosis.

[c]Another five patients developed venous thrombosis in association with a positive HIPA assay, but the platelet count did not fall by >50%.

[d]Ineffective testing was used (platelet aggregation without heparin).

Abbreviations: ATE, arterial thromboembolism; EIA, PF4-heparin enzyme-linked immunosorbent assay; HIPA, heparin-induced platelet activation test (aggregation of washed platelets); HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; MI/ACS, myocardial infarction or acute coronary syndromes; PRP, HIT assay using citrated platelet-rich plasma (PRP/SR, with serotonin release); SRA, serotonin release assay using washed platelets; UFH, unfractionated heparin; VTE, venous thromboembolism.

This risk did not differ whether the heparin had been discontinued, or whether warfarin had been substituted for the heparin. Similar findings were reported from a much smaller earlier study performed in Europe (Boon et al., 1994). This high risk for thrombosis in HIT is also supported by a prospective study (Warkentin et al., 1995), in which five of six HIT patients developed thrombosis either on the first day that their platelet count fell below 150×10^9/L or within the next few days despite the discontinuation of heparin.

Subsequent to the Hamilton study, a report by Wallis and colleagues (1999) from Loyola University confirmed the high risk for thrombosis among patients in whom HIT is identified by platelet count monitoring, even with discontinuation of heparin (Table 7). Overall, the 30-day thrombotic event rate was 43/113 (38%), with a ratio of venous to arterial thrombosis of just 1.4. The relatively low predominance of venous thrombosis could be explained by the large number of patients (59%) in this study who developed HIT following cardiac surgery (i.e., a patient population had relatively high risk for arterial thrombosis).

An intriguing finding of the Wallis report is that early cessation of heparin did not appear to improve clinical outcomes. For 40 of the 113 patients with HIT (35%), heparin was discontinued within 48 h of onset of thrombocytopenia (defined as a platelet count fall to less than 100×10^9/L, or a greater than 50% fall from the peak platelet count after initiating heparin). Indeed, there was a trend to a *higher* rate of thrombosis in the patients with early heparin cessation, compared with the remaining 65% of patients in whom heparin was stopped later (45% vs. 34%; $p = 0.26$) (Table 7).

Further evidence supporting an unfavorable natural history of untreated HIT was provided by a large prospective cohort study (Greinacher et al., 2000). These investigators found that the thrombotic event rate was 6.1% per day during the mean 1.7-day interval between diagnosis of HIT (and cessation of heparin) and initiation of lepirudin therapy. This event rate ($6.1 \times 1.7 = 10.4\%$) corresponds closely to the 10% rate of thrombosis observed in the Hamilton study in the first 48 h following diagnosis of isolated HIT (Warkentin and Kelton, 1996) (Fig. 2).

Most recently, Zwicker and colleagues (2004) noted a high frequency of thrombosis (~36%) among patients with isolated HIT and a "strong" anti-PF4/polyanion EIA result (>1.0 OD units).

Taken together, these studies of the natural history of isolated HIT provide the basis for the recent recommendation that prophylactic anticoagulant therapy (in therapeutic doses) is appropriate for most patients strongly suspected (or confirmed) to have isolated HIT (Hirsh et al., 2001; Warkentin and Greinacher, 2004) (see Chapters 1 and 12–15). Other data to support this concept include the high probability of detecting subclinical deep-vein thrombosis by duplex ultrasonography in patients with isolated HIT (Tardy et al., 1999), as well as the persistence of marked in vivo thrombin generation for several days in patients with acute HIT even following discontinuation of heparin (Warkentin et al., 1997; Greinacher et al., 2000).

B. Summary of Observations from Prospective and Retrospective Studies

Observations emerging from these studies include the following:

1. The risk of thrombosis in patients with HIT is higher than previously recognized (up to 50%), and remains high despite the discontinuation of heparin. Mortality in patients with HIT is significant, although it remains

TABLE 7 Frequency of Thrombosis Complicating HIT in Retrospective Studies

Study	Patients with HIT	Mean platelet count nadir ($\times 10^9$/L)	Number of patients with thrombosis (%)	Ratio of venous/ arterial thrombosis	Number of deaths (%)
Warkentin and Kelton, 1996	127	59[a]	97 (76%)	4.3	26 (20%)
Subgroup with "isolated" thrombocytopenia	62	57	32 (52%)[b]	4	13 (21%)
Nand et al., 1997	108	58	32 (29%)	2.5	5 (5%)[c]
Wallis et al., 1999	113	54	43 (38%)	1.4	31 (27%)
Subgroup with heparin cessation <48 h	40	56	18 (45%)	1.4	10 (25%)
Subgroup with heparin cessation >48 h	73	54	25 (34%)	1.4	21 (29%)
Silver et al., 1983	62	Range: 5–83	38 (61%)	0.6	20 (32%)[d]
Laster et al., 1987	169	57	30 (18%)	0.5	20 (12%)
Almeida et al., 1998b	94[e]	>108	7 (7%)	0.6[f]	0 (0%)
Sturtevant et al., 2006	22	39	14 (67%)	5.5[g]	7 (32%)[h]
Greinacher et al., 2005b	408	41	227 (56%)	2.4	Not stated
Mureebe et al., 2004	45	Not stated	Not stated	1.5[i]	10/35 (29%)[j]
Murray et al., 2006	6	35	1 (17%)	Not stated[k]	3/6 (50%)

Note: Where there was uncertainty over the numbers of patients with HIT, the higher estimated value was indicated in the table, to minimize the bias toward a high frequency of HIT-associated thrombosis (contrast analysis shown in Table 2).

[a]The mean platelet count nadir for 127 patients with HIT and platelet count $<150\times10^9$/L, and the median platelet count nadir for all 142 patients diagnosed with HIT (including those whose platelet count nadir was $>150\times10^9$/L), were both 59×10^9/L (Warkentin, 1998a) (see Figure 6 in Chapter 2).

[b]The cumulative 30-day frequency of new thrombosis in patients with isolated thrombocytopenia following recognition of HIT was 52.8% by Kaplan-Meier analysis.

[c]Only deaths in patients who developed thrombosis were reported. Total number of deaths in the HIT cohort was not reported.

[d]Fourteen of the 20 deaths were judged to be caused by HIT-associated thrombosis.

[e]Of 100 consecutive patients with positive in vitro testing, six were previously known to have heparin-dependent antibodies and were not subsequently reexposed to heparin.

[f]Two thromboses of arteriovenous grafts were excluded from classification into arterial or venous thrombosis.

[g]Two thromboses on the dialysis membrane were excluded from classification into arterial or venous thrombosis.

[h]In four of the seven deaths, HIT was judged to be contributory.

[i]Thrombosis of temporary (4) or permanent (10) dialysis access excluded from classification into arterial or venous thrombosis.

[j]Complete data available on 35 patients.

[k]Type of thrombosis not stated.

Abbreviation: HIT, heparin-induced thrombocytopenia.

uncertain what proportion is related to HIT-associated thrombosis, and to what extent these can be prevented by effective treatment.

2. Most thrombotic events are venous, rather than arterial, although this predominance may not be observed in patient populations at high risk for arterial disease. Pulmonary embolism may be the most frequent life-threatening consequence of HIT.

V. POPULATION-BASED STUDIES OF HIT ANTIBODY SEROCONVERSION

Usually, serological investigation for HIT antibodies is performed on patients who develop thrombocytopenia during heparin treatment. Since 1995, however, many studies have systematically assessed heparin-dependent antibody seroconversion

using sensitive assays (EIA, SRA, or both), irrespective of whether or not thrombocytopenia occurred. Some interesting insights into the pathogenesis of HIT have emerged from these reports.

As shown in Table 8, three main types of patient population have been investigated: medical patients receiving therapeutic-dose UFH; orthopedic patients receiving UFH or LMWH; and cardiac surgical patients receiving UFH or LMWH. There appear to be distinct frequencies of HIT antibody formation, as well as varying risks of "breakthrough" of HIT, among these different populations (see Fig. 3). Several observations emerge from these studies:

1. The prevalence of seroconversion depends on the diagnostic assay used. The PF4-heparin EIA is more sensitive than the SRA for the detection of anti-PF4/heparin antibodies (Bauer et al., 1997; Pouplard et al., 1999; Warkentin et al., 2000, 2005a; Warkentin and Sheppard, 2006a); however, this increase in sensitivity does not necessarily translate into greater predictive value for clinical HIT (see Chapter 10).
2. With use of PF4/polyanion EIA, the frequency of seroconversion following cardiac surgery ranges to as high as about 75% (Visentin et al., 1996; Bauer et al., 1997; Warkentin et al., 2000; Warkentin and Sheppard, 2006a). A high frequency of seroconversion (13–20%) was also observed using the SRA. Despite the highest frequency of HIT seroconversion reported in this patient population, the likelihood of developing HIT appears to be less than in orthopedic patients also treated with postoperative UFH.
3. Seroconversion occurs frequently without thrombocytopenia or thrombosis. Indeed, most patients who form HIT antibodies do not develop HIT. The proportion who develop HIT, however, is highest among the patients who have a positive SRA. This suggests that HIT antibodies "strong" enough to activate platelets are more likely to be clinically significant. Patient-dependent factors also must be important, however, because the probability of a positive SRA indicating clinical HIT ranges from about <10% (cardiac surgery) to approximately 50% (orthopedic surgery patients receiving UFH).
4. Regardless of which diagnostic assay is used, new seroconversion occurs more frequently after exposure to UFH than LMWH (Warkentin et al., 1995, 2000, 2003, 2005a; Amiral et al., 1996; Lindhoff-Last et al., 2002; Ahmad et al., 2003a) (Fig. 3).

A. Iceberg Model of HIT

The interrelationship between antibody formation and clinical HIT (with or without thrombosis) can be illustrated using the "iceberg model" (see Fig. 3). In this model, only anti-PF4/heparin antibodies of IgG class that possess platelet-activating properties are associated with risk of HIT. Analysis of RCT data illustrates that the difference in risk of HIT between UFH and LMWH can be explained by the combination of a lower frequency of antibody formation (whether detected by EIA or SRA), as well as lower risk of "breakthrough" to clinical HIT among patients who have formed heparin-dependent platelet-activating antibodies.

B. HIT in Patients Undergoing Cardiac Surgery

Three prospective studies have evaluated the frequency of HIT in postoperative cardiac surgical patients who also have received postoperative antithrombotic

(Text continues on p. 97)

TABLE 8 Studies Describing Systematic Screening for HIT Antibodies Using Sensitive Assays in Patients Receiving Heparin

Study	Trial design	Heparin (porcine UFH unless otherwise indicated)	Number of patients	HIT assay used	Patients with HIT antibodies (%)	Patients with HIT, n (%)
Medical patients						
Amiral et al., 1996	Retrospective	iv ther UFH	109	EIA-IgM/A/G	19 (17.4)	1 (0.9)[a]
				EIA-IgG	3 (2.8)	
Kappers-Klunne et al., 1997	Prospective	iv ther UFH	358	EIA-IgG	9 (2.5)	2 (0.6)
				HIPA	30 (8.4)	0 (0)
Harbrecht et al., 2004	Prospective	iv ther, sc proph UFH	200	EIA-IgM/A/G	41 (20.5)[b]	5 (2.5)
Pohl et al., 2005	Prospective	sc proph LMWH	111	EIA-IgM/A/G	2/111 (1.8)	0 (0)
Hemodialysis patients						
Greinacher et al., 1996	Prevalence study	iv ther UFH	165	HIPA	7 (4.2)	0 (0)
de Sancho et al., 1996	Prevalence study	iv ther UFH	45	EIA-IgM/A/G	0 (0)	0 (0)
Boon et al., 1996	Prevalence study	iv ther UFH	128	EIA-IgM/G	4 (3.1)	0 (0)[d]
				EIA-IgG	3 (2.3)	
		LMWH	133	EIA-IgM/G	1 (0.8)	0 (0)[c]
				EIA-IgG	1 (0.8)	
Sitter et al., 1998	Prevalence study	iv ther UFH	70	EIA-IgG	2 (2.8)	0 (0)
Luzzatto et al., 1998	Prevalence study	iv ther UFH	50	EIA-IgG	6 (12.0)	0 (0)
O'Shea et al., 2002	Prevalence study	iv ther UFH	88	EIA-IgM/A/G	1 (1.1)	0 (0)
Yu et al., 2002	Prevalence study	iv ther UFH	100	EIA-IgM/A/G	6 (6.0)	0 (0)
			71[e]		2 (2.8)	0 (0)
Lee et al., 2003	Prevalence study	iv ther UFH	91	EIA-IgM/A/G	8 (8.8)	0 (0)
Pena de la Vega et al., 2005	Prevalence study	iv ther UFH	57	EIA-IgM/A/G	2 (3.5)	0 (0)
Palomo et al., 2005	Prevalence study	iv ther UFH	207	EIA-IgM/A/G	37 (17.9)[f]	Not stated
Nakamoto et al., 2005	Prevalence study	iv ther UFH	105	EIA	2 (1.9)	Not stated
Skouri et al., 2006	Prospective study	iv ther UFH	38	EIA-IgM/A/G	8 (21)[g]	0 (0)
				HIPA	5 (13)	
Carrier et al., 2007	Prevalence study	iv ther UFH	419	EIA-IgM/A/G	54 (12.9)	Not stated
				EIA-IgG	9 (2.1)	
				SRA	0 (0)	

(Continued)

TABLE 8 Studies Describing Systematic Screening for HIT Antibodies Using Sensitive Assays in Patients Receiving Heparin *(Continued)*

Study	Trial design	Heparin (porcine UFH unless otherwise indicated)	Number of patients	HIT assay used	Patients with HIT antibodies (%)	Patients with HIT, *n* (%)
Cardiac patients						
Vonderheide et al., 1998	Prospective	iv ther UFH	58	EIA-IgM/A/G	0 (0)[h]	0 (0)
Yeh et al., 2006[i]	Prospective	iv ther UFH	311	EIA-IgM/A/G	25 (8.0)	Not stated
Foo et al., 2006[i]	Prospective	iv ther UFH	357	EIA-IgM/A/G	36 (10.1)	Not stated
Gluckman et al., 2005	Prospective	iv ther UFH	94	EIA-IgM/A/G	16 (17.0)[j]	0 (0)
				SRA	1 (2.1)	
Matsuo 2005	Prospective	iv ther UFH	252[k]	EIA	22 (8.7)[k]	4 (1.6)
Orthopedic postoperative surgical patients						
Warkentin et al., 1995, 2000	Substudy of RCT	sc proph UFH	205	EIA-IgG	29 (14.1)	10 (4.9)
				SRA	19 (9.3)	
		sc proph LMWH	182	EIA-IgG	11 (6.0)	2 (1.1)
				SRA	5 (2.7)[l]	
Amiral et al., 1996	Retrospective	sc proph LMWH	100	EIA-IgM/A/G	8 (8.0)	0 (0)
				EIA-IgG	2 (2.0)	
Marx et al., 1999	Prospective	sc proph LMWH	265	EIA	13 (4.9)[m]	0 (0)
Warkentin et al., 2000	Prospective	sc proph LMWH	257	EIA-IgG	22 (8.6)	2 (0.8)
				SRA	9 (3.5)[l]	
Warkentin et al., 2005	Substudy of RCT	sc proph LMWH	1349	EIA-IgM/A/G	30 (2.2)	0 (0)
				EIA-IgG	6 (0.4)	
				SRA	1 (0.1)	
		sc proph fondaparinux	1377	EIA-IgM/A/G	26 (1.9)	0 (0)
				EIA-IgG	9 (0.7)	
				SRA	3 (0.2)	
Greinacher et al., 2005a	Prospective	sc proph UFH	231	EIA-IgM/A/G	46 (23.5)	12 (5.2)
				HIPA	25 (12.4)	
		sc proph LMWH	271	EIA-IgG/A/M	19 (8.3)	0 (0)
				HIPA	13 (5.5)	

Cardiac postoperative surgical patients (all received porcine UFH at cardiopulmonary bypass						
Visentin et al., 1996	Retrospective	CPB: UFH (NPH)	44	EIA-IgM/G	27 (61.4)[n]	0 (0)
				EIA-IgG	23 (52.3)	
Bauer et al., 1997	Prospective	CPB: bovine UFH (NPH)	111	EIA-IgM/A/G	56 (50.5)[o]	0 (0)
				SRA	14 (12.6)[o]	
Trossaert et al., 1998	Retrospective	CPB: UFH; sc proph UFH	51	EIA-IgM/A/G	14 (27.5)[p]	0 (0)
				EIA-IgG	9 (17.6)	
				PRP	2 (3.9)	
Pouplard et al., 1999	Prospective	sc proph UFH	157	EIA-IgM/A/G	46 (29.3)	6 (3.8)
				EIA-IgG	24 (15.3)	
				SRA	6 (3.8)	
		sc proph LMWH	171	EIA-IgM/A/G	37 (21.6)	0 (0)
				EIA-IgG	24 (14.0)	
				SRA	2 (1.2)	
Warkentin et al., 2000	Prospective	CPB: UFH; sc proph UFH	100	EIA-IgG	50 (50.0)	1 (1.0)
				SRA	20 (20.0)	
Koster et al., 2001	Prospective	CPB; VAD: porcine UFH (all)	100	EIA-IgM/A/G	63 (63)	Not stated
		Subset with heparin-coated VAD	57		32 (56)	
		Subset with non-coated VAD	43		31 (72)	
Francis et al., 2003	RCT	CPB: bovine UFH	99	EIA-IgM/A/G	44 (44.4)[q]	0 (0)
		CPB: porcine UFH	108	EIA-IgM/A/G	33 (30.6)[r]	0 (0)
Kannan et al., 2005	Prospective	CPB: UFH	33	EIA-IgM/A/G	4 (12.1)	5 (15.2)
				PRP	2 (6.1)	
				SRA	5 (15.2)	
Schenk et al., 2006	Retrospective	CPB, VAD	113	EIA-IgM/A/G	74 (65)	12 (10.6)
				HIPA	12 (10.6)	
Vascular surgical patients						
Jackson et al., 1998	Prospective	iv ther UFH	54	EIA-IgM/G	3 (5.6)[s]	
Lindhoff-Last et al., 2000	Prospective	iv ther UFH × 7 days,	50	EIA-IgM/A/G	17 (34.0)	0 (0)
		then sc proph LMWH	48	EIA-IgG	6 (12.5)	
			48	HIPA	3 (6.3)[t]	

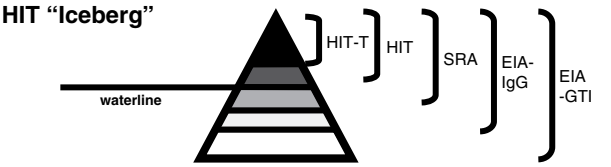
(Continued)

TABLE 8 Studies Describing Systematic Screening for HIT Antibodies Using Sensitive Assays in Patients Receiving Heparin *(Continued)*

Study	Trial design	Heparin (porcine UFH unless otherwise indicated)	Number of patients	HIT assay used	Patients with HIT antibodies (%)	Patients with HIT, *n* (%)
Screening pre-cardiac surgery						
Boning et al., 2005[u]	Retrospective	Previous UFH exposure	144	EIA-IgM/A/Gu	2 (1.4)	0 (0)
Bennett-Guerrero et al., 2005	Prospective	Previous UFH exposure	466	EIA-IgM/A/G	59 (12.7)	Not stated
Trauma patients						
Lubenow et al., 2006	RCT	sc proph UFH	314	EIA and HIPA	21 (6.7)	4 (1.3)
		sc proph LMWH	295		5 (1.7)	1 (0.3)

[a]Thrombocytopenia defined as platelet count fall >50% from baseline.
[b]HIPA positive in 3/41 (7.3%) of EIA-positive sera.
[c]Two patients with HIT antibodies had mild thrombocytopenia, but a causal relation to heparin was not stated.
[d]Results of platelet aggregation testing are not included since there was a false positive rate of 33% in control uremic patients not yet exposed to heparin.
[e]Repeat samples on 71 patients were taken 6–8 month after initial sampling of 100 patients.
[f]Twelve of 22 EIA positive sera also tested positive by SRA
[g]Pediatric HD patients—seropositivity seems to be highest in earlier months of HD, then antibody gradually disappears.
[h]Incidence of HIT antibodies may have been underestimated since testing was performed on samples from day 3–4.
[i]Yeh et al., 2006, and Foo et al., 2006, describe similar inception cohorts with a baseline prevalence of HIT-Ig of 15/500 (3.0%).
[j]Values listed indicate new seroconversions; of the inception cohort of 154, 7 (4.5%) were EIA-positive at baseline.
[k]Of patients undergoing PCI, 20/163 (12.3%) were EIA-positive; Of the non-PCI patients, 2/89 (2.2%) were EIA-positive; 22/252 represents new seroconversions (2/254 [0.8%] EIA positive at baseline).
[l]20% serotonin release used in Warkentin et al., 2000, rather than 50% serotonin release cutoff used in Warkentin et al., 1995.
[m]10/265 (3.8%) were found to have HIT-Ig prior to surgery.
[n]Ten patients had HIT-IgG preoperatively: incidence of new seroconversion was 17/44 (38.6%).
[o]Incidence of new seroconversion was 43% for EIA and 9% for SRA.
[p]Two patients had HIT-IgG preoperatively: incidence of new seroconversion was 12/51 (23.5%) for EIA-IgM/A/G and 7/51 (13.7%) for EIA-IgG. None had a positive aggregation assay preoperatively.
[q]80.2% underwent CPB, remainder "off-pump" surgery; 18.8% received postoperative UFH, LMWH, or both.
[r]Excludes patients testing positive for HIT antibodies at baseline.
[s]Two patients had HIT-IgG preoperatively. Incidence of new seroconversion was 1/54 (1.9%).
[t]Includes only HIPA patients who also tested positive in EIA.
[u]Pediatric cardiac surgery; EIA testing was performed preoperatively in patients prior to a second surgery for congenital heart defects.

Abbreviations: CPB, cardiopulmonary bypass; EIA, PF4-heparin enzyme-linked immunosorbent assay (-IgM/A/G, one or more of IgM, IgA, and IgG antibodies present; -IgG, IgG antibodies only present); HIPA, heparin-induced platelet activation assay; HIT, heparin-induced thrombocytopenia; iv ther, intravenous therapeutic-dose heparin; LMWH, low molecular weight heparin; MI, myocardial infarction; NPH, no postoperative heparin; PCI, percutaneous coronary intervention; PRP, HIT assay using citrated platelet-rich plasma; RCT, randomized controlled trial; sc proph, subcutaneous prophylactic dose heparin; SRA, serotonin release assay using washed platelets; UFH, unfractionated heparin; VAD, ventricular assist device; VTE, venous thromboembolism.



	Orthopedic Surgery			Cardiac Surgery
	UFH	**LMWH (enoxaparin)**	***P***	**UFH**
HIT-T	12/332 (**3.6%**)	1/333 (**0.3%**)	<0.001	1/100 (**1.0%**)
HIT	16/332 (**4.8%**)	2/333 (**0.6%**)	<0.001	1/100 (**1.0%**)
+ SRA	19/192 (**9.9%**)	5/170 (**2.9%**)	0.010	20/100 (**20.0%**)
+ EIA-IgG	29/192 (**15.1%**)	11/170 (**6.5%**)	0.011	50/100 (**50.0%**)
+ EIA-GTI	56/188 (**29.8%**)	26/169 (**15.4%**)	0.0015	76/98 (**77.6%**)

FIGURE 3 Iceberg model of HIT. The top panel depicts a generic "iceberg", illustrating the interrelationship of clinical HIT with formation of anti-PF4/heparin antibodies detected by the platelet SRA, an EIA-IgG, and a commercial EIA (from GTI, Inc.) that detects antibodies of all three major immunoglobulin classes (IgG, IgA, and IgM) against PF4/polyanion (EIA-GTI). The lower panel illustrates the event rates for clinical HIT (HIT), including the subgroup with HIT-T, in relation to the frequencies of antibody formation, in three clinical settings: UFH or LMWH thromboprophylaxis during orthopedic surgery (data from an RCT) and UFH thromboprophylaxis postcardiac surgery (prospective cohort study). + indicates positive test. *Abbreviations*: EIA-IgG, enzyme-immunoassay that detects IgG antibodies against PF4/heparin complexes; HIT, heparin-induced thrombocytopenia; HIT-T, HIT-associated thrombosis; LMWH, low molecular weight heparin; SRA, serotonin release assay; UFH, unfractionated heparin. *Source*: Warkentin et al., 1995, 2000, 2003, 2005a; Warkentin and Sheppard, 2006a.

prophylaxis with UFH (Trossaert et al., 1998; Pouplard et al., 1999, 2002, 2005; Warkentin et al., 2000; Warkentin and Sheppard, 2006a) (see Fig. 3). Pooling the data, the frequency of HIT appears to be about 2%. This frequency is consistent with a number of retrospective studies (Glock et al., 1988; Walls et al., 1992a,b; Singer et al., 1993) that reported a frequency of HIT of up to 5%, but overall, also noted a frequency of about 2% (Table 9). Furthermore, HIT was associated with a risk of thrombosis of 38–81%, and with an overall mortality of 18–43% in these studies. In contrast to the orthopedic patient population, the predominant thrombotic event appears to be arterial.

Only one group has examined the influence of postoperative antithrombotic prophylaxis with UFH or LMWH on the frequency of HIT antibody formation and HIT following heart surgery (Pouplard et al., 1999, 2002, 2005). In their multi-year observational studies involving nonrandomized comparisons between UFH and LMWH, a significant difference in risk of HIT was observed: UFH = 11/437 (2.5%) versus LMWH = 8/1874 (0.4%); $p < 0.0001$. However, differences in patient composition prevent firm conclusions.

TABLE 9 Frequency of HIT and Thrombosis in Retrospective Studies of HIT in Cardiovascular Surgery Patients

Study	Patients at risk *N*	Patients with HIT *n* (%)	Patients with HIT and thrombosis *n* (% of HIT)	Ratio of venous:arterial thrombosis	Total deaths in patients with HIT *n* (% of HIT)
Walls et al., 1992a	4261	82 (1.9)	31 (38)	0.3:1	23 (28)
Walls et al., 1992b	764	35 (4.5)	17 (49)	0.3:1	15 (43)
Visentin et al., 1996	51	0 (0)	–	–	–
Glock et al., 1988	–	21	17 (81)	0.7:1	8 (38)
Singer et al., 1993	1500	11 (0.75)	7 (64)[a]	0.3:1[b]	2 (18)
Wan et al., 2006	–	33	15 (46)	0.6:1[c]	4 (22)

[a]In seven patients, 17 thrombotic events occurred.
[b]Precise number of arterial and venous events is unclear from the published data. For this analysis, of six limb amputations associated with intravascular catheters or devices, five were assumed to be arterial and one venous, based on the type of intravascular catheter or device associated with the amputated limb.
[c]Two events (encephalopathy and quadriplegia) were not classified.
Abbreviation: HIT, heparin-induced thrombocytopenia.

Overall, frequency of antibody formation was similar in the 2 patient groups, using a commercial EIA that detects IgM, IgA, and IgG antibodies. This is consistent with "point immunization" from intraoperative UHF use, rather than any major influence from postoperative UFH or LMWH. The studies from this group also show that detectability of platelet-activating antibodies (by SRA) is far more predictive of thrombocytopenia than a positive anti-PF4/heparin EIA. Thus, in summary:

1. The frequency of HIT antibody formation following heart surgery is influenced primarily by UFH given at CPB, rather than the type of heparin preparation given postoperatively.
2. Among patients who form HIT antibodies following heart surgery, the risk of HIT likely is higher among those receiving postoperative UFH compared with LMWH.
3. The study indicates a greater clinical usefulness of the SRA, compared with the anti-PF4-heparin EIA (see Chapter 10).

The potential to reduce risk of "breakthrough" of HIT among postcardiac surgery patients who form HIT antibodies is a major reason why antithrombotic agents with low (danaparoid) or negligible (fondaparinux) cross-reactivity against PF4-heparin might be ideal anticoagulants for this clinical situation (Warkentin et al., 2005b) (see Chapter 7).

B. Fondaparinux

Fondaparinux (Arixtra) is a novel antithrombin-binding pentasaccharide that inhibits factor Xa without inhibiting thrombin. It has been shown to be safe and effective for antithrombotic prophylaxis following orthopedic surgery (see Chapter 17). Fondaparinux likely does bind to PF4 through charge interactions, and in suprather-apeutic concentrations will even disrupt PF4/heparin complexes (Greinacher et al., 2006). Further, in systematic studies of anti-PF4/heparin antibody formation performed using blood samples from RCTs comparing fondaparinux and enoxaparin

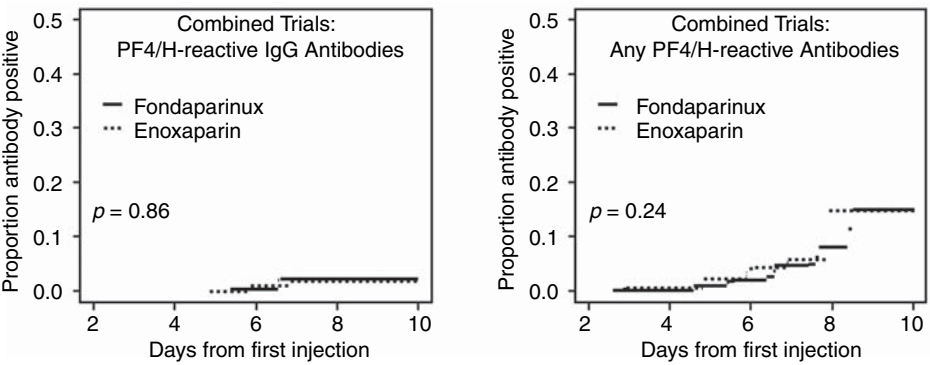


FIGURE 4 Anti-PF4/heparin antibody formation in patients receiving fondaparinux or enoxaparin after orthopedic surgery (current status analysis). Data are combined for patients undergoing knee and hip replacement. (*Left*) Anti-PF4/heparin antibodies of IgG class. There is no significant difference between the study drug groups ($p = 0.86$). (*Right)* All anti-PF4/heparin antibodies. There is no significant difference between the study drug groups ($p = 0.24$). *Abbreviation*: PF4/H, platelet factor/heparin. *Source*: From Warkentin et al., 2005b.

thromboprophylaxis after elective hip or knee replacement therapy, similar frequencies of anti-PF4/heparin antibody formation were seen with both anticoagulants (Warkentin et al., 2005b) (Fig. 4). However, anti-PF4/heparin antibodies (whether obtained from fondaparinux-treated patients or from patients with HIT) fail to cross-react in vitro with PF4/fondaparinux, even though they react strongly with PF4/UFH or PF4/LMWH (see Fig. 2 in Chapter 17). Further, induction of HIT by fondaparinux seems rare, based upon the dearth of such reports to date (Warkentin et al., 2007). Thus, this novel anticoagulant offers the possibility of negligible risk of causing HIT, a concept that can be illustrated using the iceberg model (Fig. 5) (Warkentin, 2006b).

C. Adverse Prognosis of Anti-PF4/Heparin Antibodies

An emerging issue is whether anti-PF4/heparin antibodies confer adverse prognosis even in the absence of clinically-overt HIT. Mattioli and coworkers (2000) found a higher 1 yr event-rate (death, MI, recurrent angina, revascularization, or stroke) among patients who formed anti-PF4/heparin antibodies following UFH treatment for unstable angina (66% vs. 44%; $p < 0.01$). Williams and colleagues (2003), using blood samples obtained 48 h after entry into a clinical trial of non-ST-segment elevation MI, found that death or MI was increased at 1 mo (OR, 4.0; $p = 0.0093$) among patients with a positive anti-PF4/polyanion EIA. Bennett-Guerrero et al. (2005) observed a higher risk of in-hospital death or hospitalization beyond 10 days (OR, 1.98; $p = 0.0284$) among postcardiac surgery patients with a positive EIA for anti-PF4/heparin antibodies prior to cardiac surgery. Finally, Peña de la Vega and coworkers (2005) found in a cross-sectional study of chronic HD patients that the presence of anti-PF4/polyanion antibodies corresponded to a higher mortality at mean 620-day follow-up (hazard ratio, 2.47; $p = 0.03$). In none of these studies did clinically evident HIT appear to explain these differences. Despite the implication of these studies that these antibodies might be pathogenic, an alternative view is that unrecognized confounders explain their apparent adverse prognosis. For example,

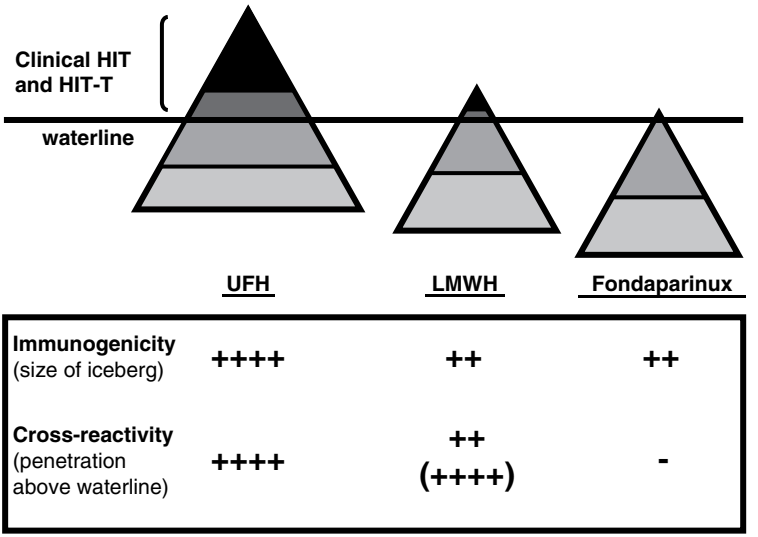


FIGURE 5 Dissociation in immunogenicity and cross-reactivity: comparison of UFH, LMWH, and fondaparinux. Of the three sulfated polysaccharide anticoagulants, UFH is most immunogenic (largest iceberg), whereas LMWH and fondaparinux exhibit similar immunogenicity. However, in contrast to UFH and LMWH, which can form well the antigens recognized by HIT antibodies, fondaparinux only poorly forms antigens with PF4 in vitro that are recognized by HIT antibodies. (Note: LMWH is indicated by ++ and ++++ to indicate that its cross-reactivity appears to differ in vivo [++] and in vitro [++++].) *Abbreviations*: HIT, heparin-induced thrombocytopenia; HIT-T, HIT-associated thrombosis; LMWH, low molecular weight heparin; UFH, unfractionated heparin. *Source*: From Warkentin, 2006b.

these antibodies could represent a *surrogate marker* for another key risk factor, such as inflammation—a known risk factor for atherosclerosis-associated mortality (Ridker et al., 1997). This distinction is critical: if these antibodies are truly pathogenic ("forme fruste HIT"), avoiding heparin might be beneficial, whereas this is unlikely to be the case if the antibodies are merely a marker for other vascular risk factors (Warkentin and Sheppard, 2006b).

VI. VARIABLE FREQUENCY OF HIT: IMPLICATIONS FOR PLATELET COUNT MONITORING

Until recently, studies of HIT frequency have yielded seemingly confusing and inconsistent results. However, as argued in this chapter, by taking into consideration (1) type of heparin used, (2) patient population treated, and (3) laboratory and clinical evidence to distinguish (immune) HIT from nonimmune HAT, distinct profiles for HIT antibody seroconversion, HIT itself, and HIT-associated thrombosis can be discerned (Fig. 3). New research questions will be generated in the search for the biological basis for these intriguing differences in HIT risk. But perhaps the most important insight to emerge from these collective studies is the simple and clinically relevant observation that new, progressive, or recurrent thrombosis occurs in at least 35–50% of patients who develop proven HIT, even after heparin is discontinued (Warkentin and Kelton, 1996; Wallis et al., 1999;

Zwicker et al., 2004). This underscores the need for prompt recognition and urgent therapy in all patients suspected of having this adverse drug reaction.

Practically, these findings suggest strategies for platelet count monitoring in patients receiving heparin. Some physicians are hesitant to institute regular platelet count monitoring for HIT. One explanation is the almost ubiquitous use of heparin in hospitalized patients. Thus, a requirement that regular, perhaps even daily, platelet count monitoring be performed seems excessive. Additionally, there is no convincing evidence that regular platelet count monitoring can prevent the thrombotic complications of HIT if the physician response is merely to stop the heparin (Wallis et al., 1999). However, a noteworthy consideration is that instituting alternative, parenteral anticoagulation likely will prevent thrombosis in patients recognized as having isolated HIT.

These comments notwithstanding, marked differences in risk for HIT are apparent among different patient populations. Thus, it seems prudent to recommend that patients at the highest risk of HIT, and for HIT-associated thrombosis (e.g., postoperative patients receiving UFH), should have platelet counts monitored regularly, perhaps at least every other day. For patients whose risk for HIT

TABLE 10 Recommendations for Platelet Count Monitoring for HIT

1. Monitoring for typical-onset HIT: stratifying the intensity of platelet count monitoring for HIT based upon its risk
   A. Patients at highest risk for HIT (1–5%) (e.g., postoperative patients receiving prophylactic-dose UFH after major surgery, patients receiving therapeutic-dose UFH): monitoring during heparin therapy, at least every second day from day 4 to day 14[a,b]
   B. Patients at intermediate risk for HIT (0.1–1%) (e.g., medical/obstetrical patients receiving prophylactic-dose UFH, or postoperative patients receiving prophylactic-dose LMWH, or postoperative patients receiving intravascular catheter "flushes" with UFH): monitoring during heparin therapy, at least every 2 or 3 days from day 4 to day 14[a], when practical[c]
   C. Patients at low risk for HIT (<0.1%) (e.g., medical/obstetrical patients receiving prophylactic- or therapeutic-dose LMWH, or medical patients receiving only intravascular catheter "flushes" with UFH): routine platelet count monitoring is not recommended[d]
2. Monitoring for rapid-onset HIT: for a patient recently exposed to heparin (within the past 100 days), a repeat platelet count within 24 h following reinitiation of heparin
3. When to suspect HIT
   A relative (proportional) platelet count fall of 50% or greater that is otherwise clinically unexplained should be considered suspicious for HIT, even if the platelet count nadir remains above 150×10^9/L.
   For any patient who develops thrombosis during (day 5 to 14) or within several days after stopping heparin therapy, or who develops an unusual clinical event in association with heparin therapy (e.g., inflammatory or necrotic skin lesions at heparin injection sites, acute systemic reaction post-intravenous heparin therapy), a repeat platelet count should be measured promptly and compared with recent values.

Note: These recommendations parallel those of the Seventh American College of Chest Physicians (ACCP) Concersus Conference on Antithrombotic and Thrombolytic Therapy (Warkentin and Greinacher, 2004).

[a]The crucial time period for monitoring "typical-onset" HIT is between days 4 to 14 (first day of heparin = day 0), where the highest platelet count from day 4 (inclusive) onwards represents the "baseline." Platelet count monitoring can cease before day 14 when heparin is stopped.

[b]Once-daily platelet count monitoring is reasonable in patients receiving therapeutic-dose UFH given that daily blood draws required for aPTT monitoring are usually required.

[c]Frequent platelet count monitoring may not be practical when UFH or LMWH is given to outpatients.

[d]Monitoring as per "intermediate" risk is appropriate if UFH was given before initiating LMWH.

Abbreviations: HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; UFH, unfractionated heparin.

Source: Adapted from Warkentin and Greinacher, 2004.

appears to be 0.1–1% (e.g., medical patients receiving UFH, surgical patients receiving LMWH), less frequent monitoring may be appropriate. Since HIT is unlikely to occur before day 5, or after day 14, the monitoring could be performed two or three times per week from days 4 to 14. Most patients have frequent complete blood counts performed during the first few days of hospitalization, so comparative platelet count results for days 0–3 are usually available.

Several consensus conferences have examined the issue of platelet count monitoring for HIT (Warkentin, 2002; Warkentin and Greinacher, 2004). These have in common the concept of stratifying the intensity of platelet count monitoring based upon the risk of developing HIT and focusing the monitoring during the time when HIT usually occurs. Table 10 summarizes the recommendations (Warkentin and Greinacher, 2004).

Regardless of the intensity of surveillance, all physicians who monitor platelet counts need to understand how to distinguish HIT from nonimmune HAT, because diagnostic confusion may lead to inappropriate decisions to discontinue heparin therapy in patients with nonimmune HAT who otherwise require anticoagulation because of high risk for thrombosis. Irrespective of whether platelet count monitoring is being performed, HIT should be considered promptly in the differential diagnosis of any patient who develops symptoms or signs of new, progressive, or recurrent thrombosis during or within a few days of discontinuing heparin treatment.

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4 Nonimmune Heparin–Platelet Interactions: Implications for the Pathogenesis of Heparin-Induced Thrombocytopenia

McDonald K. Horne III
Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland, U.S.A.

I. INTRODUCTION

Almost as soon as heparin was introduced into clinical medicine, the new drug was reported to cause immediate small, but consistent, reductions in platelet count (Sappington, 1939). Later it was also found to produce platelet dysfunction (Heiden et al., 1977), accounting for at least some of its hemorrhagic risk (Hirsh, 1984; John et al., 1993). These effects, which most likely result from direct contact between heparin and platelets, are distinct from the role heparin plays in immune-mediated, heparin-induced thrombocytopenia (HIT). However, direct heparin–platelet binding is critical in the pathogenesis of HIT as well (Horne and Hutchison, 1998). Therefore, the various "nonimmune" heparin–platelet interactions will be reviewed.

II. HEPARIN BINDING TO PLATELETS

Appreciation of the functional effects of heparin on platelets led to studies of heparin binding to these cells, which was found to be specific and saturable (Sobel and Adelman, 1988; Horne, 1988; Horne and Chao, 1989). The negative charge density of the ligand (heparin) largely determines its binding specificity (Horne, 1988; Horne and Chao, 1990). Polysaccharide molecules with various primary structures can displace heparin from platelets if they are sufficiently charged (Horne, 1988; Greinacher et al., 1993) (Table 1). The identity of the platelet-binding site(s), which provides a complementary positive charge, is uncertain. One report indicates that glycoprotein IIb/IIIa (integrin $\alpha_{II\beta}\beta_3$) contains a heparin-binding site (Sobel et al., 2001), but this is inconsistent with other studies (Horne, 1988, 1991).

Next to negative charge density, molecular size has the greatest effect on polysaccharide binding to platelets. Heparin molecular weight, for example, affects both its platelet-binding affinity and capacity (Horne and Chao, 1990). Since medicinal heparin is a mixture of molecules varying in mass from about 4000 to about 30,000 Da, the mass of a mole of heparin (i.e., approximately 6×10^{23} molecules) depends upon the mean size of the molecules in the sample. The maximum number of molecules bound per platelet is approximately the same for heparin species with molecular weights between about 5000 and 15,000 Da (Table 1). However, larger molecules bring more glycosaminoglycan (GAG) mass to the platelet surface than smaller molecules (Fig. 1). Therefore, when heparin-binding capacity is expressed in terms of mass rather than moles or molecules, the capacity of larger heparins is greater than that of smaller heparins.

TABLE 1 Platelet-Binding Parameters for Heparin Fractions of Different Molecular Mass

Heparin M_r range (Da)	Sulfate/carboxylate (mol/mol)	Dissociation constant		Binding capacity	
		(mg/L)	(nM)	(mg/10^{15} cells)	(molecules/cell)
14,000–16,000	2.0 ± 0.29[a]	4.6 ± 1.1	310 ± 73	66 ± 2.5	2600 ± 100
9500–10,500	1.8 ± 0.26	3.9 ± 2.1	390 ± 210	56 ± 8.4	3400 ± 500
4500–5500	1.9 ± 0.15	3.2 ± 1.0	640 ± 200	23 ± 5.7	2800 ± 680
2700–3300	1.7 ± 0.25	4.0 ± 2.0	1300 ± 650	10 ± 5.4	2000 ± 1100

[a]Values are means ±1 standard deviation.
Source: Horne and Chao, 1990.

Similar distinctions apply to the parameters of binding affinity. Longer heparin molecules contain more potential platelet-binding domains than shorter molecules. Therefore, a large heparin species can half-saturate platelets at a lower molar concentration (K_d) than a smaller heparin species, although the concentration of heparin platelet-binding domains in the suspension is the same for both species at half-saturation (Horne and Chao, 1990) (Fig. 1).

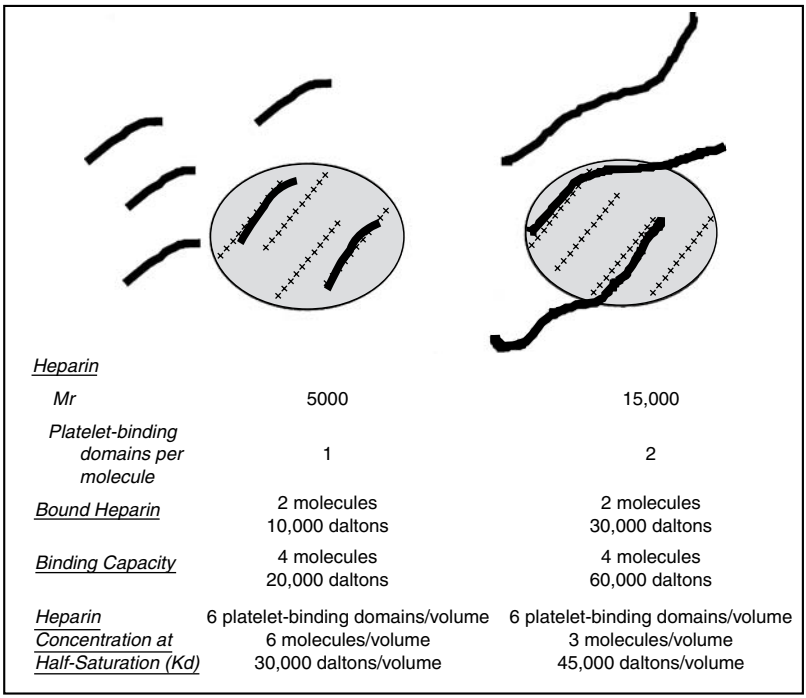

FIGURE 1 Schematic binding of heparin to platelets comparing heparin of M_r 5000 with heparin of M_r 15,000. Each "platelet-binding domain" of heparin is hypothesized to have $M_r > 3000$, whereas heparin-binding sites on the platelets (indicated by ++++) can bind 7000 Da heparin. Therefore, each binding site is not quite filled with M_r 5000 heparin but is too occupied to allow the binding of a second heparin molecule. In contrast, M_r 15,000 heparin has adequate length to occupy two binding sites on platelets, but physical constraints, such as limited heparin flexibility and the spacial distribution of binding sites, allow it to occupy only one site at a time. The scheme is consistent with the binding parameters shown in Table 1.

Because of its high charge density as well as the high linear flexibility conferred by its constituent L-iduronic acid residues, heparin also binds to a variety of plasma proteins, which theoretically could compete with platelets for heparin (Casu et al., 1988; Young et al., 1994). However, heparin binding to only two plasma proteins, antithrombin and fibronectin, interferes with heparin-induced platelet activation (Salzman et al., 1980; Chong and Ismail, 1989) or with binding of heparin to platelets (Horne and Chao, 1990).

III. NONIDIOSYNCRATIC HEPARIN-INDUCED PLATELET ACTIVATION

The functional consequence of heparin binding to platelets is subtle cell stimulation. Antibody-independent activation of platelets by heparin in vitro has been reported from many laboratories. However, the results of these studies have varied, presumably because of differences in experimental conditions. In plasma, for example, heparin alone causes slight platelet aggregation, whereas platelets suspended in laboratory buffers are reported to aggregate either briskly or not at all in response to heparin (Eika, 1972; Salzman et al., 1980; Westwick et al., 1986; Chong and Ismail, 1989). In citrate-anticoagulated plasma, heparin also potentiates platelet activation by agonists such as ADP and collagen (Holmer et al., 1980; Chen and Sylvén, 1992; Xiao and Théroux, 1998; Aggarwal et al., 2002; Klein et al., 2002), and this effect is more pronounced in patients with acute illness, arterial disease, and anorexia nervosa (Mikhailidis et al., 1985; Reininger et al., 1996; Burgess and Chong, 1997).

The platelet proaggregatory effect of heparin does not appear to be an artifact of low ionized calcium concentration due to citrate anticoagulant: Chen and colleagues (1992) observed that heparin enhanced collagen-induced platelet aggregation in a dose-dependent fashion even in whole blood anticoagulated with hirudin (i.e., physiological calcium concentrations). On the other hand, the responsiveness of washed platelets to agonists when resuspended in buffers containing physiological calcium has been reported to be both increased and decreased by heparin (Saba et al., 1984; Westwick et al., 1986). Although the data are not always consistent, this much seems clear: direct heparin-induced platelet aggregation requires metabolic energy and is mediated by fibrinogen; therefore, it depends on platelet fibrinogen receptors (platelet glycoprotein IIb/IIIa) and divalent cations (Chong and Ismail, 1989). There is also evidence that heparin can antagonize platelet inhibition by prostacyclin (Saba et al., 1979; Eldor and Weksler, 1979; Fortini et al., 1985; Berglund and Wallentin, 1991).

The properties of heparin that influence its platelet binding also influence its stimulating effect on platelets: heparin of a high molecular weight is more active than low molecular weight heparin (LMWH), and heparin with low affinity for antithrombin and fibronectin is more active (because it is more available) than heparin with high affinity for these plasma proteins (Salzman et al., 1980; Holmer et al., 1980; Westwick et al., 1986; Chong and Ismail, 1989; Brace and Fareed, 1990; Xiao and Théroux, 1998; Aggarwal et al., 2002; Klein et al., 2002). The latter observation implies that the anticoagulant (antithrombin-dependent) activity of heparin is distinct from its platelet stimulatory effects. Furthermore, nonheparin polysaccharides can mimic the effect of heparin on platelets if they are sufficiently large and charged (Tiffany and Penner, 1981). In contrast, heparan sulfate (the predominant anticoagulant GAG in danaparoid) has negligible platelet-activating properties, as it has a relatively low degree of sulfation, despite sharing a

carbohydrate backbone similar to that of heparin (Lindahl and Kjellen, 1991; Burgess and Chong, 1997).

IV. PLATELET-RELATED PROHEMORRHAGIC EFFECTS OF HEPARIN

Paradoxically, despite the in vitro evidence that heparin stimulates platelets, there is evidence that heparin causes bleeding partly because of its effects on platelet function (Hirsh, 1984; John et al., 1993). Heparin, for example, causes prolongation of the skin bleeding time unrelated to any effects on platelet counts. Also, the structural characteristics of heparin that enhance platelet stimulation in vitro (i.e., increased heparin size or sulfation; decreased affinity for antithrombin) are associated with enhanced bleeding in animal models (Hjort et al., 1960; Carter et al., 1982; Ockelford et al., 1982; Fernandez et al., 1986; Borowska et al., 1988; Van Ryn-McKenna et al., 1989).

The apparent inhibition of platelet function in vivo may be related to two specific actions of heparin: inhibition of thrombin-induced platelet activation and reduction of von Willebrand factor (vWf)–dependent platelet function. Thrombin is a "strong" platelet activator (i.e., it stimulates platelet secretion without intermediate platelet aggregation [Ware and Coller, 1995]). However, in the presence of antithrombin, heparin essentially eliminates stimulation of platelets by thrombin (Westwick et al., 1986; Cofrancesco et al., 1988). This effect is likely responsible for the marked prolongation of bleeding time seen in patients receiving high doses of heparin during heart surgery (Kestin et al., 1993). Heparin also binds to vWf, preventing vWf binding to platelets (Sobel et al., 1991, 1992). This reduces vWf-mediated subendothelial adhesion of platelets flowing at high shear rates, perhaps also contributing to the heparin-related prolongation of the bleeding time.

V. NONIMMUNE HEPARIN-ASSOCIATED THROMBOCYTOPENIA

Nonimmune heparin-associated thrombocytopenia (HAT) describes the common clinical situation in which a patient develops a fall in platelet count within the first few days of receiving heparin. Often, there are concomitant clinical factors to explain the thrombocytopenia (e.g., hemodilution, bacteremia, or disseminated intravascular coagulation [DIC]). In some patients, however, it is possible that a direct proaggregatory effect of heparin is responsible for the drop in platelet count (Salzman et al., 1980). The designation *associated* helps to convey the uncertain role of heparin in causing thrombocytopenia in this setting, and the term *nonimmune* distinguishes this syndrome from immune-mediated HIT (Warkentin et al., 1998).

Nonimmune HAT is typically mild, often transient, and clinically inconsequential (Gollub and Ulin, 1962; Johnson et al., 1984; Chong, 1988; Warkentin and Kelton, 1994). There is debate whether this represents a real in vivo phenomenon or whether the apparent thrombocytopenia is instead related to ex vivo platelet aggregation (Davey and Lander, 1968). Indeed, some investigators were unable to show this phenomenon at all (Heinrich et al., 1988; Xiao and Théroux, 1998). Sometimes, however, nonimmune HAT is a dramatic clinical syndrome that can be confused with HIT (Chong et al., 1982) (see Chapter 11).

Balduini et al. (1993) observed that an early fall in platelet count was more frequent and of greater magnitude in patients receiving heparin following

streptokinase therapy for acute myocardial infarction compared with control patients who received streptokinase alone. The heparin-treated patients also showed greater ex vivo spontaneous platelet aggregation, suggesting that heparin may have had a direct proaggregatory effect.

VI. HEPARIN–PLATELET INTERACTIONS IN THE PATHOGENESIS OF HIT

Nonimmune heparin–platelet interactions are central to the pathogenesis of HIT because of the key role of platelet factor 4 (PF4). This cationic chemokine is secreted from activated platelets and binds to GAGs on the surface of platelets and endothelium (Dawes et al., 1982; Rao et al., 1983; O'Brien et al., 1985; Capitanio et al., 1985; Cines et al., 1987; Visentin et al., 1994). PF4 also binds to soluble GAGs, especially highly anionic heparin, leading to a competition between cell-bound and soluble GAGs for PF4 (Horne, 1993; Newman et al., 1998).

When complexed with GAG, PF4 exposes one or more neoantigens that stimulate formation of HIT antibodies (Amiral et al., 1992, 1995; Kelton et al., 1994; Newman and Chong, 1999). Recent evidence suggests that the neoantigen is formed by close approximation of two PF4 tetramers, which can happen when the positive charge of the PF4s is neutralized by GAGs (Greinacher et al., 2006). However, to be immunogenic, the PF4-GAG complexes presumably must be soluble and thereby accessible to the immune system. Perhaps this explains why PF4-GAG that is constitutively present on the endothelial surface is not immunogenic, but soluble PF4-heparin complexes are.

Once stimulated by exposure to PF4-heparin, HIT antibodies can bind to PF4 complexed with other GAGs (e.g., heparan sulfate and chondroitin sulfate) on cell membranes. By this mechanism, they could stimulate platelets (Rauova et al., 2006) and also (directly or indirectly) endothelial expression of tissue factor (Cines et al., 1987; Herbert et al., 1998). Such heparin-independent binding of HIT antibodies to platelets and endothelium may explain the appearance or persistence of thrombocytopenia in HIT after heparin exposure has ceased (see Chapter 2). Activation of platelets in the absence of heparin, however, appears to require extensive saturation of the platelet surface with PF4, since antibody binding in vitro is observed only with PF4 concentrations >300 nM, whereas the K_d for the binding of PF4 to platelets is reported to be about 30 nM (Loscalzo et al., 1985; Rauova et al., 2006). Such concentrations would be rarely, if ever, achieved in vivo. On the other hand, PF4 binds to endothelium even at normal plasma concentrations less than 1 nM and is readily displaced by heparin. Therefore, stimulation of endothelium by HIT antibodies seems a more probable mechanism for appearance or persistence of HIT after heparin has been discontinued.

When heparin is present, it forms soluble complexes with PF4 that it displaced from endothelium or that was secreted by activated platelets. These complexes also bind HIT antibodies, and they have the potential for docking at the platelet surface, attaching via their heparin at cationic sites rather than at the GAG (chondroitin sulfate) naturally found in the platelet cell membranes (Greinacher et al., 1993; Horne and Hutchison, 1998).

The ability of HIT-immune complexes to stimulate platelets appears to depend on the size of the PF4-heparin component (Rauova et al., 2005). The largest PF4-heparin complexes have the greatest chance of binding to platelets, and they can also carry several HIT-IgG molecules (Rauova et al., 2005). Therefore, when

such a complex attaches to a platelet, it brings an especially rich trove of HIT-IgG to activate the cell through its Fcγ receptors (Horne and Alkins, 1996).

The size of PF4-heparin complexes depends upon the length of the heparin chains and upon the molar ratio of heparin to PF4. A heparin molecule of M_r ~11,000 can bind about four PF4 molecules, only partially saturating each one (Loscalzo et al., 1985). Heparin molecules about half this size (M_r 5000–7000) can crosslink two or three PF4s (Bock et al., 1980). Therefore, LMWH (M_r 3000–10,000 Da) does not form ultralarge complexes as readily as unfractionated heparin (UFH, M_r 4000–30,000 Da), and the pentasaccharide fondaparinux (M_r 1728) does not form them at all (Rauova et al., 2005).

When UFH and PF4 are present in roughly equal molar amounts, large lattices (>670,000 Da) of heparin and PF4 can form (Bock et al., 1980; Rauova et al., 2005) (Fig. 2, middle panel). As the molar concentration of PF4 exceeds that of heparin, the size of the complexes becomes smaller and smaller until each contains only a single heparin molecule saturated with PF4 (Fig. 2, upper panel). A consequence of this is that these complexes cannot attach to platelets because the PF4 sites that might bind to platelet chondroitin sulfate are blocked by heparin, while there is no heparin free to bind to platelet cationic sites (Horne and Hutchison, 1998). If the molar ratio shifts the other way, so that heparin is in excess, the complexes will become limited to one heparin and one PF4 molecule each (Fig. 2, bottom panel). In this situation, the complexes are unlikely to bind to platelets because of competition with free heparin molecules and because the negative charge of heparin, which affects its affinity for the platelet, is partially neutralized by binding to PF4.

The critical importance of the molar ratio of heparin to PF4 probably explains why most patients who develop HIT antibodies never develop the clinical syndrome: molar ratios of PF4 and heparin favorable for platelet binding are rare or transient in most clinical situations (Amiral et al., 1996; Visentin et al., 1996; Kappers-Klunne et al., 1997; Warkentin et al., 2000; Rauova et al., 2006). While the plasma concentration of heparin is dose-dependent, the plasma concentration of PF4 depends on the level of platelet activation, which is affected by the degree of stimulation by HIT antibodies and on displacement of PF4 from the endothelial and platelet surfaces by heparin (O'Brien et al., 1985; Horne, 1993). Furthermore,

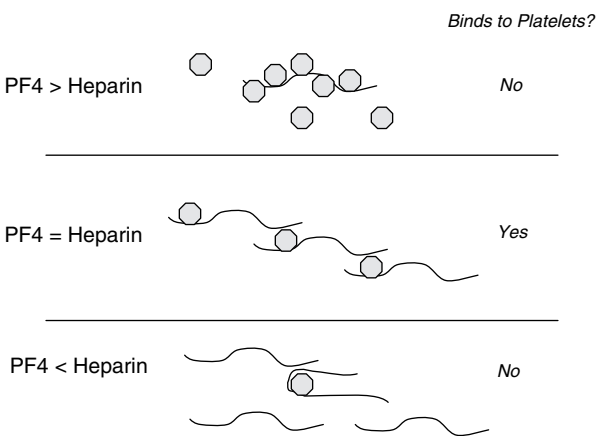

FIGURE 2 Schematic representation of the effect of the molar ratio of PF4 and heparin on the formation of complexes. *Abbreviation*: PF4, platelet factor 4.

individuals are reported to vary greatly in their platelet content of PF4 (O'Brien et al., 1984; Rauova et al., 2005).

In most clinical settings, free heparin is in considerable molar excess over PF4. Therapeutic concentrations of heparin (0.2–0.4 U/mL) correspond to about 100–200 nmol/L heparin. When heparin is given to normal individuals, plasma concentrations of PF4 from endothelial reservoirs only reach approximately 8 nM (Dawes et al., 1982). For PF4 concentrations to approach 100–200 nmol/L, marked activation of circulating platelets is necessary. Complete activation of platelets in a concentration of 250×10^9/L will generate a plasma PF4 concentration of about 200 nM (Horne, 1993). Therefore, a molar excess of PF4 over therapeutic concentrations of heparin would be highly unlikely outside extreme clinical circumstances, although in the immediate environment of an activated platelet, the concentration of PF4 could rise much higher. On the other hand, prophylactic doses of heparin (e.g., 5000 U every 8–12 h by subcutaneous injection) administered in a setting associated with a degree of platelet activation (e.g., after surgery) might well produce molar ratios of heparin and PF4 that would favor platelet binding of heparin-PF4 complexes, and—if an immune response has occurred—platelet binding of heparin-PF4-IgG complexes. Indeed, such scenarios are the ones in which HIT is reported most frequently (Boshkov et al., 1993; Warkentin et al., 1995, 2000) (see Chapter 3).

VII. IMPLICATIONS OF NONIMMUNE HEPARIN BINDING TO PLATELETS FOR THE PREVENTION OR TREATMENT OF HIT

The fact that heparin's molecular size determines its platelet-binding affinity and capacity and its ability to assemble ultralarge complexes with PF4 explains why LMWH preparations are associated with a lower incidence of HIT than standard UFH and why, in some instances, LMWH has been given to patients with HIT without adverse consequences (Warkentin et al., 1995; Slocum et al., 1996). Indeed, there is increasing evidence that the very smallest heparin, the synthetic pentasaccharide, fondaparinux (M_r 1728), may be a promising medication for patients with HIT (Elalamy et al., 1995; Walenga et al., 1997) (see Chapter 17). Although fondaparinux apparently can bind well enough to PF4 to be immunogenic and the anti-PF4-heparin antibodies identified in patients who have received this drug can promote platelet activation in vitro in the presence of UFH or LMWH, they are not active in the presence of fondaparinux (Warkentin et al., 2005). In theory, this is because fondaparinux is either too small to form a stable complex with PF4 or to mediate the binding of complexes to the platelet surface (Elalamy et al., 1995; Walenga et al., 1997; Ahmad et al., 1999; Warkentin et al., 2005).

Similarly, the safety and efficacy of treating HIT patients with danaparoid, a so-called heparinoid, can be explained by the fact that its major component (approximately 84% heparan sulfate) does not bind to platelets (Horne, 1988; Magnani, 1993). On the other hand, danaparoid sometimes cross-reacts with HIT antibodies in laboratory tests for HIT (see Chapter 13). This is perhaps mediated by a minor component of danaparoid (about 12% dermatan sulfate) that does have weak affinity for both platelets and PF4 (Barber et al., 1972; Horne, 1988).

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5 Role of Heparin-Dependent Antigens in Immune Heparin-Induced Thrombocytopenia

Jean Amiral and Anne Marie Vissac
Hyphen Biomed Research, Zac Neuville-Université, Neuville-Sur-Oise, France

I. INTRODUCTION

Immune (type II) heparin-induced thrombocytopenia (HIT) remains a major iatrogenic complication of heparin therapy. It is triggered by "heparin-dependent" antibodies targeted to protein-heparin—mainly platelet factor 4 (PF4)—complexes. Its frequency could still be underestimated (Francis, 2005). HIT develops more frequently during therapy with unfractionated heparin (UFH) (Poncz, 2005; Greinacher, 2006), especially in the setting of vascular alteration, blood activation, and inflammation. PF4 complexed to heparin (PF4-H) was identified as the major target antigen for heparin-dependent antibodies involved in the pathogenesis of immune HIT 15 yr ago (Amiral et al., 1992, 1995) and confirmed by various investigators (Gruel et al., 1993; Greinacher et al., 1994, 1995; Kelton et al., 1994; Visentin et al., 1994). However, today, presence of anti-PF4-H antibodies (especially when they are detected using assays that recognize all three major immunoglobulin classes, IgG, IgA, and IgM) must be understood to be just a risk factor for HIT rather than an absolute indicator of this clinical complication (see Chapter 10). Conversely, when thrombocytopenia (and/or thrombosis) develops 5 or more days after beginning heparin therapy, the presence of these anti-PF4-H antibodies—especially when they are of IgG class and found at high levels—essentially confirms the diagnosis of HIT (Lindhoff-Last et al., 2001; Warkentin, 2004, 2005; Warkentin and Sheppard, 2006; Warkentin et al., 2005; Greinacher, 2006).

Occasionally, other antigens can be involved in HIT pathogenesis, such as interleukin-8 (IL-8) or neutrophil-activating peptide-2 (NAP-2), two CXC chemokines of the PF4 superfamily that exhibit affinity for heparin (Amiral et al., 1996a; Regnault et al., 2003). There is increasing evidence that the risk of HIT depends on the type of heparin used, its sulfation grade (Greinacher et al., 1995), the duration of therapy, and the patient's clinical context (Kelton, 1992; Warkentin and Kelton, 1996) (see Chapters 2 and 3). However, many questions remain unresolved: How are these antibodies generated? Why are they observed in only a subgroup of patients receiving heparin? Why do they become pathogenic in only a few of these patients? Why are antibodies formed so often, but with a (relatively) low incidence of thrombocytopenia and thrombosis, particularly in some clinical contexts such as extracorporeal circulation (Bauer et al., 1997) or hemodialysis? What is the explanation for "delayed-onset" HIT in some patients (Rice et al., 2002; Smythe et al., 2005)? Indeed, antibodies to PF4-H develop surprisingly often in many heparin-treated patients, especially in the context of platelet activation, such as heart surgery using cardiopulmonary bypass (CPB) (Amiral et al., 1996b; Visentin et al., 1996). Clinical complications of HIT are especially associated with high-titer anti-PF4-H antibodies of the IgG isotype, usually in patients with comorbid disease who are

receiving UFH. Experimentally, presence of prothrombotic factors has recently been demonstrated to enhance occurrence of HIT and thrombosis in a mouse model (Reilly et al., 2006). The frequency of HIT is less with low molecular weight heparin (LMWH) (Greinacher, 2006; Warkentin, 2004, 2005; Warkentin et al., 1995, 2006a). However, some studies suggest that this complication might also develop rarely in the absence of IgG isotypes (Amiral et al., 1996c; Meyer et al., 2006). In a few patients with clinically apparent HIT and with positive testing for heparin-dependent, platelet-activating antibodies, only IgA (Meyer et al., 2006) or IgM isotypes are present, though usually at very high concentrations.

In this chapter, we will highlight the role of PF4 as the major antigen for the generation of heparin-dependent antibodies, although we will discuss also the contribution of other antigens such as IL-8. We will also focus on the current understanding of anti-PF4-H antibody generation and its contribution to the complications of HIT. Formation of the PF4-H antigen complexes and their binding to blood and endothelial cells (ECs), which targets the immune response onto these cells (Cines et al., 1987; Visentin et al., 1994; Visentin and Aster, 1995; Horne and Hutchison, 1998; Arepally and Mayer, 2001, Pouplard et al., 2001; Blank et al., 2002), thereby inducing their activation and release of tissue factor (TF) and procoagulant microparticles, will be outlined. Finally, the possibility that HIT can be caused without detectable antibodies against PF4-H is reviewed, including the hypothesis that preexisting antibodies to other chemokines, such as IL-8, or even NAP-2, are involved. These antibodies could become pathogenic during heparin treatment (Amiral et al., 1996a; Regnault et al., 2003), thereby mimicking the clinical picture of rapid-onset HIT (see Chapter 2).

II. HOW DOES HEPARIN TRANSFORM PF4 INTO AN ALLOANTIGEN?

PF4 is a 7.8 kDa CXC chemokine protein present in platelet α-granules as a tetramer of about 30 kDa. Upon platelet activation or lysis, PF4 is released into blood as a high molecular weight complex (350 kDa) consisting of a proteoglycan dimer carrying eight PF4 tetramers. PF4 is rapidly cleared from blood through binding to EC glycosaminoglycans (GAGs), for which it has a greater affinity than for the platelet proteoglycan dimer. During heparin therapy (UFH or LMWH), PF4 tetramers are displaced from the EC storage sites (because heparins have a higher affinity for PF4 than do other GAGs) and they are released into blood at concentrations that depend on the patient's platelet activation status (Fig. 1). In some cases, these PF4-H complexes induce the generation of heparin-dependent antibodies (Poncz, 2005).

Antibodies to self-antigens, including certain autologous plasma proteins, can develop as a result of immune dysfunction, triggering autoimmune disease. Sometimes, however, formation of complexes between an autologous protein and a foreign substance leads to new antigens on the self protein, which can be described as *cryptic alloantigens* or *neoantigens*. Figure 2 shows how the PF4 tetramer can be modified by its binding to heparin, thereby exposing neoepitopes that were masked on native PF4. The immune stimulation resulting from such an altered self-epitope abates quickly once the inducing foreign substance is no longer present. Such a model explains some of the clinical events observed in HIT (see Chapter 2). In HIT, PF4 constitutes the self antigen, forming an "alloantigen" when complexed with heparin, particularly when both PF4 and heparin are present at the stoichiometric concentrations that allow formation of multimolecular

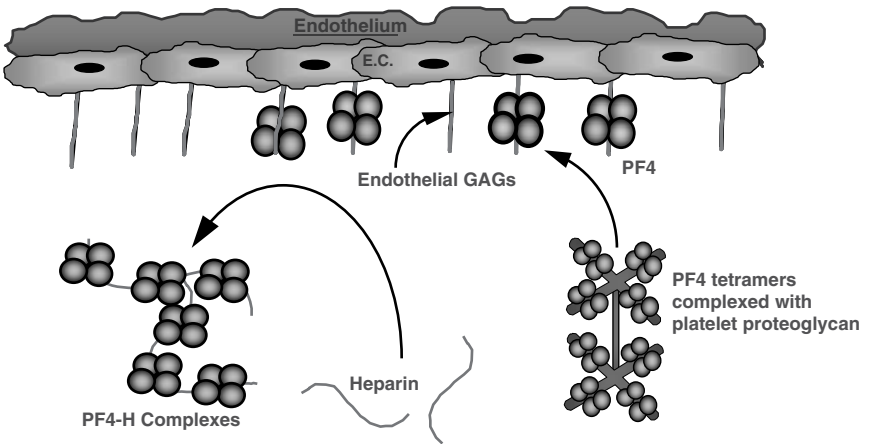

FIGURE 1 (*See color insert*) Release of PF4 from platelets as a high molecular weight complex of eight tetramers with a proteoglycan dimer; PF4 binds to endothelial cell GAGs, for which it has a greater affinity, but it is displaced by heparin, which exhibits a higher affinity for PF4. *Abbreviations*: GAGs, glycosaminoglycans; PF4, platelet factor 4; PF4-H, PF4 complexed to heparin.

complexes and, consequently, exposure of one or more cryptic neoepitopes. Thus, the antibodies to PF4-H complexes essentially can be considered to be autoantibodies or alloantibodies (Shoenfeld, 1997). However, as a foreign substance (heparin) is involved, and as there is little evidence that complexes between PF4 and naturally occurring GAGs generate antibodies, it seems more appropriate to call those heparin-dependent antibodies, "*alloantibodies*."

As mentioned, PF4 is a positively charged tetrameric glycoprotein (GP) member of the CXC chemokine family (Brandt and Flad, 1992). The tetramer forms by sequential noncovalent association of identical PF4 monomers: two dimers are formed that self-associate into the fundamental tetrameric structure. As found within platelet α-granules, PF4 is released into blood only after platelet activation, such as seen with trauma, surgery, atherosclerosis (Dunlop et al., 1987), diabetes, CPB, inflammation, cancer, infections, and so on. In vivo, PF4 has many different biological functions, including immunoregulation, inhibition of megakaryocytopoiesis and angiogenesis, and mediation of cell response (Nesmelova et al., 2005; Slungaard, 2005). As summarized in Figure 1, PF4 released from platelets is in a 350 kDa complex comprised of eight PF4 tetramers linked to a chondroitin-containing proteoglycan dimer (Barber et al., 1972; Luscombe et al., 1981; Huang et al., 1982). These PF4 complexes then bind to EC proteoglycans (heparan sulfate), and heparin, when present, displaces PF4 from the EC GAGs due to its greater affinity for PF4. The resulting PF4-H complexes are released into the circulating blood.

The interaction between heparin and PF4 has been intensively studied (Bock et al., 1980; Cowan et al., 1986; Stuckey et al., 1992; Maccarana and Lindahl, 1993). In the presence of a stoichiometric concentration of heparin and PF4 (which corresponds to about 27 international units [IU], i.e., about $175 \pm 25\,\mu g$ of heparin per milligram of PF4), multimolecular PF4-H complexes are generated (Greinacher et al., 1994; Amiral et al., 1995). With stoichiometric concentrations, heparin tightly wraps around the PF4 molecule (Fig. 2a), altering its structure and rendering it antigenic through the exposure of neoantigens. Figure 2b shows the different

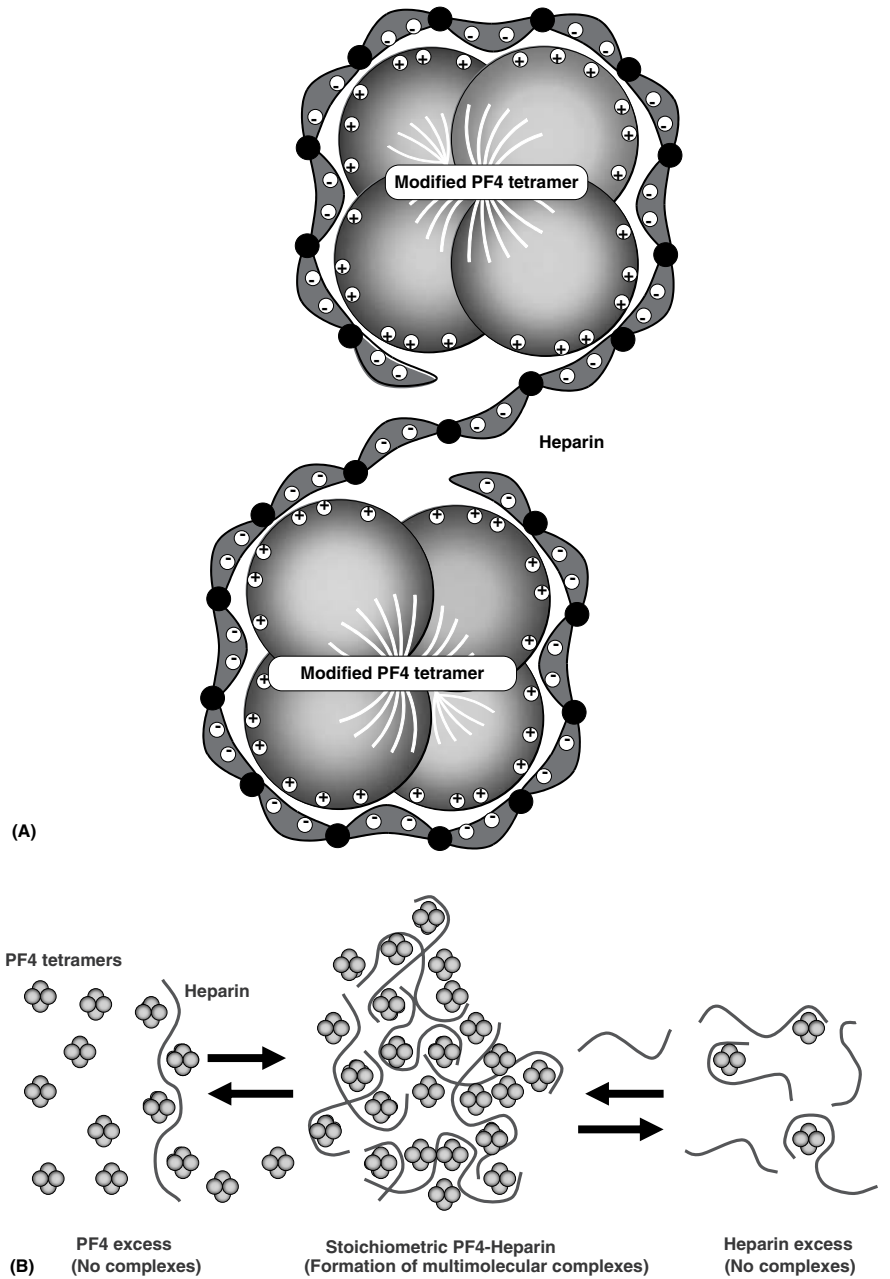

FIGURE 2 (*See color insert*) Schema showing the "modifications" of the PF4 tetramer following its tight binding with heparin at stoichiometry and exposure of neoepitopes (**A**) and depicting the formation of heparin and PF4 complexes at different concentrations of heparin and PF4 (**B**). In the presence of stoichiometric concentrations of both substances, multimolecular complexes are formed. Heparin then wraps around the PF4 tetramer, altering its structure and rendering it antigenic. *Abbreviation*: PF4, platelet factor 4.

complexes that can be formed between heparin and PF4, depending on the respective concentrations of both substances. Only multimolecular complexes, formed when well-defined ratios between PF4 and heparin exist, are believed to be antigenic. The larger PF4-H complexes formed with UFH, compared with LMWH, are believed to be more immunogenic and more pathogenic (Poncz, 2005; Rauova et al., 2005; Greinacher, 2006). Since complex formation depends strictly on the respective concentrations of heparin and PF4, if we consider the usual therapeutic range for heparin to be 0.1–1 IU/mL, then the amount of PF4 required for the generation of multimolecular PF4-H complexes ranges from 3 to 40 μg/mL. In patients undergoing CPB, who receive higher heparin concentrations (up to 3 IU/mL), the correspondingly higher PF4 concentrations required to form immunogenic PF4-H complexes results from the intense platelet activation produced when blood is exposed to the CPB circuit. In general, the existence of favorable conditions permitting formation of multimolecular PF4-H complexes may depend as much upon the underlying disease promoting platelet activation as on the dose of heparin given (see Chapter 3). Indeed, PF4 can be present at very high concentrations (exceeding the expected serum concentration of about 5 μg/mL) at pathological sites where platelets and leukocytes are chemoattracted and then activated (Fig. 3), such as during major surgery (orthopedic, cardiac), acute infection or inflammation, malignancy, etc.

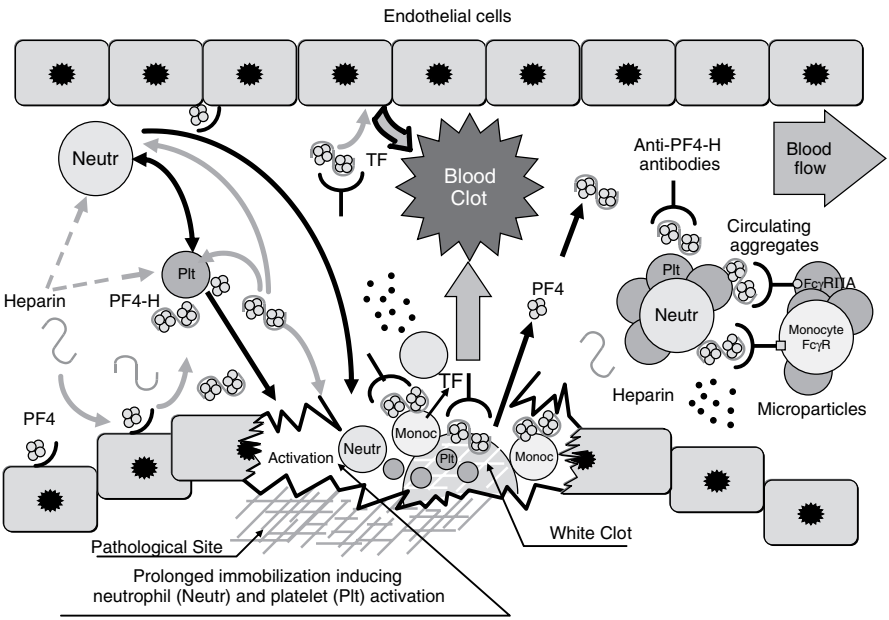

FIGURE 3 (*See color insert*) Cell–cell interactions in the neighborhood of blood activation or inflammation sites: Presence of heparin-dependent antibodies increases the amount of cells available at these sites, amplifies cell–cell interactions and cellular activation, and can lead to blood clotting or release of circulating cell aggregates. The procoagulant effect is enhanced by release of tissue factor (from endothelial cells and monocytes) and generation of microparticles. *Abbreviations*: IL-8, interleukin-8; PF4, platelet factor 4; PF4-H, platelet factor 4-heparin complex; TF, tissue factor.

The intensity of the heparin-dependent immune response thus depends on the presence and, probably, the persistence of multimolecular PF4-H complexes. In particular, presence of high concentrations of PF4-H complexes may be important in triggering an immune response. However, heparin concentrations can vary considerably even in an individual patient during the course of heparin therapy, and so the stoichiometric concentrations allowing for PF4-H complex formation may occur frequently. But, if only low PF4 concentrations are present, formation of immunogenic complexes occurs only at corresponding low levels of heparin, e.g., 0.027 IU/mL of heparin for 100 ng/mL of PF4, which is the approximate highest PF4 concentration in normal subjects receiving heparin. The chances of developing a significant immune response in this setting would be low. In contrast, PF4 concentrations are expected to be high at sites where platelets and leukocytes converge and are activated (Fig. 3), thus improving the chances of an immune response. The potentially important role of individual immune responsiveness to a given PF4-H antigenic stimulus is unknown. Recent studies have demonstrated that the immunological response to a PF4-H immunological stimulus is T-cell dependent (Bacsi et al., 1999; Suvarna et al., 2005).

We have reported that although antibodies to PF4-H complexes are present in most patients who develop HIT, they are absent in some patients with apparent HIT, including patients with positive platelet activation assays. Antibodies to IL-8 or to NAP-2 have been observed in some of these patients (Amiral et al., 1996a; Regnault et al., 2003), but in a few, no specific heparin-dependent antibodies have yet been identified. As discussed later, antibodies to IL-8 or to NAP-2 can be generated by mechanisms different from those involved in PF4-H antibody formation. These antibodies can predate the heparin therapy, and could have a regulatory role for inflammation (Reitamo et al., 1993). Administration of heparin is then the trigger for their pathologic effect, as heparin then promotes binding of these chemokines onto some blood cells (including platelets and ECs) to which they do not bind (or bind poorly) in the absence of heparin. In contrast to anti-PF4-H antibodies, these anti-IL-8 or anti-NAP-2 antibodies might be true autoantibodies (Bendtzen et al., 1995). They can be generated in many different clinical situations, and their pathological incidence still remains unknown.

III. PATHOLOGICAL MECHANISMS OF HEPARIN-DEPENDENT ANTIBODIES

In our experience, anti-PF4-H antibodies of the IgG isotype are present in at least 85% of patients with clinical HIT. In the remaining cases, IgA, IgM, or both isotypes—but only when present at high concentrations—could be involved. This is based on studies of HIT in which the anti-PF4-H antibodies were fully isotyped (Amiral et al., 1996c). Although the clinical picture and positive platelet aggregation tests supported the diagnosis of immune HIT, only IgM and/or IgA isotypes of anti-PF4-H antibodies were found, and no IgG was detected. This finding is nevertheless controversial, as recent studies tend to demonstrate the preeminent role of the IgG isotype in development of HIT (Lindhoff-Last et al., 2001; Warkentin, 2004, 2005; Warkentin et al., 2005; Greinacher, 2006). However, HIT cases associated with high concentrations of IgM and/or IgA isotypes (Amiral et al., 1996c; Meyer et al., 2006) could be underdiagnosed, depending on the study inclusion criteria. In any event, these intriguing observations require explanation for how IgM and IgA antibodies could trigger thrombocytopenia, with or without thrombosis.

It is well accepted that IgG antibodies to PF4-H can become pathogenic when they interact with platelets, particularly when PF4-H-IgG complexes bind to the platelet Fc receptors (FcγRIIa) (Kelton et al., 1988) (see Chapter 8). Another group proposed that, in addition, an IgG receptor polymorphism on leukocyte FcγRIIIa, different from that of FcγRIIa, could also be involved (Gruel et al., 2004). Our observations indicate that other mechanisms for PF4-H-antibody complexes binding onto blood cells could be involved. These could result not only if the PF4-H complexes bind to the cell surfaces through their heparin-binding sites (Van Rijn et al., 1987; Horne and Alkins, 1996; Horne and Hutchison, 1998) but possibly also through PF4-binding sites (Capitanio et al., 1985; Rybak et al., 1989). Although HIT antibodies recognize PF4-H complexes in the fluid phase (Newman et al., 1998), it is uncertain whether this typically occurs in vivo before interaction of PF4-H-IgG complexes with the platelet surface, or whether HIT antibodies only bind after PF4-H complexes first become attached to the platelet surface. Recent reports have shown that anti-PF4-H antibodies from patients with HIT can activate ECs (especially microvascular ECs) and also monocytes, and thereby induce release of TF (Pouplard et al., 2001; Arepally and Mayer, 2001; Blank et al., 2002).

Regardless, the clinical state of the patient, determining the extent of platelet and EC activation, seems to be a key factor for determining whether clinical HIT results (Boshkov et al., 1993; Reininger et al., 1996). This contribution occurs in several ways: activated platelets release high amounts of PF4 that can complex with heparin, and activated platelets also expose a higher density of heparin-binding sites (Horne and Chao, 1989). Consequently, these platelets may be even more readily activated by heparin-dependent antibodies. This situation occurs in patients with acute or chronic platelet activation associated with CPB, atherosclerosis, inflammation, infections, cancer, diabetes, and orthopedic surgery, among others.

Another factor determining HIT antibody formation is the type of heparin that binds to PF4, which depends on its oligosaccharide composition, polysaccharide length, and grade of sulfation (Lindahl et al., 1994; Greinacher et al., 1995). Formation of PF4-H complexes requires a heparin molecule containing at least 12–14 oligosaccharide units and a high sulfation grade (more than three sulfate groups per disaccharide) (Amiral et al., 1995). Furthermore, binding of heparin to blood and ECs also increases with heparin molecule length and sulfation grade (Sobel and Adelman, 1988; Horne and Chao, 1990; Harenberg et al., 1994). Heparin structure thus has a dual effect in HIT: it is required not only to form PF4-H complexes but also to target these complexes onto cell surfaces. These factors could explain the higher frequency of PF4-H antibody development and of HIT in patients receiving UFH, compared with those receiving LMWH (Poncz, 2005; Greinacher, 2006). With UFH, PF4-H complexes are larger and are more easily formed, requiring a lower heparin concentration than with LMWH. For the latter drug, only the subset of molecules containing at least 12–14 oligosaccharide units (MW > 3600 Da) can generate immunoreactive PF4-H complexes. Thus, because LMWH has a lower propensity to form PF4-H complexes and binds less readily to platelets and ECs, LMWH therapy is also less likely to result in thrombocytopenia even when pathologic HIT antibodies are already present.

PF4-H-reactive antibodies targeted at platelets induce platelet activation, resulting in thrombocytopenia and, often, thrombosis. Occasionally, heparin-induced thrombosis occurs in the absence of thrombocytopenia (Hach-Wunderle et al., 1994; Bux-Gewehr et al., 1996). Platelet activation by the IgG isotype antibodies

is mediated by interaction with the platelet FcγRIIa receptors (Kelton et al., 1988; Denomme et al., 1997). Some studies suggest an important role for an FcγRIIa polymorphism (Brandt et al., 1995; Burgess et al., 1995). However, the role of the FcγRIIa receptor polymorphism is controversial (Arepally et al., 1997; Denomme et al., 1997; Suh et al., 1997; Bachelot-Loza et al., 1998) (see Chapter 8).

Platelet activation might also occur through other mechanisms such as direct antibody binding to exposed cell antigens (Rubinstein et al., 1995), a phenomenon that is dependent on the antigen electric charge (Schattner et al., 1993). Heparin is highly electronegative. Evidence for direct activation through antigen binding is supported by the positive platelet aggregation response produced by some patient plasma samples containing only anti-PF4-H antibodies of the IgM and/or IgA isotypes. Formation of heparin-containing immune complexes on cell surfaces can initiate blood and EC interactions, and this can enhance their activating effects. Cell–cell interactions may occur and be amplified through release products that chemoattract and activate cells or through transcellular metabolism (Nash, 1994; Marcus et al., 1995). Platelet products (e.g., PF4) and platelet-derived microparticles (Warkentin et al., 1994) can induce activation of leukocytes (Aziz et al., 1995; Jy et al., 1995; Petersen et al., 1996). Leukocyte-release products, such as cathepsin G, can directly activate platelets and cleave β-thromboglobulin to the active chemokine, NAP-2, thus establishing an amplification loop. Platelet-leukocyte aggregates can form in vivo, contributing to vascular occlusion, especially in limb vessels (Fig. 3). In a recent study, antibodies to PF4-H from patients with HIT were shown to induce synthesis of TF by monocytes in the presence of PF4 and heparin (Pouplard et al., 2001) or by microvascular ECs (Blank et al., 2002). This could be a complementary pathway for inducing thrombosis.

Variability in certain biologic characteristics of anti-PF4-H antibodies influences their potential for inducing HIT. Platelet activation caused by anti-PF4-H antibodies is usually weak and is only pathogenic when amplification mechanisms are involved. This is demonstrated by the variable lag phase observed in platelet aggregation studies performed with different plasmas or sera from HIT patients. Antibody concentration is another important factor for determining the extent of platelet activation. Antibody affinity is also crucial: the higher the affinity, the lower the concentration of antibodies required for activating platelets. Recently, a subset of antibodies to PF4-H complexes that had platelet-activating properties was isolated in three patients with HIT (Amiral et al., 2000). These platelet-activating antibodies had the highest avidity for PF4-H. In contrast, the bulk of antibodies against PF4-H in these patients had no effect on platelet activation. Also, when IgM or IgA isotypes are present, affinity for PF4-H complexes is usually lower than that of IgG isotypes and, consequently, high concentrations are necessary for pathogenicity. Lastly, HIT antibodies do not all bind to the same epitopes on PF4-H complexes, and this specificity could be an additional important factor (Horsewood et al., 1996; Pouplard et al., 1997; Suh et al., 1998). At least two neoepitopes have been identified on PF4 that are distinct from the "region of positive charge" to which heparin binds (Ziporen et al., 1998; Li et al., 2002) (see also Chapter 6). Thus, anti-PF4-H antibodies are not equivalent, and those with the strongest affinity are most pathogenic.

Platelet activation in HIT involves amplification through ADP receptors (Polgár et al., 1998) and involves GPIIb/IIIa (Hérault et al., 1997; Jeske et al., 1997). These findings further emphasize the importance of platelet activation amplification loops for producing the clinical manifestations of HIT.

IV. ROLE OF PREEXISTING ANTIBODIES TO CXC CHEMOKINES

Preexisting antibodies to chemokines, such as IL-8 or NAP-2, or possibly even to PF4 itself, may be present in some patients before heparin therapy (Sylvester et al., 1992; Bendtzen et al., 1995; Warkentin et al., 2006b). These antibodies may occur naturally or be induced in pathologic states, where they might have a regulatory role in inflammation (Reitamo et al., 1993). In some diseases, they are present at high concentrations. Antibodies to IL-8 are the most common (Reitamo et al., 1993). However, in some patients, true autoantibodies to PF4 alone can also be observed. In the absence of heparin, these antibodies usually do not demonstrate any clear pathogenicity. However, during heparin therapy, PF4 and other chemokines are released into blood from their storage pools. Heparin may further localize these chemokines onto blood cells and endothelium, with deleterious consequences. The amount of chemokine-heparin complexes bound to blood cells and ECs depends on different factors: the amount of releasable chemokines (i.e., the patient's clinical state); the type and dose of heparin used; the presence of activated cells with an increased capacity to bind chemokines; and, if present, the heparin-dependent antibodies, through their binding to chemokine-heparin complexes. As with antibodies against PF4-H complexes, these natural antichemokine antibodies could initiate cell activation and cell–cell interactions as well as generate circulating cell aggregates that could lead to vessel occlusion. Figure 4 shows the possible mechanism for pathogenic effects of these antichemokine antibodies, as antibody localization to the target cells is enhanced by heparin therapy. Finally, we can speculate that protamine sulfate, used for neutralizing heparin after CPB, could generate an immunologic stimulus for heparin-dependent immunization (Al-Mondhiry et al., 1985), especially as both protamine and heparin can induce thrombocytopenia, and protamine can induce specific antibody formation.

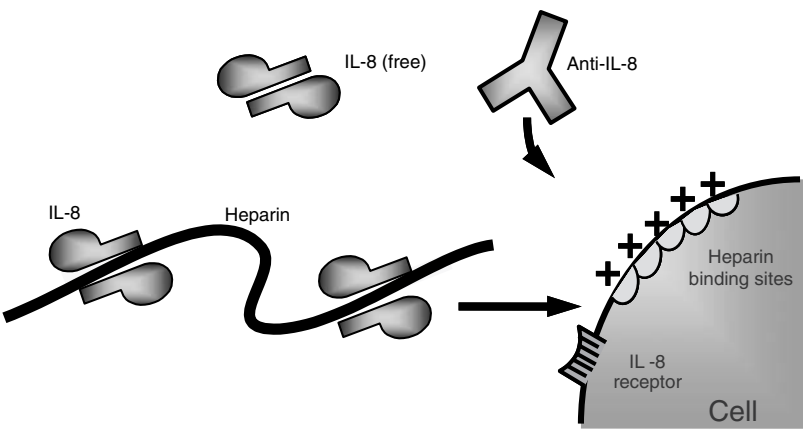

FIGURE 4 (*See color insert*) Possible effect of heparin for carrying preexisting antibodies to IL-8 onto platelets (and other blood cells), through the heparin binding sites or through the IL-8 receptors, targeting the deleterious consequences of these antibodies onto these cells. *Abbreviation*: IL-8, interleukin-8.

V. NEW ASSAYS FOR HIT BASED ON HEPARIN-DEPENDENT ANTIGENS

The present understanding of how heparin-dependent antibodies contribute to the development of HIT allowed us to develop new assays for measuring these antibodies, by mimicking closely the conditions thought to occur in vivo. For this approach, functionally available heparin is coated onto a solid surface such as an enzyme-immunoassay (EIA) plate or any other surface. This can be achieved by different means: coating protamine sulfate in the presence of a large excess of heparin; coating streptavidin/biotinylated heparin; or coating heparin covalently bound to a carrier protein (such as albumin) or a polymer. The patient plasma or serum is incubated with this heparinized surface. If chemokines that exhibit heparin affinity are present, they bind to the coated heparin, exposing neoepitopes, and thereby capturing heparin-dependent antibodies (Fig. 5). In addition, if antigenic heparin-protein complexes are present, they can also directly bind to heparin through the heparin-binding protein. Using an anti-IgG/A/M or an anti-IgG or a combination of anti-IgG, -IgA and -IgM peroxidase conjugates allows measurement of all the antibody isotypes (useful for screening for HIT) or only the IgG isotype (the preferred assay for confirming the diagnosis of HIT) or proceeding to a full isotyping of those antibodies (which remains a convenient tool for research studies). This approach is flexible, very sensitive, and highly specific for heparin-dependent antibodies. It allows identification of the antibody isotypes of clinical relevance.

An interesting improvement consists in supplementing the reaction milieu with platelet lysates or with lysates from leukocyte-platelet concentrates, or, when required, directly with PF4, IL-8, or any other high affinity heparin-binding protein. This provides PF4 or other chemokines in excess for forming the heparin-dependent antigenic target for HIT antibodies. Using platelet lysates, the assay correlates fully with the conventional EIA for measuring anti-PF4-H antibodies of

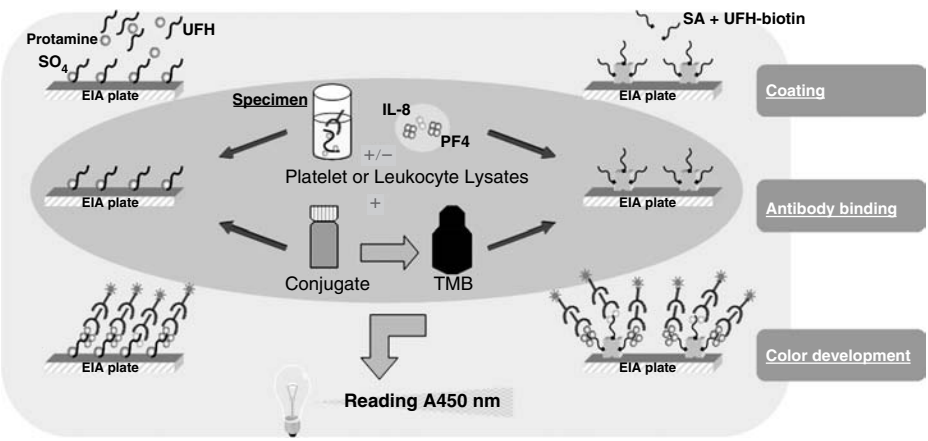

FIGURE 5 (*See color insert*) Assay for testing heparin-dependent antibodies, associated with HIT, by their binding to functionally available heparin through the heparin cofactor antigen (usually PF4); heparin is coated in a large excess as a complex with aprotinin or biotinylated and reacted with coated streptavidin. *Abbreviations*: EIA, enzyme-immunoassay; IL-8, interleukin-8; PF4, platelet factor 4; SA, streptavidin; TMB, tetramethyl benzidine; UFH, unfractionated heparin.

IgG isotype using plasmas from patients with clinically-suspected HIT ($r^2 = 0.89$), although some IgG positive patients were negative with the conventional PF4-H EIA, possibly because additional antigenic complexes are only measured with the new assay. When comparing the assay performed with or without platelet lysates, two groups of patients with HIT are identified: those for whom the antibody binding is totally dependent on the presence of platelet lysates and those in whom antibodies in the dilute patient plasma (or serum) bind "directly" to functionally active heparin. At this time, no clinical significance has yet been identified regarding these differences.

VI. CONCLUSIONS

PF4 complexed with heparin is the major antigen involved in HIT. Understanding the mechanisms for generating heparin-dependent antibodies, and how these antibodies become pathogenic, offers new approaches for diagnosis and management of HIT. Only a subset of antibodies to PF4-H exhibit a pathogenic effect by triggering thrombocytopenia and/or thrombosis, particularly IgG antibodies with the highest affinity for PF4-H. The conditions that permit formation of the PF4-H target antigens involve the type of heparin used, the dose and duration of therapy, and the clinical context of the treated patient. Immunoreactive complexes between PF4 and heparin are formed only under certain conditions, with their formation in high concentrations facilitated if underlying disease favors platelet activation and release. Similar conditions enhance the pathogenicity of the HIT antibodies generated. These considerations help to unravel the seemingly random generation of HIT antibodies among heparin-treated patients as well as the apparent chance occurrence of thrombocytopenia and thrombotic events. In addition, some HIT episodes could be associated with preexisting antibodies to chemokines such as IL-8, NAP-2, or possibly, even PF4. The use of heparin then only constitutes the abrupt trigger of pathogenicity by focusing these antibodies onto targeted blood cells and ECs. This could be the explanation for atypical presentations of HIT that can occur with pathological states such as malignancy, major surgery/inflammation, or infections. Understanding how heparin-dependent antigens can induce antibody generation, how these antibodies can become pathogenic in a subset of patients, and how heparin can trigger pathological effects for naturally-occurring antichemokine antibodies, can improve recognition and management of these complications of heparin therapy. This includes the development of more appropriate diagnostic laboratory assays for this life-threatening iatrogenic complication of heparin therapy.

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6 Molecular Immunopathogenesis of Heparin-Induced Thrombocytopenia

Gian Paolo Visentin and Chao Yan Liu
University at Buffalo, The State University of New York, Buffalo, New York, U.S.A.

Richard H. Aster
Medical College of Wisconsin and Blood Research Institute, The Blood Center of Wisconsin, Milwaukee, Wisconsin, U.S.A.

I. INTRODUCTION

Thrombocytopenia occurs commonly during heparin therapy, usually as a transient fall in platelet count 1–3 days after initiation of treatment. In most patients, this is of no clinical significance and platelet levels return to normal within 3 days, with or without discontinuing the heparin administration. In contrast, a relatively small group of patients develop thrombocytopenia, with a characteristic delay that is usually 5–10 days after starting heparin therapy, although some patients recently exposed to heparin develop an abrupt onset of thrombocytopenia. Paradoxically, many of these patients experience venous or arterial thromboembolism (see Chapter 2). To early investigators, this profile of a delay in onset of thrombocytopenia, as well as abrupt recurrence on rechallenge, suggested an immune pathogenesis (see Chapter 1).

Today, it is recognized that this immune-mediated syndrome, designated heparin-induced thrombocytopenia (HIT), is an important and sometimes life- and limb-threatening complication of heparin therapy. HIT may be more common in patients receiving certain types of heparin such as unfractionated heparin (UFH) of beef lung (rather than porcine) origin and unfractionated (rather than low molecular weight) forms of heparin (see Chapter 3). HIT can be triggered by standard therapeutic-dose heparin, low-dose (prophylactic) treatment (Hrushesky, 1978), low molecular weight heparin (LMWH) (Lecompte, 1991; Tardy, 1991), and even by minute quantities given to "flush" intravascular catheters (Heeger and Backstrom, 1986; Ling and Warkentin, 1998; Kelton and Warkentin, 1998). Various aspects of the pathogenesis of this disorder are also summarized in Chapters 4, 5, 7, 8, and 9.

Early investigations showed that IgG antibodies associated with HIT could induce platelet activation in the presence of pharmacological (0.2–1 U/mL) or even lower doses of heparin. By taking advantage of this property, two "platelet activation-dependent" diagnostic tests were developed for HIT: the platelet aggregation test (PAT) and washed platelet assays such as the serotonin release assay (SRA) (see Chapter 10). Because activation of platelets by IgG from patients with HIT in the presence of heparin could be inhibited by a monoclonal antibody that blocks the platelet FcγRIIA receptor (Kelton et al., 1988), it was assumed that the antibodies react with heparin to form immune complexes (ICs), which, in turn,

activate platelets. However, early studies failed to demonstrate binding of heparin-induced antibodies to platelets in the presence of heparin, in contrast to the behavior of platelet-reactive antibodies induced by other drugs such as quinidine and quinine. Moreover, the putative heparin-IgG complexes could not be identified in most studies (Green et al., 1978; Warkentin and Kelton, 1991; Greinacher et al., 1992). Thus, it remained unclear how heparin induces platelet activation and thrombocytopenia in patients with HIT.

A new understanding of pathogenesis emerged when Amiral and coworkers (1992) suggested that antibodies in HIT are specific to complexes of heparin and platelet factor 4 (PF4) rather than for heparin alone. We (Visentin et al., 1994) and others (Greinacher et al., 1994; Kelton et al., 1994) confirmed these findings. We added the observation that HIT antibodies recognize PF4 bound to heparan sulfate, normally found on the surface of endothelial cells (ECs) in the form of proteoglycan, and speculated that binding of antibodies to PF4 on ECs might promote EC damage, predisposing one to thrombosis (Visentin et al., 1994). These advances enabled the development of hypotheses to explain thrombocytopenia and thrombosis in HIT, but our understanding of the pathogenesis of this disorder is still incomplete.

II. HEPARIN AND PF4

Heparin and heparan sulfate constitute a distinct class of glycosaminoglycans (GAGs). GAGs are long, linear polymers composed of repeating disaccharide subunits. Heparin and heparan sulfate belong to a family of polysaccharide species, the chains of which are made up of alternating 1-4-linked and variously sulfated residues of hexuronic (D-glucuronic or *L*-iduronic) acid and D-glucosamine. The two substances differ in their hexuronic acid composition and pattern of substitution, with heparin having a higher content of sulfates and, consequently, a greater linear charge density. Commercially available heparin preparations are heterogeneous and polydisperse, consisting of polysaccharide fragments ranging in length from 3000 to 30,000 Da (10–100 saccharide residues) (see Chapter 7).

Heparan sulfate, together with chondroitin sulfate and dermatan sulfate, is widely distributed in the body, whereas heparin is found only in lung, ileum, skin, lymph nodes, thymus, and appendix, sites where mast cells are concentrated (Gomes and Dietrich, 1982). Metachromatic granules of mast cells are the major reservoir of heparin (Metcalfe et al., 1979). Heparan sulfate and other GAGs are also found in mast cell granules but are expressed mainly on the surface of nearly all adherent mammalian cells in the form of proteoglycans, consisting of oligosaccharides covalently linked to a core protein (syndecan) (Höök et al., 1984).

PF4, a heparin-binding protein normally found in platelet α-granules, is secreted when platelets are activated by various stimuli. Human PF4 is a member of a large family of homologous proteins, encoded by genes located on chromosomes 4 and 17, which have been designated "chemokines," and they are involved in chemotaxis, coagulation, inflammation, and cell growth (Zlotnik et al., 2000; Rollins, 1997; Luster, 1998). This family has been separated into four branches, designated CX_3C, CXC, CC, and C, based on the relative position of the first two conserved cysteines. PF4 belongs to the CXC family, which includes, among others, interleukin-8 (IL-8), interferon-γ-inducible protein (IP-10), platelet basic

protein (PBP), and two proteins derived from PBP by proteolytic cleavage: β-thromboglobulin (β-TG) and neutrophil-activating protein-2 (NAP-2). Human PF4 is a symmetrical, tetrameric molecule made up of identical subunits, each containing 70 amino acid residues of known sequence (Poncz et al., 1987), including two disulfide bonds, a single tyrosine, but no tryptophan. The molecule is positively charged at physiological pH (Handin and Cohen, 1976). The crystal structure of human PF4 has been resolved (Zhang et al., 1994). Lysine residues on the exterior faces of α-helices at the COOH-terminus of each monomer are critical for heparin binding (Loscalzo et al., 1985). However, residues located elsewhere on the tetramer are probably also important for this interaction (Maccarana and Lindahl, 1993; Mayo et al., 1995a).

III. NATURE OF THE EPITOPES RECOGNIZED BY HIT ANTIBODIES

A. The Role of Polyanion

The HIT antibodies fail to recognize PF4 or heparin alone but bind avidly to the PF4-heparin complex (Visentin et al., 1994). Antibody epitopes, therefore, could be composed of either combinatorial epitopes, consisting partly of heparin and partly of PF4, or conformational epitopes on the PF4 molecule induced by heparin binding. The epitopes could even be formed when two PF4 tetramers become closely apposed due to polysaccharide charge neutralization (Greinacher et al., 2006). Alternatively, a conformational change elsewhere on the PF4 molecule, created when the complex forms, could be targeted.

Heparin is a linear polyanion, and Maccarana and Lindahl (1993) have suggested that it binds to positively charged PF4 by nonspecific, electrostatic interactions rather than by specific oligosaccharide sequence recognition. However, Stringer and Gallagher (1997) have described a sequence on heparan sulfate consisting of a 9 kDa fragment, with sulfated domains at each end separated by a central, *N*-acetylated region, which may confer some specificity for PF4 binding. Regardless of whether PF4-heparin interaction is to some extent structure specific, non-GAG molecules can be substituted for heparin in detecting HIT antibodies. Kelton et al. (1994) found that highly sulfated polysaccharides, including heparan sulfate, pentosan polysulfate, and dextran sulfate, could be used, provided that they contained 1.0–1.5 sulfate groups per saccharide residue. Chondroitin sulfates A, B, and C, containing an average of only 0.5 sulfates per saccharide residue, were inactive. Highly sulfated but low molecular weight substances such as glucose-l,3,6-trisulfate, 1,2-cyclohexanediol disulfate, and heparin disaccharide, were likewise inactive. Greinacher and colleagues (1992, 1995) also characterized the structural requirements of polysaccharides active in generating HIT antibody epitopes. They showed that the β 1, 4-linkage between disaccharides, characteristic of heparin and other GAGs, was not essential, that heparin fractions containing fewer than 10 residues were unable to promote platelet serotonin release by HIT antibodies, and that branched glucan sulfates were more effective than linear glucan sulfates of the same molecular weight. Similarly, Amiral and coworkers (1995) found that the extent of polysaccharide sulfation is positively correlated with the ability to interact with PF4 in facilitating the binding of HIT antibodies.

It has been shown that in the presence of UFH, PF4 forms ultralarge complexes that are strongly antigenic, bind multiple IgG antibodies, and promote platelet activation (Rauova et al., 2005, 2006). However, recent data suggest that PF4 released from platelets can react directly with endogenous GAGs on the

surface of platelets (Newman and Chong, 2000; Prechel et al., 2005; Rauova et al., 2006). Recognition of these complexes by high titer antibodies could explain "delayed HIT" occurring days or weeks after the last exposure to heparin in some individuals (Aster, 2005).

Studies conducted in our laboratory (Visentin et al., 2001) showed that UFH of bovine and porcine origin, as well as LMWH, formed complexes with PF4 that were recognized equally well by a panel of HIT antibodies. In studies with heparin fragments of known size, a length of at least 10 saccharide residues was required to form complexes with PF4 that reacted (weakly) with this antibody panel. For optimal antibody recognition, fragments containing at least 12 saccharide residues were required. Also, sulfated GAGs other than heparin (e.g., heparan sulfate) as well as non-GAG sulfated polysaccharides (e.g., fucoidan and dextran sulfate) behaved similarly to heparin in their ability to form antibody-binding complexes with PF4. However, the "heparinoid" anticoagulant danaparoid (Orgaran), a mixture of nonheparin low molecular weight GAGs having a low degree of sulfation, formed complexes that reacted with only about one-third of patient samples tested (Visentin et al., 2001). Our findings, together with those of Kelton, Greinacher, and Amiral already cited, indicate that the ability of GAGs and other sulfated polysaccharides to substitute for heparin in promoting platelet activation by HIT antibodies and to form complexes with PF4 to which the antibodies bind is directly related to the size and degree of sulfation of the polysaccharide.

To determine whether or not a polysaccharide structure is necessary for the formation of HIT antibody epitopes, we evaluated a series of linear, nonsaccharide, polyanionic compounds and found unexpectedly that polyvinyl sulfate, polyvinyl sulfonate, polyvinyl phosphate, polyvinyl phosphonate, and polyanethole sulfonate all react with PF4 to produce complexes recognized by HIT-associated antibodies (Visentin et al., 2001) (Fig. 1). Thus, neither a saccharide chain nor

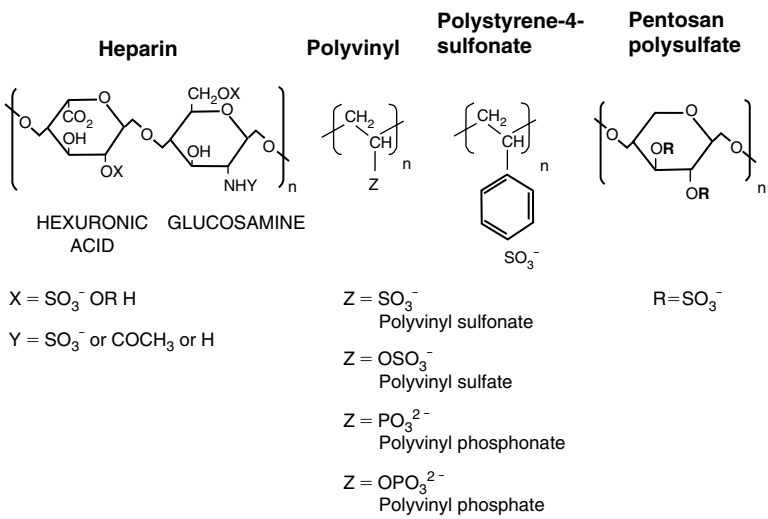


FIGURE 1 Nonspecific role of heparin and other polyanions that lead to neoepitope formation on platelet factor 4. *Source*: From Warkentin, 2003.

sulfate side groups is essential for a polyanion to react with PF4 and to create sites for antibody binding, arguing strongly against the possibility that HIT antibodies are specific to "compound epitopes" consisting partially of GAG and partially of peptide sequence at sites where the molecules making up the complex come into close contact. This observation, together with the finding that HIT antibodies fail to recognize heparin complexed with protamine (unpublished observation), excludes the possibility that they recognize a configuration of the sulfated saccharide that is stabilized on binding to a small, positively charged, spherical protein.

It appears likely, therefore, that sites for antibody binding are created when linear polyanionic compounds bind to PF4 and alter its 3D configuration. Heparin-induced antibodies associated with HIT bind avidly to complexes formed between PF4 and heparin fragments attached by end-linkage to agarose beads but fail to recognize PF4 complexed with heparin molecules immobilized by multiple cross-linkages (Suh et al., 1998). Thus, another requirement for the formation of PF4-heparin complexes for which HIT antibodies are specific appears to be that the saccharide chain making up the heparin molecule must be in a flexible, relatively unconstrained state prior to reacting with PF4.

Although PF4-heparin complexes have not yet yielded to structural analysis, some informative data about the nature of PF4-heparin interaction and its effect on PF4 structure are available. Both bovine (St. Charles et al., 1989) and human (Zhang et al., 1994) PF4 tetramers have been crystallized and have similar structure. Each PF4 monomer consists of a COOH-terminal amphiphilic α-helix overlying a three-stranded antiparallel β-sheet, a structure typical of CXC chemokine family members (Luster, 1998). Two PF4 monomers associate side by side to produce a six-stranded antiparallel β-sheet, with overlying antiparallel α-helices (AB dimer). Each AB dimer associates with an identical CD dimer through surface interaction between the β-sheets. The elements of PF4 structure are shown schematically in Figure 2a.

Crystallographic studies have shown that both bovine (St. Charles et al., 1989) and human (Zhang et al., 1994) PF4 contain a ring of positively charged

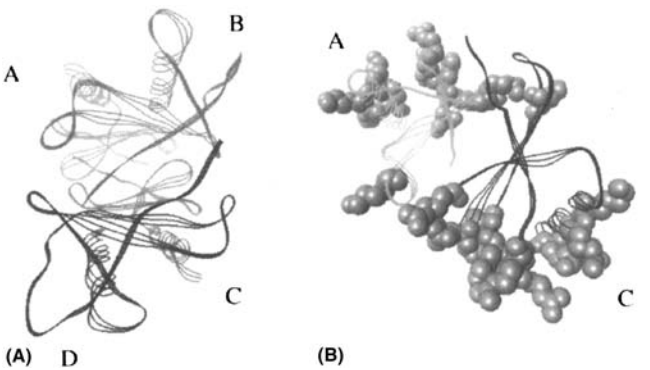


FIGURE 2 (*See color insert*) (**A**) Computer-generated model (WebLab ViewerPro; Molecular Simulation Inc., San Diego, CA) of the human PF4 tetramer, based on the crystallographic coordinates. (**B**) AC dimer view of human PF4: the amino acid residues crucial for heparin binding are displayed. *Abbreviation*: PF4, platelet factor 4. *Source*: (**A**) from Zhang et al., 1994; (**B**), from Loscalzo et al., 1985; Mayo et al., 1995a.

lysine, arginine, and histidine residues that encircle the tetramer along a line perpendicular to the α-helices and are available for interaction with solvent. Modeling studies (Stuckey et al., 1992) support the possibility that a negatively charged heparin molecule, containing 18 saccharide residues (MW $\sim$ 5.4 kDa), interacts with these positively charged residues spanning about half the tetramer. Mayo et al. (1995a,b) created a PF4 mutant (PF4-M2) in which the NH_2-terminal 11 residues were replaced by eight residues from the homologous CXC chemokine IL-8 to produce a tetramer that binds heparin with the same avidity as native PF4 but is more nearly symmetrical around all three axes, facilitating nuclear magnetic resonance (NMR) structural analysis. Their data, contrary to PF4-heparin-binding models that center around COOH-terminal α-helix lysines, indicate that arginines 20, 22, and 49, and to a lesser extent, histidine 23, threonine 25, and lysine 46, are also important for heparin binding (Fig. 2b and Fig. 3). On the basis of these findings, it was speculated that heparin does not bind perpendicularly to the α-helices of the AB dimer as had been suggested (Stuckey et al., 1992) but instead reacts with the α-helix at an angle, interacting preferentially with PF4 along the AD dimer, where it would encounter arginine and other positively charged residues. In either model, it is plausible that binding of a linear polyanion of sufficient length and linear charge density to positively charged residues on the surface of PF4 could cause the structural rearrangement throughout the entire tetramer necessary for generation of HIT antibody epitopes.

On the basis of these reports, it is possible to propose a model of how heparin and other linear polyanions react with PF4 to produce configurational changes in the tetramer and create sites for HIT antibody binding. We suggest that linear polyanions, such as heparin, that carry appropriately spaced, strong negative charges interact with PF4 by binding to the ring of positive charges extending between the A and D or B and C subunits or both. The minimum length for a fully active polyanion is about 50 Å, equivalent to six disaccharide subunits (12-mer), with each disaccharide measuring about 8.4 Å in length (Visentin et al., 2001). Reconfiguration of the tetramer, resulting from binding of the polyanion, creates the neoepitope(s) for which HIT antibodies are specific.

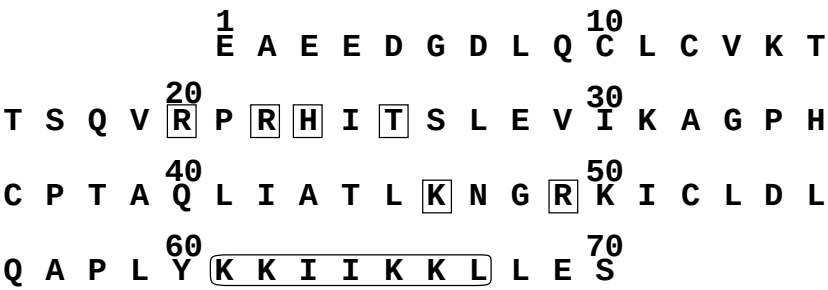


FIGURE 3 Amino acid composition of human PF4 monomers: Residues crucial for heparin binding (COOH-terminal α-helix residues encompassing lysines 61–62 and 65–66 [Loscalzo et al., 1985], arginines 20, 22, and 49, histidine 23, threonine 25, and lysine 46 [Mayo, 1995b]) are boxed. Filled circles identify the six residues (37, 38, 39, 49, 55, and 57) in the 47 COOH-terminal region of PF4 at which human and rat PF4 differ. *Abbreviation*: PF4, platelet factor 4.

B. The Role of Protein

Only a few investigators have attempted to map the actual epitopes on PF4-heparin complexes recognized by HIT antibodies. Horsewood et al. (1996) studied a total of 29 antibodies from patients with HIT that were positive in the PF4-heparin enzyme immunoassay (EIA) and in the SRA. Five of these antibodies also reacted with reduced alkylated PF4 in the presence of heparin. The same five antibodies also recognized a peptide containing the 19 COOH-terminal amino acid residues of the PF4 monomer, a region that encompasses a positively charged α-helical domain thought to be critical for heparin binding (Loscalzo et al., 1985). However, neither reduced PF4 nor the COOH-terminal peptide could inhibit binding of HIT antibodies to PF4-heparin complexes, even at high concentrations. Therefore, the clinical significance of the five antibodies is uncertain.

Amiral and coworkers (1996a) studied a subgroup of 15 patients thought to have HIT, whose antibodies were positive in a PAT but negative in PF4-heparin EIA. Nine of these patients had antibodies that recognized NAP-2 or IL-8, or both, two members of the CXC chemokine family that are homologous with PF4. These findings are of interest because five of the nine patients had thrombotic episodes. However, reactions of these antibodies against NAP-2 or IL-8 in their normal configurations (not immobilized on plastic) were not described, and their relation to antibodies that recognize PF4-heparin complexes is uncertain.

Ziporen et al. (1998) studied the binding of antibodies from 50 HIT patients to different constructs of PF4 containing a single amino acid substitution and chimeric proteins containing various portions of human PF4 and NAP-2. Mutation to alanine of three (K62, K65, K66) of the four lysine residues in the COOH-terminal α-helix had only minimal effect on the binding of HIT antibodies, and the K61 → A mutation reduced antibody binding by only about 50%, indicating that the COOH-terminal lysines of PF4 do not constitute the major antigenic site for HIT antibodies. NH_2-terminal PF4-NAP-2 chimeras exhibited only slightly reduced antibody binding. In contrast, the PF4-NAP-2 chimera, in which the portion of PF4 lying between the third and fourth cysteine residue (amino acids 37–47) was substituted by the corresponding NAP-2 sequence, was almost totally nonreactive.

With a different approach, we found that, although human PF4 has 74% protein sequence identity to bovine and rat PF4, neither bovine nor rat PF4 complexed to heparin is recognized by HIT antibodies (Visentin, 1999). Yet, rat PF4 differs from its human counterpart at only six of its 47 COOH-terminal amino acids (Doi et al., 1987; Poncz et al., 1987) (Fig. 3). To characterize the binding sites for HIT antibodies on PF4-heparin, we constructed seven PF4 mutants in which the human sequence (reactive) was converted to the corresponding residues of rat PF4 (nonreactive) and determined the effect of each change on HIT antibody binding to the construct complexed with heparin. The PF4 constructs tested were comparable with wild-type PF4 in their avidity for heparin. Each of the 15 antibodies from HIT patients recognized PF4-heparin complexes containing PF4 constructs bearing mutations: E4 → S, L11 → V, and T16 → S at the NH_2-terminus, or A57 → V at the COOH-terminus just as well as the wild-type human PF4-heparin complexes. In contrast, complexes containing other COOH-terminal mutants: P37 → A / T38 → V/A39 → P, R49 → S, and L55 → R exhibited varying degrees of reduced binding. The HIT antibodies tested recognized PF4 mutated at positions 49 and 55 only at a higher ratio of heparin to PF4 (0.8 U/mL vs. 0.5 U/mL). None of the 15 antibodies recognized peptides comprising the 26 or 15

COOH-terminal amino acid residues of the PF4 monomer or reduced alkylated human PF4 either in the presence or in the absence of heparin.

These results, together with the observations by Ziporen and associates (1998), point to the region of PF4 between the third and fourth cysteine residues as the major antigenic site for HIT antibody binding (Fig. 4). Li et al. (2002), using a series of mouse/human PF4 chimeras, identified another antigenic site on PF4-heparin that requires both P34 and an intact *N*-terminus (Fig. 4). The latter results, together with our studies utilizing biotin-labeled, affinity-purified HIT antibodies in a competitive inhibition assay (Suh et al., 1998), indicate that at least three dominant HIT antibody recognition sites can be distinguished and further support the idea that HIT antibodies recognize conformation-dependent "neoepitopes" formed on PF4 when it binds to heparin.

IV. THE IMMUNE RESPONSE TO HEPARIN

The finding that anti-PF4-heparin antibodies can be of the IgM, IgG, or IgA isotype (Visentin et al., 1994; Greinacher et al., 1994; Kelton et al., 1994; Amiral et al., 1995, 1996b; Arepally et al., 1997; Suh et al., 1997) indicates that class switching, likely requiring helper T cells, may occur in patients mounting a humoral immune response to PF4-heparin. Although HIT is a drug-induced disorder, parallels for the role of T cells in HIT may be drawn from studies of autoimmune

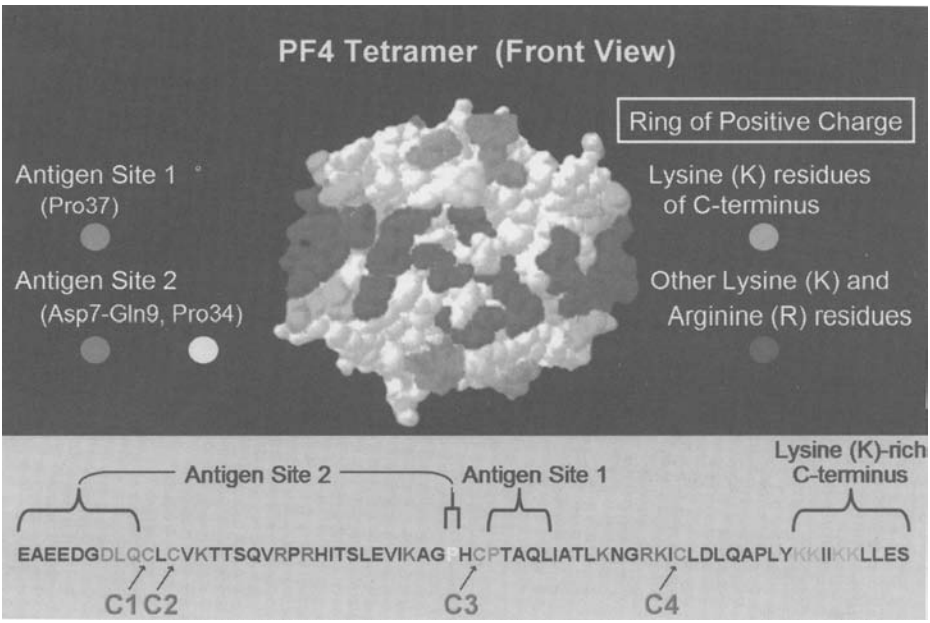


FIGURE 4 (*See color insert*) Primary and secondary structure of PF4 in relation to HIT neoepitopes. (*Top*) 3D representation of the PF4 tetramer, indicating two neoepitope sites (per monomer). The "ring of positive charge" is formed by lysine residues in the C-terminus (*light blue*) and other lysine and arginine residues (*dark blue*). (*Bottom*) The linear sequence of the 70-amino acid polypeptide of a single PF4 molecule is shown. *Abbreviation*: PF4, platelet factor 4. *Source*: From Li et al., 2002.

conditions such as systemic lupus erythematosus, systemic sclerosis, and insulin autoimmune syndrome (Ito et al., 1993; Crow et al., 1994; Kuwana et al., 1995a). In both lupus and scleroderma, T-helper cells mediate antigen-specific autoantibody production by B cells (Adams et al., 1991; Mohan et al., 1993; Kuwana et al., 1995b).

We hypothesized that the PF4-heparin complex not only is the target for antibody but also is the stimulus for T-cell activation, and have used T-cell receptor (TCR) spectratyping (Maslanka et al., 1995), also called immunoscope (Cochet et al., 1992; Pannetier et al., 1993), and clonotyping (Maslanka et al., 1996) to characterize the T-cell response to PF4-heparin complexes in HIT.

Culture of peripheral blood mononuclear cells (PBMC) from patients experiencing HIT incubated with PF4-heparin complexes, but not PF4 or heparin alone, leads to selective expansion of T-cell subsets (Liu et al., 2000; Bacsi et al., 2001). On in vitro culturing of PBMC from two HIT patients, the PF4-heparin complexes preferentially stimulated CD4 T cells expressing TCR with β-chains of the V 5.1 family, with a shared core CDR3 region amino acid motif (PGTG) (Bacsi et al., 1999). In a study of a third HIT patient, we found PF4-heparin-specific expansion of several βV 17 TCR clonotypes with yet another shared core CDR3 region amino acid motif (TSG) (Bacsi et al., 2001). However, T-cell lines derived from this third patient and maintained in culture in the presence of PF4-heparin demonstrated selective expansion of the β 6.1 and 17 families sharing the conserved core GTG motif previously identified in the βV 5.1 family of the first two HIT patients (Liu et al., 2001).

These findings provide evidence for the existence of T-cell subpopulations specific to PF4-heparin complexes in the peripheral blood of patients experiencing HIT and suggest that a common CDR3 TCR motif may be important for recognition of a peptide derived from PF4 processed by antigen-presenting cells (APCs) in the presence of heparin.

Despite the intriguing implications of these findings, however, T-cell lines and clones derived from these cultures and from those derived from two more HIT patients proliferated very poorly or not at all in the presence of PF4-heparin. Several reports have identified PF4 as an inhibitor of EC proliferation and angiogenesis (Broxmeyer et al., 1993; Strieter et al., 1995). Furthermore, PF4 recently has been shown to inhibit strongly T-cell proliferation, interferon-γ (IFN-γ), and IL-2 release of isolated T cells (Fleischer et al., 2002). We wondered if similar inhibitory effects of PF4 could be observed on specific T-cell subsets, e.g., T regulatory (Tr) cells.

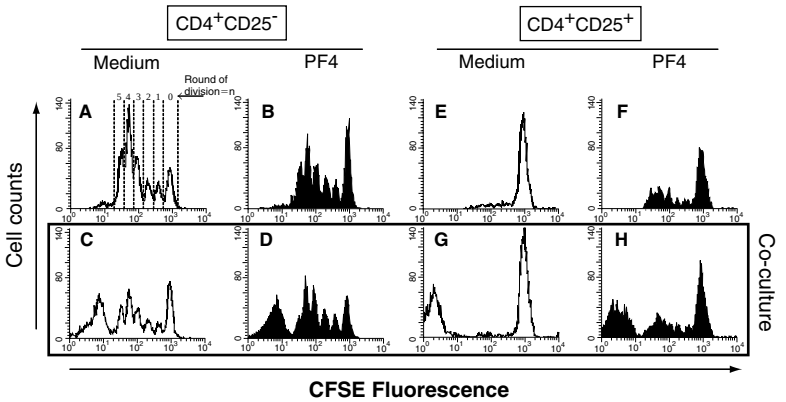
It has been recently reported in a murine model of HIT that the induction of anti-PF4-heparin antibodies requires involvement of T cells that have been educated in the thymus (Suvarna et al., 2005). On the contrary, a reported clinical evidence of lack of immunologic memory (Warkentin and Kelton, 2001) together with the antigenicity of high molecular weight PF4-heparin complexes (Visentin et al., 2001; Rauova et al., 2005, 2006) is consistent with the possibility that HIT can be induced independently of T-cell help.

Autoreactive T and B cells can be detected in healthy individuals but are normally kept in check by regulatory mechanisms. Among those is active suppression of naïve T cells by endogenous Tr cells. Several types of Tr cells exist, including $CD4^+$ T cells that express the IL-2 receptor α chain (CD25) constitutively, do not secrete IL-10, and suppress immune responses via direct cell-to-cell interactions. $CD4^+CD25^+$ Tr cells represent 5–10% of the endogenous $CD4^+$ T-cell

subset and are able to suppress $CD4^+$ and $CD8^+$ T-cell responses in vitro and in vivo upon TCR engagement. The recent finding that human PF4 inhibits the proliferative response of human $CD4^+CD25^-$ T cells, while inducing expansion of $CD4^+CD25^+$ Tr cells, and that PF4-induced $CD4^+CD25^+$ Tr cells lose their potent suppressor function in vitro (Fig. 5), suggests a previously unrecognized role of PF4 in the regulation of immune responsiveness (Liu et al., 2005).

It is presently unclear why patients with HIT mount a brisk humoral immune response to an autologous protein (PF4). PF4 is undoubtedly processed, under normal circumstances, by antigen-APCs without triggering immunity.

It appears that multiple factors influence the formation of antibodies specific to PF4-heparin complexes in patients receiving heparin. Currently, there is no evidence to support genetic predisposition as a basis for antibody formation in patients receiving heparin. Unlike the situation in alloimmune thrombocytopenia (de Waal et al., 1986; Mueller-Eckhardt et al., 1989), no connection between HIT and human leukocyte antigens (HLA) has been found (Greinacher and Mueller-Eckhardt, 1993). IgM antibodies specific to PF4-heparin complexes are a common finding in HIT (Visentin et al., 1994), indicating a primary immune response, and it could be speculated that patients who received UFH previously may be at



	Culture Condition							
	CFSE-labeled $CD4^+CD25^-$				CFSE-labeled $CD4^+CD25^+$			
			Co-culture				Co-culture	
Division	Medium (A)	PF4(B)	Medium (C)	PF4(B)	Medium (F)	PF4(F)	Medium (G)	PF4(H)
% Div.	43.43±0.6	27.69±0.8	27.95±0.8	39.78±0.7	2.51±1.1	9.54±0.9	2.32±1.2	10.93±0.9

FIGURE 5 PF4 induces proliferation of $CD4^+CD25^+$ Tr cells and impairs their ability to suppress the response of $CD4^+CD25^-$ T cells to anti-CD3. CFSE labeled $CD4^+CD25^-$ T cells were cultured alone (A-B) or with an equal number of unlabeled $CD4^+CD25^+$ Tr cells (C-D). In the converse experiment, CFSE labeled $CD4^+CD25^+$ Tr cells were cultured alone (E-F) or with an equal number of unlabeled $CD4^+CD25^-$ T cells (G-H). Dashed lines (shown only on panel A) indicate the boundaries of each division cycle. The numbers appearing above each peak (0–5) denote each division population, with the undivided T cells (0) residing in the rightmost peak, and the T cells that have divided five times (5) residing in the leftmost peak. The leftmost peak represents unlabeled $CD4^+CD25^+$ (C and D) and $CD4^+CD25^-$ T cells (G and H). The percent divided (% Div.) represents the portion of the original CFSE labeled T-cell population induced into cell division. *Abbreviations*: CFSE, carboxyfluorescein diacetate succinimidyl ester; PF4, platelet factor 4; Tr, regulatory cells; T, cells. *Source*: From Liu et al., 2005.

greater risk to produce PF4-heparin-specific antibodies and to develop HIT, if rechallenged with heparin. However, Cadroy et al. (1994) described a patient with a history of HIT, who mounted a brisk IgM response when challenged again with UFH 3 yr later. A report by Warkentin and Kelton (2001) suggests that there is no anamnestic immune response in HIT (i.e., patients either have typical" HIT [onset at days 5–10] or "rapid" HIT, the latter apparently caused by residual circulating HIT antibodies rather than a secondary immune response). Furthermore, HIT did not necessarily recur in patients who were exposed to heparin a second time.

Our findings provide the first evidence that the CXC chemokine PF4 can interfere with Tr-suppressive capacity. We hypothesize that in HIT, PF4 is not only the target for the antibody (when complexed with heparin) but is also a modulator of T-cell activation.

We propose that the modulatory effect of PF4 on $CD4^+CD25^+$ Tr cells is not a "physiological" effect on the immune system homeostasis but is rather a pathologic complication of heparin therapy, since heparin scavenges PF4 from EC GAGs and directly from circulating platelets (Zucker, 1975). Moreover, platelet activation that occurs during and after specific invasive procedures associated with heparin administration (such as cardiopulmonary bypass) leads to increased PF4 release into the circulation (Wan et al., 1997). Therefore, a transient impairment of the Tr suppressor activity might be postulated in otherwise healthy individuals experiencing acute platelet destruction, thus providing a possible explanation for why different patient groups exposed to heparin become sensitized at different rates (Visentin et al., 1996; Yamamoto et al., 1996).

V. IMPLICATIONS

The identification of mutations of human PF4 that lead to loss of HIT antibody binding does not necessarily localize the epitopes at which antibodies attach because the actual binding site(s) could be elsewhere in the PF4 tetramer. Moreover, HIT antibodies appear to recognize multiple sites on PF4-heparin (Suh et al., 1998). Because the PF4 molecule is a nearly symmetrical tetramer (Ibel et al., 1986), the HIT epitope could be expressed four times on each PF4-heparin heterodimer, creating the potential for even a single antibody clone to react with four sites on a PF4 tetramer complexed with heparin. Studies from our group (Visentin et al., 1994, 1996) and others (Amiral et al., 1995; Arepally et al., 1995) have shown that although antibodies reactive with PF4-heparin complexes are nearly always present in patients with HIT, not all patients who form such antibodies experience thrombosis or even thrombocytopenia. Factors that could predispose antibody-formers to develop the HIT syndrome include the formation of unusually potent (high-titer) antibodies (Suh et al., 1997) and the presence of underlying conditions, congenital or acquired, that predispose one to thrombosis. It can be speculated that antibodies recognizing certain sites on PF4-heparin form ICs that are particularly effective in activating platelets. The same antibodies might be more likely to promote vessel injury when they bind to PF4 complexed with GAGs on ECs. Alternatively, patients who make antibodies that recognize multiple sites on PF4-heparin may be more likely to produce pathogenic ICs, leading to more severe symptomatology.

The recent finding that human PF4 reverts the anergic Tr cell phenotype and impairs their suppressive activity (Liu et al., 2005) suggests a previously unrecognized role of PF4 in the regulation of immune responsiveness.

On the basis of findings made by our group and many others, we propose the following model for HIT and associated thrombosis in patients mounting an immune response to PF4-heparin (Fig. 6): (1) Injected heparin recruits PF4 from EC GAGs and releases PF4 directly from circulating platelets (Zucker, 1975); (2) APCs, including B cells, process PF4-heparin, which binds to PF4 with high avidity, may alter the processing of PF4 in such a way that one or more peptides not ordinarily seen by the immune system ("neoantigens") are generated; thus "conventional" and "cryptic" PF4-derived peptides could be presented to T cells in the context of Class II HLA; (3) Released PF4 induces loss of suppressive activity of Tr cells (Liu et al., 2005), allowing PF4-specific T-cell clones engaged on APCs to expand, thus activating $CD4^+$ effector T cells and B cells; (4) Activated B cells secrete antibodies reactive with PF4-heparin complexes formed on the platelet surface (Newman and Chong, 2000; Rauova et al., 2006) to produce ICs that engage platelet Fc receptors (Amiral et al., 1992; Visentin et al., 1994; Greinacher et al., 1994); Engagement of the Fc receptor by ICs leads to platelet activation (Visentin et al., 1994; Greinacher et al., 1994; Kelton et al., 1994) and initiation of a vicious cycle of platelet-derived procoagulant microparticle formation, PF4 release, platelet-derived CD154 (CD40 ligand) exposure/release (Henn et al., 1998), and formation of additional PF4-heparin complexes, triggering even more platelet activation and thrombocytopenia (Warkentin et al., 1994; Visentin, 1999); (6) Soluble CD40 ligand (sCD154) released from activated platelets induces tissue factor (TF)

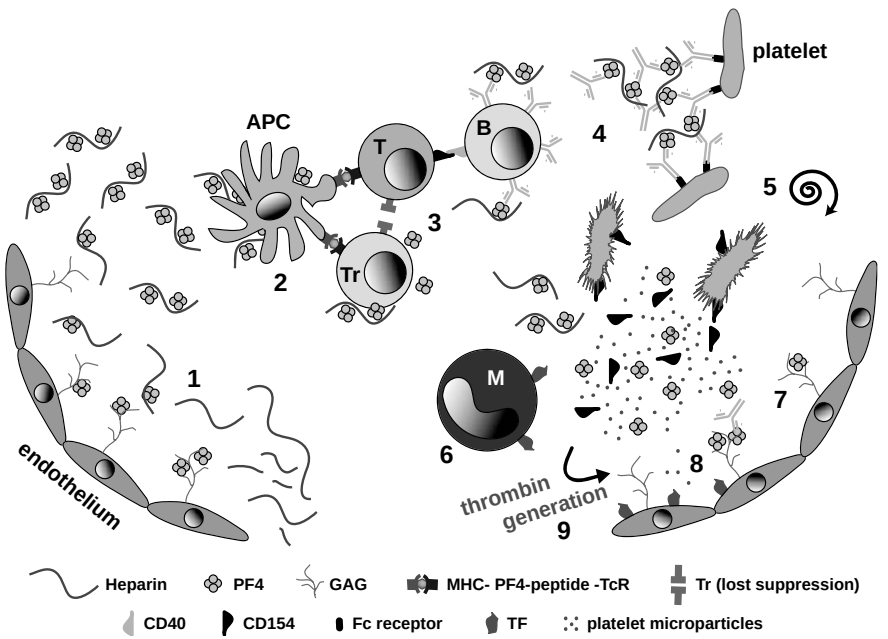


FIGURE 6 (*See color insert*) Proposed model of pathogenesis in HIT and thrombosis. PF4 is postulated to be both the target for the antibody (when complexed with heparin) and a modulator of T-cell responsiveness (see text for additional details). *Abbreviations*: APC, antigen-presenting cell; GAG, glycosaminoglycan; HIT, heparin-induced thrombocytopenia; MHC, major histocompatibility complex; PF4, platelet factor 4; TCR, T-cell receptor; Tr, T regulatory; TF, tissue factor.

expression on monocytes and ECs (Henn et al., 1998; Mach et al., 1997); (7) Following clearance of heparin, released PF4 reassociates with GAGs on ECs and PBMCs; (8) Antibodies bind to these newly formed targets to cause EC damage and/or activation (Visentin et al., 1994) and more TF expression (Arepally and Mayer, 2001; Pouplard et al., 2001), thus (9) enhancing—together with the released platelet-derived microparticles—thrombin generation with resulting thrombosis or disseminated intravascular coagulation (Warkentin et al., 1998; Visentin, 1999).

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7 Role of Sulfated Polysaccharides in the Pathogenesis of Heparin-Induced Thrombocytopenia

Susanne Alban
Christian-Albrechts-University of Kiel, Kiel, Germany

Andreas Greinacher
Ernst-Moritz-Arndt-University-Greifswald, Greifswald, Germany

I. INTRODUCTION

Unfractionated heparin (UFH) and low molecular weight heparin (LMWH) are the anticoagulants of choice when parenteral anticoagulation is required. Both can be given subcutaneously or intravenously, and both are effective in a variety of clinical settings (Hirsh and Raschke, 2004). UFH, in particular, has several limitations. These include its poor bioavailability after subcutaneous injection as well as the marked variability in the anticoagulant response to UFH treatment in patients with acute thromboembolism (Hirsh and Raschke, 2004; Young et al., 1992). Another problem is the risk of inducing heparin-induced thrombocytopenia (HIT). These limitations are closely linked (Greinacher, 1995): the underlying cause is the high density of negative charges of the heparin molecule, leading to nonspecific interactions of heparin with cells and plasma proteins other than antithrombin (AT). This results in reduced anticoagulant effects of heparin as well as in conformational changes of the proteins bound to heparin, with the potential for exposure of neoepitopes, or cryptic epitopes, which may induce an immune response.

In this chapter, the mechanism and structural requirements for complex formation between sulfated carbohydrates, especially heparin, and proteins, such as platelet factor 4 (PF4), are reviewed. The pathophysiological consequences of these interactions in causing HIT are summarized. From these considerations, the prospects for development of carbohydrate-based heparin alternatives with a lower risk for immune thrombocytopenia are discussed.

II. INTERACTIONS OF PF4 WITH SULFATED CARBOHYDRATES

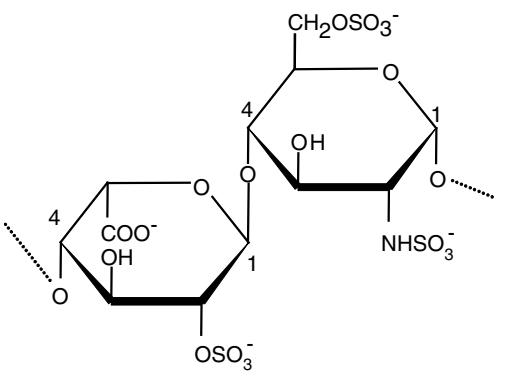
A. Structure of PF4

Heparin activity is neutralized by PF4, a protein released from the α-granules of activated platelets (Sear and Poller, 1973; Klener and Kubisz, 1978; Luscher and Kaser-Glanzman, 1975; Niewiarowski, 1976), which attaches to the endothelial surface by binding to glycosaminoglycans (GAGs) (Novotny et al., 1993). PF4 is a compact homotetrameric globular protein with a subunit molecular weight (MW) of 7780 Da (70 amino acid residues per subunit) (Kaplan and Niewiarowski, 1985; Mayo et al., 1995). With its content of 6.0% arginine, 3.2% histidine, and 12.3% lysine, PF4 is a basic (positively charged) protein (Moore et al., 1975). The NH_2 terminal residues form antiparallel β-sheet-like structures that induce non-covalent

associations between dimers and also contribute to the cohesion of the tetrameric unit. Furthermore, electrostatic interactions between charged amino acid side chains and hydrogen-bonding interactions at the AB/CD dimer interface serve to stabilize the tetrameric structure. The COOH-terminal α-helices, which contain four lysine residues, are arranged as antiparallel pairs on the surface of each extended β-sheet (St. Charles et al., 1989). The lysine residues are predominantly on one side, resulting in a "ring of positive charge" that runs perpendicularly across the helices (Stuckey et al., 1992; Zhang et al., 1994) (Figs. 2 and 4; see also Chapter 6).

B. Structure of Heparin

Heparin is a polydisperse mixture of GAGs with MWs ranging from 5 to 30 kDa, with an average MW of 13 kDa (Linhardt and Toida, 1997). It is composed of alternating β-D-glucosamine residues $1 \rightarrow 4$-linked to either α-L-iduronic acid or β-D-glucuronic acid (Casu, 1985). The principal repeating unit in heparin is the trisulfated disaccharide [$\rightarrow$4)-α-L-iduronic acid-2-*O*-sulfate ($1 \rightarrow$4)-α-D-glucosamine-2-*N*, 6-*O*-disulfate ($1 \rightarrow$] (Fig. 1), which represents 75–90% of the heparin chain (Linhardt et al., 1992). The remaining 10–25% of disaccharide units differ in their degree and positions of sulfation (Linhardt et al., 1988). Besides, there are disaccharides consisting of unsulfated glucuronic acid and/or N-acetylglucosamine. With a SO_4^-: COO^- ratio of 2.0–2.5, heparin is the GAG with the highest charge density. By binding to domains containing positively charged amino acids, especially arginine and lysine, it interacts with many proteins, resulting in manifold biological activities. The most prominent example is a well-defined pentasaccharide sequence with a central α-D-glucosamine-2-N, 3-*O*, 6-*O*-trisulfate unit, which binds specifically to AT (Choay, 1989). About 30% (range 10–50%) of the heparin chains contain this pentasaccharide (Fig. 2). These molecules are called high-affinity heparin in contrast to the low-affinity heparin without this AT-binding site (Casu, 1990). AT is a natural serine protease inhibitor that controls blood coagulation by forming equimolar covalent complexes with certain coagulation enzymes. The anticoagulant action of heparin is mainly based on accelerating the slow rate of factor Xa and thrombin inhibition by AT (Bjork et al., 1989). Whereas the heparin pentasaccharide is sufficient for factor Xa inhibition, thrombin inhibition



[4)-α-L-Ido*p*A2S-(1→4)-α-D-Glc*p*N2S,6S-(1→]$_n$

FIGURE 1 Main disaccharide unit of heparin composed of 75–90% heparin. *Abbreviation*: AT, antithrombin.

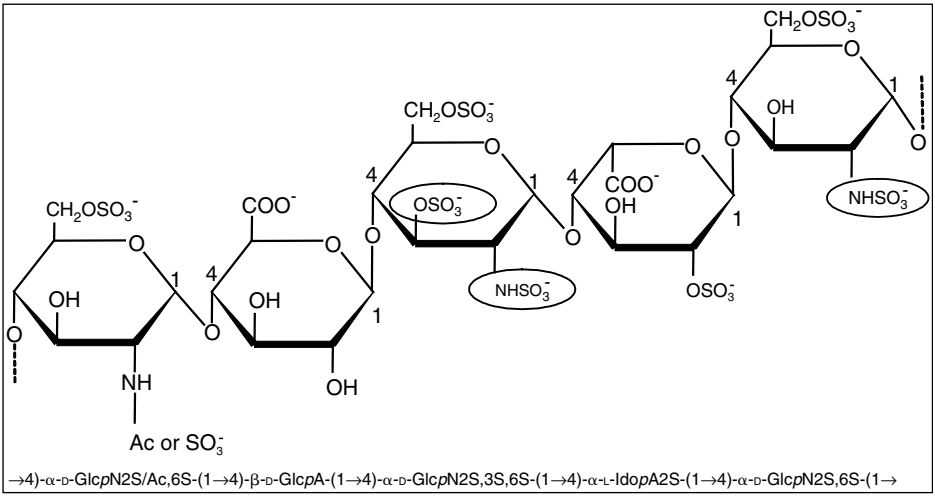


FIGURE 2 Pentasaccharide sequence of the AT-binding site of heparin, sulfate groups essential for the AT-binding are encircled. *Abbreviation*: AT, antithrombin.

requires a minimum heparin chain length of 18 monosaccharides (5400 Da) to permit simultaneous binding of heparin to both AT and thrombin.

C. PF4-Sulfated Polysaccharide Complexes

PF4 has the highest affinity to heparin among proteins stored within the platelet α-granules. Heparin molecules bind to PF4 by interactions with the positively charged residues on the surface of PF4 (see Chapter 6). Stuckey and coworkers (1992) suggested that heparin is bound to PF4 by being wrapped around the tetramer along the ring of positive charges. A heparin molecule with 16-18 monosaccharides interacts with PF4 by spanning about half of the tetramer. As a consequence, only very long molecules are able to wrap around the complete tetramer. Mayo and coworkers (1995) identified a loop containing Arg-20, Arg-22, His-23, and Thr-25, as well as Lys-46 and Arg-49, which are more relevant for heparin binding than the COOH-terminal lysines. For optimal interaction with PF4, a heparin molecule should consist of at least 12 monosaccharides (Visentin, 1999; Mikhailov et al., 1999). At low concentrations (0.1-1.0 IU/mL) of heparin and high concentrations of PF4, several PF4 tetramers compete for heparin binding. This permits binding of a heparin chain to more than one PF4 tetramer. Particularly, if a heparin molecule is longer than 16 monosaccharides, it is able to bind to and thereby bridge two PF4 tetramers. Thus, at certain concentrations of heparin and PF4, large, multimolecular PF4-heparin complexes are formed. UFH forms larger complexes than LMWH (Rauova et al., 2005; Greinacher et al., 2006). These complexes can be dissociated in the presence of high heparin concentrations (Bock et al., 1980; Greinacher et al., 1994a, 1995).

In vitro, only heparin molecules containing 16 or more monosaccharides completely bind to immobilized PF4, resulting in total neutralization of their antifactor Xa (anti-Xa) and anti-thrombin (anti-IIa) activities, whereas progressively smaller oligosaccharides (without anti-IIa activity) become increasingly

TABLE 1 Main Disaccharide Units of Mammalian Glycosaminoglycans, Arranged by Increasing Affinity to Platelet Factor 4

Glycosaminoglycan	Main disaccharide unit	DS[a]
Hyaluronic acid	[4)-β-D-Glc*p*A-(1 → 3)-β-D-Glc*p*NAc-(1 →]	0
Keratan sulfate	[3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc6S-(1 →]	~0.6
Chondroitin sulfate A	[4)-β-D-Glc*p*A-(1 → 3)-β-D-Glc*p*NAc4S-(1 →]	~0.8
Chondroitin sulfate C	[4)-β-D-Glc*p*A-(1 → 3)-β-D-Glc*p*NAc6S-(1 →]	~0.8
Dermatan sulfate = ChS B	[4)-α-L-Glc*p*A-(1 → 3)-β-D-Glc*p*NAc4S-(1 →]	~1.4
Heparan sulfate	[4)-β-D-Glc*p*A-(1 → 4)-α-D-Glc*p*NAc6S-(1 →]	0.8–1.8
Low molecular weight heparins[b]	[4)-α-L-Ido*p*A2S-(1 → 4)-α-D-Glc*p*N2S,6S-(1 →]	2.0–2.5
Unfractionated heparin	[4)-α-L-Ido*p*A2S-(1 → 4)-α-D-Glc*p*N2S,6S-(1 →]	2.0–2.5

[a]DS, degree of sulfation (sulfate groups per disaccharide unit).
[b]Produced by degradation of unfractionated heparin.

resistant to neutralization of their anti-Xa activity by PF4 (Denton et al., 1983; Lane et al., 1984). Also in plasma, the anti-IIa activity of LMWHs, which is mediated by molecules with MW > 5400 Da, but not their anti-Xa activity, can be completely neutralized by higher PF4 concentrations (Padilla et al., 1992; Bendetowicz et al., 1994). The reduced sensitivity of LMWHs to inactivation by PF4 also explains why LMWHs are more active than UFH in platelet-rich plasma (Beguin et al., 1989).

Low- as well as high-affinity heparin binds to PF4 with a similar apparent K_d (Loscalzo et al., 1985). The interaction appears to be mediated by electrostatic interactions as shown by studies of heparin oligosaccharides with different charge densities (Maccarana and Lindahl, 1993). Therefore, the complexes are dissociable. Indeed, heparin can be displaced from PF4 by sulfated polysaccharides, such as other GAGs (Handin and Cohen, 1976), dextran sulfate (Loscalzo et al., 1985), or xylan sulfate (Campbell et al., 1987). The molar ratios required for complex formation increase in the order: UFH < LMWH < heparan sulfate < dermatan sulfate < chondroitin-6-*O*-sulfate < chondroitin-4-*O*-sulfate (Handin and Cohen, 1976). Besides the degree of sulfation (DS) and MW, other structural parameters, such as the type of the uronic acid and the position of the sulfate groups within the monosaccharide in the case of GAGs, influence the affinity of a polysaccharide to PF4 (Table 1).

In contrast to the earlier findings on a minimum heparin chain length (Visentin, 1999; Mikhailov et al., 1999), heparin molecules as small as the pentasaccharide were recently shown to interact with PF4 as well (Greinacher et al., 2006). By atomic force microscopy and photon correlation spectroscopy, it has been demonstrated that at very high concentrations and in the absence of AT, the pentasaccharide forms clusters in which PF4 tetramers become closely apposed, even though this tendency is much lower than that of UFH and LMWH.

D. Interactions of PF4 with Sulfated Polysaccharides In Vivo

Intravenous injection of heparin causes an increase in plasma PF4 level, whereas subcutaneous injection does not (O'Brien et al., 1985). The maximum amount of PF4 released corresponds to only about 5% of total platelet PF4 (Dawes et al., 1982).

In vivo, heparin and some other GAGs are able to increase plasma PF4 levels (Cella et al., 1986). Endothelial bound, rather than platelet-stored, PF4 seems to be the predominant source of the PF4 released by heparin. Most likely, heparin and other high-sulfated polysaccharides are able to displace PF4 from endothelial heparan sulfate in relation to their affinity for PF4 (O'Brien et al., 1985).

III. PF4-HEPARIN COMPLEXES AS THE MAJOR ANTIGEN RECOGNIZED BY HIT ANTIBODIES

A. Formation of Antigens Recognized by HIT Antibodies

The strong anionic character of heparin plays the predominant pathogenic role in formation of the HIT antigens. Heparin adheres to both cell surface-bound and free PF4, and may additionally increase free PF4 by its platelet-activating effects (Horne and Hutchison, 1998; Newman and Chong, 2000). Heparin binding to PF4 results in clustering of PF4 (Amiral et al., 1992; Rauova et al., 2005) forming linear, ridge-like, multimolecular complexes (Greinacher et al., 2006). From experiments using the pentasaccharide, it has been shown that antigenic PF4 complexes are formed not only by bridging of two PF4 molecules, but also when charge neutralization by polyanion allows positively charged PF4 tetramers to undergo close approximation (Greinacher et al., 2006). Some patients develop antibodies (HIT antibodies) against these complexes. Most HIT antibodies recognize noncontiguous conformational epitopes on the PF4 molecule (three distinct binding sites for HIT antibodies on the PF4-heparin complexes have been identified [Suh et al., 1998; Li et al., 2002]) that are produced when at least two PF4 tetramers are bound together by heparin (Horsewood et al., 1996; Newman and Chong, 1999; Greinacher et al., 2006). Differences in the relative size, amount, and stability of the complexes may be responsible for the observed differences in immunogenicity (UFH > LMWH $\approx$ fondaparinux) (Warkentin et al., 2005a) and clinically relevant cross-reactivity (UFH > LMWH $\gg$ fondaparinux) (Savi et al., 2005; Warkentin et al., 2005a) (Fig. 3).

Differing optimal ratios of heparin and PF4 observed to form the HIT epitopes are reported (Rauova et al., 2005; Greinacher et al., 2006), perhaps resulting from differences in heparin preparations used and in experimental design. The reported optimal molar ratios between heparin and PF4 are debatable because exact molar concentrations cannot be indicated for polydisperse mixtures of molecules such as heparin.

In a few cases, PF4 alone can be recognized by the HIT antibodies, as shown by the reaction of purified HIT antibodies with either PF4-heparin complexes or PF4 in the absence of heparin (Greinacher et al., 1994a; Newman and Chong, 1999; Amiral et al., 2000; Prechel et al., 2005). Since pretreatment of platelets with chondroitinase abolishes the platelet-activating effects of these heparin-independent antibodies (Rauova et al., 2006), endogenous platelet-associated GAGs are thought to take the role of heparin. This may also be an explanation for "delayed-onset HIT," where thrombocytopenia and thrombosis begin several days after heparin has been stopped (Warkentin and Kelton, 2001). One such patient developed high levels of antibodies against PF4-heparin complexes, together with thrombocytopenia and multiple thromboses, beginning about 1 wk after a single 5000-unit injection of heparin (Warkentin and Bernstein, 2003). Sera from patients with delayed-onset HIT activate platelets strongly in vitro even in the absence of added heparin. The most likely explanation is that these patients develop autoantibodies that recognize PF4 bound to platelet surface GAGs.

Potentially, other obscure factors can induce the HIT antigen. This hypothesis is strengthened by the observation that on exceptionally rare occasions, patients can be identified with a thrombocytopenic, prothrombotic disorder that serologically mimics HIT but in whom no previous heparin exposure can be identified (Warkentin et al., 2006a). A related observation could be the finding

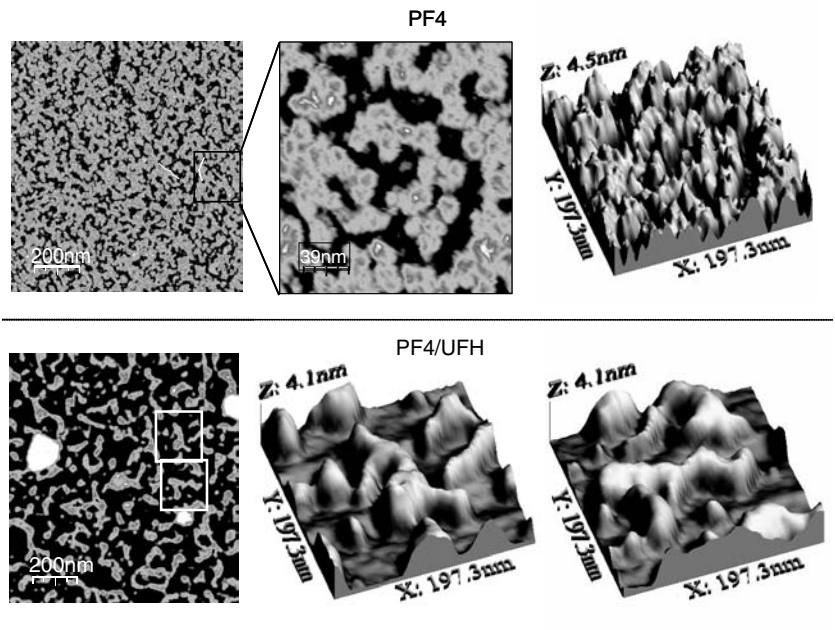


FIGURE 3 Atomic force microscopy of PF4 (*upper panel*) and PF4/UFH complexes (*lower panel*) shows the structural changes that PF4 undergoes when it complexes to UFH. PF4 molecules maintain a certain distance to each other due to their strong positive charges. When charges are neutralized by negatively charged heparin, PF4 molecules form linear, ridge- like clusters. *Abbreviations*: PF4, platelet factor 4; UFH, unfractionated heparin. *Source*: From Greinacher et al., 2006.

that blood obtained from some patients with acute myocardial infarction contains anti-PF4/heparin antibodies, despite apparent absence of previous exposure to heparin (Suzuki et al., 1997). Perhaps, antibodies with cross-reactivity to PF4–heparin complexes may have been generated against such endogenous GAG-PF4 complexes, even without preceding heparin treatment. Whether such antibodies contribute to the pathogenesis of cardiac ischemia is not established. A retrospective analysis of patients with acute coronary syndrome found that the presence of anti-PF4/heparin antibodies at onset was associated with a higher risk of acute myocardial infarction and death (Williams et al., 2003). This observation requires prospective confirmation.

B. Effects of HIT Antibody-Containing Immune Complexes

HIT antibodies bind to PF4-heparin complexes by their $F(ab')_2$ domains (Horne and Alkins, 1996; Newman and Chong, 2000). The predominant immunoglobulin isotype in clinical HIT is IgG (Amiral et al., 1996b; Warkentin et al., 2005b; Juhl et al., 2006). Thus, divalent IgG binding to multimolecular PF4-heparin complexes, leads to the formation of large immune complexes containing HIT-IgG on the platelet surface. The interaction of the HIT-IgG Fc with the platelet FcγIIa receptors leads to cross-linking of these receptors on the same or adjacent platelets, which triggers platelet activation (Kelton et al., 1988, 1994; Chong et al., 1989a) (see Chapter 8). The HIT antibody–mediated platelet activation can be inhibited by a

monoclonal antibody specific for the FcγIIa receptor, by high concentrations of Fc fragments derived from normal IgG, and by excess heparin saturating all binding sites on PF4, and thus preventing the formation of multimolecular complexes (Greinacher et al., 1994b; Visentin et al., 1994).

Besides these effects on platelets, polyclonal HIT antibodies bind to endothelial cells (Cines et al., 1987; Visentin et al., 1994). The most convincing evidence demonstrating that these antibodies are the same ones that cause platelet activation was provided by classic adsorption-elution experiments (Greinacher et al., 1994a). Purified IgG obtained from sera of HIT patients gave positive reactions in both activation (serotonin release) and antigen (anti-PF4/heparin) assays. This IgG fraction was then incubated with cultured endothelial cells and, after extensive washing, the antibodies were eluted from the endothelial cells by low pH. The eluate again tested positive in both activation and antigen assays. Thus, these experiments showed that the antibodies recognize the same epitope on platelets, endothelial cells, and PF4-heparin complexes coated onto a microtiter plate. It appears most likely that the epitope on endothelial cells comprises surface GAGs (Cines et al., 1987; Greinacher et al., 1994a; Visentin et al., 1994).

In addition to platelet and endothelial cell activation, there is concomitant activation of coagulation, as shown by marked elevations in thrombin-AT complex levels (Warkentin et al., 1997; Greinacher et al., 2000). The simultaneous activation of platelets, endothelium, and coagulation factors is in line with the development of thrombocytopenia combined with thrombosis and disseminated intravascular coagulation in patients with HIT (see Chapter 2).

C. Importance of HIT Antibodies in Clinical HIT

HIT antibodies occur commonly in heparin-treated patients. However, as many patients develop neither thrombocytopenia nor thrombosis (Amiral et al., 1996a; Kappers-Klunne et al., 1997; Arepally et al., 1997), it is evident that pathogenicity requires additional factors. Possible factors are number and size of the antigen complexes (Rauova et al., 2005; Greinacher et al., 2006), antibody class (Warkentin et al., 2005b; Juhl et al., 2006), and titer of the HIT antibodies (Suh et al., 1997), as well as optimal concentrations of heparin and PF4 in the blood circulation, which enable the formation of macromolecular PF4-heparin antigen complexes (Horne and Alkins, 1996; Horne and Hutchison, 1998). Thus, during low-dose heparin prophylaxis in a setting of minimal platelet activation, clinical HIT may occur less often than in patients with activated platelets receiving high heparin doses (Fondu, 1995). Accordingly, HIT antibodies are most frequently induced by UFH in patients following cardiopulmonary bypass surgery (∼50%), followed by patients undergoing major orthopedic surgery (∼15%), and least frequently in medical patients (∼3%) (see Chapter 3).

Besides MW, DS and concentration of heparin, additional factors favoring the development of clinical HIT are prethrombotic or inflammatory situations (e.g., open heart surgery) (Visentin et al., 1996), greater susceptibility of the platelets to activation by HIT antibodies (Salem and van der Weyden, 1983), perhaps mediated by increased PF4 binding to platelets (Capitanio et al., 1985), or expression of PF4 (Rauova et al., 2006) and FcγIIa receptors (Chong et al., 1993) on the platelet surface. Further, the polymorphism of the FcγIIa receptor at position Arg-His^{131} seems to be associated with a predisposition to HIT (Carlsson et al., 1998). Another poorly understood phenomenon is gender imbalance in HIT,

TABLE 2 Characteristics of Commercial Low Molecular Weight Heparins

INN (brand name)	Degradation method	Mean MW (kDa)	Anti-Xa (U/mg)	Anti-Xa:anti-IIa ratio[a]
Ardeparin sodium (Normiflo®)	Peroxidation at elevated temperature	4.0–6.0	120 ± 25	1.7–2.4
Bemiparin sodium[b] (Hibor®)	Basic degradation in a nonaqueous media and fractionation	3.6	80–90	8.1
Certoparin sodium (Mono-Embolex® NM)	Hydrolysis with isoamylnitrite	4.2–6.2	80–120	1.5–2.5
Dalteparin sodium[c] (Fragmin™)	Hydrolysis with HNO_2 and fractionation	5.6–6.4	110–210	1.9–3.2
Enoxaparin sodium[c] (Clexane®, Lovenox®)	Benzylation and alkaline β-elimination	3.8–5.0	95–125	3.3–5.3
Nadroparin sodium[c] (Fraxiparin®)	Hydrolysis with HNO_2 and fractionation	3.6–5.0	95–135	2.5–4.0
Parnaparin sodium[c] (Fluxum®)	Radical-catalyzed degradation with H_2O_2 and Cu salts	4.0–6.0	75–110	1.5–3.0
Reviparin sodium (Clivarin®)	Hydrolysis with HNO_2	3.5–4.5	105	3.6–6.3
Tinzaparin sodium[c] (Innohep®)	Enzymatic (heparinase) β-elimination	5.6–7.5	70–120	1.5–2.5

[a]Ratio of anti-Xa activity (IU/mg) to anti-IIa activity (IU/mg).
[b]Bemiparin is the first example of the second-generation LMWHs, which are defined to have mean MW < 4.0 kDa, a proportion of fragments > 6.0 kDa < 15%, and an anti-Xa:anti-IIa ratio > 4 : 1.
[c]From Monographs in European Pharmacopoeia, 5th ed.
Abbreviations: INN, International Nonproprietary Name; MW, molecular weight.

with females having a higher risk of HIT than males, especially with UFH treatment (Warkentin et al., 2006b).

IV. CROSS-REACTIVITY OF HIT ANTIBODIES WITH OTHER SULFATED CARBOHYDRATES

A. Interactions with LMWHs

Generation of the HIT antigen depends not only on the concentration, but also on the chain length of heparin. LMWH preparations (Table 2) have reduced affinity for platelets, endothelial cells, and plasma proteins, such as PF4 (Horne, 1993; O'Brien et al., 1985; Turpie, 1996). Accordingly, LMWHs are less likely to form multimolecular complexes with PF4 (Greinacher et al., 1993; Rauova et al., 2005). Hence, LMWHs are less likely than UFH to induce an immune response, resulting in a lower frequency of HIT (Warkentin et al., 1995; Greinacher et al., 2005; Martel et al., 2005; Warkentin et al., 2006b).

Despite their lower immunogenicity, LMWHs exhibit nearly 100% in vitro cross-reactivity to HIT antibodies using sensitive assays (Greinacher et al., 1994b,c; Warkentin et al., 1995; Amiral et al., 1996b; Amiral, 1997). The small variations found with different LMWH preparations in vitro are probably based on their individual composition of molecules with different chain length (Fareed et al., 1988). Homogeneous heparin molecules consisting of 20, 18, 16, 14, and 12 monosaccharides form antigenic multimolecular complexes to which HIT antibodies bind strongly. Fragments containing 10 residues form complexes with PF4 which are recognized by the antibodies only weakly as judged by platelet-activation assay, while fragments containing eight and six residues are even

less reactive (Amiral et al., 1995; Greinacher et al., 1995) or non-reactive (Visentin et al., 2001).

B. Interactions with Other Sulfated Carbohydrates

The formation of platelet-activating immune complexes is not limited to heparin (Greinacher et al., 1992, 1993). Various other sulfated polysaccharides, and even polyvinylsulfonate, bind PF4 to form antigen complexes recognized by HIT antibodies. This cross-reaction depends on their structure, especially on their DS and MW (Greinacher et al., 1992, 1995; Kelton et al., 1994; Amiral et al., 1995). In vitro assays demonstrate that pentosan polysulfate, dextran sulfate, as well as a high-sulfated chondroitin sulfate, and a highly sulfated polysaccharide (PI-88) developed for anti-tumor treatment (Rosenthal et al., 2002) can substitute for heparin. In contrast, neither dextran, dermatan sulfate, *N*-desulfated heparin, sulfated glucosamine (Weimann et al., 2001), nor the AT-binding pentasaccharide react in these assays. Accordingly, pentosan polysulfate, high-sulfated chondroitin sulfate, and PI-88 have induced thrombocytopenia and thrombosis in vivo (Greinacher et al., 1993; Tardy et al., 1994; Rosenthal et al., 2002). The corresponding antibodies can be detected by conventional PF4-heparin enzyme immunoassay (PF4-H EIA) demonstrating the cross-reactivity with heparin (Gironell et al., 1996).

C. Relation Between the Anticoagulant Activity of β-1, 3-Glucan Sulfates and Their Cross-Reaction with HIT-Associated Antibodies

To establish the structural requirements for the anticoagulant activity of sulfated carbohydrates, as well as for the development of platelet-activating immune complexes in the presence of HIT antibodies, we synthesized structurally well-defined sulfated polysaccharides (Greinacher et al., 1995). The resulting β-1,3-glucan sulfates (GluS) varied in their DS, MW, sulfation pattern, and chemically introduced glycosidic side chains (Fig. 4). Although these compounds differ structurally from heparin, they exhibit structure-dependent anticoagulant as well as antithrombotic activities (Alban et al., 1995; Franz and Alban, 1995). They also induce platelet activation in the presence of HIT antibodies (Greinacher et al., 1995). Therefore, neither uronic acids, amino groups, nor the α-1,4- or β-1, 4-glycosidic linkages found in heparin are essential for these biological properties.

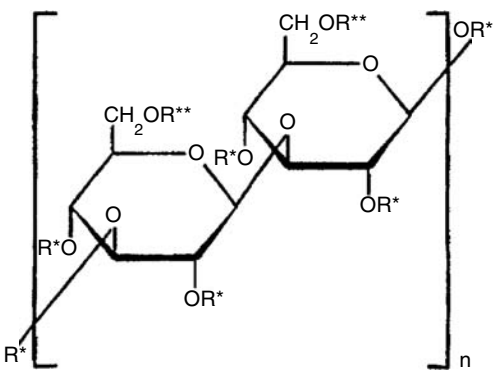


FIGURE 4 Repeating unit of β-1, 3-glucan sulfates: The primary OH group in position 6(**) is preferentially sulfated. Glycosidic-branched β-1, 3-glucan sulfates are substituted by a glucose, rhamnose, or arabinose unit, respectively, in position 6.

An increase in the DS results in improved anticoagulant activity and, after binding to PF4, in increased formation of HIT antibody-binding sites. The MW is a second important structural parameter for anticoagulant potency of a sulfated polysaccharide, as well as its capacity to cause platelet activation in the presence of HIT antibodies. Fractions with hydrodynamic volumes between 38 and 60 kDa showed the most prominent effects (Alban and Franz, 1994a; Greinacher et al., 1995) (the hydrodynamic volumes were determined by gel filtration chromatography using neutral pullulans as MW standards; since these have lower hydrodynamic volumes owing to the missing sulfate groups, the measured hydrodynamic volumes are higher than the real MW; e.g., UFH had a mean hydrodynamic volume of 30 kDa). Therefore, this MW range, which corresponds to that of the so-called extra large material of UFH, seems to represent the optimal chain length both for the interaction with proteins involved in the coagulation cascade as well as with PF4 to form HIT antigens. Beyond the optimal chain length, higher concentrations are required to form multimolecular PF4-GluS complexes (Greinacher et al., 1995).

Compared with linear GluS having similar DS and MW, glycosidically branched compounds generally exhibit higher anticoagulant activity than the respective linear derivatives (Alban, 1993, 1997). Glycosidic substitution changes the 3D structure of the polysaccharide chain, resulting in enhanced flexibility, and improved the interaction with proteins (Kindness et al., 1980). In addition, as the side chains are more accessible to sulfation, they represent clusters of negative charges (Alban and Franz, 1994b) facilitating binding to PF4, which results in an increased cross-reactivity with HIT antibodies.

V. IMPLICATIONS FOR THE DEVELOPMENT OF CARBOHYDRATE-BASED HEPARIN ALTERNATIVES

A. Structural Requirements of Carbohydrate-Based Heparin Alternatives

A carbohydrate-based antithrombotic drug with a reduced risk of inducing HIT antigen(s) should meet the following criteria (Greinacher et al., 1995):

The molecule should not be branched to reduce its flexibility and to minimize charge clusters.

Its DS should be lower than 1.0 per monosaccharide, if its chain length exceeds 10 monosaccharides.

Its MW should be lower than 2.4 kDa (about seven monosaccharides), if its DS is higher than 1.0.

If the MW is higher than 2.4 kDa and the DS higher than 1.0, then at least the therapeutic concentration must be lower than that exhibiting cross-reactivity with HIT antibodies.

B. Danaparoid

Danaparoid sodium (Orgaran) is an alternative anticoagulant that is effective for treating patients with HIT (see Chapter 13). This heparinoid consists of a depolymerized mixture of GAGs extracted from porcine intestinal mucosa, with a mean MW of 4–7 kDa. Its components are approximately 80% low molecular weight heparan sulfate, 10% dermatan sulfate, 5% chondroitin sulfate, and a small proportion of heparan sulfate (4%) with high affinity for AT (Meuleman, 1992). Apart from the minor AT-binding heparan sulfate component, the constituents of

danaparoid have a DS per monosaccharide between 0.5 and 0.7, as well as a low MW. Thus, the two important requirements to form multimolecular complexes with PF4 are not met. This is consistent with the low cross-reactivity rate of danaparoid (about 10%) (Wilde and Markham, 1997) (see Chapters 10 and 13). As danaparoid inhibits platelet activation by HIT antibodies even in the presence of heparin (Chong et al., 1989b), it is possible that the GAG mixture binds to PF4 without producing the antigen. Consequently, less PF4 is available for the small amount of higher-sulfated heparan sulfate molecules responsible for AT binding and, presumably, PF4 binding resulting in cross-reactivity with HIT antibodies (Greinacher et al., 1992).

C. Pentasaccharides

Within the scope of developing new carbohydrate-based antithrombotics, fondaparinux, a fully synthetic, chemically defined pentasaccharide (formerly named Org31540/SR90107A, MW = 1728 Da; DS = 1.6; 700 anti-Xa U/mg), has been developed, which corresponds to the AT-binding site of heparin (Petitou et al., 1997) (Fig. 5; see also Chapter 17). By its highly specific binding to AT, fondaparinux selectively inhibits factor Xa and thus prevents thrombin generation (Bauer et al., 2002). Fondaparinux is approved for thrombosis prophylaxis and treatment (see Chapters 1 and 17).

Fondaparinux does not cross-react with HIT antibodies in any concentration tested, either in the PF4-H-EIA or in the serotonin-release assay (Amiral et al., 1997; Greinacher et al., 1995; Ahmad et al., 1999). Immune thrombocytopenia attributable to fondaparinux has not been observed in any of the clinical studies. However, patients treated with fondaparinux in clinical trials generated anti-PF4/heparin antibodies at a similar frequency as observed in patients treated with LMWH (enoxaparin). These antibodies tested positive in a PF4-dependent EIA and caused platelet activation in vitro in the presence of added heparin, although no cross-reactivity with fondaparinux itself could be shown (Warkentin et al., 2005a). It has

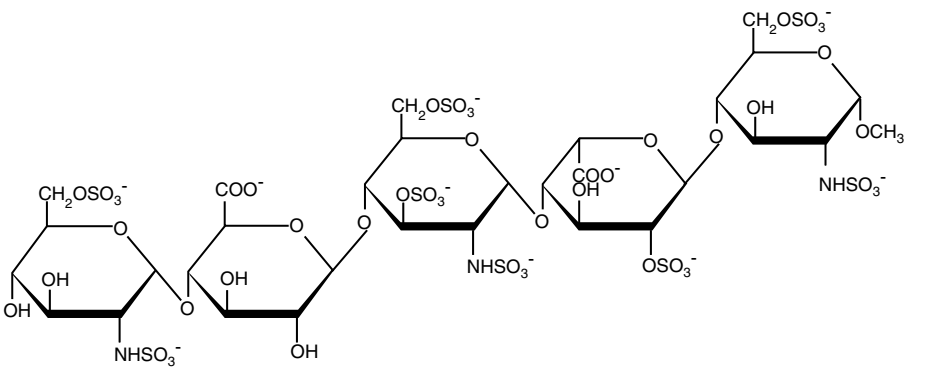


FIGURE 5 Chemical structure of the synthetically produced pentasaccharide, fondaparinux (formerly, Org31540/SR90107A; MW = 1728 Da; DS = 1.6; 864 anti-Xa U/mg), with eight sulfate groups corresponding to the natural antithrombin-binding site. *Abbreviations*: DS, degree of sulfation; MW, molecular weight.

been hypothesized that PF4 forms only few and potentially relatively unstable multimolecular complexes in the presence of fondaparinux. These few complexes can trigger the immunization but are too sparse to mediate relevant platelet activation (Greinacher et al., 2006).

In support of this concept, we have observed in our laboratory that a more highly sulfated pentasaccharide, Org 32701 (MW = 1991 Da; DS = 2. 0) (Herbert et al., 1996) (Fig. 6), induces platelet activation in the presence of HIT antibodies. This demonstrates that certain highly sulfated oligosaccharides are indeed able to bind to PF4 and thus to form the HIT neoantigen. But, whether such a highly sulfated pentasaccharide itself could induce clinical HIT cannot yet be answered.

D. Specifically Designed Oligosaccharides

Pentasaccharides such as fondaparinux or the long-acting idraparinux (Herbert et al., 1998) have minimal, if any, undesirable interactions with blood and vessel components, but their anticoagulant activity is limited to AT-mediated factor Xa inhibition. Additional thrombin inhibitory properties might further improve the anticoagulant efficacy of heparin-related oligosaccharides. Unfortunately, as with heparin, lengthening the sulfated oligosaccharide chain increases nonspecific binding that could have undesirable effects, such as binding to PF4 and associated risk of HIT. Thus, Petitou and coworkers (1999) synthesized "heparin mimetics" that inhibited thrombin, but failed to bind other proteins, particularly PF4. The most promising structure is the hexadecasaccharide SR123781A, which is undergoing clinical evaluation (Herbert et al., 2001). It is obtained from glucose through a convergent synthesis and consists of an AT-binding pentasaccharide sequence linked to a thrombin-binding domain via a neutral methylated hexasaccharide "spacer." It specifically catalyzes the AT-mediated inhibition of factor Xa (IC50 = 77 ± 5 ng/mL, 297 ± 13 U/mg) and thrombin (IC50 = 4.0 ± 0.5 ng/mL, 150 ± 30 U/mg), without any effect on heparin cofactor II and without binding to PF4. Compared with UFH and LMWH in animal studies, SR123781A exhibited a highly favorable antithrombotic bleeding ratio. This compound did not activate platelets in the presence of plasma from HIT patients.

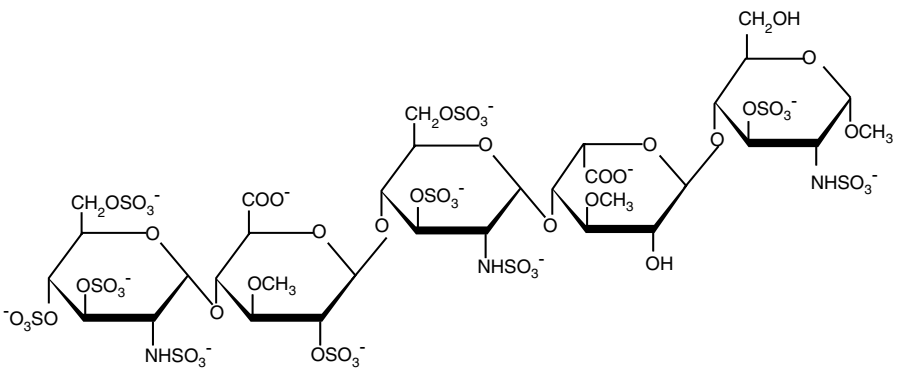


FIGURE 6 Chemical structure of the synthetically produced pentasaccharide, Org 32701 (MW = 1991 Da; DS = 2; 1150 anti-Xa U/mg), with a higher degree of sulfation (10 sulfate groups) than the natural antithrombin-binding site. *Abbreviations*: DS, degree of sulfation; MW, molecular weight.

E. Conclusions

From experiments with well-defined GluS, the various structural requirements for a sulfated carbohydrate to form the HIT antigen have become clear. Given this detailed knowledge, at least three carbohydrate-based anticoagulant options can be proposed that should have a negligible risk for inducing clinical HIT:

- Mixtures of GAGs consisting predominantly of low-sulfated carbohydrates with correspondingly limited capacity to form antigenic complexes with PF4: A prototype of such an anticoagulant is danaparoid.
- Oligosaccharides with antithrombotic activity similar to the AT-binding pentasaccharide: A prototype is fondaparinux.
- GAGs with highly sulfated, but short, regions that are connected by nonsulfated "spacers": a prototype is the hexadecasaccharide SR123781A (Petitou et al., 1999).

The increasing use of LMWH already seems to have reduced the incidence of HIT. We propose that the problem of HIT can be avoided further by using anticoagulants meeting the foregoing outlined criteria in our treatment arsenal.

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8 Platelet and Leukocyte Fcγ Receptors in Heparin-Induced Thrombocytopenia

Gregory A. Denomme
University of Toronto, Canadian Blood Services and Mount Sinai Hospital, Toronto, Ontario, Canada

I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a unique immune-mediated disorder. HIT is common, occurring in as many as 5% of certain patient populations. Affected patients can develop a paradoxical thrombotic episode, rather than bleeding, despite having thrombocytopenia. One possible reason for the unique clinical profile is the central role of platelet Fcγ receptor (FcγR) IIa in mediating platelet activation in HIT. Indirect evidence suggests a crucial role for platelet activation in the pathogenesis of HIT since thrombocytopenia and the presence of HIT antibodies are strongly associated with thrombosis, whereas formation of antibodies without thrombocytopenia is not (Warkentin et al., 1995). Notwithstanding the role that platelet FcγRs play in platelet activation, leukocyte FcγRs could also contribute to the pathogenesis of HIT.

It has been known for several years that HIT results from a predominant IgG immune response to antigenic determinants involving platelet-bound heparin (Green et al., 1978). Thus, the pathogenesis of HIT resembles a type II immune reaction, i.e., a cytotoxic antibody response (Roitt et al., 1985), and a murine model of HIT supports a T-cell dependent immune response (Suvarna et al., 2005). The typical features of a type II immune response, such as phagocytosis, killer-cell activity, or complement-mediated lysis, do not seem to predominate in HIT. Instead, thrombocytopenia results primarily from FcγRIIa-mediated platelet activation, aggregation, and granule release (Chong et al., 1981) as a result of IgG binding to platelet factor 4-heparin (PF4-H) complexes on the platelet surface. Furthermore, HIT antibodies activate endothelium in vitro by interaction with PF4-heparan sulfate complexes (Cines et al., 1987; Greinacher et al., 1994a; Visentin et al., 1994). However, unlike platelets, human endothelium (with the exception of placental villous endothelial cells and a subset of endothelial cells found in the superficial dermal vascular plexus) does not express any FcγRs, either constitutively or in the setting of immune complex diseases (Sedmak et al., 1991; Gröger et al., 1996). Thus, platelet activation and endothelial activation in HIT probably arise from fundamentally distinct processes. Other effects of HIT include the formation of platelet-leukocyte aggregates, the release of tissue factor (TF) from monocytes, and the FcγRIIIa-dependent phagocytosis or natural killer (NK) cell destruction of antibody-sensitized platelets (Khairy et al., 2001; Pouplard et al., 2001; Gruel et al., 2004).

One of the most important unanswered questions in the pathophysiology of this disorder is an explanation for why only a few patients who develop HIT antibodies become thrombocytopenic. This problem has led investigators to study the role of FcγRIIa in explaining, at least partly, the heterogeneous clinical sequelae

among patients with HIT. This chapter will (1) review the structure and function of the platelet FcγRIIa; (2) describe the mechanism of HIT antibody-induced platelet activation by FcγRIIa; and (3) summarize the studies that have attempted to identify the role of FcγRs in modifying the clinical manifestations of HIT.

II. FcγR STRUCTURE, DISTRIBUTION, AND FUNCTION

FcγRIIa is a member of a family of structurally related glycoproteins (GPs), many of which are expressed on hematopoietic cells (Table 1). Twelve different transcripts have been reported, derived from eight genes grouped into three different classes: I, II, and III (van de Winkel and Capel, 1993; Rascu et al., 1997; Gessner et al., 1998). Allelic polymorphic variants add yet another level of diversity for FcγRIIa, FcγRIIIa, and FcγRIIIb. The genomic organization of the FcγR genes on chromosome lq23 was resolved by Su and coworkers (2002). The multigenic region at 1q21-23 is approximately 1 mb in size and is in the following gene order and orientation: centromere—*FCGR2A*(5′-3′)—*FCGR3A*(3′-5′)—*FCGR2C*(3′-5′)—*FCGR3B*(3′-5′)—*FCGR2B*(5′-3′)—telomere.

The affinity for IgG varies among isoforms and variants. FcγRIIa-His^{131} has a higher affinity for human IgG2 than FcγRIIa-Arg^{131} (Warmerdam et al., 1991). Furthermore, FcγRIIIa-Val^{158} and FcγRIIIb-NAl bind nearly twice as much IgG as FcγRIIIa-Phe^{158} and FcγRIIIb-NA2, respectively (Salmon et al., 1990; Koene et al., 1997). One nonfunctional FcγRIIc variant has a nonsense codon, which results in a null phenotype (Metes et al., 1998). When cross-linked, each isoform participates in biological activities through distinct signal transduction pathways that affect cell functions including antigen presentation, immune complex clearance, phagocytosis and the oxidative burst, release of cytokines and intracellular granular mediators, antibody-dependent cellular cytotoxicity (ADCC), and downregulation of antibody production or phagocytosis.

Only FcγRIIa is expressed on platelets (Rosenfeld et al., 1985; Kelton et al., 1987). The receptor is a single α-chain, 40-kDa GP, with an extracellular region consisting of two immunoglobulin-like, disulfide-linked domains responsible for ligand binding, a transmembrane region, and an intracellular domain that incorporates an immunoreceptor tyrosine-based activation motif (ITAM) essential for intracellular signal transduction (Qiu et al., 1990; Brooks et al., 1989). A soluble form of the receptor is produced by alternative splicing of primary RNA transcripts to exclude exon 5 containing the transmembrane region (Rappaport et al., 1993).

The extracellular domain of FcγRIIa shares 96% amino acid identity with FcγRIIb and FcγRIIc (Brooks et al., 1989). A G → A polymorphism at nucleotide position 519 of the cDNA (position 535 in Genbank Reference Sequence NM_021642) is responsible for the Arg/His^{131} functional variants (Clark et al., 1989). An additional polymorphism, an A → G at nucleotide 207 of the cDNA, results in a Gln-Trp^{27} substitution in the mature polypeptide (Warmerdam et al., 1991). However, the Arg-His^{131} position is near or within the binding region for IgG Fc (Hulett et al., 1995), and it is this polymorphism that is associated with the affinity differences for human IgG2 (Warmerdam et al., 1991). More recently, another polymorphism proximal to Arg^{131} that affects IgG2 binding has been found in a single healthy individual (Norris et al., 1998): A lysine substitution for glutamine at position 127 demonstrated a significant increase in FcγR-mediated phagocytosis in this homozygous FcγRIIa-Arg^{131} individual.

TABLE 1 The Family of Fcγ Receptors: Molecular and Structural Characteristics and Tissue Distribution

	FcγRI (CD64)	FcγRII (CD32)	FcγRIII (CD16)
Genes	IA, IB, IC	IIA, IIB, IIc	IIIA, IIIB
Functional variants[a]	None	IIa: Gln/Lys^{127} IIa: Arg/His^{131}	IIIa: Leu/Arg/His^{48} IIIa: Phe/Val^{158}
		IIc: Gln/stop codon^{13}	IIIb: NA1/NA2
			IIIb: Ala/Asp^{60} (SH)
RNA transcripts[b]	IA1 IB1, IB2	IIA1, IIA2 IIB1, IIB2, IIB3	IIIA IIIB
	IC	IIC	
Glycoprotein expressed[c]	Ia	IIa1, sIIa2 IIb1, sIIb2, IIb3	IIIa IIIb (GPI linked)
		IIc	
Molecular weight (kDa)	72	40	50–80
Extracellular Ig-like domains	3	2	2
Intracellular tyrosine motif[d]	None	ITAM (IIa, IIc) ITIM (IIb)	None
Noncovalent-associated subunit	γ-chain	None	β-chain, γ-chain, ζ-chain
Affinity constant	10^8 M^{-1}	$<10^7$ M^{-1}	IIIa: 3×10^7 M^{-1} IIIb: $<10^7$ M^{-1}
IgG subclass avidity[e]	3 = 1 > 4 >>>> 2	IIa-Arg^{131}3 > 1 >>> 2 > 4 IIa-His^{131}3 > 1 = 2 > 4	IIIa-Val^{158}1 > 3 >>> 2,4 IIIa-Val^{158} > IIIA-Phe^{158} for 1 and 3
		IIb1 3 > 1 > 4 >>>> 2	IIIb-NA1 > IIIb-NA2 for 1 and 3
Hematopoietic cell distribution	CD34 progenitor cells, monocytes, macrophages, dendritic cells	IIa: platelets, endothelial cells, monocytes, macrophages, eosino-/baso-/neutrophils, Langerhans/dendritic cells	IIIa: monocytes, macrophages, NK cells, T cells
		IIb: B cells, monocytes	IIIb: neutrophils
		IIc: NK cells	

[a]Allelic polymorphisms that show differences in IgG binding; NA1/NA2 variants have multiple amino acid differences (Ory et al., 1989); SH^+ individuals (Bux et al., 1997) carry three copies of FcγRIIIb (Koene et al., 1998); the FcγRIIc null phenotype is due to a nonsense mutation (Metes et al., 1998).
[b]Multiple mRNA transcripts from FcγRIb, IIa, and IIb are the result of alternative splicing of primary transcripts.
[c]Soluble forms of FcγRIIa and IIb (sIIa, sIIb) are devoid of the hydrophobic transmembrane exon; GPI, glycosyl-phosphatidylinositol.
[d]ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif.
[e]Numbers represent the relative order of IgG subclass binding to variants of FcγRIIa (Warmerdam et al., 1990), FcγRIIIa (Koene et al., 1997; Wu et al., 1997), and FcγRIIIb (Salmon et al., 1990; Bredius et al., 1994a).
Abbreviation: NK, natural killer.

FcγRIIa has low affinity for IgG ($<10^7$ M^{-1}) and interacts mainly with antigen-antibody complexes (Warmerdam et al., 1991; Parren et al., 1992). The copy number of FcγRIIa expressed on resting platelets varies among healthy individuals but is stable (Rosenfeld et al., 1987). There is roughly a 3-fold variation among individuals,

with the copy number ranging from 600 to 1500 molecules per platelet when assayed using an intact murine monoclonal antibody IV.3 (McCrae et al., 1990) and 1500 to more than 4500 when tested with a Fab preparation (Tomiyama et al., 1992; Brandt et al., 1995). There are no major differences in FcγRIIa copy number between sexes, among platelets from persons of different ages, or for the three genotypic classes of FcγRIIa-Arg/His^{131} (Brandt et al., 1995).

III. IMMUNOGLOBULIN G AGONISTS AND PLATELET ACTIVATION

A. FcγIIaR-Mediated Platelet Activation

Murine monoclonal IgG1 anti-CD9 were among the first studied for their platelet-activating properties. Subsequently, it was determined that monoclonal antibodies to GPIIb/IIIa, β_2-microglobulin, GPIV (CD36), and other selected antigens can activate platelets (Rubinstein et al., 1995). In each instance, platelet activation occurs by a consistent mechanism: first, the variable region of the antibody binds to its cognate antigen; then the Fc portion of the antibody interacts with platelet FcγRIIa. Evidence for FcγRIIa dependency includes the inhibition of platelet activation by the murine monoclonal anti-FcγRIIa antibody (IV.3). However, some platelet GPs (e.g., GPIb) do not support activation by monoclonal antibodies; others support activation despite their usual sequestered location within platelets (e.g., GPIa*), and still other GPs (e.g., GPIIb/IIIa) support activation by only certain monoclonal antibodies (Horsewood et al., 1991). These observations suggest that specific factors such as target protein membrane mobility and localization of the epitope, which permit formation of multimolecular GP antigen-IgG-FcγRIIa complexes, are crucial for FcγRIIa-mediated platelet activation. The IgG can interact with either FcγRIIa on the same platelet (intraplatelet activation) or with FcγIIaRs located on other platelets in close proximity (interplatelet activation) (Anderson et al., 1991; Horsewood et al., 1991).

Complexed human IgG is also a potent stimulator of platelet activation. Karas and coworkers (1982) showed that trimeric human IgG and larger immune complexes had significant affinity for platelet FcγRIIa. King et al. (1990) showed that a minimum of trimeric IgG molecules are necessary for platelet activation. Heat-aggregated IgG also is a potent agonist for platelet activation (Warkentin et al., 1994; Warkentin and Sheppard, 1999), as are streptokinase–anti-antistreptokinase complexes (Lebrazi et al., 1995) and PF4-H immune complexes (Greinacher et al., 1994a).

HIT-IgG causes the generation of thromboxane A2 and associated platelet granule release (Chong et al., 1981). Indeed, several different "activation assays" have been developed that detect HIT antibodies by their ability to cause resting platelets to aggregate (Greinacher et al., 1991), effect granule release (Sheridan et al., 1986), or generate platelet-derived microparticles (Warkentin et al., 1994) (see Chapter 10).

B. Procoagulant, Platelet-Derived Microparticles

Platelet activation by various agonists leads to procoagulant alterations of the platelet membrane. This includes loss of the usual membrane asymmetry (i.e., with platelet activation, there is increased transbilayer movement of phosphatidylserine from the inner to the outer leaflet of the platelet plasma membrane). The membrane "flip-flop" is a consequence of a calcium-dependent enzyme ("scramblase") that serves to undo the membrane asymmetry actively maintained

in resting platelets by other enzymes (aminophospholipid translocase and "floppase") (Bevers et al., 1999). Additionally, platelet activation also leads to profound morphological changes that include the generation of procoagulant platelet-derived "microparticles" (Sims et al., 1989).

Serum and purified IgG from patients with HIT, as well as immune complexes and murine platelet-activating monoclonal IgG, also generate platelet-derived microparticles via the platelet FcγRIIa; in contrast, quinine- and quinidine-dependent sera do not produce microparticles, even though they lead to far greater drug-dependent binding of IgG to platelets, compared with HIT samples (Warkentin et al., 1994). Indeed, HIT serum is superior in generating platelet-derived microparticles and in producing platelet procoagulant activity than thrombin, collagen, and adenosine diphosphate (ADP); only the nonphysiological agonist calcium ionophore produces greater numbers of microparticles and procoagulant activity than does HIT sera (Warkentin and Sheppard, 1999).

Flow cytometry using particle size ("forward scatter") and fluorescein-labeled platelet, GP-specific monoclonal antibodies can detect platelet-derived microparticles generated by HIT antibodies (Warkentin et al., 1994; Hughes et al., 2000). This technique has been used as a diagnostic assay for HIT (Lee et al., 1996). Alternatively, Tomer (1997) used fluorescent-labeled annexin V, a protein that binds to phosphatidylserine, to detect activated platelets and microparticles (see Chapter 10).

There is some uncertainty as to whether the "microparticles" detected by flow cytometry represent true microparticles or rather platelets that have undergone considerable morphological changes during activation. Use of orthogonal light scatter, combined with fluorescence gating on platelet antigens, detects significant increases in total particle count, suggesting that at least some microparticles are generated (Bode and Hickerson, 2000). Moreover, microparticles being generated by HIT antibodies is suggested by a study that used confocal microscopy and scanning/transmission electron microscopy for their detection (Hughes et al., 2000) (Fig. 1).

C. ADP Potentiation of Platelet Activation

ADP is an important autocrine stimulator of platelet activation by HIT-IgG (Chong et al., 1981). This observation was confirmed by Anderson and Anderson (1990) who showed that in vitro FcγRIIa-mediated platelet activation was augmented by ADP. Although ADP potentiates platelet activation by many agonists, Polgár and coworkers (1998) found that pretreatment of platelets with a potent ADP receptor antagonist completely blocked the activity of HIT sera. This observation indicates that ADP and a functional ADP receptor are crucial to FcγRIIa activation by HIT-IgG. However, it should be pointed out that patients receiving ADP receptor antagonists (e.g., clopidogrel) can still develop HIT and HIT-associated thrombosis (Selleng et al., 2005).

D. FcγR-Mediated Signal Transduction

FcγRIIa activation, as a result of IgG binding and by action of phosphatidylinositol 3-kinase (PI 3-kinase) and phospholipase C-γ2 (PLCγ2), leads to release of diacylglycerol (DAG) and inositol triphosphate (IP3), mobilization of internal calcium stores, and, subsequently, platelet aggregation (Anderson and Anderson, 1990). Initially, IgG binding leads to FcγRIIa clustering, resulting in the phosphorylation of FcγRIIa ITAMs by Src family protein tyrosine kinases (Chacko et al., 1994;

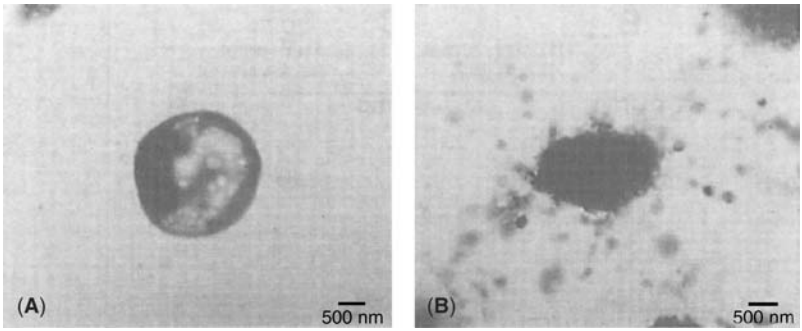

FIGURE 1 Electron microscopy of negatively stained platelets activated in situ with HIT serum. Platelets were allowed to settle on bovine serum albumin-coated Formvar grids and then incubated with (**A**) serum testing negative for HIT antibodies or heparin (not shown) or (**B**) HIT serum in the presence of heparin, 0.1 U/mL. Platelets were then fixed with 2% glutaraldehyde and negatively stained with 2% phosphotungstic acid. Whereas unactivated platelets demonstrated round or discoid shapes (see A), platelets activated by HIT serum demonstrated numerous surrounding microparticles ranging in size from <0.1 to 1.0 μm in diameter. (Original magnification ×13,000.) *Abbreviation*: HIT, heparin-induced thrombocytopenia. *Source:* From Hughes et al., 2000.

Huang et al., 1992). Following FcγRIIa phosphorylation, tyrosine kinase activity (e.g., $p72^{syk}$) and PI 3-kinase activity increase through the noncovalent interaction of their SH2 domains with phosphorylated FcγRIIa ITAMs (Greinacher et al., 1994b; Yanaga et al., 1995; Chacko et al., 1996). Subsequently, PLCγ2 is phosphorylated by $p72^{syk}$ (Blake et al., 1994), which is dependent on phosphatidylinositol-trisphosphate (PtdIns[3,4,5]P3) (Gratacap et al., 1998). PLCγ2 activation is crucial for the generation of DAG and IP3.

More recently, Gratacap and coworkers (2000) showed that FcγRIIa activation alone does not produce sufficient levels of PtdIns(3,4,5)P3 by PI 3-kinase to cause PLCγ2 activation, platelet release, and aggregation. Additionally, ADP receptor activation by Gi-protein signaling is required to generate PtdIns(3,4,5)P3 via PI 3-kinase, which combined with activation by FcγRIIa generates optimal levels of PtdIns(3,4,5)P3, leading to efficient PLCγ2 phosphorylation. Activated PLCγ2 then generates DAG and IP3 from PtdIns(4,5)P2, mobilizing calcium and effecting platelet aggregation (Fig. 2). Moreover, lipid rafts appear to play an important role in the organization of the FcγRIIa/ADP receptor/PLCγ2 signaling pathway (Bodin et al., 2003). These findings help to explain the previous observations that ADP scavengers (e.g., apyrase) fully inhibit platelet aggregation by HIT-IgG (Polgár et al., 1998).

IV. FcγRIIa ACTIVATION IN HIT

Although an association between heparin treatment and paradoxical thrombosis was first suspected about 40 yr ago (Weismann and Tobin, 1958; Roberts et al., 1964), it was Rhodes and colleagues (1973) who first provided evidence that serum from HIT patients contained a substance, most likely IgG, that aggregated normal platelets in the presence of heparin. This observation was confirmed by Fratantoni et al. (1975) who reported a simple indirect aggregation method for detecting HIT antibodies. In 1986, Sheridan and coworkers (1986) reported a washed platelet

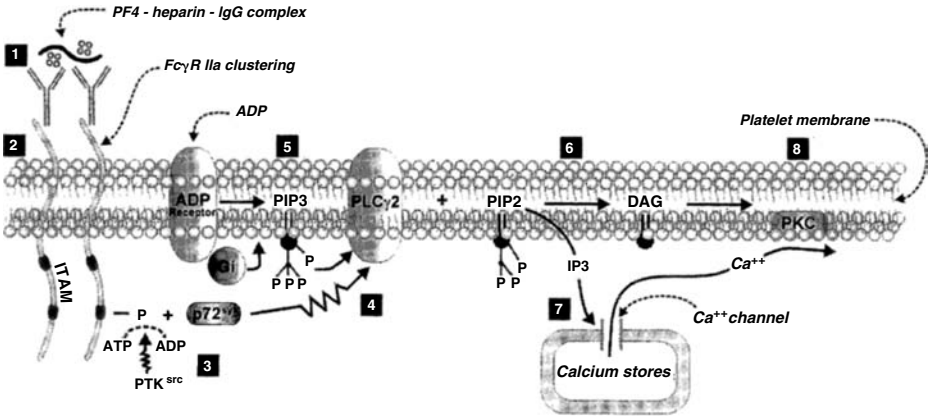

FIGURE 2 Fcγ receptor–mediated signal transduction. PF4-heparin-IgG complexes (1) bind to FcγRIIa, causing receptor clustering (2). The ITAMs on FcγRIIa are phosphorylated by PTK^{src} (3). The phosphorylated ITAMs interact with SH domains on $p72^{syk}$ to phosphorylate PLCγ2 (4). ADP receptors, activated via ADP and Gi proteins, generate PIP3 via PI 3-kinase (not shown) (5), which helps phosphorylate PLCγ2. Activated PLCγ2 acts on PIP2 to generate IP3, and DAG from phosphatidylinositol-bisphosphate (6). IP3 mobilizes Ca^{++} to the intracellular space via Ca^{++} channels (7) and together with DAG activates downstream PKC signaling pathways (8). *Abbreviations*: DAG, diacylglycerol; IP3, inositol triphosphate; ITAMs, immunoreceptor tyrosine activation motifs; PF4, platelet factor 4; PKC, protein kinase C; PLCγ2, phospholipase Cγ2; PIP3, phosphatidylinositol-trisphosphate; PTK^{src}, src protein tyrosine kinases.

activation assay, employing radiolabeled serotonin, as an activation endpoint that was sensitive and specific for detecting clinically significant HIT antibodies. This same group later reported that platelet activation by HIT antibodies was platelet FcγRIIa dependent, as it could be completely abrogated by a murine monoclonal anti-FcγRIIa antibody, IV.3 (Kelton et al., 1988). Other workers confirmed the central importance of the platelet Fc receptor in mediating platelet activation in HIT (Adelman et al., 1989; Chong et al., 1989a,b). Subsequently, Amiral and colleagues (1992) reported that the major target antigen for HIT-IgG was PF4 complexed to heparin, a finding quickly confirmed by other workers (Greinacher et al., 1994a; Kelton et al., 1994; Visentin et al., 1994).

A. Dynamic Model of Platelet Activation in HIT

The initial event in HIT is the binding of HIT-IgG to PF4-H complexes on the platelet surface. HIT-IgG binds to platelets even if the Fc receptors are blocked (Newman and Chong, 2000). Platelet activation by HIT-IgG is a dynamic process: initially, tiny amounts of PF4-H complexes form on the platelet surface. HIT-IgG binds to these complexes then engaging and cross-linking FcγRIIa by their Fc moiety. FcγRIIa ligation triggers platelet activation and degranulation (including release of the crucial potentiator, ADP). The released PF4 binds heparin and forms more complexes containing antigen on the platelet surface. Thus, positive feedback accelerates platelet activation. HIT-IgG also causes the release of TF and interleukin-8 (IL-8) from monocytes (Arepally and Mayer, 2001). In addition, antibodies to IL-8 (a chemokine structurally related to PF4) have been reported in some HIT patients. It appears that these antibodies can activate platelets (Regnault et al., 2003).

B. Platelet FcγRIIa Numbers

Variable expression of FcγRIIa numbers among individuals could affect susceptibility to immune complex diseases (Rosenfeld et al., 1987) or even to HIT. The number of platelet surface-expressed FcγRIIa molecules is increased dramatically in HIT (Chong et al., 1993b). However, increased FcγRIIa expression is also seen after in vitro activation of platelets by HIT antibodies. Elevated FcγRIIa numbers may be a consequence of platelet activation in HIT, rather than a proximate cause. This notion is supported by the fact that increased platelet FcγRIIa levels are seen in patients with atherothrombosis and diabetes mellitus (Calverley et al., 2002).

C. Plasma-Soluble FcγRIIa

Soluble FcγRIIa, which is released from α-granules on platelet activation by thrombin, has been demonstrated in plasma (Gachet et al., 1995). However, the relative amount of membrane versus soluble FcγRIIa is fixed (Keller et al., 1993). Gachet and colleagues (1995) reported that approximately 2 ng of soluble FcγRIIa is produced from 10^9 platelets. This value equals 0.002 ng, or 300 molecules per platelet compared with roughly 5–10 times as many molecules on the platelet surface. Due to the low affinity, a much larger amount of plasma-soluble FcγRIIa would be needed to inhibit significantly PF4-H immune complexes from binding to platelet FcγRIIa. Moreover, plasma levels of soluble FcγRIIa are higher in patients with HIT than in heparin-treated or other nonthrombocytopenic controls, presumably as a marker of in vivo platelet activation in HIT (Saffroy et al., 1997).

D. Plasma IgG Concentrations

Plasma IgG levels appear to influence platelet activation and aggregation by HIT sera. With a platelet-rich plasma (PRP) aggregation test to detect HIT antibodies, Chong et al. (1993a) showed variable platelet sensitivity to aggregation that was stable over time among different platelet donors. Chong and coworkers showed that the addition of purified human IgG to the PRP inhibited platelet aggregation by HIT sera, with complete inhibition at 40 mg/mL. It is possible that the effect of purified IgG is due to the presence of small IgG oligomers, because Karas et al. (1982) demonstrated that monomeric IgG does not bind to the platelet FcγRIIa. Furthermore, Greinacher et al. (1994b) showed that different preparations of intravenous IgG (ivIgG) for therapeutic use varied in their ability to inhibit HIT antibody-induced platelet serotonin release. Although the use of ivIgG to treat HIT does not appear to be common, it has some rationale in certain clinical settings (see Chapter 12).

E. FcγRIIa-Arg/His^{131} Polymorphism

The Arg/His amino acid variation at position 131 of FcγRIIa affects the ability of murine monoclonal IgGl as well as human IgG2 to activate platelets (Horsewood et al., 1991; Tomiyama et al., 1992; Parren et al., 1992; Bachelot et al., 1995). These observations prompted Burgess et al. (1995) to suggest that FcγRIIa variants could be a risk factor for developing HIT. In a small cohort of patients, they found an overrepresentation of the FcγRIIa-His^{131} variant. They hypothesized that IgG2 might be an important IgG subclass among HIT-IgG, as this could explain an apparent association between HIT and the FcγRIIa-His^{131} variant. However, subsequent reports argued against this hypothesis: IgGl rather than IgG2 was

the predominant subclass among HIT-IgG (Arepally et al., 1997; Denomme et al., 1997; Suh et al., 1997). Nevertheless, in support of a biological basis for a possible increased frequency of FcγRIIa-His^{131}, two groups found that HIT antibodies, including those that were predominantly IgGl, preferentially activated washed platelets of the His^{131} variant in vitro (Denomme et al., 1997; Bachelot-Loza et al., 1998). However, Brandt et al. (1995) found the opposite activation profile in platelet aggregation studies using citrated PRP (i.e., the FcγRIIa-Arg^{131} variant was preferentially activated by HIT plasma). No consensus has emerged from the six studies that investigated the frequency of FcγRIIa-Arg/His^{131} variants for patients with HIT: three studies show an overrepresentation of FcγRIIa-His^{131} (Burgess et al., 1995; Brandt et al., 1995; Denomme et al., 1997); two studies found no correlation with either variant (Arepally et al., 1997; Bachelot-Loza et al., 1998); and one study (the largest) showed the reverse correlation (Carlsson et al., 1998).

F. Animal Models of HIT

One of the earliest animal models of HIT used the natural immune process of anti-idiotypic antibody production to invoke expression of HIT-IgG in mice (Blank et al., 1997, 1999). Mice immunized with HIT-IgG developed anti-idiotypic IgG that now recognized PF4-H. Unfortunately, this model has limited use, as the mice did not develop thrombosis, perhaps because murine platelets lack FcγRIIa.

Other investigators (Arepally et al., 2000) developed a murine monoclonal antibody, termed KKO, by immunizing mice with PF4-H. This murine $IgG_{2b}\kappa$ monoclonal antibody mimics HIT-IgG, as it requires both PF4 and heparin to activate human platelets through their FcγRIIa. However, besides lacking FcγRIIa, mouse PF4 is not recognized by HIT-IgG or KKO. To overcome these problems, Reilly and colleagues (2001) produced transgenic mice that express both human FcγRIIa and human PF4. In these animals, addition of KKO caused thrombocytopenia and death, including thrombosis of the lung vasculature. This murine model has proven useful to address immunological questions related to HIT. First, large macromolecular complexes are a necessary component in the development of HIT (Rauova et al., 2005). Second, a preexisting prothrombotic condition may influence the development of HIT. Mice fed a hypercholesterolemic diet had increased platelet and endothelial-cell activation and were predisposed to HIT to a greater extent than healthy, diet-fed syngeneic control mice (Reilly et al., 2006).

When platelet-activating (anti-CD9) IgG was administered to FcγRIIa transgenic mice, more severe thrombocytopenia resulted, compared with a previously studied anti-mouse platelet (nonactivating) IgG (Taylor et al., 2000). Severe thrombosis, shock, and death developed in FcγRIIa transgenic mice crossed with FcRγ-chain knockout mice. Moreover, splenectomy facilitated anti-CD9–mediated shock in FcγRIIa transgenic mice. The authors concluded that the clearance of antibody-sensitized platelets by phagocytic cells in the spleen may play a protective role in preventing thrombosis.

Unlike mice, primate platelets do possess FcγRIIa. Thus, a primate model for HIT may be feasible, as suggested by a recent report (Ahmad et al., 2000). The animals *(Macaca mulatto)* used do not express the human Arg-His polymorphism, perhaps explaining why less variability in platelet activation response to HIT-IgG was observed in these in vitro studies. The primate model may have value in evaluating therapeutic agents for HIT (Untch et al., 2002).

G. Monocyte FcγRs in HIT

Monocytes and macrophages possess several different classes of FcγR (Table 1), and thus may play a part in influencing the frequency and severity of both thrombocytopenia and thrombosis in HIT. One role, discussed in the previous section, involves their potential to influence the balance between platelet activation and reticuloendothelial-mediated platelet clearance in HIT. Another function recently proposed for monocytes is that of contributing to the procoagulant state in HIT (a role posited previously for endothelial cells) (see Chapter 9). Pouplard and colleagues (2001) found that by adding HIT-IgG and PF4 (or PF4-H) directly to isolated monocytes or to whole blood, the monocytes produced TF, an effect that could be inhibited by high concentrations of heparin. Arepally and Mayer (2001) found that monocytes expressed surface TF when incubated with PF4 in the presence of either HIT-IgG or the HIT-mimicking murine monoclonal antibody, KKO. Because monocytes express sulfated proteoglycans on their surface, PF4 binding to monocytes can occur in the absence of added heparin. These studies raise the possibility that monocytes play an important role in the pathogenesis of the procoagulant state characteristic of HIT. Animal models suggest there may be a balance between platelet activation by HIT-IgG (predisposing one to thrombosis) and clearance of platelets by monocytes-macrophages (protecting somewhat against thrombosis). However, phagocytosis or NK cell destruction of antibody-sensitized platelets likely contribute to the thrombocytopenia since HIT is associated with an overrepresentation of FcγRIIIa-Val^{158} (Gruel et al., 2004), an FcγR with higher affinity for IgG1 and IgG3 (Table 1).

V. FcγRIIa POLYMORPHISMS IN DISEASE

A. Determining the FcγRIIa Polymorphism

The FcγRIIa-Arg/His^{131} polymorphism was first identified on the basis of functional differences effected by anti-CD3 monoclonal antibodies of the murine IgGl subclass (Tax et al., 1983, 1984). Proliferation assays distinguished "high" and "low" responders relative to the effects of these anti-CD3 murine monoclonal antibodies on T-cell-dependent mitogenesis. Subsequently, individuals bearing the FcγRIIa-Arg^{131} phenotype were identified as the "high responders" and the functional differences between the two variants were later confirmed using other FcγRIIa-dependent assays such as erythrocyte antigen-rosetting, phagocytosis, and platelet activation (Clark et al., 1989; Warmerdam et al., 1991; Parren et al., 1992; Salmon et al., 1992). Murine monoclonal IgGl activate platelets of all three Arg/His^{131} phenotypes, but the homozygous FcγRIIa-Arg^{131} variant requires less murine monoclonal antibody for platelet activation to occur.

The high-affinity binding of human IgG2 to FcγRIIa results when histidine is substituted at amino acid 131 of the mature protein (Warmerdam et al., 1991). FcγRIIa-His^{131} has a greater affinity for human IgG2 but a lower affinity for murine IgGl. Therefore, the terms high and low responder, used historically for the effects of murine monoclonal antibodies on Arg^{131} and His^{131} FcγRIIa phenotypes, respectively, is confusing, as the opposite reaction profile is observed with human IgG2. The high/low responder terminology has been largely replaced in favor of referring simply to the amino acid polymorphism.

The FcγRIIa-Arg/His^{131} variant polymorphism can be determined in three ways: (1) by functional assay such as T-cell-dependent proliferation or murine monoclonal antibody activation; (2) by specific binding using 41H16, a monoclonal

antibody that binds exclusively to the FcγRIIa Arg^{131} variant; and (3) by molecular genotyping. Several DNA-based methods have been developed to genotype for the FcγRIIa-Arg/His^{131} nucleotide substitution (Clark et al., 1991; Osborne et al., 1994; Bachelot et al., 1995; Burgess et al., 1995; Jiang et al., 1996; Denomme et al., 1997; Flesch et al., 1998; Carlsson et al., 1998).

B. Influence of FcγRIIa Polymorphism in Infectious or Autoimmune Disease

A few early studies have examined whether expression of the FcγRIIa-Arg/His^{131} polymorphism influences susceptibility to infectious or autoimmune disease. In theory, the weaker binding of human IgG2 to the FcγRIIa-Arg^{131} variant suggests that this gene might be overrepresented among patients with recurrent infections characterized by certain microbes with polysaccharide coats (i.e., involving an IgG2 antibody response) and overrepresented in disease characterized by circulating immune complexes (because phagocytic cells bearing the FcγRIIa-His^{131} variant would clear these complexes more readily). Certainly, a skewed genotypic distribution favoring the FcγRIIa-Arg^{131} variant has been noted in patients with *Haemophilus influenzae* infections (Sanders et al., 1994) and meningococcal septic shock (Bredius et al., 1994b). Furthermore, there is also predominance of FcγRIIa-Arg^{131} in patients with elevated levels of immune complexes and glomerulonephritis complicating systemic lupus erythematosus (Duits et al., 1995) (Table 2).

C. FcγRIIa Polymorphism in HIT

It was logical to hypothesize that the platelet FcγRIIa-Arg/His^{131} polymorphism would influence the clinical expression of HIT. First, platelets from normal

TABLE 2 Role of FcγRIIa-Arg/His^{131} Polymorphism in Disease

Disease	Predominant FcγRIIa variant	Comment
Infections by encapsulated bacteria	Arg^{131}	Reduced binding of IgG2 by FcγRIIa–Arg^{131}-MPS cells reduces phagocytosis, conferring susceptibility to infections with bacteria bearing polysaccharide capsules *(Haemophilus,* meningococcus)
Immune complex nephritis (SLE)	Arg^{131}	Reduced clearance of IgG-containing immune complexes by FcγRIIa–Arg^{131}-MPS cells leads to greater glomerular deposition of immune complexes
HIT with thrombosis	Arg^{131}	Reduced clearance of IgG-containing immune complexes by FcγRIIa–Arg^{131}-MPS cells leads to greater immune complex-dependent activation of platelets and endothelial cells (Carlsson et al., 1998)
HIT with or without thrombosis	His^{131}	Increased activation by HIT-IgGl and HIT-IgG2 of FcγRIIa–His^{131} platelets, without significant role for MPS cells (Denomme et al., 1997)

Abbreviations: Arg, arginine; FcγRIIa, FcγIIa receptor; His, histidine; HIT, heparin-induced thrombocytopenia; MPS, mononuclear phagocytic system (reticuloendothelial system); SLE, systemic lupus erythematosus.

individuals exhibit considerable variability in their activation by HIT sera (Salem and Van der Weyden, 1983; Pfueller and David, 1986; Warkentin et al., 1992). Second, many patients who form HIT antibodies during heparin treatment do not develop thrombocytopenia (Warkentin et al., 1995, 2005; Amiral et al., 1996; Suh et al., 1997). Third, the inciting role of heparin, a sulfated carbohydrate, suggested that there could be an important role for HIT antibodies of IgG2 subclass, which is the subclass with higher affinity for FcγRIIa-His^{131} that is predominantly formed in response to carbohydrate antigens (Herrmann et al., 1992). However, HIT epitopes form on the protein PF4 when it undergoes conformation change bound to heparin (see Chapters 5–7). Consequently, it was speculated that the FcγRIIa variant distribution in HIT would differ significantly from a random control population and especially differ from patients who did not develop thrombocytopenia during heparin treatment (Denomme et al., 1997; Bachelot-Loza et al., 1998).

The six studies investigating the role of the FcγRIIa-Arg/His^{131} variants have not yielded uniform results (Fig. 3). Three studies showed a predominance of the His^{131} variant in patients with HIT that was significant, compared with control

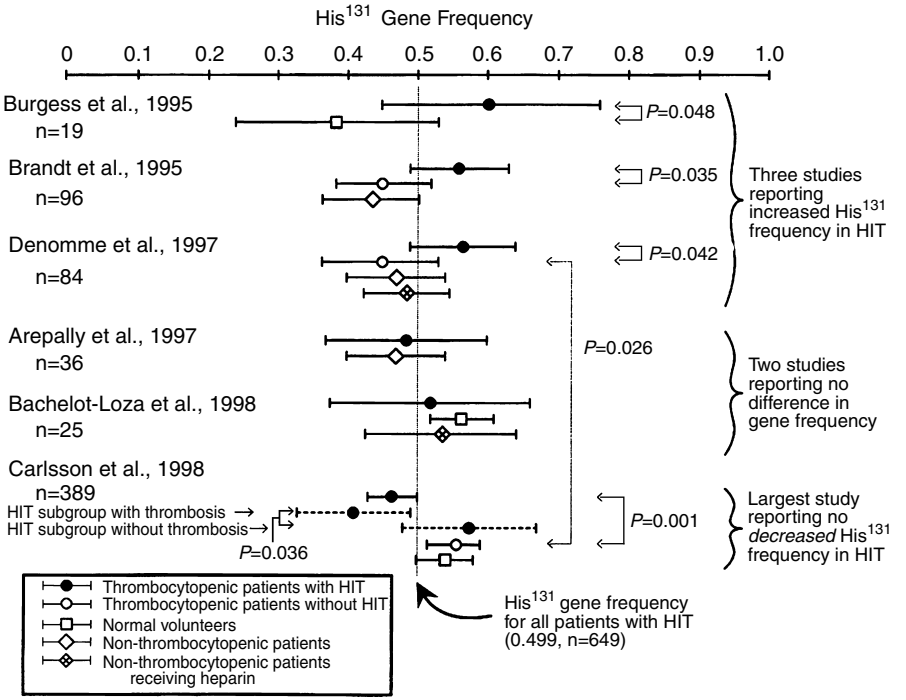

FIGURE 3 FcγRIIa-His^{131} gene frequencies in six studies of HIT are shown: The first four studies were from North American centers and the last two from Europe. Although the first three studies showed predominance of His^{131} in patients with HIT, the last study showed predominance of Arg^{131} in patients with HIT complicated by thrombosis. A complicating feature is the difference in gene frequencies between certain control populations [e.g., between Denomme et al., (1997) and Carlsson et al. (1998)]. Not shown in the figure is the significant difference between control patients in the studies by Carlsson and Brandt ($p = 0.013$).

patients. Together with evidence that HIT antibodies preferentially activate platelets in vitro from individuals bearing the FcγRIIa-His^{131} variant (Denomme et al., 1997; Bachelot-Loza et al., 1998), it was suggested that FcγRIIa-His^{131} predominance could reflect a greater potential for these platelets to be activated in vivo by HIT antibodies (Table 2). Two relatively small studies did not show any significant differences in the Arg/His^{131} variants between HIT patients and controls.

However, the largest of the six studies, involving 389 patients (i.e., more than the 260 HIT patients reported in the previous five studies combined), showed an *increase* in the frequency of the Arg^{131}, rather than the His^{131}, variant in patients with HIT (Carlsson et al., 1998). Moreover, these workers observed that the increase in FcγRII-Arg^{131} variant occurred only in the subset of patients whose HIT was complicated by thrombosis. These investigators proposed that reduced clearance of IgG-containing immune complexes by phagocytic cells bearing FcγRIIa-Arg^{131} leads to greater immune complex-dependent activation of platelets, thus predisposing one to thrombosis (Table 2). Although Arepally et al. (1997) did not observe a significant increase in the FcγRIIa-Arg^{131} variant among HIT patients with thrombosis, their subset of HIT patients with thrombosis was much smaller than that reported by Carlsson (23 vs. 68 patients). On the other hand, when Pouplard and colleagues (1999) examined the FcγRIIa-Arg/His^{131} variant frequency among patients who formed antibodies against PF4-H following cardiac surgery, they noted that platelet levels were significantly lower only in the homozygous FcγRIIa-Arg/Arg^{131} group, when compared with patients who did not form antibodies.

The explanation for the differences among these various studies is not readily apparent. However, a complicating aspect is noted in Figure 3: the frequency of the FcγRIIa-His^{131} variant is higher in the European control populations (Bachelot-Loza et al., 1998; Carlsson et al., 1998), compared with the North American and Australian controls (Burgess et al., 1995; Brandt et al., 1995; Denomme et al., 1997; Arepally et al., 1997), an observation consistent with population allele frequencies reported by Rascu et al. (1997). Indeed, pairwise X^2 analysis for the frequency of the FcγRIIa-His^{131} variant among the various controls shows that the control population of Carlsson's study differs from that reported by Denomme and Brandt (Fig. 3). The FcγRIIa-Arg/His^{131} variants differ among populations: in whites and African Americans, the allele frequencies have roughly a 50:50 balance (Osborne et al., 1994; Lehrnbecher et al., 1999). In contrast, in the Japanese and Chinese populations, the FcγRIIa-His^{131} allele frequency is approximately 75% (Rascu et al., 1997; Osborne et al., 1994). It is possible that unrecognized differences in population between HIT patients and controls could be important. For example, whereas samples from HIT patients could be referred from a wider geographic area, control patients might have been obtained from a localized area. None of the six studies reported on the FcγRIIa-Arg/His^{131} variant distribution among heparin-treated patients who formed HIT antibodies but who did not develop thrombocytopenia (i.e., the ideal control group for assessing the influence of the FcγRIIa polymorphism).

In summary, the role of the FcγRIIa-Arg/His^{131} variants in contributing to the pathogenesis of HIT remains controversial. Regardless of its ultimate resolution, the elucidation of the biological basis for differences in frequency of FcγRIIa phenotype between HIT patients, with or without thrombosis, and control subjects will provide new insights into the pathogenesis of immune-mediated disease.

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9 Immune Vascular Injury in Heparin-Induced Thrombocytopenia

Gowthami M. Arepally
Duke University Medical Center, Durham, North Carolina, U.S.A.

Mortimer Poncz
University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, U.S.A.

Douglas B. Cines
University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

I. INTRODUCTION

The most important complication of heparin-induced thrombocytopenia (HIT) is thrombosis. Clinically overt arterial or venous thrombi have been observed in 50% or more patients with HIT in some series (see Chapters 2 and 3), a frequency that far exceeds any other drug-induced immune platelet disorder. The propensity for thrombosis is in part attributable to platelet activation through FcγIIa receptors by IgG-containing complexes comprised of platelet factor 4 (PF4) and heparin (see Chapter 8). Strong support for this concept comes from recent studies involving mice transgenic for human FcγIIA and human PF4 (hPF4) (Reilly et al., 2001; Rauova et al., 2006) (see Chapter 8). However, the "nonpermissive" FcγIIa phenotype affords limited protection against thrombosis (Trikalinos et al., 2001), perhaps because of the high incidence of IgGl antibodies (Denomme et al., 1997). Furthermore, the occasional HIT patient in whom only IgM or IgA antibodies are detected (Amiral et al., 1996; Meyer et al., 2006) suggests that additional factors, acting at the level of the platelet or elsewhere, contribute to the thrombotic diathesis. In addition, thrombosis is usually restricted to large arteries and veins and occurs at a limited number of sites (Greinacher et al., 2005) (see Chapter 2), although HIT antibodies and activated platelets circulate in asymptomatic patients. These considerations point to alterations in the local vascular milieu as influencing the clinical expression of disease.

There are several other reasons to suspect that immune complex-mediated injury of endothelial cells (ECs) lining the vasculature may contribute to the thrombotic complications of HIT. First, the ECs help maintain the balance between blood fluidity and clotting. Second, ECs express heparan sulfate-containing proteoglycans that help regulate coagulation and contribute to the metabolism of PF4. Third, anti-EC antibodies (AECA) have been identified in patients with other disorders characterized by thrombosis and thrombocytopenia. And fourth, there is some direct evidence that patients with HIT form antibodies that recognize PF4-heparin complexes on the endothelium, and thereby promote prothrombotic reactions.

II. THE ENDOTHELIUM IN HEMOSTASIS

The role of the endothelium in regulating blood fluidity and trafficking of circulating hematopoietic cells has been the subject of several reviews (Cines et al., 1998; Aird, 2003). ECs express a variety of factors that inhibit coagulation, including soluble substances, such as nitric oxide and prostacyclin (acting to inhibit platelet activation), and tissue-type plasminogen activator (t-PA, acting to promote fibrinolysis), among many others. EC surface-bound molecules with anticoagulant activity include heparan sulfate-containing proteoglycans (see below), thrombomodulin (TM), complement regulatory proteins, as well as receptors for activated protein C (APC), urokinase, and plasminogen.

Unperturbed ECs also do not express several moieties that promote platelet and leukocyte adhesion, such as endothelial leukocyte adhesion molecule (ELAM), P-selectin, and platelet-activating factor (PAF). These can be induced, however, when the cells are stimulated by agonists, such as cytokines, thrombin (Drake et al., 1993; Kaplanski et al., 1998), or when the cells are injured by immune factors, atherosclerosis, or shear stress (Yu et al., 2005). Additionally, ECs exposed to such factors express a reduced content of heparan sulfate, internalize and degrade APC, elaborate tissue factor, and secrete abundant plasminogen activator inhibitor-1 (PAI-1), each of which may promote thrombus formation (Cines et al., 1998). Histochemical studies of the endothelium in murine models of inflammation have confirmed many of these observations, predicated in cell culture (Fries et al., 1993), affirming the notion that the endothelium undergoes multifaceted changes from an antithrombotic to a procoagulant phenotype in response to injury.

Also relevant to the pathogenesis of HIT is the remarkable heterogeneity of ECs, within and among different vascular beds, owing to genetic differences and acquired changes in phenotype (for reviews: see Cines et al., 1998; Aird, 2003). For example, only a small fraction of ECs constitutively expresses t-PA or urokinase-type plasminogen activator (u-PA) in vivo (Levin et al., 1994), whereas a different subset expresses tissue factor when exposed to endotoxin (Drake et al., 1993). ECs from different organs express tissue-specific promoters that regulate the expression of von Willebrand factor (vWF) in vivo (Aird et al., 1997). ECs also show regional variation in the synthesis of prostacyclin and expression of leukocyte adhesion molecules and Fcγ receptors, among many other phenotypic differences.

There is also evidence to indicate that protein C activation on macrovascular ECs is mediated predominantly through the protein C receptor, whereas TM may dominate in the microvasculature (Laszik et al., 1997; Van de Wouwer et al., 2004). TM changes thrombin from a procoagulant to an anticoagulant enzyme (i.e., TM-bound thrombin activates the natural anticoagulant zymogen, protein C) (Esmon, 2001). Targeted disruption of the endothelial TM gene leads to juvenile onset of thrombosis (Isermann et al., 2001). The anticoagulant function of TM in the microvasculature may contribute to the pathogenesis of warfarin-associated venous limb gangrene that can complicate HIT. This syndrome has been attributed to the coincidence of persistent thrombin generation and acquired protein C deficiency that may occur during the first few days of anticoagulation with warfarin (Warkentin et al., 1997; Srinivasan et al., 2004) (see Chapters 2 and 12).

The behavior of ECs can also be modified during the evolution of vascular disease. For example, atherosclerotic vessels produce less nitric oxide in response to a variety of stimuli than do healthy vessels (Shaul, 2003). Atherosclerotic vessels may also undergo alterations in their expression of glycosaminoglycans (GAGs)

(Talusan et al., 2005) and an increase in expression of various cell adhesion molecules (for review: see Fuster et al., 1998). The binding of advanced glycation end products to specific EC receptors during normal aging and diabetes mellitus increases vascular permeability, exposing the subendothelial matrix to lipoproteins and other injurious substances (Basta et al., 2004). It is also likely that genetic variation in EC behavior contributes to the host response to antibody- and platelet-mediated EC injury, although the methods to identify or monitor such risk factors remain to be developed. Thus, any inquiry into the reason why only a subset of patients who develop anti-PF4-heparin antibodies develop thrombosis, or why thrombi occur at restricted vascular sites, must take into consideration the specific attributes of the affected endothelial vascular bed.

III. HEPARAN SULFATE-CONTAINING PROTEOGLYCANS, HEPARIN, AND THE ENDOTHELIUM

The expression and anticoagulant function of heparan sulfate-type proteoglycans (HSPGs) by ECs may be central to the pathogenesis of vascular thrombosis in patients with HIT. The biochemistry and function of these GAGs and the proteoglycans to which they bind have been the subject of extensive study (for review: see Rosenberg et al., 1997; Esko and Lindahl, 2001; Forsberg and Kjellen, 2001). The involvement of heparan sulfate in the development of HIT is considered elsewhere (see Chapter 7). HSPGs expressed by ECs bind antithrombin (AT) in vitro and in vivo, and accelerate the inactivation of thrombin and factor Xa approximately 20-fold, an effect that is biologically equivalent to 0.1–0.5 U/mL of heparin (Marcum and Rosenberg, 1984). Yet less than 1% of the HSPGs isolated from cultured ECs express anticoagulant activity (Marcum and Rosenberg, 1984). Active species are characterized by an approximately twofold enrichment in glucuronyl 3-*O*-sulfated glucosamine residues, compared with inactive species (Marcum and Rosenberg, 1984). Interestingly, targeted deletion of the murine 3-*O*-sulfo-transferase-1 enzyme (the enzyme responsible for generating this anticoagulant modification of HSPGs) does not lead to a prothrombotic phenotype (HajMohammadi et al., 2003). The physiological mechanisms that control the synthesis and postsynthetic modifications of HSPG remain an active area of investigation (Forsberg and Kjellen, 2001).

Microheterogeneity in the composition of HSPG in arteries, veins, and capillaries has been noted (Lowe-Krentz and Joyce, 1991), but the significance of these differences is unknown. Expression of HSPG by ECs undergoes developmental changes (David et al., 1992), and its composition varies after the cells are exposed to thrombin (Benezra et al., 1993), homocysteine (Nishinaga et al., 1993), heparin (Nader et al., 1989), wounding and migration (Kinsella and Wight, 1986), and after induction by activated platelets (Yahalom et al., 1984), among other stimuli. ECs also bind heparin (for review: see Patton et al., 1995), which alters their proliferation, matrix composition, and many other vascular functions. It has also been reported that AT is displaced from ECs by heparin, and its binding is inhibited by PF4 (Stern et al., 1985). Whether HIT antibodies promote the capacity of PF4 to neutralize AT activity has not been reported.

IV. PLATELET FACTOR 4 AND THE ENDOTHELIUM

The biochemistry of PF4 and its involvement in HIT is reviewed elsewhere (see Chapter 5). The metabolism of the protein is regulated by its interactions with the

endothelium. PF4 is stored in the α-granules of platelets as a tetramer bound to chondroitin sulfate (Barber et al., 1972). The tetramer may dissociate from the GAG as the platelets are activated, but more likely, dissociation occurs subsequent to binding to EC HSPG, which contains a higher charge density. [^{125}I]PF4 is cleared from the circulation with an α-elimination phase approximating 2 min, which primarily represents binding to the endothelium, and a β-elimination phase approximating 40 min, corresponding to uptake and degradation, predominantly by hepatocytes (Rucinski et al., 1986, 1990).

The endothelium binds approximately 50 pmol PF4/10^5 cells (Rybak et al., 1989). Several classes of binding sites have been identified, including a high-capacity, low-affinity site on HSPG, as well as higher-affinity binding sites involving specific chemokine receptors and certain coagulant proteins (see below). Binding of PF4 to the endothelium is attenuated by pretreatment with heparinase (Marcum et al., 1984), and plasma concentrations are increased 10- to 20-fold after heparin is infused intravenously (Dawes et al., 1982). Binding of PF4 to EC GAGs is electrostatic (Wu et al., 1984) and is independent of the pentasaccharide involved in the binding of AT (Loscalzo et al., 1985). The affinity of PF4 binding to ECs is lower than to purified heparin ($K_d = 2–3\,\mu mol/L$ vs. $2\,nmol/L$, respectively) (Rybak et al., 1989), consistent with the biochemical heterogeneity of vascular matrix. PF4 has a 10- to 100-fold greater affinity for EC HSPG than does AT (Jordan et al., 1982) and thus markedly attenuates the antiprotease cofactor activity of AT on intact vessels (Busch et al., 1980; Stern et al., 1985).

The involvement of PF4 in hemostasis is mediated in part by charge-dependent interactions with EC proteoglycans (Eslin et al., 2004). Recent in vivo studies utilizing murine PF4 knock-out (mPF4$^{-/-}$) and human PF4 (hPF4^{+}) transgenic animals show that PF4 helps stabilize clots formed in response to EC injury. Both mice lacking and those that overexpress PF4 show delayed and unstable clot formation, indicating that a narrow range of PF4 concentrations is needed for efficient clot formation (Fig. 1). The defect in thrombus formation in mPF4$^{-/-}$ can be corrected by infusion of human PF4 or protamine sulfate. On the other hand, the overexpression of PF4 seen with hPF4^{+} animals as well as infusions of protamine into wild-type animals is associated with impaired thrombus formation, which can be reversed through charge neutralization using heparin (Eslin et al., 2004). These studies suggest that PF4 facilitates clot formation by neutralizing negatively charged surfaces on platelets and ECs, perhaps allowing closer approximation of platelets to each other and to the endothelial lining. In settings where insufficient or excessive PF4 is released, cell surfaces may retain a net negative or positive charge, respectively, which prevents optimal approximation of cellular elements (Eslin et al., 2004).

The existence of an "optimal" concentration range of PF4 for hemostasis mirrors the binding of HIT antibody to complexes formed at various molar ratios of PF4 to heparin (Bock et al., 1980; Greinacher et al., 1994; Rauova et al., 2005). PF4 and heparin form ultra-large complexes (ULCs, MW > 670 kDa) over a narrow range of molar ratios, approximating 1 mole of PF4 tetramer to 1 mole of unfractionated heparin, the ratio at which HIT antibody binding is optimal (Rauova et al., 2005) (see Chapter 5). These ULCs are preferentially recognized by KKO, a murine HIT-like antibody. Recent in vivo studies affirmed the importance of these findings. When KKO is administered to double transgenic mice expressing platelet hFcγRIIA and varying levels of hPF4 (hPF4^{high}/hFcγRIIA, hPF4^{mid}/hFcγRIIA, or hPF4^{low}/hFcγRIIA), the severity of thrombocytopenia is proportionate

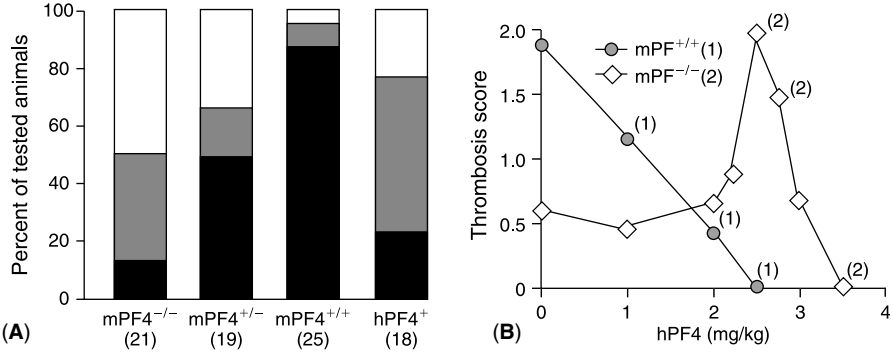

FIGURE 1 Biologic role of PF4 in stabilizing thrombus formation after endothelial injury. (**A**) Animals were subjected to $FeCl_3$-induced carotid injury and formation of a stable thrombus was determined. The percentage of animals developing a stable occlusive thrombus (■), transient occlusive thrombus (■), and no occlusive thrombus is shown. The animals are arranged from no PF4 expression on the left to excess PF4 expression on the right. The number tested of each phenotype is indicated at the bottom ($P < 0.005$ $mPF4^{+/-}$ vs. $mPF4^{+/+}$; $P < 0.0001$ for $mPF4^{-/-}$ vs. $mPF4^{+/+}$ and $hPF4^{+}$ vs. $mPF4^{+/+}$). (**B**) Mean thrombosis score of wild-type and littermate $mPF4^{-/-}$ mice treated with increasing doses of recombinant hPF4 infusion ($n = 5$ animals at each point). A thrombosis score of 2 = stable occlusive thrombus, 1 = transient occlusive thrombus, and 0 = no occlusive thrombus developed. The zero concentration data points are extrapolated from panel (**A**). Numbers indicate levels of heparin infusion in which occlusive thrombi occurred at a significantly different rate from non–heparin-treated mice of that phenotype ($P < 0.01$, Student t test; 1 = $mPF4^{+/+}$, and 2 = $mPF4^{-/-}$. *Abbreviations*: PF4, platelet factor 4; hPF4, human PF4; mPF4, murine PF4. *Source*: Reproduced from Eslin et al., 2004. (Blood, 2004 vol. 104, p. 3176 by copyright permission of the author and American Society of Hematology.)

to the level of PF4 expression and does not require exogenous heparin. Interference with cell-surface PF4/GAG assembly with high dose heparin or protamine sulfate, ameliorates the severity of the thrombocytopenia (Rauova et al., 2006). These findings suggest that the level of PF4 on the vasculature and other cell surfaces may affect antigen assembly and thereby contribute to the clinical variability seen in patients with anti-PF4-heparin antibodies. Thrombosis may be fostered in patients who release relatively large amounts of PF4 from their platelets, either due to constitutive overexpression or due to platelet-activating effects of atherosclerosis and vascular injury. Heparin may stabilize or propagate thrombosis by neutralizing the charge effects of "excess" cell-surface PF4. Assembly of cell-surface antigenic complexes capable of binding HIT antibodies in the absence of drug may contribute to the development of HIT when only low sensitizing doses of heparin are employed and to the persistent hypercoagulable state after heparin has been withdrawn.

Anticoagulant properties of PF4 have also been described. PF4 binds to TM, a 60.3 kDa protein constitutively expressed on the surface of ECs. Binding of thrombin to TM alters its substrate specificity, such that proteolytic cleavage of protein C is accelerated 20,000-fold (Esmon, 1989). TM is posttranslationally modified by association with a chondroitin sulfate A-like GAG, which invests it with the capacity to bind cationic peptides at physiological pH. The binding of eosinophilic cationic protein, major basic protein, and histidine-rich glycoprotein to these GAG residues inhibits the function of TM, whereas the binding of PF4

(but not β-thromboglobulin or thrombospondin) increases protein C-cofactor activity 25-fold in a cell-free system (Slungaard and Key, 1994; Dudek et al., 1997). Addition of PF4 to cultured ECs accelerates APC generation approximately 5- to 10-fold depending on vascular origin (Slungaard et al., 2003). Injection of PF4 into primates infused with thrombin increases APC generation two- to three-fold and prolongs the baseline aPTT (Slungaard et al., 2003). Additional studies should clarify whether HIT antibodies interfere with the anticoagulant function of PF4 and thereby may predispose to warfarin-associated venous limb gangrene.

Not all of PF4's biologic effects are mediated by electrostatic interactions. Two putative PF4 cellular receptors have been identified. Like other chemokines, PF4 binds to the Duffy antigen/receptor for chemokines (DARC) (Tournamille et al., 1997), which has been identified on ECs in postcapillary venules and in the splanchnic bed, even in individuals who do not express the antigen on their erythrocytes (Peiper et al., 1995). The distribution of Duffy on ECs in other vascular beds is less well studied. The binding site of PF4 on DARC likely coincides with the binding sites of other chemokines located on the NH_2-terminal domain (Tournamille et al., 1997). The role of DARC on red cell surfaces and on various vascular beds in the natural clearance of PF4 remains to be defined.

PF4 also binds to an alternatively spliced isoform of the chemokine receptor CXCR3, CXCR3B (Lasagni et al., 2003). CXCR3, now termed CXCR3A, is a seven transmembrane chemokine receptor that binds Mig (CXCL9), IP-10 (CXCL10), and I-TAC (CXCL11). CXCL -9, -10 and -11 are chemokines that regulate lymphocyte chemotaxis and inhibit angiogenesis. CXCR3B, derived from an alternative splicing site within the intron of the CXCR3 gene, has high affinity for PF4 (K_d = 4 nmol/L) and mediates the anti-angiogenic effects of PF4. CXCR3B is expressed on microvascular ECs of the heart, kidney, liver, and skeletal muscle (Lasagni et al., 2003). Whereas overexpression of CXCR3A into human microvascular ECs increases survival, overexpression of CXCR3B is associated with low proliferation and increased apoptotic cell death. It is presumed that binding of PF4 to the CXCR3B receptor mediates the described anti-angiogenic properties of PF4 (Yamaguchi et al., 2005; Strieter et al., 2006) though the need for micromolar concentrations of PF4 to see an antigenic effect with PF4 in most settings is more consistent with its binding to surface HSPG.

A pathogenic role of PF4 in atherogenesis has been recently elucidated. Human atherosclerotic lesions are invested with PF4 (Pitsilos et al., 2003) (Fig. 2; see color insert). PF4 is found not only along the overlying endothelium, but also in foam cells and acellular portions of the plaque. In vitro, PF4 binds to the low density lipoprotein (LDL) receptor and to proteoglycans, forming ternary complexes that show limited migration into clathrin-coated pits, thereby retarding endocytosis and catabolism of LDL (Sachais et al., 2002). PF4 binds directly to oxidized LDL, promoting foam cell formation (Nassar et al., 2003) and upregulating expression of E-selectin, an adhesion molecule implicated in atherogenesis (Yu et al., 2005). In mice, activated platelets deposit PF4 on endothelium and monocytes, potentiating effects of P-selectin on platelet-leukocyte aggregate formation and atherosclerotic development (Huo et al., 2003). In a murine HIT model, hypercholesterolemic diet is associated with increased platelet reactivity and EC activation, as indicated by elevated levels of soluble vascular cell adhesion molecule (VCAM) (Reilly et al., 2006). Antibodies to PF4-heparin complexes have recently been identified as an independent predictor of myocardial infarction at 30 days in patients presenting with acute coronary ischemic syndromes (Williams

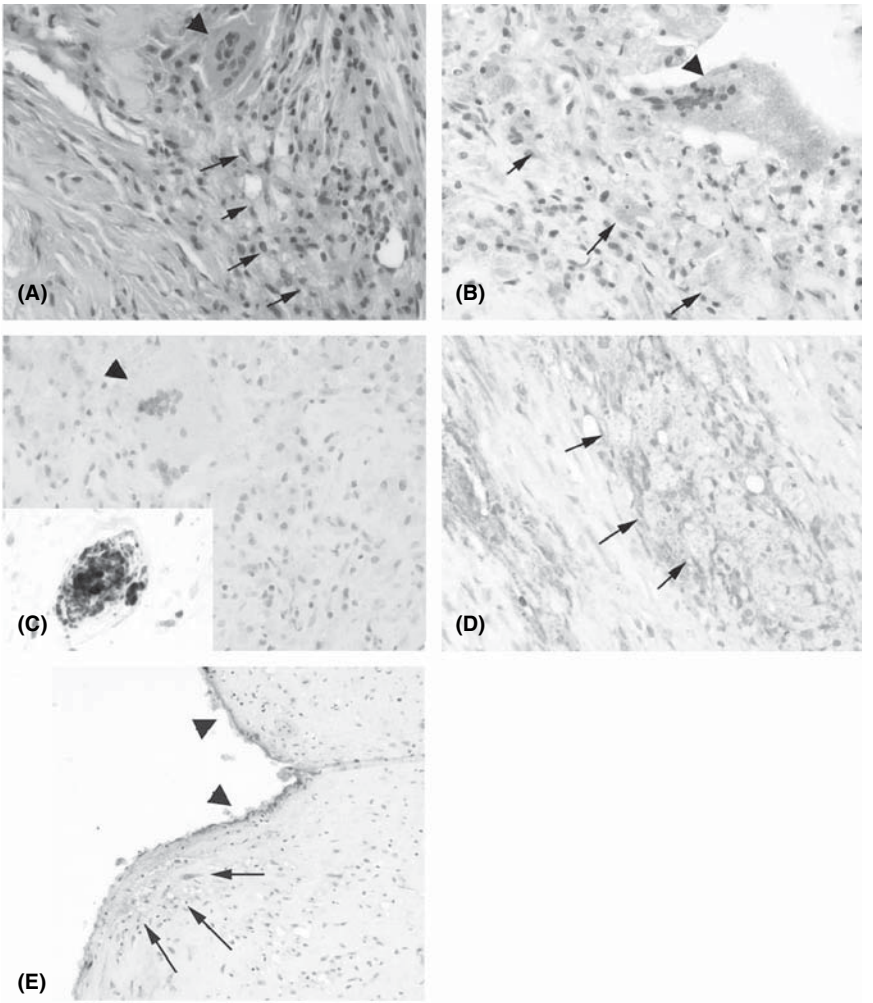

FIGURE 2 (*See color insert*) Atherosclerotic tissue is invested with PF4. (**A**) Photomicrograph (H&E stain) demonstrating the appearance of a group of foamy macrophages, which contain bubbly, vacuolated cytoplasm and indistinct nuclei (*arrows*). A giant cell is indicated by the arrowhead. (**B**) Photomicrograph of immunohistochemical staining for CD68, which is specific for cells of the macrophage lineage. Foamy macrophages (*arrows*) and a giant cell (*arrowhead*) is indicated. (**C**) Photomicrograph of immunohistochemical staining for CD41, specific for platelets. A small vessel filled with red blood cells and platelets serves as an internal positive control (*inset*). (**D**) This photomicrograph demonstrates the anti-PF4 staining the foamy macrophages. Note that the morphology of these cells is identical to those in panels (**A**–**C**), with vacuolated cytoplasm that stains for PF4 (*arrows*). (**E**) Photomicrograph demonstrating PF4 staining of an early atherosclerotic lesion from a carotid artery obtained at autopsy. The endothelium (*arrowheads*) and subendothelial macrophages (*arrows*) of an early lesion stain intensely positive with anti-PF4. Images are 40X (**A**–**D**) or 20X (**E**) original magnification. *Abbreviation*: PF4, platelet factor 4. *Source*: Reproduced from Pitsilos et al., 2003. (Thrombosis and Haemostasis, 2003, vol. 90, p. 1117, by copyright permission of the author and Schattauer GmbH.)

et al., 2003). Thus, HIT antibodies may modify the interactions of PF4 with diseased endothelium by (1) binding to PF4/proteoglycan complexes in atherosclerotic lesions, (2) inducing formation of platelet-leukocyte aggregates (Khairy et al., 2001), or (3) binding to circulating monocytes (Arepally et al., 2001; Pouplard et al., 2001), thereby increasing local inflammation and stimulating procoagulant processes.

V. IMMUNE EC INJURY

EC-reactive antibodies have been found in patients suffering from disorders characterized by vasculitis or thrombosis (for review: see Praprotnik et al., 2001a; Meroni et al., 2005). The best-studied example is allograft rejection, a setting in which there is extensive evidence for AECA, in part directed at carbohydrate antigens that regulate procoagulant activity in vitro (Saadi and Platt, 1995; Diujvestijn et al., 2000). AECA have also been identified in patients with hyperacute and acute graft rejection, systemic lupus erythematosus (Constans et al., 2003), antiphospholipid antibody syndrome (Cesarman-Maus et al., 2006), and thrombotic thrombocytopenic purpura (Praprotnik et al., 2001b). What is curious is the extraordinary diversity of the clinical syndromes associated with AECA. Also of interest, the target cells used in most assays (i.e., ECs derived from human umbilical vein [HUVEC]), are not known to be affected by immune vascular injury in the clinical setting. This suggests that the expression of the target antigen(s) is highly restricted in vivo, reflecting either regional differences in the composition of the vascular bed (microvascular or macrovascular) or, perhaps, indicating distinct responses to injury and inflammation. Alternatively, these AECA could represent a surrogate marker for other pathogenic antibodies, or the capacity of the affected vasculature is a critical determinant of whether thrombosis develops.

Several effects of AECA that could contribute to thrombosis include cell lysis or apoptosis (Bordron et al., 2001), induction of cytokine secretion and promotion of leukocyte adhesion (Del Papa et al., 1997), enhancement of vascular permeability (Tinckam and Chandraker, 2006), acceleration of procoagulant reactions (Saadi et al., 1995), and reduction in the expression of heparan sulfate (Nathan and Ihrcke, 1996). The reason why some antibodies promote thrombosis (e.g., HIT antibodies), whereas others induce primarily a necrotizing vasculitis, remains unresolved, but may relate directly to the biological functions of heparin and PF4.

VI. EC ANTIBODIES IN HIT

There are limited experimental data to indicate that EC-reactive antibodies or immune complexes contribute to the development of thrombosis in patients with HIT. Over 10 yr ago, one group reported that sera from essentially all patients with HIT deposit increased amounts of IgG, IgM, or IgA on HUVEC (Cines et al., 1987). Binding was reduced when the cells were pretreated with enzymes that degrade heparin or heparan sulfate, whereas addition of chondroitinase was without effect. HIT sera induced ECs to express the procoagulant tissue factor, and the expression of procoagulant activity was enhanced further in the presence of platelets. These observations were confirmed and extended by Visentin and colleagues (1994), who demonstrated that the binding of HIT antibodies of HUVEC was dependent on PF4, but not on exogenous heparin, in contrast to the requirement for both to be added for antibody binding to platelets. This is consistent with the concept that PF4, released from activated platelets, can form a competent antigenic complex on the pericellular matrix of the endothelium.

The role of EC-reactive antibodies was also explored in an animal model that simulates certain aspects of HIT (Blank et al., 1997). Mice injected with IgG fractions obtained from HIT patients developed anti-idiotypic antibodies that recognized complexes between hPF4 and heparin. Furthermore, the anti-idiotypic antibodies competed with the immunizing antibodies for binding to the antigenic complex. These effects were not noted when antibodies obtained from mice immunized with control IgG were studied. Additionally, mice immunized with HIT-IgG developed thrombocytopenia when exposed to heparin. Affinity-purified anti-PF4-heparin antibodies bound to murine endothelioma cells in the presence of PF4, but not β_2GPI. Of interest, immunized mice did not develop overt thrombi on exposure to heparin, possibly because of insufficient circulating PF4, intrinsic differences in the balance between the procoagulant and fibrinolytic systems compared with humans, or differences in signal transduction through murine and human platelet Fcγ receptors. However, it is also possible that the difference lies in a reduced capacity of otherwise healthy mouse ECs to respond to the procoagulant stimulus induced by these antibodies, compared with the responsiveness of patients with HIT, who, in the main, comprise a more elderly population with underlying vascular disorders.

Recent studies suggest that requirements for EC activation by HIT antibodies may differ based on the vascular origin. Blank et al. (2002) reported that anti-PF4-heparin IgG could activate certain microvascular ECs directly. However, antibody-mediated platelet activation (Herbert et al., 1998) or stimulation with cytokines such as tumor necrosis factor-alpha (TNF-α) (Blank et al., 2002) may be necessary to activate macrovascular ECs. In the presence of platelets or TNF-α, sera or IgG from patients with HIT stimulated expression of E-selectin, VCAM, intercellular adhesion molecule-1 (ICAM-1), and tissue factor, release of IL-β1, IL-6, TNF-α, and PAI-1, and adhesion of platelets and monocytes to the activated ECs. EC activation was inhibited by SR121566a (a platelet glycoprotein IIb/IIIa antagonist) and to some extent by apyrase and ATPγS, implicating expression of endogenous platelet fibrin(ogen) and release of adenosine diphosphate (ADP). The mechanism(s) by which platelet or cytokine activation facilitate binding of HIT antibodies to cell-associated PF4-heparin requires further investigation, but may be related to released stores of platelet PF4.

VII. IMMUNE VASCULAR INJURY AND MONOCYTE ACTIVATION IN HIT-ASSOCIATED THROMBOSIS

The studies described above support the notion that platelet activation and/or an inflammatory milieu contribute to EC dysfunction, predisposing to HIT-associated thrombosis. This view of HIT as an inflammatory disorder has gained additional experimental support by recent findings that monocytes are activated as well. HIT plasma or IgG stimulates monocytes to elaborate tissue factor-dependent procoagulant activity in monocytes (Pouplard et al., 2001). This procoagulant effect required small amounts of heparin to activate the monocytes in whole blood, but appeared to be heparin-independent when isolated mononuclear cells were studied, presumably by exposure of cell-associated proteoglycans (Pouplard et al., 2001). Heparin-independent upregulation of monocyte tissue factor activity by HIT antibodies was confirmed using human and murine antibodies (Arepally et al., 2001). Maximal tissue factor expression was detected at 4–6 h, suggesting that synthesis rather than de-encryption was primarily responsible for increased

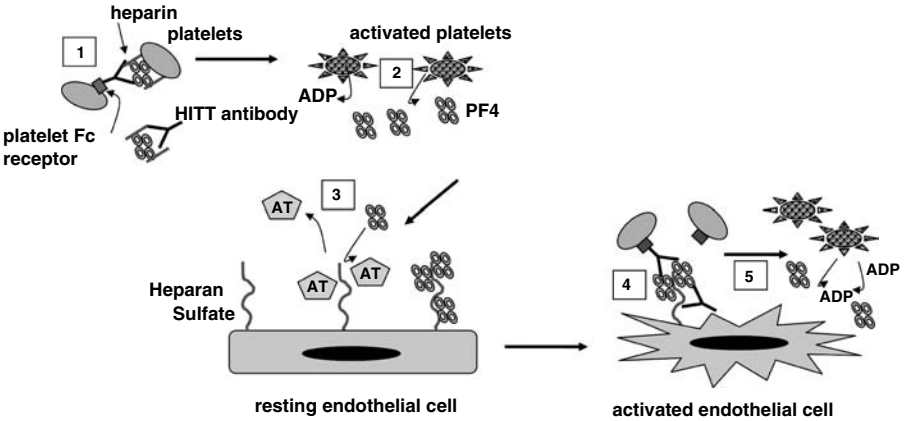

FIGURE 3 (*See color insert*) Model of HIT antibody interactions with endothelial cells. (1) HIT antibodies bind to antigen (multimolecular PF4/heparin complexes) localized to platelets. (2) Platelet activation occurs after Fc receptor binding, leading to platelet granule release. (3) Released PF4 binds to platelets and endothelial cell HS displacing AT from endothelial cells. (4) Antigenic complexes on endothelial cells bind HIT antibodies. (5) HIT antibody binding to endothelial cells leads to endothelial cell activation and further platelet activation. *Abbreviations*: ADP, adenosine diphosphate; AT, antithrombin; HIT, heparin-induced thrombocytopenia; HS, heparan sulfate; PF4, platelet factor 4.

procoagulant activity. Monocyte surface proteoglycans likely also bind PF4 to form antigenic complexes and following activation, macrophages express increasing hypersulfated surface GAGs (Ghiselli et al., 1998). The development of monoclonal antibodies against PF4 and PF4/heparin (Arepally et al., 2000) and murine models (Reilly et al., 2001; Suvarna et al., 2005) should help to delineate the contribution of monocyte activation to the immune pathogenesis of thrombocytopenia and thrombosis in HIT.

VIII. PERSPECTIVE AND FUTURE DIRECTIONS

HIT continues to pose several enigmas including the fundamental issue of how heparin induces the formation of self-reactive antibodies to a native protein in such a high proportion of immunologically competent individuals (Visentin et al., 1996; Bauer et al., 1997). It also remains unclear why only a subset of patients with anti-PF4-heparin antibodies develops thrombocytopenia, and fewer still develop thrombosis. It is possible that characteristics of HIT antibodies, such as their subtype, specificity, and affinity for portions of the PF4 molecule, may provide some of the answers. However, it is also likely that part of the propensity for thrombosis, and the localization of clotting observed in HIT, relate to antigen expression and response to injury at the level of the vessel wall itself (Fig. 3).

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10 Laboratory Testing for Heparin-Induced Thrombocytopenia

Theodore E. Warkentin
Michael G. DeGroote School of Medicine, McMaster University, and Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada

Andreas Greinacher
Ernst-Moritz-Arndt-University-Greifswald, Greifswald, Germany

I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is caused by heparin-dependent antibodies that usually recognize multimolecular complexes of platelet factor 4-heparin (PF4-H). HIT can be viewed as a clinicopathologic syndrome (Warkentin et al., 1998). Thus, a diagnosis of HIT should be based on two criteria: (1) clinically evident abnormalities, most commonly thrombocytopenia with or without thrombosis (see Chapter 2), and (2) detection of HIT antibodies. In some ways, HIT resembles another clinicopathologic disorder, the antiphospholipid (lupus anticoagulant) syndrome (Table 1).

Two major classes of assays—platelet activation (functional) and PF4-dependent antigen—have been developed to detect HIT antibodies (Warkentin and Sheppard, 2006a) (Table 2).

II. PLATELET ACTIVATION ASSAYS FOR HIT ANTIBODIES

A. Washed Platelet Assays

The classic washed platelet assay for HIT is the serotonin release assay (SRA) (Sheridan et al., 1986; Warkentin et al., 1992). This assay was a modification of platelet-washing techniques in use at McMaster University that resuspended washed platelets in buffer containing physiological concentrations of calcium. The purpose was to avoid platelet activation artifacts associated with low calcium concentrations (Mustard et al., 1972; Kinlough-Rathbone et al., 1983) (see Chapter 1). The use of washed platelets is also central to certain other functional assays, such as the heparin-induced platelet activation (HIPA) assay (Greinacher et al., 1991). Figure 1 summarizes washed platelet assays for HIT.

Preparation of Platelets for Washed Platelet Assays

1. Collect 8.4 volumes of blood from a normal donor into 1.6 volumes of acid-citrate-dextrose (ACD).

Comment. Aspirin-free normal blood donors whose platelets are known to respond well to HIT sera should be selected, as there is considerable heterogeneity to platelet activation by HIT sera among platelets obtained from different normal individuals (Warkentin et al., 1994). In Hamilton, platelets from two donors are

TABLE 1 Two Clinicopathologic Syndromes: HIT and Antiphospholipid Syndrome

Laboratory features	Heparin-induced thrombocytopenia	Antiphospholipid syndrome
Structure of the major antigen	"Cryptic" autoepitope on PF4 expressed when complexed with heparin	"Cryptic" autoepitope on β_2-GPI expressed when bound to anionic phospholipid (Pengo et al., 1995)
Nonspecific nature of anionic component of antigen (cross-reactivity)	Variable reactivity when heparin substituted by LMWH, danaparoid, pentosan polysulfate, and others	Variable reactivity when cardiolipin[a] substituted by phosphatidylserine[b] or other molecules (e.g., irradiated plastic)
Sequestered location of antigen	PF4 within platelet α-granules	Anionic phospholipids of inner leaflet of bilipid membranes
Functional assay	Heparin-dependent activation of platelets by patient serum or plasma	Prolongation of phospholipid-dependent coagulation assay by patient's plasma
Inhibition of functional assay	Inhibition by high-dose heparin	Inhibition of lupus anticoagulant assays by excess phospholipid
Antigen assay	PF4-H-EIA	Anticardiolipin EIA
Relative sensitivity of assays for antibodies	Antigen > functional	Antigen > functional
Relative specificity of assays for disease state	Functional > antigen	Functional > antigen

Note: Information on relative sensitivity and specificity of functional and antigens assays for HIT and antiphospholipid antibody syndrome are provided elsewhere (Visentin et al., 1994; Ginsberg et al., 1995; Berube et al., 1998; Warkentin et al., 2000).

[a]Cardiolipin is found primarily in the inner leaflet of the mitochondrial membrane.

[b]Phosphatidylserine is located in the inner leaflet of platelet membranes; thus, antiphospholipid antibodies with antiphosphatidylserine activity could be relatively more important in the pathogenesis of thrombocytopenia.

Abbreviations: EIA, enzyme immunoassay; β_2-GPI, beta$_2$-glycoprotein I; LMWH, low molecular weight heparin; PF4-H, platelet factor 4-heparin; HIT, heparin-induced thrombocytopenia.

combined. In Greifswald, platelets from four different donors selected randomly are prepared and tested individually. ABO blood group discrepancies do not affect the results of these assays (Greinacher et al., 1991).

2. Perform differential centrifugation to obtain ACD-anticoagulated platelet-rich plasma (PRP).

Comment. Low-speed centrifugation prepares ACD-anticoagulated PRP. Additional ACD (111 µL/mL PRP) is added (Greifswald) to ensure that the pH of the PRP is sufficiently low (<6.5) to prevent platelet aggregation that otherwise would occur during platelet pelleting: the platelet release reaction is triggered by close platelet contact in low calcium concentrations at physiological pH (Kinlough-Rathbone et al., 1983). If the serotonin release method is used, the PRP is incubated at 37°C for 30 min with [^{14}C]serotonin (0.1 µCi/mL of PRP added from a stock solution of 50 µCi/mL of [^{14}C]serotonin) (Lee et al., 1996).

3. Wash the platelets by pelleting them from PRP, then gently resuspend the platelets in calcium- and magnesium-free Tyrode's buffer, pH 6.3, containing glucose (5.6 mmol/L) and apyrase (2.5 U/mL).

Comment. Tyrode's buffer consists of physiological concentrations of sodium chloride (NaCl, 137 mmol/L), potassium chloride (2.7 mmol/L), calcium chloride

TABLE 2 Classification of Laboratory Tests for HIT

Platelet activation (functional) assays
Washed platelet assays
Serotonin release assay (SRA): quantitation of ^{14}C-radiolabeled serotonin released from dense granules of activated platelets (Sheridan et al., 1986); chemical and chromatographic detection of serotonin also described (Fouassier et al., 2006)
Heparin-induced platelet activation (HIPA) test: visual assessment of platelet aggregation (Greinacher et al., 1991; Eichler et al., 1999)
ATP release detected by luminography (Stewart et al., 1995)
Platelet microparticle assay: quantitation of platelet-derived microparticles by flow cytometry (Lee et al., 1996)
Platelets in citrated platelet-rich plasma (c-PRP)
Platelet aggregation test (PAT): assessment of platelet aggregation using conventional aggregometry (Fratantoni et al., 1975; Chong et al., 1993a)
Annexin V-binding assay: quantitation by flow cytometry of annexin V binding to anionic phospholipids expressed by activated platelets (Tomer, 1997; Tomer et al., 1999)
Serotonin release detected by flow cytometry (Gobbi et al., 2003)
Antigen assays (PF4-dependent)
Enzyme immunoassays (EIAs)
Target antigen: PF4-H complexes (Amiral et al., 1992)
Target antigen: PF4-polyvinylsulfonate complexes (Visentin et al., 2001)
Fluid-phase immunoassay (Newman et al., 1998)
Rapid assays (based on PF4- or PF4/heparin-coated particle agglutination)
Particle gel immunoassay (PaGIA) (Meyer et al., 1999)
Particle immunofiltration assay (PIFA)

($CaCl_2$, 2 mmol/L), magnesium chloride ($MgCl_2$, 1.0 mmol/L), and sodium dihydrogen phosphate (NaH_2PO_4, 3.3 mmol/L); however, calcium-free and magnesium-free Tyrode's is used in this wash step to avoid activating the coagulation factors and platelets. The low pH prevents platelets from aggregating during pelleting. Apyrase is an enzyme that degrades adenine nucleotides (i.e., accumulation of the ADP from the platelets is prevented). Azide-free bovine serum albumin (3.5 mg/mL) and hirudin (1 U/mL) are included in the wash buffer in Greifswald, but not Hamilton, although HEPES (5 mmol/L) is added to this buffer in Hamilton. Following resuspension, the platelets are incubated for 15 min at 37°C (Greifswald).

4. Pellet the washed platelets as before, and then gently resuspend the platelets into calcium- and magnesium-containing Tyrode's buffer, pH 7.4, without apyrase or hirudin.

Comment. Following resuspension, the platelets should "rest" for 45 min at 37°C (Greifswald). The final resuspension buffer (Tyrode's buffer at physiological pH) contains calcium (2 mmol/L) and magnesium (1 mmol/L Hamilton; 2 mmol/L Greifswald). The platelet count is adjusted to a minimum of 300×10^9/L; thus, after addition of washed platelets (75 µL) to the microtiter wells containing test serum (20 µL) and heparin-buffer (5 µL in Hamilton, 10 µL in Greifswald), the final platelet concentration will be at least 215×10^9/L. Apyrase must not be included in this buffer, as the ADP released during assessment of HIT-induced platelet activation is an important potentiator of platelet Fc receptor–mediated platelet activation (Polgár et al., 1998).

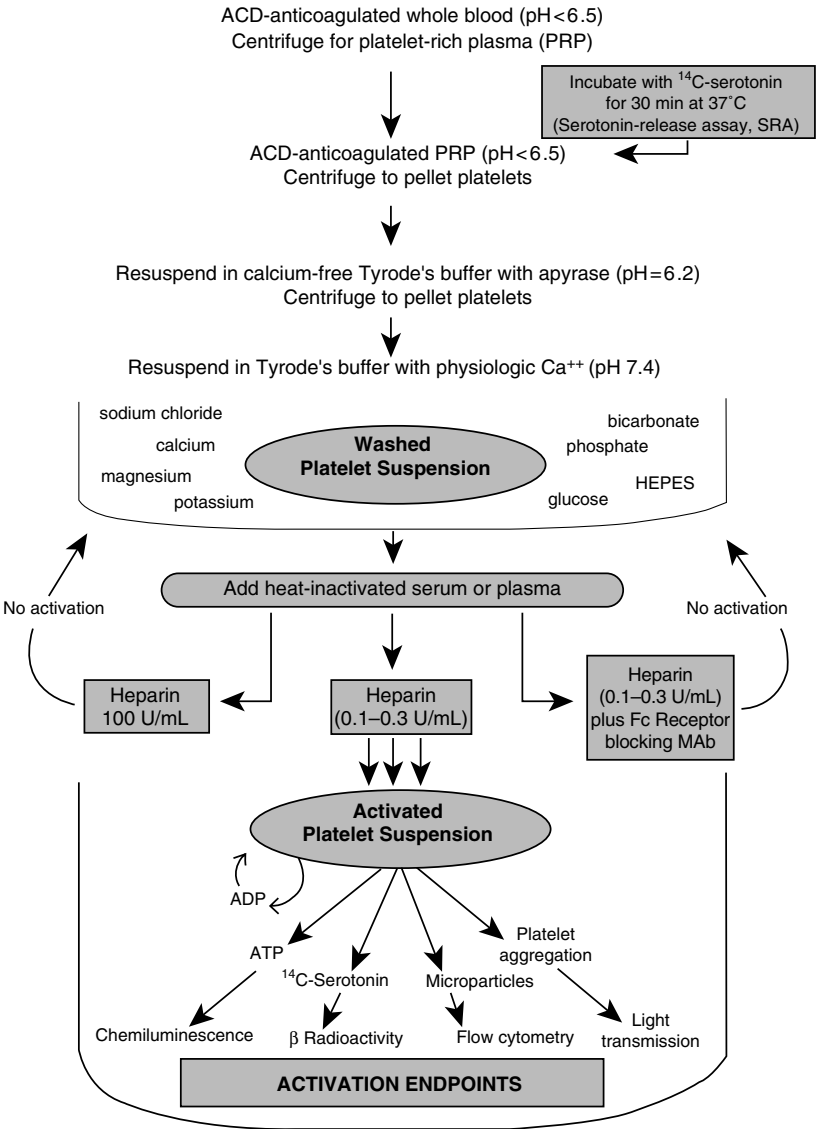

FIGURE 1 Schematic overview of washed platelet assays: HIT serum causes platelet activation at therapeutic (0.1–0.3 U/mL) heparin concentrations, but not in the presence of Fc receptor–blocking monoclonal antibody or high (100 U/mL) heparin concentrations. Platelet activation by HIT serum is potentiated by ADP release from platelet dense granules. Various platelet activation endpoints can be used. False-positive results can be avoided if typical reaction profiles of non-HIT platelet activation triggers are recognized: for example, (1) residual thrombin (activation at low but not high heparin concentrations, including activation in the presence of Fc receptor–blocking monoclonal antibody); (2) immune complexes (activation at low and high heparin concentrations, both of which are inhibited by Fc receptor–blocking monoclonal antibody); and (3) TTP serum (variable activation in the presence of heparin that is not inhibited by Fc receptor–blocking monoclonal antibody). *Abbreviations*: ACD, acid-citrate-dextrose; ADP, adenosine diphosphate; ATP, adenosine triphosphate; PRP, platelet-rich plasma; TTP, thrombotic thrombocytopenic purpura.

Test Conditions of Heparin-Dependent Platelet Activation: Perform Platelet Activation Studies Under Various Test Conditions

Comment. In Hamilton, sera are studied using six different reaction conditions: (1) buffer; (2) unfractionated heparin (UFH), 0.1 U/mL; (3) UFH, 0.3 U/mL; (4) UFH, 100 U/mL; (5) low molecular weight heparin (LMWH), enoxaparin, 0.1 U/mL; and (6) UFH, 0.3 U/mL plus a monoclonal antibody (IV.3) that inhibits platelet Fc receptor–mediated platelet activation. In Greifswald, routine testing is performed using (1) buffer; (2) LMWH (reviparin), 0.2 U/mL; (3) UFH, 100 U/mL; and (4) danaparoid, 0.2 U/mL (to assess cross-reactivity); (5) sometimes LMWH 0.2 U/mL plus IV.3 is performed to resolve unclear results. In Greifswald, the LMWH preparation reviparin (Clivarine) is used because of its narrow molecular weight (MW) range (80% of its chains have molecular mass of 2.4–7.2 kDa, i.e., 4–12 disaccharide units) (Jeske et al., 1997); this results in more consistent formation of PF4-H complexes, enhancing sensitivity of the assay (Greinacher et al., 1994b). Platelets are incubated with various test and positive and negative control sera under these various reaction conditions for up to 30 min (Greifswald) or 60 min (Hamilton). Addition of hirudin avoids thrombin-dependent platelet activation. The order of pipetting is important in optimizing assay results (Eichler et al., 1999) (Table 3). After adding serum to the microtiter plate wells, high heparin concentrations are added to the appropriate wells: this will disrupt PF4—heparin complexes that may be present in the serum. After adding washed platelets, buffer, LMWH (low concentrations), and danaparoid (for cross-reactivity testing, if desired) are added. If inhibition by monoclonal antibody IV. 3 is tested, this reagent is added before addition of the washed platelet suspension. In Hamilton, the pipetting order for the SRA is (1) addition of buffer-heparin, (2) serum, and (3) platelets.

Interpretation of the Obtained Test and Control Data

Comment. Several techniques can be used to assess activation of washed platelets (Fig. 1). The actual method of detection of platelet activation is probably less important than the technique of platelet preparation itself, including the selection of suitable platelet donors.

A positive test result is one in which heparin-dependent platelet activation occurs at therapeutic concentrations of heparin (0.1–0.3 U/mL) but is inhibited at very high (100 U/mL) heparin concentrations and in the presence of platelet Fc receptor–blocking monoclonal antibody. By assessing activation in the presence of different LMWH compounds or danaparoid, studies of in vitro cross-reactivity can be performed (discussed subsequently). It is important to ensure that control HIT sera, including one or more weak positive controls, react as expected. Given the experience from a workshop on testing for HIT antibodies (Eichler et al., 1999), we recommend exchange of weak positive control sera among laboratories for quality control.

Platelet Activation Endpoints

[^{14}C]Serotonin release was the first activation endpoint described using washed platelets (Sheridan et al., 1986). In this method, the washed platelets are incubated with test and control serum or plasma and heparin-buffer in flat-bottomed polystyrene microtiter wells (in duplicate or triplicate), performed on a platelet shaker (shaken, not stirred). After 1 h, the reaction is halted with 100 µL of 0.5% EDTA in phosphated-buffered saline (PBS). The microtiter plates are centrifuged at 1000 *g*

TABLE 3 Pipette Scheme for the HIPA Test

	Add first	Add second	Add third	Add fourth	Add last		
	Hirudin (50 U/mL)	Heat-inactivated patient or control serum	UFH 1050 U/mL = 100 U/mL (final)	Washed platelet suspension (300,000 platelets/μL)	Suspension buffer	LMWH (2.1 anti-Xa U/mL) = 0.2 U/mL (final)	Danaparoid (2.1 anti-Xa U/mL) = 0.2 U/mL (final)
Control with buffer	10 μL	20 μL	–	75 μL	10 μL	–	–
Low heparin concentration	10 μL	20 μL	–	75 μL	–	10 μL	–
High heparin concentration	10 μL	20 μL	10 μL	75 μL	–	–	–
Cross-reactivity with danaparoid	10 μL	20 μL	–	75 μL	–	–	10 μL

Abbreviations: LMWH, low molecular weight heparin; HIPA, heparin-induced platelet activation; UFH, unfractionated heparin.

for 5 min, and 50 μL of supernatant fluid is transferred to tubes containing scintillation fluid for detection of [^{14}C]serotonin released during platelet activation.

Carbon-14 is a radioisotope with a long half-life (5730 yr) that emits β-particles (electrons). Laboratories require special licenses to handle radioisotopes, thus limiting widespread use of this platelet-activation marker. However, it is also possible to quantitate serotonin by nonradioactive analysis (Gobbi et al., 2003; Fouassier et al., 2006). Results are expressed as percentage of serotonin released. This is calculated based on comparison with maximal possible release (determined following detergent-induced platelet lysis), and adjusted for background release (determined by quantitating serotonin release from a sample incubated with buffer alone). Acceptable experiments should have less than 5% background release, with both buffer and negative control serum testing being negative for HIT antibodies.

Aggregation of Washed Platelets. A convenient and useful activation endpoint—platelet aggregation—was reported in the HIPA assay (Greinacher et al., 1991; Eichler et al., 1999). Test serum and heparin buffer are placed in U-bottomed polystyrene microtiter wells containing two stainless steel spheres, and the platelets are stirred at approximately 500 rpm, using a magnetic stirrer. At 5-min intervals, the wells are examined against an indirect light source: a change in appearance of the reaction mixture from turbidity (nonaggregated platelets) to transparency (aggregated platelets) is a positive result. Although the activation endpoint is evaluated subjectively, interobserver agreement is good. A further advantage of this technique is its repeated evaluation of platelet activation over time. Thus, strong HIT sera that cause the typical activation profile of HIT (i.e., activation at low, but not high, heparin concentrations) within 15–30 min are readily identified. In contrast, such a strong HIT serum might eventually cause platelet activation even at the high heparin concentration and thus cause an "indeterminate" reaction pattern (activation at both low and high heparin concentrations) if activation is assessed at a later time point only. Occasionally there is interference with visual interpretation (e.g., a lipemic serum).

Luminography. Stewart et al. (1995) reported luminography to detect platelet activation, using a commercially available lumiaggregometer. Adenosine triphosphate (ATP) is released from platelet-dense granules during platelet activation. In the presence of luciferin–luciferase reagent, a light flash is generated in the presence of ATP, which is detected and quantitated. Another group reported similar results using a standard scintillation counter (Teitel et al., 1996). It is uncertain how the sensitivity and specificity of these assays compare with other markers of platelet activation.

Platelet-Derived Microparticle Generation. Generation of platelet-derived microparticles occurs when washed platelets are activated by HIT sera (Warkentin et al., 1994). With the use of a fluorescein-labeled anti-GPIα murine monoclonal antibody, a method for quantitating microparticles using flow cytometry was reported by Lee et al. (1996). Although both platelets and microparticles bind fluorescein-labeled anti-GPIα monoclonal antibodies, they can be distinguished by their size and scatter parameters using flow cytometry, with microparticles quantitated in relation to platelet numbers (Lee et al., 1996).

Heat Inactivation of Patient Serum or Plasma

To avoid thrombin-induced platelet activation in buffer containing physiological calcium, steps are taken to inactivate residual thrombin. Thus, plasma and serum must first be heat inactivated before use in these assays. Heating at 56°C for

30–45 min inactivates thrombin and complement. Fibrin and other precipitates are removed by high-speed centrifugation (8000 g for 5 min). More intense heating of serum (63°C for 20 min) forms platelet-activating immune complexes (Warkentin et al., 1994); thus, if a patient sample shows heparin-independent platelet activation (indeterminate result), another sample aliquot should be heat inactivated, and the HIT assay repeated. Often, this will result in disappearance of the initial artifact that presumably was caused by too intense heat inactivation. Serum is preferred for use in functional HIT assays in our laboratories, as serum contains more PF4, thereby facilitating initial formation of the antigen.

Biological Basis for High Sensitivity of Washed Platelets to Activation by HIT Antibodies

Table 4 lists differences between using washed platelets and platelets suspended in citrate-anticoagulated plasma to study HIT antibody–mediated platelet activation. Some of these differences may be important in explaining the greater sensitivity and specificity of washed platelets in detecting HIT antibodies.

1. Baseline platelet activation, including platelet-granule release, occurs during preparation of washed platelets. This enhances the platelet-binding capacity of heparin (Home and Chao, 1989) and may also increase the availability of PF4 to form the target antigen.

TABLE 4 Comparison Between Citrated Platelet-Rich Plasma and Washed Platelet Assays

Technical aspects	Washed platelet assay	Platelet-rich plasma assay	Comments
Platelet preparation	High *g* centrifugation during washing: increased baseline platelet activation	Low *g* centrifugation: less baseline platelet activation	Availability of PF4 may be higher using washed platelets (greater formation of PF4-heparin antigen complexes)
Apyrase	Apyrase added to wash solution, but not to the final resuspension (reaction) buffer	No apyrase used (no wash steps)	Apyrase degrades ADP, and prevents its accumulation; thus, platelet refractoriness to ADP-mediated potentiation of HIT serum-induced platelet activation is avoided by apyrase
Reaction milieu	Physiological calcium concentration (2 mmol/L)	Low (micromolar) calcium owing to citrate	IgG-mediated platelet activation optimal with physiological calcium concentrations
IgG levels	Reduced IgG levels during final reaction	Normal plasma IgG levels	Reduced inhibition of Fc receptor–mediated platelet activation by IgG in washed platelet assays
Plasma protein	Reduced plasma protein levels	Normal plasma protein levels	Reduced nonidiosyncratic platelet activation by heparin using washed platelets (?)
Temperature	Room temperature	37°C	Unknown significance
Reaction assessment	Microtiter plates	Conventional aggregometer	Many assays performed simultaneously using microtiter plates

Note: See text for further details on differences between washed platelet and citrated plasma assays (pp. 234–235).

2. Apyrase is used to prevent accumulation of ADP during platelet washing. This prevents platelets from becoming refractory to subsequent ADP-mediated platelet activation (Ardlie et al., 1970). Empirically, apyrase grade III (Sigma) is acceptable for use: grades I and II are too impure, and grades IV and higher are expensive.
3. Physiological calcium concentrations are present when washed platelets are used. Under these conditions, ADP produces only primary platelet aggregation. However, as observed by Packham et al. (1971), traces of immunoglobulin complexes in amounts too low to cause aggregation themselves will cause secondary aggregation to occur following the addition of ADP. The importance of ADP in mediating HIT antibody–induced platelet activation has been reported by Polgár and colleagues (1998). Thus, the reaction conditions that exist when washed platelets are used appear to maximize HIT antibody–induced platelet activation because the platelets retain sensitivity to ADP-mediated platelet activation.
4. Low concentrations of IgG are present in the final washed platelet reaction mixture: there is a fivefold reduction in IgG compared with citrated platelet-rich plasma (c-PRP) assays, because only IgG from the test serum is present in the final reaction mixture. Chong and colleagues (1993a) showed that high plasma IgG levels in one platelet donor's blood seemed to explain the discrepancy between studies using donor c-PRP (poor reactivity) and donor washed platelets (good reactivity). These and other investigators (Greinacher et al., 1994c) also observed that addition of IgG inhibits HIT serum-induced activation of washed platelets in a dose-dependent fashion.
5. Low concentrations of fibrinogen and other plasma proteins could reduce the potential for nonidiosyncratic heparin-induced platelet aggregation (Salzman et al., 1980; Chong et al., 1993a). In contrast, low concentrations of heparin rarely cause significant activation of washed platelets. It is possible that acute-phase reactant proteins such as fibrinogen could lead to false-positive activation assays for HIT using c-PRP.
6. Room temperature conditions are used for washed platelet assays; in contrast, c-PRP studies are performed at 37°C. Although this is a major difference between the assays, it is unknown whether there are advantages or disadvantages of performing washed platelet assays at room temperature. In Greifswald, all buffers are warmed to 37°C, and all incubation steps are performed at this temperature; only the final incubation on the microtiter plates is performed at room temperature.
7. Multiple serum-platelet reactions in microtiter plates can be performed, and even several hundred reactions studied in parallel. Quality control is thereby enhanced by the large number of control and test reaction conditions that can be analyzed, and the long incubation period employed (up to 60 min). The incubation period in HIT assays should be at least 20–30 min (Stewart et al., 1995).

Quality Control in Washed Platelet Assays for HIT

The variable reactivity of donor platelets to HIT sera is an important issue in activation assays for HIT. It has long been recognized that inconsistent results can be obtained using these assays (Salem and van der Weyden, 1983; Pfueller and David, 1986; Warkentin et al., 1992).

TABLE 5 Reactivities of 10 HIT Sera with Platelets from 10 Normal Donors

HIT sera (S_1–S_{10})	Normal platelet donors: strongest (P_1) to weakest (P_{10})									
	P_1 84.3	P_2 71.2	P_3 68.4	P_4 53.6	P_5 52.5	P_6 41.3	P_7 39.7	P_8 38.9	P_9 36.9	P_{10} 29.9
S_1 85.4	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	+ + + +	+ +	+ + +
S_2 84.4	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	+ + +	+ + + +	+ + +	+ + +
S_3 69.1	+ + + +	+ + + +	+ + + +	+ + +	+ + +	+ +	+ + +	+ +	+ +	+ +
S_4 61.0	+ + + +	+ + + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+ +	+
S_5 56.4	+ + + +	+ + +	+ + +	+ +	+ + +	+ +	+ +	+	+	+
S_6 50.7	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+ +	+	+
S_7 44.1	+ + + +	+ + +	+ + +	+	+ +	+	+	+	+ +	−
S_8 30.1	+ + + +	+ +	+ + +	+	+	+	−	−	−	−
S_9 24.2	+ + +	+ +	+	+ +	+	−	−	−	−	−
S_{10} 11.3	+ +	+	+	−	−	−	−	−	−	−

Serum samples and platelet donors are ranked from strongest to weakest (S_1–S_{10} and P_1–P_{10}, respectively), according to the mean percentage of [^{14}C]serotonin release when considering all 100 serum-platelet donor pairs (10 pairs corresponding to each HIT serum and each normal platelet donor). For each serum-platelet donor pair, the individual amount of serotonin release is summarized as follows: 80–100% release, + + + +; 60–79% release, + + +; 40–59% release, + +; 20–39% release, +; <20% release, −.
Overall, there is a graded pattern of reactivity among the individual reaction pairs that is hierarchical (i.e., there are no unexpected weak or strong reactions among the pairs). All negative reactions (<20% release) were found in the lower right portion of the table. Conversely, the strongest reactions (>80% release) were found in the upper left portion of the table.
Source: Warkentin et al., 1992.

Hierarchical Versus Idiosyncratic Platelet Activation by HIT Sera. The results of a systematic investigation, summarized in Table 5, showed that both HIT sera and platelet donors exhibit variable reactivity in a hierarchical, rather than an idiosyncratic, manner. The strongest reactions were produced by strong HIT sera against strongly reactive platelet donors. All of the negative reactions occurred when the weakest sera were mixed with the weakest platelets. Importantly, no unexpected negative reactions occurred elsewhere in the 10×10 serum-platelet grid (Table 5). Furthermore, the relative ranking of platelet donors appeared to be stable over time, an observation also reported by Chong and colleagues (1993a) in their study of platelet donor variability using c-PRP.

The finding of a hierarchical pattern of reactivity has important implications for quality control in diagnostic testing for HIT using activation assays. First, it indicates that platelets from certain donors who tend to respond well to HIT sera should be chosen. Second, relatively weak HIT sera should be included as positive controls (~20–50% serotonin release, or 25 min lag time in the HIPA).

Heparin-Independent Platelet Activation: Indeterminate Results. About 5% of test sera or plasma give an indeterminate result in an activation assay. This is defined as platelet activation that occurs at both therapeutic (0.1–0.3 U/mL) and supratherapeutic (10–100 U/mL) heparin concentrations. Often an interpretable result is obtained when the assay is repeated using another heat-inactivated aliquot. This suggests that the first result may have been an artifact caused by heat-aggregated IgG generated ex vivo. However, some serum and plasma samples repeatedly demonstrate heparin-independent platelet activation. Biological explanations include circulating immune complexes (e.g., systemic lupus erythematosus), high-titer HLA class I alloantibodies, and, possibly, other platelet-activating factors (e.g., thrombotic thrombocytopenic purpura). An antigen assay is required for further investigation when an indeterminate result is consistently obtained.

Inhibition by High Heparin Concentrations. Sheridan and colleagues (1986) first emphasized that there was a relatively specific activation profile triggered by HIT sera and plasmas: activation at therapeutic heparin concentrations (maximal at 0.1–0.3 U/mL) that progressively diminished with increasing heparin concentrations, typically falling to background activation at very high (100 U/mL) heparin concentrations. Classically, a positive test was deemed as greater than 20% serotonin release at 0.1 U/mL heparin and less than 20% serotonin release at 100 U/mL heparin. These criteria should not be applied indiscriminately, however. For example, a very strong HIT serum could produce more than a 90% release at 0.1 U/mL heparin and 25% release at 100 U/mL heparin. Alternatively, a serum or plasma sample that was not adequately heat-inactivated could produce a similar reaction profile (i.e., residual thrombin is inhibited by the high, but not low, heparin concentration). The strength of reactivity caused by patient serum can be helpful: clinically significant HIT antibodies almost always cause more than 50% serotonin release using optimally reactive platelets (Warkentin et al., 2000; Warkentin and Heddle, 2003). In the HIPA test, differences in the lag time to platelet aggregation provide useful information.

Inhibition by Fc Receptor Blockade. Platelet activation by HIT antibodies is inhibited in the presence of a murine IgG2b monoclonal antibody (IV.3) that recognizes the platelet FcγIIa receptor (Kelton et al., 1988; Chong et al., 1989) and can be used to enhance test specificity.

Interpretation of Platelet Activation by HIT Serum in the Absence of Added Heparin

With activation assays, it is not uncommon for HIT serum or plasma to cause platelet activation, even in the absence of added heparin (Warkentin and Kelton, 2001; Prechel et al., 2005). However, even greater platelet activation occurs in the presence of added heparin. When strong serum-dependent platelet activation occurs with buffer and at a 0.1–0.3 U/mL heparin concentration, it is important to ensure that the other reactions (at 100 U/mL heparin, and 0.1–0.3 U/mL heparin together with Fc receptor blockade) are as expected. This is because residual thrombin could produce strong platelet activation in both the absence and presence of low heparin concentration, thereby causing the potential for a false-positive result.

There are at least two potential explanations for strong platelet activation in the absence of added heparin. First, there may be residual heparin in the sample (White et al., 1992; Pötzsch et al., 1996). However, this phenomenon can persist despite attempts to remove heparin using binding resins. Further, heparin-independent platelet activation can be a feature of serum obtained from patients with "delayed-onset HIT," in which the presence of residual heparin is unlikely because onset of thrombocytopenia and thrombosis begins several days after the patient's last exposure to heparin (Warkentin and Kelton, 2001) (see Chapter 2). A second explanation is that some HIT antibodies recognize platelet-bound PF4 in the absence of an exogenous source of heparin, perhaps by PF4 bound to platelet glycosaminoglycans such as chondroitin sulfate (Rauova et al., 2006). Alternatively, as HIT antibodies are heterogeneous, there may be pathogenic antibody subpopulations that bind relatively well to PF4 even in the absence of heparin or heparin-like molecules (Newman and Chong, 1999; Amiral et al., 2000). This phenomenon has implications for the interpretation of tests of cross-reactivity of LMWH and danaparoid, as discussed later.

Advantages and Disadvantages of Washed Platelet Assays

High sensitivity and specificity is the major advantage of the washed platelet activation assays. In our hands, utilizing "weak positive" control HIT sera for quality control, the sensitivity of the SRA for clinical HIT is very high (>95%) (Warkentin et al., 2000, 2005a), and only reduced by the small number (<5%) of patient samples that yield "indeterminate" results (Smith et al., 2006). The diagnostic specificity is also high in most clinical settings, especially if the test gives a very strong result (>80% serotonin release) and all the controls react as expected.

The major disadvantage of washed platelet assays for detecting HIT antibodies is that they are technically demanding and labor intensive. A workshop that compared a washed platelet assay (the HIPA test) and an antigen assay showed greater variability in activation assay results among the participating laboratories (Eichler et al., 1999). Washed platelet activation assays are best suited for reference laboratories assessing many HIT sera, as this facilitates acquisition of sufficient technical experience to perform the assay successfully on a consistent basis. Assay-specific disadvantages include the requirement for radioactivity (SRA), the use of a subjective, visual endpoint (HIPA), and expensive equipment (flow cytometry–based assays).

B. Activation Assays Using Citrate-Anticoagulated Blood

The first reports describing the use of normal donor c-PRP to detect platelet activation caused by HIT serum or plasma appeared in the 1970s (Rhodes et al., 1973; Fratantoni et al., 1975; Babcock et al., 1976). A ratio of serum (or plasma) to c-PRP between 0.66 and 1.0 was used (e.g., 200 μL serum added to 200–300 μL c-PRP). No standardized method has evolved, however, although a survey of 54 laboratories in France (Nguyen et al., 1994) found some practices to be more common. For example, most laboratories test patient citrated platelet-poor plasma (c-PPP) rather than heat-inactivated serum. Variable heparin concentrations are used, most commonly between 0.5 and 1.0 U/mL. The ratio of patient c-PPP to donor c-PRP is usually 1:1, and ABO discrepancies are usually ignored. About 75% of the laboratories use at least two platelet donors for diagnostic testing.

Testing for HIT Antibodies Using c-PRP

The following description of the assay taken from Chong and colleagues (1989, 1993a) has the highest reported sensitivity and specificity among c-PRP methods. Blood is obtained from normal blood donors whose platelets respond well to serum or plasma from HIT patients, and c-PRP is prepared. Testing involves addition of 150 μL of patient heat-inactivated c-PPP or serum to 340 μL of c-PRP (final platelet concentration, 250–350 $\times$ 10^9/L) at 37°C. The platelets are monitored for a few minutes to exclude nonspecific platelet aggregation. After addition of 10 μL heparin-saline, aggregation is monitored over the next 15 min or until aggregation has occurred. A positive result is an increase in light transmission of more than 25% above baseline in the presence of therapeutic-dose heparin (0.5 U/mL) and patient serum or c-PPP and inhibition of aggregation in the presence of patient serum or plasma and supratherapeutic-dose heparin (100 U/mL). Use of such a two-point assay reduced the false-positive rate, as serum or plasma from some patients without HIT caused platelet aggregation at all heparin concentrations tested. To ensure that the platelets are functional, platelets are also tested with collagen (2 μg/mL). Details on methodology of c-PRP assays are also given elsewhere (Kapsch and Silver, 1981; Almedia et al., 1998).

Some workers report that platelets from a patient with HIT are very reactive to heparin-dependent activation by their own serum or plasma (Kappa et al., 1987; Chong et al., 1993b). Use of autologous c-PRP can sometimes be limited by the patient's thrombocytopenia, however. Potential explanations for the high sensitivity of autologous platelets include persisting high Fc receptor expression on platelets of patients with acute HIT (Chong et al., 1993b) and baseline platelet activation (Chong et al., 1994), with the potential for higher PF4 availability.

Disadvantages of c-PRP Aggregation Assays

Problems with these assays include (1) potential for false-positive interpretation if heparin produces nonspecific aggregation of donor platelets, an effect that could be enhanced nonspecifically by proaggregatory factors in the patient serum or plasma, and (2) risk of false-negative interpretation if HIT serum–induced platelet aggregation begins even before addition of heparin.

Nonspecific activation of platelets by heparin occurs with some normal donor c-PRP (Chong et al., 1993a), rendering these donors unsuitable for diagnostic testing. It is also possible that plasma from very sick patients may be more likely to cause nonspecific aggregation of platelets in c-PRP in the presence of heparin (Goodfellow et al., 1998).

An important practical disadvantage is that only a limited number of platelet aggregation tracings can be performed using conventional aggregometers. Thus, relatively few reactions with a limited number of patient and control samples can be evaluated.

Other Assays Using Citrated-Anticoagulated Whole Blood or PRP

Tomer (1997) reported a c-PRP activation assay for HIT antibodies in which the activation endpoint is quantitation of binding of fluorescein-labeled recombinant annexin V to platelets, as detected using flow cytometry. Annexin V, a placental protein, interacts with the prothrombinase-binding anionic phospholipids expressed on the surface of activated platelets and correlates with platelet procoagulant activity. It is uncertain whether the reaction conditions employed (e.g., 30-min incubation at 26°C) or the high sensitivity of annexin V binding (300-fold increase over baseline) overcomes the inherent limitations of sensitivity observed with other assays using c-PRP. Gobbi and colleagues (2003) developed a flow cytometry assay modeled after that of Tomer (1997), except that loss of serotonin from platelet granules was used as the platelet activation endpoint. Vitale et al. (2001) found P-selectin to be a better marker of platelet activation than annexin V.

C. Comparison of Washed Platelet and c-PRP Activation Assays

It became evident during the mid-1980s that the sensitivity of c-PRP aggregation assays for HIT was relatively poor (Kelton et al., 1984; Pfueller and David, 1986). Favaloro and colleagues (1992) first compared the c-PRP aggregation assay with the washed platelet SRA. They observed that only 6 of 13 HIT sera or plasmas that tested positive in the SRA also tested positive in the c-PRP aggregation assay. In contrast, no sample was identified that tested positive only in the aggregation assay. Chong and colleagues (1993a) also found a higher sensitivity for the SRA method. However, considerable variability in sensitivity for HIT antibodies among the various platelet donors was seen, ranging from 39–81% (c-PRP assay) to 65–94% (washed platelet SRA).

Strong evidence in favor of a higher sensitivity for washed platelet assays was provided by direct comparison using platelets prepared and tested in parallel that were obtained simultaneously from the same platelet donors (Greinacher et al., 1994a). Only 23 of 70 HIT sera that tested positive by the HIPA assay also tested positive using c-PRP aggregation. In contrast, all but one of 24 sera testing positive in the c-PRP aggregation assay also tested positive in the HIPA test.

More recently, Walenga and colleagues (1999) also found a lower sensitivity of the c-PRP aggregation test compared with the SRA. In contrast, Pouplard et al. (1999) reported a similarly high sensitivity of the c-PRP as the SRA (91% vs. 88%), but with a lower specificity (77% vs. 100%).

III. ANTIGEN ASSAYS FOR HIT ANTIBODIES

A. Solid-Phase PF4-H Enzyme Immunoassay

The solid-phase enzyme immunoassay (EIA) has been described in detail (Amiral et al., 1992; Visentin et al., 1994; Greinacher et al., 1994a; Amiral et al., 1995; Horsewood et al., 1996; Juhl et al., 2006). Methods differ in the way that PF4-H complexes are coated on the microtiter wells. A general scheme is shown in Figure 2. In this assay, stoichiometric concentrations of PF4 and heparin (e.g., 50 μL each of 20 μg/mL PF4 and 1 U/mL UFH) dissolved in phosphate buffer are added

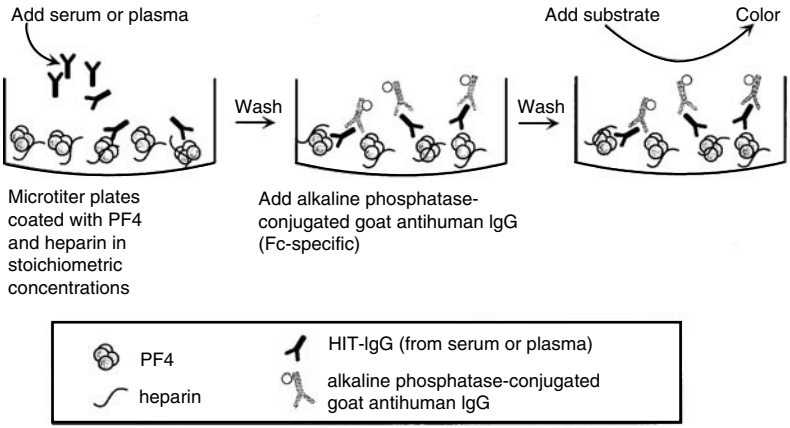

FIGURE 2 Schematic figure of solid-phase PF4-heparin-EIA. *Abbreviations*: EIA, enzyme immunoassay; HIT, heparin-induced thrombocytopenia; PF4, platelet factor 4.

together to the wells of a microtiter plate and incubated at 4°C overnight. After washing with phosphate buffer saline Tween 20 (PBS-Tw), the wells are "blocked" with a protein-containing solution such as PBS-Tw containing either 10% normal goat serum (NGS) or 20% fetal calf serum, followed by washing with PBS-Tw. To perform the assay, 50–100 µL of test or control plasma or serum diluted 1:50 in PBS containing 2% NGS is added to duplicate wells for 1 h at room temperature. After thorough washing with PBS-Tw, bound immunoglobulin is detected by adding alkaline phosphatase–conjugated goat antihuman immunoglobulin (e.g., affinity-purified goat antihuman IgG Fc diluted 1:1000 in PBS-Tw-2% NGS) followed by incubation for 1 h at room temperature. After thorough washing, *p*-nitrophenyl phosphate in 1 M diethanolamine buffer is added. After incubation in the dark, the reaction is stopped with 1 N NaOH, and absorbance is read at 405 nm using an automated microplate reader. The upper limit of the normal range is usually set at the mean +3 SD obtained using normal sera. Some laboratories set an indeterminate range for samples that are only minimally above the upper normal range (e.g., up to 1.0 units).

B. Solid-Phase PF4-Polyvinylsulfonate Antigen Assay

Several negatively charged substances can cause the cryptic autoepitope within PF4 to become recognizable to HIT antibodies (see Chapters 5–7). Indeed, a commercial assay for HIT using PF4 complexed with polyvinylsulfonate has been developed (Collins et al., 1997; Visentin et al., 2001). Sensitivity and reproducibility were high using polyvinylsulfonate that had been fractionated to a relatively uniform MW (5000 ± 500 Da). Some technical advantages of this assay include the observation that the ratio of PF4/PVS is not critical (cf. PF4-H), with acceptable concentrations of PVS ranging from 0.1 to 100 mmol/L for a corresponding concentration of 10 µg/mL PF4. The antigen complex is also stable for long periods.

The manufacturer of the PF4/polyvinylsulfonate EIA (Genetics Testing Institute [GTI], Waukesha, WI) recommends that a confirmatory step be performed, in

TABLE 6 Comparison of Two Commercial Antigen Assays for HIT Antibodies

	Asserachrom® (Stago)	GTI-PF4 EIA (GTI)
Target antigen	PF4-heparin complex	PF4-polyvinyl sulfonate complex
Source of PF4	Recombinant PF4	PF4 purified from outdated platelets
Microwell strips provided	Six strips of eight wells, in three nonresealable pouches	Twelve strips of eight wells (in resealable pouches)
Sample required	Plasma (sodium citrate) 200 μmL diluted 1:100 (=2μL plasma per well)	Serum or plasma 50 μL diluted 1:50 (=1 μL serum/plasma per well)
Controls supplied	Positive and negative control lyophylate,[a] calibrating standard	Positive and negative control sera[a]
Covers supplied	One provided	Multiple provided
Incubation times and conditions	All at 22°C (~2 h total)	37°C (two steps); then 22°C (~2 h total)
Plate reader settings	492 nm	405 or 410 nm
Detecting antibody system	Goat anti-IgG/A/M (peroxidase-conjugated)	Goat anti-IgG/A/M (alkaline phosphatase-conjugated)
Reaction stopping solution	3 M sulfuric acid or 1 M hydrochloric acid	3 M sodium hydroxide
Cutoff from negative	Internal control reagent is used to calibrate	>0.4 OD (assumes controls react as expected: pos ≥1.8, neg ≥;0.2)

[a]The Asserachrom® assay provides lyophylized control sera, whereas handling of control sera in the GTI assay is similar to handling of the test sera/plasma.
Abbreviations: EIA, enzyme immunoassay; GTI, Genetics Testing Institute; PF4, platelet factor 4.
Source: Warkentin, 2000.

which inhibition by 50% or more in the presence of high heparin (100 IU/mL) is considered supportive of a positive test result. However, this maneuver does not distinguish between clinically relevant and irrelevant anti-PF4-H antibodies, so test specificity may not be meaningfully increased. Furthermore, including this step will either double test costs (by requiring that each assay be performed both in the absence and in the presence of high heparin), or will delay the reporting of a positive test result (in case an algorithm is used in which a tentative positive test result is subsequently "confirmed") (Warkentin and Sheppard, 2006b).

Table 6 compares the two commercial EIAs. In the laboratory in Greifswald, discrepant results between the two assays have been observed in about 15% of patient samples tested.

Some research laboratories perform "in-house" PF4-H EIAs to detect HIT antibodies. An advantage is that an EIA can be used that only detects HIT antibodies of the IgG class (Warkentin et al., 2000, 2005a; Lindhoff-Last et al., 2001; Untch et al., 2002). This improves test specificity, as PF4-H-reactive IgA and IgM class antibodies (which are detected in the two commercial EIAs) are unlikely to cause HIT.

C. Fluid-Phase EIA

The fluid-phase EIA for HIT antibodies (Newman et al., 1998) is an adaptation of a staphylococcal protein A antibody-capture EIA method (Nagi et al., 1993). By permitting antibody–antigen interactions to occur in a fluid phase, problems of protein (antigen) denaturation inherent in solid-phase assays are avoided.

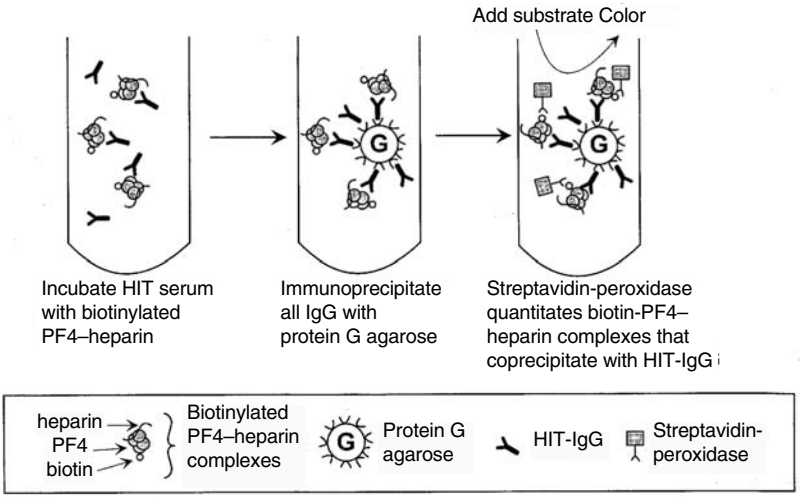

FIGURE 3 Schematic figure of fluid-phase PF4-heparin-EIA. *Abbreviations*: EIA, enzyme immunoassay; HIT, heparin-induced thrombocytopenia; PF4, platelet factor 4.

PF4 (5% biotinylated) is mixed with an optimal concentration of heparin, and this antigen mixture is incubated with diluted patient serum or plasma (Fig. 3). Subsequently, the antigen–antibody mixture is incubated with protein G-Sepharose in a microcentrifuge tube. Biotinylated antigen–antibody complexes become bound to the protein G-Sepharose by antibody Fc, and the complexes are separated from unbound antigen by centrifugation and washing. The amount of biotin-PF4-H-antibody complexes immobilized to the beads is measured using peroxidase substrate after initial incubation with streptavidin-conjugated peroxidase.

The fluid-phase EIA appears to have a lower rate of false-positive reactions. This may be because in the solid-phase-EIA, nonspecific binding of IgG to the microtiter wells can occur. Furthermore, the cryptic antigen site of PF4 can be exposed when the molecule comes into close contact with the plastic surface, even in the absence of heparin (Newman and Chong, 1999). The fluid-phase assay avoids these problems by first precipitating all reactive IgG antibodies, then detecting the antigen specifically bound to the IgG. Thus, higher concentrations of patient serum or plasma can be tested without increasing nonspecific reactivity. The advantages of this assay in performing in vitro cross-reactivity are discussed later. Because antibody is bound using protein G-Sepharose, IgM and IgA anti-PF4-H antibodies are not detected in this assay.

Wang and coworkers (1999a,b) used protein A to capture IgG antibodies from HIT patient serum. The immobilized antibodies were then incubated with normal serum (presumed to contain PF4) and fluorescence-labeled heparin. The amount of fluorescence dye bound to the protein A sepharose was used to detect HIT antibodies. The major drawbacks of this approach include the initial capturing of IgG other than HIT-IgG, as well as the unpredictable PF4/heparin ratios.

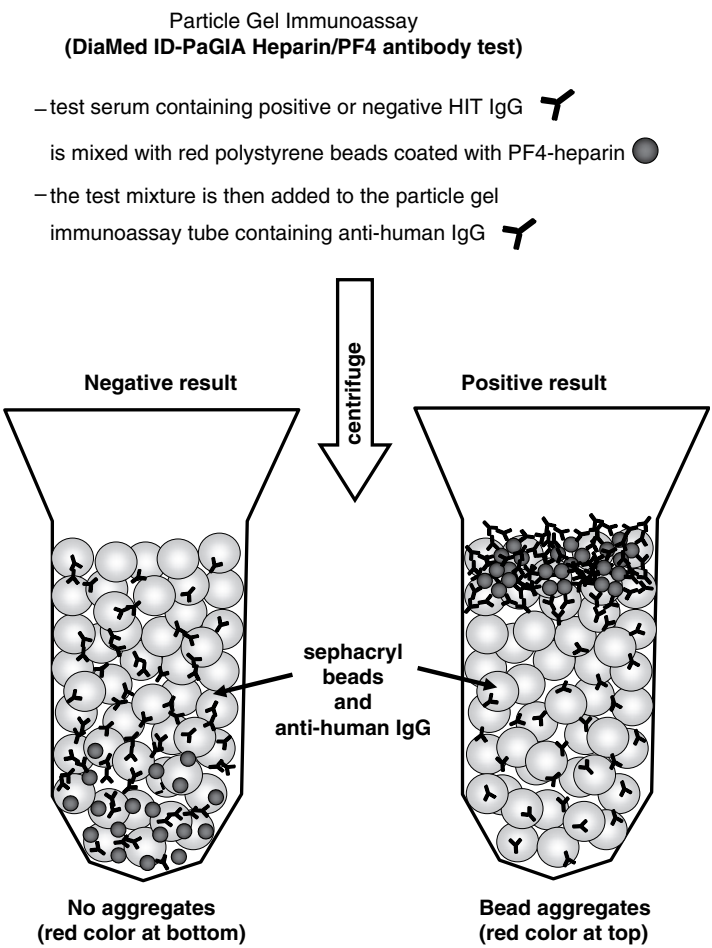

FIGURE 4 Schematic drawing showing the PaGIA using gel centrifugation technology. A secondary anti-antibody is used to facilitate particle gel agglutination. Unlike the EIA, which reaches an equilibrium state of antibody–antigen reaction, the PaGIA is a dynamic immunoassay. *Abbreviations*: HIT, heparin-induced thrombocytopenia; PaGIA, particle gel immunoassay; PF4, platelet factor 4. *Source*: From Warkentin and Sheppard, 2006a.

D. Rapid Assay: Particle Gel Immunoassay (ID-H/PF4 Test)

Figure 4 illustrates a rapid assay for anti-PF4-H antibodies, the particle gel immunoassay [(PaGIA], H/PF4-PaGIA) (DiaMed, Cressier sur Morat, Switzerland). This assay utilizes PF4-H complexes bound to red, high-density polystyrene particles; after addition of patient serum or plasma, the anti-PF4-H antibodies bind to the antigen-coated beads (Meyer et al., 1999; Eichler et al., 2001). However, IgG class antibodies do not agglutinate the polystyrene beads well, and therefore a secondary antihuman immunoglobulin antibody is added into the sephacryl gel. The principle of this (and other gel centrifugation assays) is that upon centrifugation, the agglutinated beads (indicating the presence of anti-PF4-H antibodies) do not migrate through the sephacryl gel (strong positive

result), whereas nonagglutinated beads (indicating absence of antibodies) pass through the gel (negative result), thus forming a red band at the bottom. A weak positive result is indicated by dispersal of the particles throughout the gel. The assay is technically easy, can be performed rapidly, and is readily automated. Results are read visually. The method is available to blood banks that utilize a gel centrifugation technology system. Currently, the PaGIA is available in Europe and Canada, and is under investigation in the United States.

Eichler and colleagues (2001) compared this new assay with two functional assays (HIPA test; SRA) and both commercially available solid-phase PF4-dependent EIAs. In preselected samples, the H/PF4-PaGIA had a sensitivity intermediate between that of the functional and commercial antigen assays. The specificity appeared to resemble that of the functional assays.

In contrast, Risch and coworkers (2003) found many more sera to test positive using the H/PF4-PaGIA, compared to a commercial EIA (Asserochrom®), among 42 patients sampled 10–18 days following cardiac surgery (69% vs. 26%). Since none of the patients had clinical evidence of HIT, this suggested that the diagnostic specificity of the H/PF4-PaGIA was far less than the solid-phase EIA. These authors did not test sera from patients with HIT, and therefore were unable to assess test sensitivity (Warkentin, 2003b).

The manufacturer's instructions indicate that the assay is to be read as "positive" (any agglutination within the gel), "negative" (no agglutination) using neat (undiluted) serum, or "borderline." However, when a positive or borderline test result was obtained, Alberio et al. (2003) repeated the assay with undiluted and serially diluted plasma (up to one in 1024) until the result was negative. The reported titer was the last positive result followed by either borderline or negative results. Patients judged clinically to have had "probable" or "highly probable/definite" HIT had antibody titers of four or more in 39 of 54 (72%) cases, compared with only two of 85 (2%) judged "unlikely" to have had HIT. Further, all 19 of the patient samples that tested positive in a c-PRP aggregation assay tested positive in the PaGIA (generally, in a titer of eight or higher). Among all patients studied, the percentage with associated thrombotic complications increased from 8% (negative or low titer) to 55% (positive titer 4–16) to 74% (positive titer 32–256). This study suggests that reporting quantitatively the results of the H/PF4-PaGIA—with a titer of four or more being clinically significant—may increase diagnostic usefulness.

E. Rapid Assay: Particle Immunofiltration Assay

More recently, another rapid immunoassay for anti-PF4-H antibodies, the "HealthTEST® Heparin/Platelet factor 4 Antibody Assay" (Akers Laboratories, Inc., Thorofare, NJ) received approval by the U.S. Food and Drug Administration (Fig. 5). This assay utilizes a system known as "particle immunofiltration assay" (PIFA), wherein patient serum (fresh not frozen/thawed) is added to a reaction well containing dyed particles coated with PF4 (*not* PF4/heparin). The lack of requirement for heparin presumably reflects formation of the HIT antigens through close approximation of PF4 tetramers achieved under the conditions of PF4 binding to the particles (Greinacher et al., 2006). Subsequently, nonagglutinated—but not agglutinated particles—will migrate through the membrane filter. Thus, a negative test is shown by a blue color in the result well, whereas no color indicates a positive test. FDA approval was based upon the assay being judged by

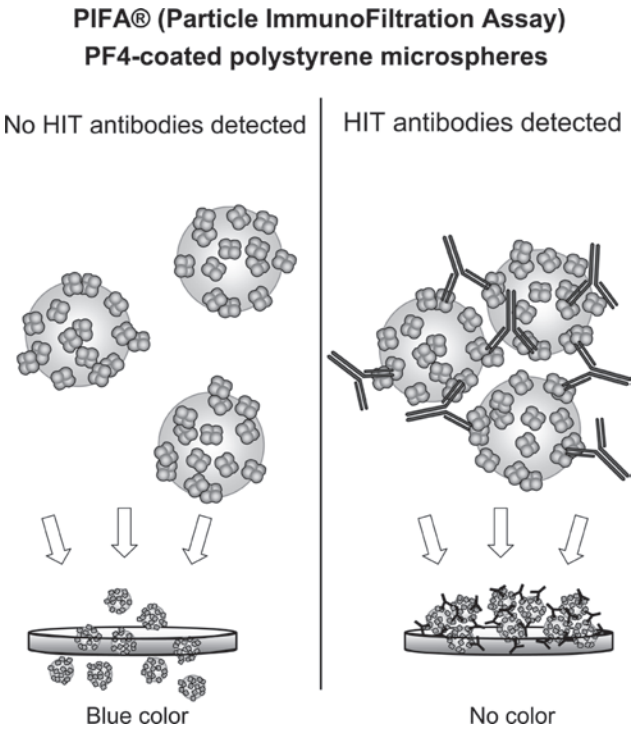

FIGURE 5 Schematic drawing showing the PIFA. *Abbreviations*: HIT, heparin-induced thrombocytopenia; PIFA, particle immunofiltration assay; PF4, platelet factor 4. *Source*: From Warkentin and Sheppard, 2006a.

the FDA as substantially equivalent to the solid-phase EIA available from GTI. However, in a preliminary report (Francis et al., 2006), and in the laboratories of both authors, this assay showed unacceptable sensitivity and specificity for detecting anti-PF4/H antibodies.

F. Comparison of Activation and Antigen Assays

Both PF4-H-EIA and washed platelet activation assays have approximately equal sensitivity for clinical HIT (Greinacher et al., 1994a; Warkentin et al., 2000, 2005). For serum or plasma samples that are known to be positive by one sensitive washed platelet activation assay (e.g., SRA or HIPA), the corresponding probability of the PF4-H-EIA for confirming the positive result is at least 75–90% (Greinacher et al., 1994a; Arepally et al., 1995), with more recent studies suggesting >95% sensitivity of the EIA (Warkentin et al., 2005; Juhl et al., 2006). Conversely, a similar percentage of referred samples with high clinical probability of HIT that test positive in the EIA will also test positive using a washed platelet activation assay (Greinacher et al., 1994a; Lo et al., 2006; Schenk et al., 2006, 2007). The sensitivity of both EIA and SRA was even higher (>95%) for detecting antibodies that caused HIT in prospectively studied postoperative patients (Warkentin et al., 2000, 2005a).

Although both antigen and activation assays have similarly high sensitivity for clinical HIT, there is evidence that antigen assays have greater sensitivity for

detecting HIT antibodies not associated with thrombocytopenia or other clinical events (Amiral et al., 1995; Arepally et al., 1995; Bauer et al., 1997; Warkentin et al., 2000, 2005a; Juhl et al., 2006; Schenk et al., 2006, 2007) (Fig. 6). Stated another way, the SRA is more specific for clinical HIT than the antigen assay. The biological explanation for greater specificity of a sensitive activation assay for clinical HIT, compared with an antigen assay, could relate to the functional heterogeneity of HIT antibodies against antigenic determinants on PF4, only some of which activate platelets strongly (Amiral et al., 2000). Data reported by Visentin and colleagues (1994) also support a higher sensitivity of antigen assays for detecting platelet-activating anti-PF4/H antibodies. These workers studied 12 HIT plasmas that tested positive in both SRA and PF4-heparin-EIA. However, at a 1:100 sample dilution, only 2 of the 12 samples still tested positive in the activation assay. In contrast, even at a 1:200 dilution, all 12 plasmas still tested positive in the EIA. Bachelot and colleagues (1998) observed that HIT plasmas that tested only weakly positive in the PF4-H-EIA tended to give negative washed platelet SRA results when using platelets with the least reactive FcγIIa receptor phenotype, Arg^{131}.

The difference in sensitivity for HIT antibodies between the PF4-H-EIA and aggregation studies using c-PRP is considerable. Only about 33–64% of samples that test positive in the PF4-H-EIA also test positive using c-PRP aggregation (Greinacher et al., 1994a; Nguyen et al., 1995; Rugeri et al., 1999). Although one laboratory reported a greater sensitivity using c-PRP aggregation than the EIA (Look et al., 1997), these workers did not employ a two-point method, and so may have observed false-positive results using the aggregation assay.

IV. INTERPRETATION OF HIT TEST RESULTS

It is important to incorporate clinical information into the interpretation of any laboratory result for HIT. This is because thrombocytopenia, whether or not caused by HIT, is common in hospitalized patients receiving heparin, and because nonpathogenic HIT antibodies are often detected by sensitive assays in patients who have received heparin for 5 or more days.

Several clinical scoring methods have been described to help estimate the probability of HIT independently of the HIT antibody test results (Greinacher et al., 1994a; Pouplard et al., 1997; Warkentin, 2003a; Warkentin and Heddle, 2003; Lo et al., 2006). Some include assessing the platelet count recovery upon stopping heparin, and so may be more useful when reviewing a case after its clinical evolution. Chapter 2 provides an example of one scoring system to estimate the pretest probability of HIT that can be applied at the time of initial diagnostic assessment.

A. How Should Clinical HIT Be Defined?

HIT is a clinicopathologic syndrome, and thus can be defined as a patient with a clinical profile consistent with HIT, and in whom heparin-dependent, platelet-activating antibodies can be detected. From an operational point-of-view, this corresponds to at least an "intermediate" pretest probability score (e.g., $\geq$4 points in the 4 T's scoring system) and having a positive washed platelet activation assay for "strong" heparin-dependent platelet-activating antibodies (generally >50% serotonin release or <25 min lag time in the HIPA). If a positive EIA is used to support the presence of antibodies, the clinician should be aware that non–platelet-activating antibodies could give a "false-positive" test result, and so if the clinical picture

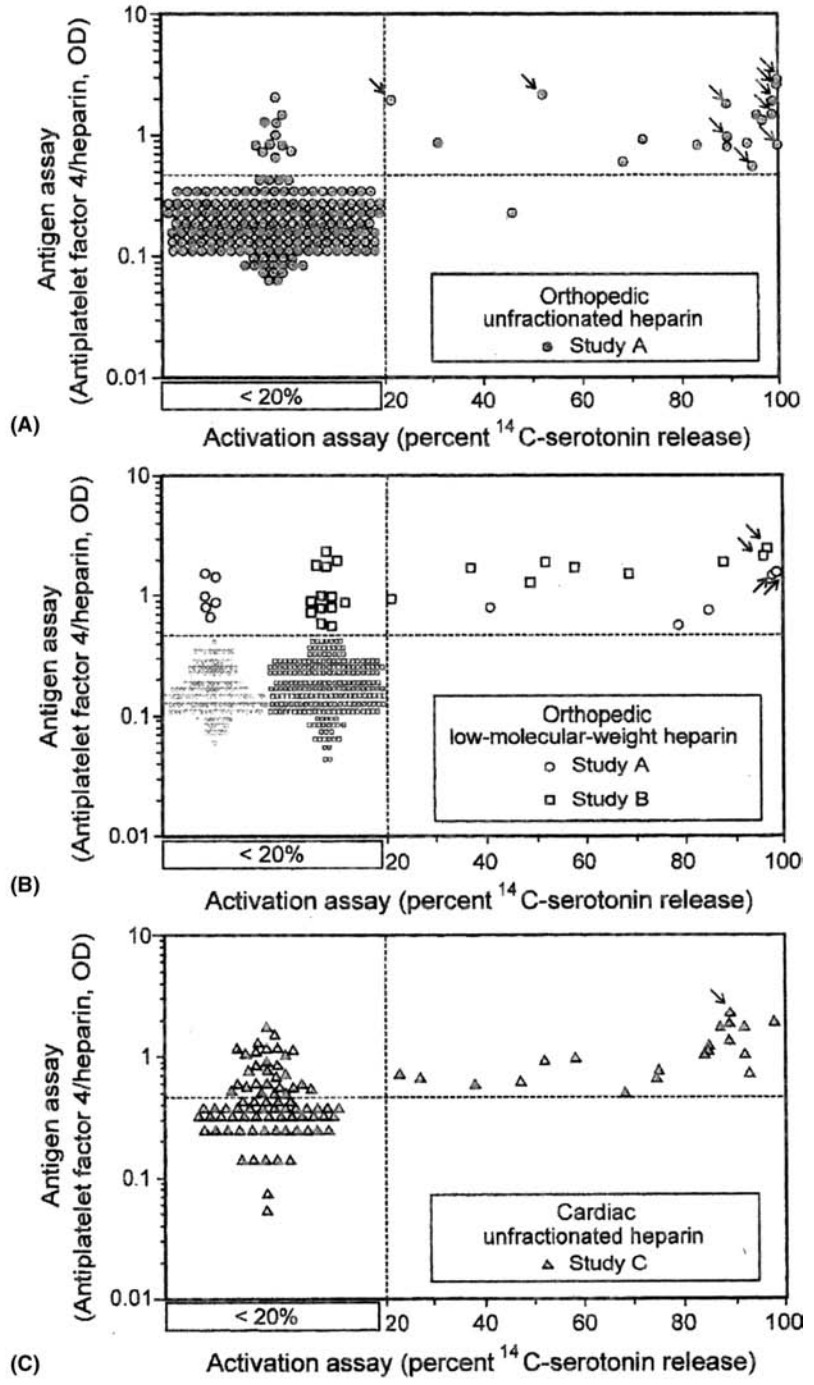

FIGURE 6 *(Caption on facing page)*

suggests alternative diagnoses, requesting the more specific washed platelet assay may be helpful. Another relevant issue is that a higher "strength" of a positive washed platelet activation assay or solid-phase EIA increases the likelihood of the patient having HIT, given a certain pretest probability (Warkentin, 2005). This is discussed in the section "Diagnostic Interpretation of Laboratory Results."

Laboratory testing for HIT antibodies is often performed in clinical situations suggesting a low probability for HIT, presumably because physicians wish to "rule out" this diagnosis (Lo et al., 2006). We have found that only about 7% to 10% of patients who undergo testing for HIT antibodies have a serological profile consistent with the diagnosis (Juhl et al., 2006; Warkentin and Sheppard, 2006a).

Rapid Versus Typical Onset of Thrombocytopenia

We will discuss the diagnostic approach to HIT based on the timing of onset of thrombocytopenia, either rapid (<5 days) or typical (≥5 days) (see Chapter 2).

In general, there are two broad pretest probabilities for patients with rapid thrombocytopenia: low and high. Patients with low pretest probability for HIT are those who have not recently been exposed to heparin (thus, they would not be expected to have circulating HIT antibodies, or to have generated them so quickly), or who have another good explanation for thrombocytopenia. (An important caveat is that sometimes a recent heparin exposure is not known to the patient or has not been documented in the medical records.) With a low pretest probability for HIT, either of the sensitive assays for HIT (washed platelet activation assay or antigen assay) can reliably rule out HIT. However, an unexpected negative result in a patient with a high pretest probability, or an unexpected positive result in a patient with a low pretest probability, should lead to repeating the test or performance of the complementary activation or antigen assay. Additionally, further clinical information should be sought. (For example, has another explanation for the thrombocytopenia become apparent? Could the patient have had an unrecognized recent heparin exposure?)

In contrast, for patients with the typical temporal onset of thrombocytopenia (i.e., a platelet count fall that begins 5–10 days after beginning heparin treatment), we believe that, in general, there are two different pretest probabilities for HIT: moderate and high. Because HIT is a relatively common explanation for thrombocytopenia that begins during this characteristic time period, it should be considered a plausible diagnosis even if another possible explanation for thrombocytopenia is identified (hence, a moderate pretest probability). In a patient without another apparent explanation for thrombocytopenia, or one in whom an unexplained new

FIGURE 6 (*Figure on facing page*) Comparison of activation and antigen assays for HIT-IgG: analysis of prospective studies. Quantitative results of an activation assay, the SRA, are shown on the *x*-axis (although samples that gave <20% serotonin release are shown without reference to the actual quantitative result obtained [see box designated <20%]); quantitative results of the antigen assay (which detected only IgG anti-PF4-H antibodies) are shown on the *y*-axis. (**A**) Orthopedic surgery patients who received UFH; (**B**) orthopedic surgery patients who received LMWH; and (**C**) cardiac surgery patients who also received postoperative UFH. The arrows indicate the data points corresponding to the 15 patients who developed clinical HIT (>50% platelet count fall from the postoperative peak). The data show similarly high sensitivity of the activation and antigen assays for clinical HIT; however, the activation assay had higher specificity for clinical HIT. Most sera (13/15, 87%) from patients with clinical HIT strongly activated platelets (>80% serotonin release). *Abbreviations*: HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; PF4, platelet factor 4; SRA, serotonin release assay; UFH, unfractionated heparin. *Source*: From Warkentin et al., 2000.

thrombotic event has occurred, the pretest probability for HIT would be considered to be high.

B. Diagnostic Interpretation of Laboratory Results

In patients with a high pretest probability of HIT who have a negative screening test, the test should be repeated and the complementary activation or antigen assay should be performed. The diagnosis of HIT is very unlikely if both activation and antigen assays are negative. In patients with a moderate pretest probability who have one or more positive tests for HIT, the final diagnosis may well rest on the overall clinical picture, rather than on the test result alone. This conclusion results from two clinical realities: (1) sensitive HIT assays frequently detect clinically insignificant anti-PF4-H antibodies in patients who have received heparin for more than 5 days, and (2) thrombocytopenia, whether caused by HIT or not, is common in clinical practice. There is evidence that positive washed platelet activation assays for HIT have greater diagnostic specificity for clinical HIT (Warkentin et al., 2000, 2005a), especially when strong, rapid platelet activation is produced by patient serum. Regardless, these considerations underscore the importance of conceptualiz-

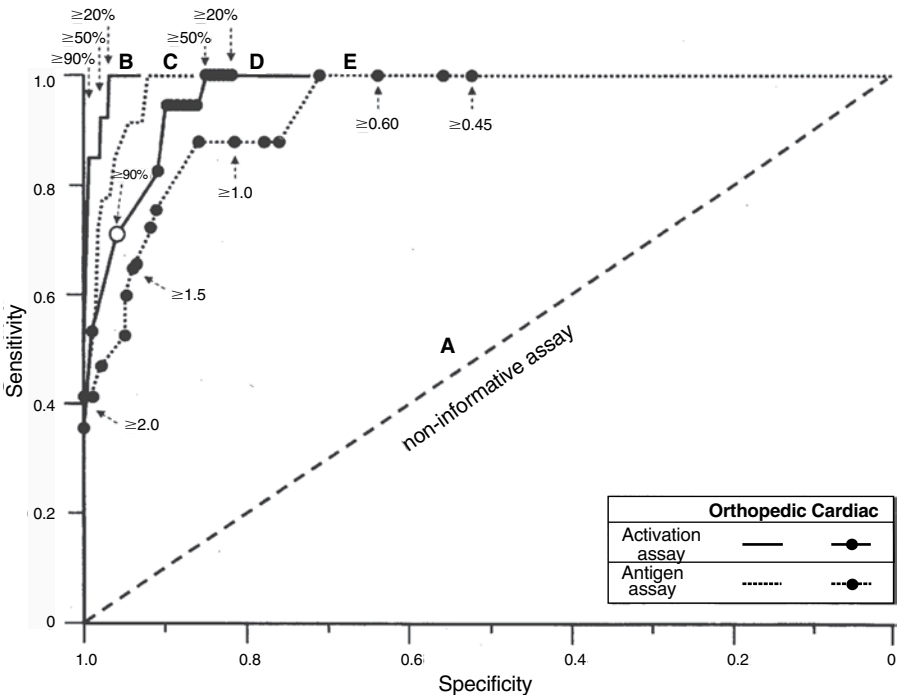

FIGURE 7 Sensitivity–specificity tradeoffs for diagnosis of HIT (receiver operating characteristic curve analysis). The arrows indicate various cut-offs between positive and negative test results; e.g., the open circle indicates 90% serotonin release (postcardiac surgery patient) using a washed platelet activation assay (serotonin release assay). The likelihood ratio for HIT for a given positive test result can be estimated from the graph, using the formula: likelihood ratio = sensitivity/(1 − specificity). Thus, for 90% serotonin release (postcardiac surgery), the estimated likelihood ratio is 0.7/(1 − 0.965) = 20. *Source*: From Warkentin, 2003b.

ing HIT as a clinicopathologic syndrome, in which both clinical information and results of HIT antibody testing are used for diagnosis.

The diagnostic usefulness of certain laboratory tests for HIT is shown in Figure 7 (Warkentin, 2003b). Both the SRA and an in-house EIA that detect only HIT-IgG were very sensitive and specific for clinical HIT in postorthopedic surgery patients. The diagnostic usefulness of these assays was somewhat less in a postcardiac surgery population. For example, among postcardiac surgery patients, the likelihood ratio of a strong-positive SRA result (90% serotonin release; see open circle in Fig. 7) is about 20. The likelihood ratio, which is defined as the extent to which a given test result alters the physician's estimate of the pretest probability of HIT, is defined as sensitivity/(1 − specificity). In this example, the corresponding likelihood ratio is $0.70/(1-0.965) = 20$. Thus, if the physician had estimated a pretest probability of 50% (odds of 0.5:0.5), then this test result would increase the posttest probability of having HIT to more than 95% (0.5:0.5 × 20:1 = 20:1, or 95.2%). In contrast, the high sensitivity of this assay to detect clinically important HIT antibodies (>95%) means that a negative test result lowers the posttest probability to less than 5%.

The diagnostic impact of such a strong-positive SRA result (90% serotonin release) is even greater in postorthopedic surgery patients, for whom the corresponding likelihood ratio is about 85, i.e., $0.85/(1-0.99)$. As before, a negative test result essentially rules out HIT.

Although the EIA that detects only HIT-IgG antibodies has lower diagnostic specificity than the SRA, it remains a useful assay. The likelihood ratios for a strong positive test result (e.g., optical density of 1.5) range from about 10 to 40 for postcardiac and postorthopedic surgery patients, respectively. Also, its high sensitivity (>98%) means that a negative test generally rules out HIT (Greinacher et al., 2007).

Thus, HIT antibody testing is among the most useful of platelet immunology assays. For comparison, Figure 7 also shows the profile of a "noninformative assay" (see line A). This is the profile for various tests of "platelet-associated IgG" for the diagnosis of autoimmune thrombocytopenia. Certain glycoprotein-specific platelet antibody tests have operating characteristics intermediate between those for HIT and a noninformative assay. For example, the monoclonal antibody immobilization of platelet antigens (MAIPA) assay has only moderate sensitivity but high specificity for diagnosis of autoimmune thrombocytopenia.

Figure 7 shows the operating characteristics for two assays: the SRA and a PF4-H-EIA that detects only IgG antibodies. Recently, Warkentin and coworkers (2005a) used archived plasma samples to compare the operating characteristics of a commercial EIA (PF4-polyvinylsulfonate EIA, GTI) that detects antibodies of all three major immunoglobulin classes (IgG, IgA, IgM) (Fig. 8). This study showed that the additional detection of IgA and IgM class antibodies *worsened* the test's operating characteristics, by detecting clinically insignificant IgA and IgM antibodies without any offsetting improvement in test sensitivity. These data are consistent with the view that HIT is usually (perhaps invariably) caused by IgG antibodies, i.e., the only antibody class able to activate platelets via their IgG (Fcγ) receptors.

A practical implication of Figure 7 is that the *magnitude* of a positive HIT antibody test provides diagnostically useful information, with a strong positive result associated with a greater likelihood of a patient having clinical HIT than a weak positive result (Warkentin, 2003b; Zwicker et al., 2004). Similarly, if two sensitive and complementary tests for HIT antibodies (washed platelet activation

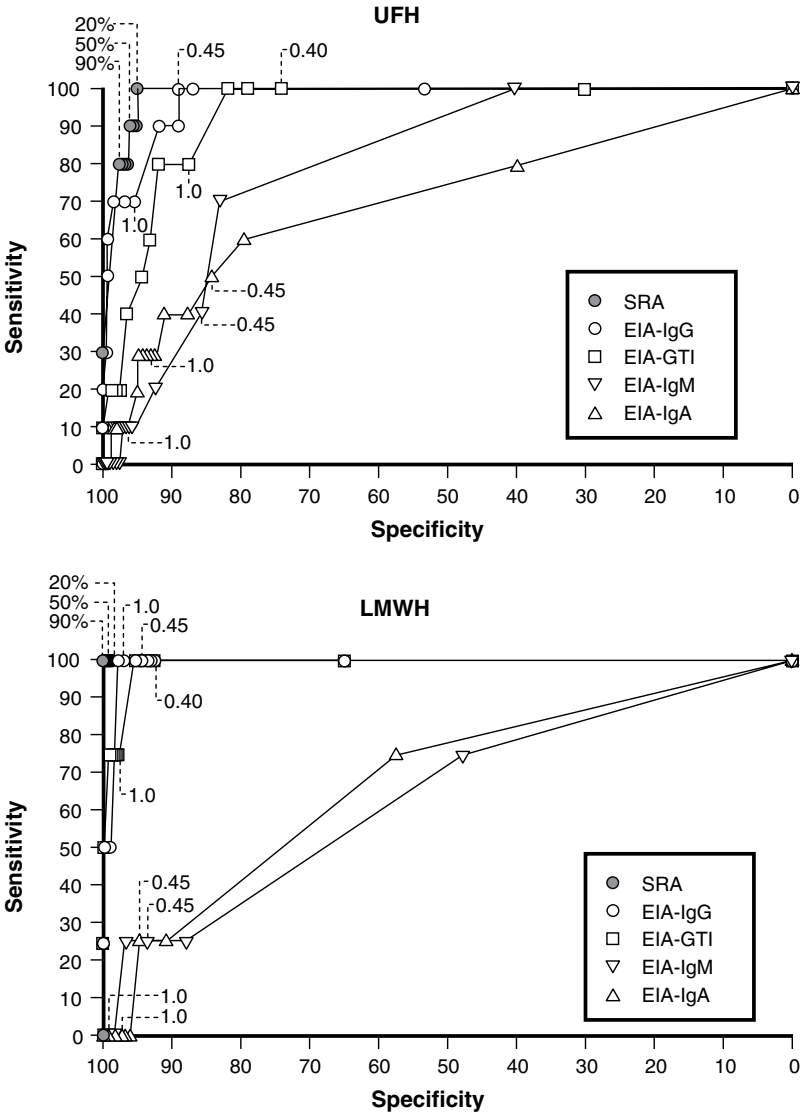

FIGURE 8 Comparisons of ROC curves among five different assays for anti-PF4/heparin antibodies. (*Top*) UFH-treated patients ($n = 192$). (*Bottom*) LMWH-treated patients ($n = 256$). The sensitivity–specificity tradeoffs at different diagnostic cut-offs for clinical HIT are shown. For the SRA, the points represent step-wise 10% changes in percent serotonin release. For the EIA-IgG and EIA-GTI, the points represent step-wise 0.20 units of optical density, except for the point labeled 0.45, which represents the usual diagnostic cut-off for the three EIAs shown (EIA-IgG, EIA-IgA, and EIA-IgM). For both UFH- and LMWH-treated patients, the ROC curves show that the operating characteristics rank as follows for the diagnosis of clinical HIT: SRA > EIA-IgG > EIA-GTI > EIA-IgA ~ EIA-IgM. *Abbreviations*: EIA, enzyme immunoassay; LMWH, low molecular weight heparin; ROC, receiver operating characteristic; UFH, unfractionated heparin. *Source*: From Warkentin et al., 2005a.

assay, PF4-dependent EIA) both give negative test results, the diagnosis of HIT is virtually excluded (even in a patient with a high pretest probability).

Some investigators advocate routine repeat testing for HIT antibodies by EIA when an initial test result is negative (Refaai et al., 2003). There is a potential pitfall to this approach: the high sensitivity (>98%) of the EIA for HIT means that the clinical events that led to initial testing almost certainly are not due to HIT. Thus, in the absence of progressive or recurrent thrombocytopenia, or subsequent development of thrombosis, a subsequent positive EIA is more likely to represent a seroconversion event involving nonpathogenic anti-PF4/heparin antibodies. When HIT is strongly suspected—despite a negative EIA—there likely is greater utility in performing the complementary platelet activation assay, as it can detect pathologic antibodies against antigens other than the PF4-H complex.

V. IN VITRO CROSS-REACTIVITY

A. Cross-Reactivity Using Activation Assays

Cross-reactivity studies have been performed most frequently using activation assays. However, there are no standard methods for, or even a standard definition of, in vitro cross-reactivity. In one study of LMWH and danaparoid cross-reactivity, an increase in platelet activation in the presence of the drug over baseline was used to determine cross-reactivity (Warkentin, 1996). This definition was used to avoid falsely attributing cross-reactivity to drug-independent platelet activation that is produced by some patients' sera. The reason for this definition was the common phenomenon that platelet activation can be caused by a patient's serum even in the absence of added heparin. In the HIPA test, comparison of the lag time to aggregation can be used to judge cross-reactivity: if a sample shows platelet aggregation with danaparoid or LMWH earlier than in the presence of buffer, then cross-reactivity is present. In general, in vitro cross-reactivity with danaparoid is usually clinically insignificant (Warkentin, 1996; Newman et al., 1998) (see Chapters 12 and 13).

Comparison of c-PRP Versus Washed Platelet Assays

Sensitive washed platelet assays generally show almost 100% cross-reactivity of HIT antibodies for LMWH (Greinacher et al., 1992; Warkentin et al., 1995a). Indeed, UFH and LMWH are essentially indistinguishable in these assays. However, very different results have been reported by investigators using c-PRP assays (Makhoul et al., 1986; Chong et al., 1989; Kikta et al., 1993; Vun et al., 1996). Here, LMWH consistently shows less cross-reactivity compared with UFH. It is possible that differences in nonidiosyncratic heparin-induced platelet activation underlie these observations (see Chapter 4): UFH is more likely to result in weak platelet activation, including some PF4 release, which leads to amplification of the platelet activation response in the presence of PF4-H-reactive HIT antibodies. In contrast, in washed platelet assays, IgG-mediated platelet activation, but not nonidiosyncratic HIPA, occurs.

B. Cross-Reactivity Using Antigen Assays

Although it is theoretically possible to perform a solid-phase EIA to assess cross-reactivity (Amiral et al., 1995), this is complicated because the antigen has to be

coated as a complex to the solid phase. This problem has been overcome in a fluid-phase EIA described by Newman and colleagues (1998). Because this assay detects binding to a defined quantity of labeled PF4-containing antigen, the assay is able to determine in vitro cross-reactivity more accurately than the solid-phase EIA. These investigators observed an in vitro cross-reactivity rate of 88% for LMWH; about half the HIT samples reacted weakly against danaparoid in their study. The fluid-phase EIA has also been used to show that the antithrombin-binding pentasaccharide, fondaparinux, does not cross-react with HIT-IgG antibodies (Warkentin et al., 2005b).

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11 Pseudo-Heparin-Induced Thrombocytopenia

Theodore E. Warkentin
Michael G. DeGroote School of Medicine, McMaster University, and Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada

I. INTRODUCTION

A. The Concept of Pseudo-Heparin-Induced Thrombocytopenia

Heparin-induced thrombocytopenia (HIT) is strongly associated with life- and limb-threatening venous and arterial thrombosis, including pulmonary embolism, venous limb gangrene, and large vessel arterial occlusion. However, HIT is by no means a unique explanation for the combination of thrombocytopenia and thrombosis (Table 1). In these pseudo-HIT disorders—so named because they strongly mimic HIT on clinical grounds—thrombocytopenia usually occurs early during the course of heparin treatment. This could reflect the prothrombotic process associated with the patient's primary diagnosis. Alternatively, heparin could exacerbate the platelet count fall by nonimmune proaggregatory effects on platelets (see Chapter 4). If the patient previously received heparin, physicians might consider HIT in the differential diagnosis of the platelet count fall.

However, one pseudo-HIT syndrome in particular closely resembles even the typical day 5–10 timing of thrombocytopenia characteristic of HIT: adenocarcinoma-associated disseminated intravascular coagulation (DIC). In these patients, the fall in platelet count begins soon after stopping heparin treatment. Because the patients usually will have received heparin for 5–10 days to treat adenocarcinoma-associated thrombosis, the timing of the onset of thrombocytopenia closely resembles immune HIT. Furthermore, the frequent occurrence of new or progressive thrombosis in this setting also suggests HIT.

The crucial concept in defining the notion of pseudo-HIT is the presumption that no matter how closely the thrombocytopenic disorder resembles HIT on clinical grounds, pathologic HIT antibodies, i.e., those characterized by strong heparin-dependent, platelet-activating properties, are *not* detectable in the patient's blood. This concept is credible given the high sensitivity of certain assays for detecting such antibodies (see Chapter 10).

This chapter draws attention to those clinical disorders that can mimic and, thereby, be confused with HIT. This is not a trivial distinction: whereas heparin is contraindicated in patients with HIT, it often is the optimal treatment of patients with pseudo-HIT. Second, the close clinical parallels between HIT and certain pseudo-HIT disorders can provide insights into the pathogenesis of thrombosis. For example, the recognition that venous limb gangrene can complicate metastatic adenocarcinoma, and the clinical parallels with a similar syndrome in HIT patients, suggests that a common factor (coumarin anticoagulation) may play a crucial pathogenic role in both disorders (Warkentin, 2001). Likewise, similarities between HIT and the lupus anticoagulant syndrome suggest that they could also share common pathogenic mechanisms (Arnout, 1996, 2000; Gruel, 2000).

TABLE 1 Pseudo-HIT Disorders

Pseudo-HIT disorders	Pathogenesis of thrombocytopenia and thrombosis	Timing
Prothrombotic disorders		
Adenocarcinoma	DIC secondary to procoagulant material(s) produced by neoplastic cells	Late[a]
Pulmonary embolism	Platelet activation by clot-bound thrombin	Early[b] or late[c]
Diabetic ketoacidosis	Hyperaggregable platelets in ketoacidosis (?)	Early[d]
Antiphospholipid antibody syndrome	Multiple mechanisms described, including platelet activation by antiphospholipid antibodies (?)	Early
Thrombolytic therapy	Platelet activation by thrombin bound to fibrin degradation products (?)	Early[e]
Septicemia-associated purpura fulminans	Symmetrical peripheral gangrene secondary to DIC with depletion of protein C and antithrombin	Early
Infective endocarditis	Infection-associated thrombocytopenia; ischemic events secondary to septic emboli	Early
Paroxysmal nocturnal hemoglobinuria	Platelets susceptible to complement-mediated damage; platelet hypoproduction	Early
Post-surgical TTP	Anti-ADAMTS13 autoantibodies modulated by the postoperative state (?)	Early or late
Prohemorrhagic disorders		
GPIIb/IIIa antagonist-induced thrombocytopenia	Usually secondary to natural anti-GPIIb/IIIa antibodies; bleeding more common than thrombosis	Early
PTP	"Pseudospecific" alloantibody-mediated platelet destruction (exception: bleeding, not thrombosis)	Late

Note: These pseudo-HIT disorders can mimic HIT by causing thrombocytopenia and thrombosis in association with heparin treatment. An exception is PTP, which causes bleeding, but not thrombosis; however, PTP can resemble HIT because both disorders usually occur about a week after major surgery requiring blood and postoperative heparin. The pseudo-HIT disorders can be categorized based on whether the onset of thrombocytopenia is typically "early" (<5 days) or "late" (>5 days) in relation to the heparin.
[a]See Fig. 1 for an example of pseudo-HIT caused by adenocarcinoma-associated DIC.
[b]See Fig. 3 for early thrombocytopenia associated with pulmonary embolism.
[c]See Fig. 4 for late thrombocytopenia associated with pulmonary embolism.
[d]See Fig. 5 for early thrombocytopenia associated with diabetic ketoacidosis.
[e]See Fig. 6 for early thrombocytopenia caused by thrombolytic therapy.
Abbrevations: ADAMTS, ***a d***isintegrin ***a***nd ***m***etalloprotease with ***t***hrombo***s***pondin-1-like domains; DIC, disseminated intravascular coagulation; GP, glycoprotein; HIT, heparin-induced thrombocytopenia; PTP, posttransfusion purpura; TTP, thrombotic thrombocytopenic purpura.

II. PSEUDO-HIT SYNDROMES

A. Adenocarcinoma

Mucin-producing adenocarcinoma is an important cause of venous and arterial thrombosis that occurs in association with thrombocytopenia. In these patients, DIC is often the predominant explanation for the thrombocytopenia. The diagnosis is suggested by reduced fibrinogen levels (or prolonged thrombin time), elevated prothrombin time, and elevated cross-linked (D-dimer) fibrin degradation products (or a positive protamine sulfate "paracoagulation" test).

Adenocarcinoma-associated DIC can strongly resemble HIT (Fig. 1). Typically, a patient presents with idiopathic deep vein thrombosis (DVT), sometimes with mild to moderate thrombocytopenia. During treatment with therapeutic-dose unfractionated or low molecular weight heparin (LMWH), the platelet count rises,

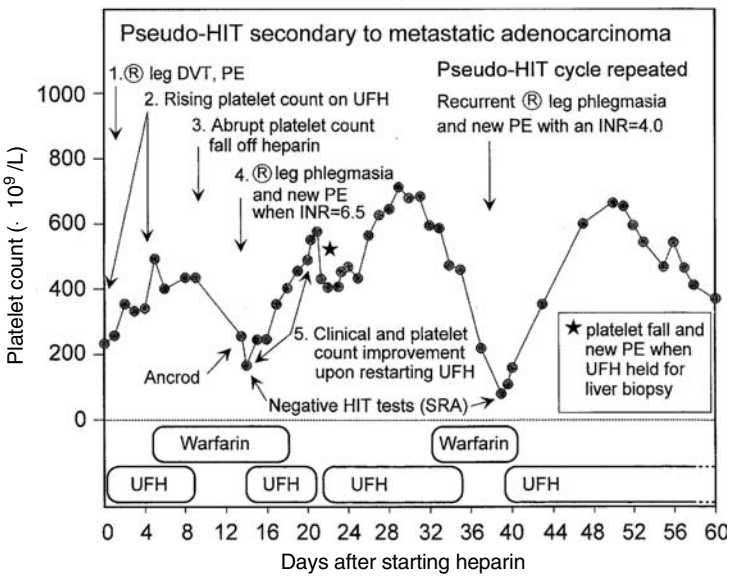

FIGURE 1 Pseudo-HIT: adenocarcinoma with thrombocytopenia and phlegmasia cerulea dolens after stopping administration of UFH. The late presentation of thrombocytopenia suggested HIT, prompting use of an alternative anticoagulant (ancrod). Heparin was restarted when HIT antibodies were not detected by SRA. Subsequently, discontinuation of heparin led to recurrence of thrombocytopenia and warfarin-associated phlegmasia cerulea dolens (repeat of pseudo-HIT cycle). *Abbreviations*: DVT, deep venous thrombosis; HIT, heparin-induced thrombocytopenia; INR, international normalized ratio; PE, pulmonary embolism; SRA, serotonin-release assay; UFH, unfractionated heparin.

likely because of improved control of DIC by the heparin. In my experience, this often dramatic rise in the platelet count during heparin treatment of "idiopathic" DVT is a clinically useful marker for adenocarcinoma-associated DIC. During the 5- to 10-day period of heparin treatment with overlapping warfarin anticoagulation, no problems are encountered. However, there is rapid recurrence of thrombocytopenia within hours or days of discontinuing the heparin, despite apparent therapeutic anticoagulation with warfarin, during which time the patient develops new or progressive venous, or even arterial, thrombosis. Thus, the onset of thrombocytopenia and thrombosis may occur within the 5- to 10-day "window" that suggests HIT.

Venous Limb Gangrene Complicating Adenocarcinoma

The venous thrombotic events complicating adenocarcinoma include DVT, phlegmasia cerulea dolens, and even venous limb gangrene (Everett and Jones, 1986; Adamson and Currie, 1993). Clinical and laboratory parallels between HIT and adenocarcinoma suggest that, paradoxically, coumarin treatment could contribute to the pathogenesis of venous gangrene in these patients through a disturbance in procoagulant–anticoagulant balance (Warkentin, 1996, 2001; Klein et al., 2004; Ng and Crowther, 2006). Figure 2 summarizes the proposed pathogenesis of this syndrome from the perspective of the characteristic clinical triad of venous limb gangrene: (1) thrombocytopenia caused by HIT or adenocarcinoma-associated DIC;

(2) acute DVT with acral (distal) microvascular thrombosis; and (3) a supratherapeutic international normalized ratio (INR) associated with coumarin therapy.

Venous limb gangrene appears to result from failure of the protein C anticoagulant pathway to down-regulate thrombin generation within the microvasculature (Warkentin 1996; Warkentin et al., 1997; see Chapter 2). Here, the elevated INR represents a surrogate marker for marked reduction in functional protein C levels (by a parallel reduction in factor VII); the thrombocytopenia is a surrogate marker for uncontrolled thrombin generation associated either with HIT or adenocarcinoma (Fig. 2). As venous limb gangrene occurs in a limb with preceding active DVT, this suggests that local factors, such as direct extension of thrombosis, as well as exacerbation of distal thrombosis by venous stasis, contribute to large- and small-vessel thrombosis characteristic of this syndrome.

Venous thrombosis complicating adenocarcinoma, especially when complicated by DIC or severe venous ischemia or necrosis, should be treated with heparin, rather than warfarin or other coumarin anticoagulants. Reversal of warfarin anticoagulation (intravenous vitamin K, with or without plasma infusion) and prompt control of DIC with heparin could salvage a limb with severe phlegmasia, or limit damage in a patient with venous gangrene. An effective agent often is LMWH (Prandoni, 1997; Lee et al., 2003). I recommend monitoring using antifactor Xa levels, because some patients with heparin resistance require high doses of heparin to achieve therapeutic anticoagulation.

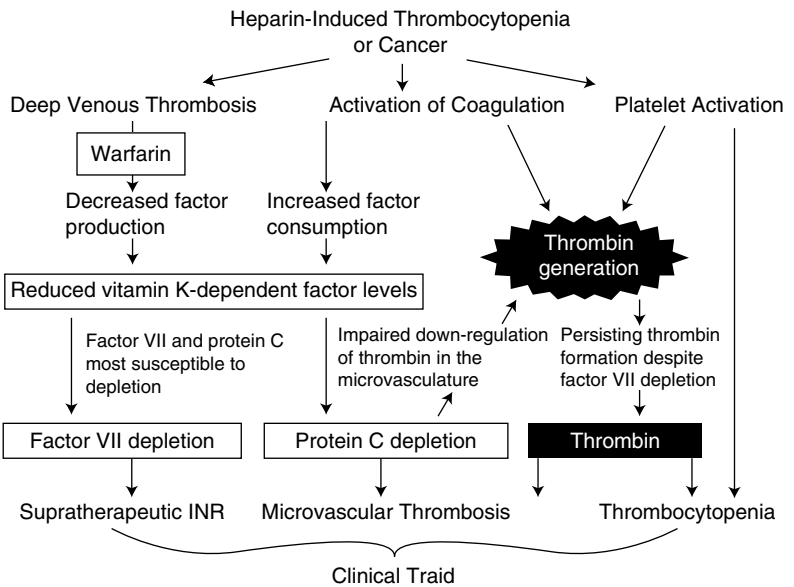

FIGURE 2 The pathogenesis of warfarin-associated venous limb gangrene is shown in relation to its typical clinical triad—supratherapeutic INR, microvascular thrombosis, and thrombocytopenia. The central paradox is persisting formation of thrombin despite markedly depleted plasma factor VII level, which is paralleled by severely depleted protein C activity, leading to impaired down-regulation of thrombin generation in the microvasculature and, consequently, microvascular thrombosis. *Abbreviation*: INR, international normalized ratio. *Source*: From Warkentin, 2001.

Ironically, one of the problems of heparin in these patients is its efficacy: thus, if heparin is discontinued for any reason, rapid recurrence of thrombocytopenia and thrombosis can result. Figure 1 shows an example in which thrombocytopenia and pulmonary embolism occurred (day 21) when heparin was held for a few hours to permit a liver biopsy to diagnose metastatic carcinoma. I have also observed a patient with lung adenocarcinoma in whom heparin was held to permit limb amputation; postanesthesia, the patient was aphasic (intraoperative stroke).

B. Pulmonary Embolism

Mild thrombocytopenia is common in patients with pulmonary embolism. Sometimes the thrombocytopenia is severe and associated with laboratory markers of DIC (Stahl et al., 1984; Mustafa et al., 1989) (Fig. 3). The thrombocytopenia presumably results from thrombin-induced platelet activation and/or platelet accretion within the thromboemboli (Welch, 1887; Kitchens, 2004). Studies of experimental venous thromboembolism in dogs show abrupt increase in plasma fibrinopeptide levels upon embolization, consistent with intensification of the thrombotic process (Morris et al., 2004). Large thromboemboli within the high-flow pulmonary vessels

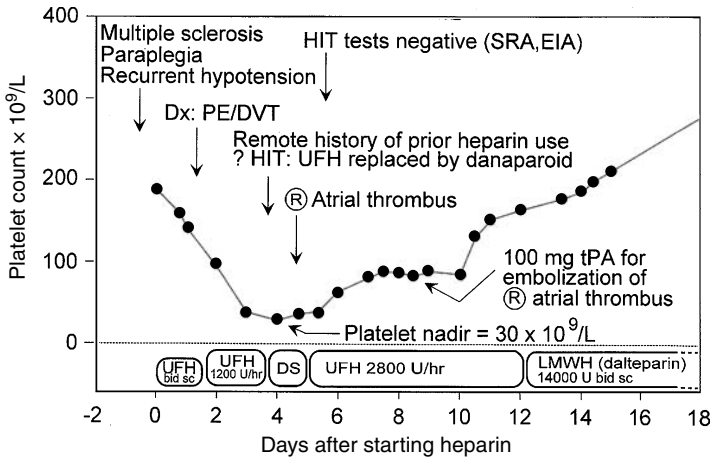

FIGURE 3 Pseudo-HIT secondary to PE and DIC: An obese, 50 yr old man with paraplegia was admitted for recurrent hypotension. He initially received b.i.d. sc UFH for antithrombotic prophylaxis, as the initial diagnosis was septicemia. DVT and PE were then diagnosed (Dx), and therapy changed to intravenous UFH, 1200 U/h. The platelet count fell over 4 days to a nadir of 30×10^9/L; DS was given because of concern over possible HIT (there was a remote history of previous heparin use). An echocardiogram showed large right atrial thrombus (likely representing a leg vein embolus), and the patient was transferred to a cardiac surgical center. The platelet count fall was judged too rapid to be HIT (see Chapter 2), a viewpoint supported by negative testing for HIT antibodies by SRA and PF4-heparin EIA. UFH administration was restarted in higher doses with antifactor Xa monitoring to overcome heparin resistance. Recurrent hypotension occurred when the right atrial thrombus embolized; full hemodynamic and platelet count recovery occurred following t-PA administration, followed by UFH, then LMWH, and (later) warfarin treatment. The patient was well at 3-yr follow-up, without evidence of carcinoma. *Abbreviations*: b.i.d., twice-daily; DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis; DS, danaparoid sodium; EIA, enzyme-immunoassay; HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; PE, pulmonary embolism; SRA, serotonin-release assay; sc, subcutaneous; t-PA, tissue plasminogen activator; UFH, unfractionated heparin.

may act as a reservoir for clot-bound thrombin that is relatively protected from inhibition by antithrombin-dependent inhibitors (Weitz et al., 1990). This view is indirectly supported by the observation that thrombocytopenia commonly occurs in patients with pulmonary embolism, but not in patients with DVT alone (Monreal et al., 1991; Warkentin et al., 2003a). Furthermore, increased heparin clearance has been demonstrated in experimental pulmonary embolism (Chiu et al., 1977), which could also contribute to increased thrombin generation.

Because HIT is also strongly associated with pulmonary embolism (Warkentin et al., 1995, 2003a), a diagnostic and therapeutic dilemma results when a patient presents with pulmonary embolism and thrombocytopenia 5 or more days after surgery managed with postoperative heparin prophylaxis (Fig. 4). Initiating therapeutic heparin could have catastrophic consequences for the patient who has circulating HIT antibodies, although in sufficient doses it is effective for a patient with pulmonary embolism and DIC without HIT. Because these two possibilities cannot be readily distinguished on clinical grounds alone, one should manage such a patient with an alternative anticoagulant until the results of HIT antibody testing become available (Warkentin, 2000).

C. Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) can be associated with acute thromboembolic complications. In vitro studies indicate that high glucose levels enhance platelet activation by adenosine diphosphate (ADP) and other platelet agonists (Sudic et al., 2006). Evidence for in vivo platelet activation was observed in one study of 10 patients who had elevated plasma levels of platelet factor 4 (PF4) and β-thromboglobulin during DKA that resolved following recovery (Campbell et al., 1985). Evidence for activation of coagulation includes elevated fibrin degradation products and reduced antithrombin (Paton, 1981). Figure 5 illustrates a patient with "white clots" in the femoral artery, leading to amputation, who was initially thought to have HIT. However, HIT antibody testing and subsequent clinical events proved that the patient did not have HIT as the initial explanation for this dramatic clinical presentation of thrombocytopenia and thrombosis complicating DKA (although HIT occurred later in the clinical course). I am also aware of a patient with essential thrombocythemia who developed postoperative DKA, thrombocytopenia, and bilateral lower-limb artery thrombosis that occurred too early (days 2–3) during thromboprophylaxis with unfractionated heparin (UFH) to have been caused by immune HIT. A similar example of early-onset severe thrombocytopenia and arterial thrombosis resulting in amputation of an arm was reported in a patient with DKA and adult respiratory distress syndrome (ARDS) (Phillips et al., 1994). Although the authors suggested HIT secondary to heparin "flushes" as the diagnosis, pseudo-HIT seems more likely based upon the temporal features of the case, as well as the negative laboratory testing for HIT antibodies. Casteels et al. (2003) observed the combination of rhabdomyolysis, thrombocytopenia, and anemia in a child presenting with DKA.

D. Antiphospholipid Antibody Syndrome or Lupus Anticoagulant Syndrome

Clinical Features

Antiphospholipid antibodies can be detected either as "lupus anticoagulants" or as anticardiolipin antibodies (Asherson et al., 1989; Ginsberg et al., 1995).

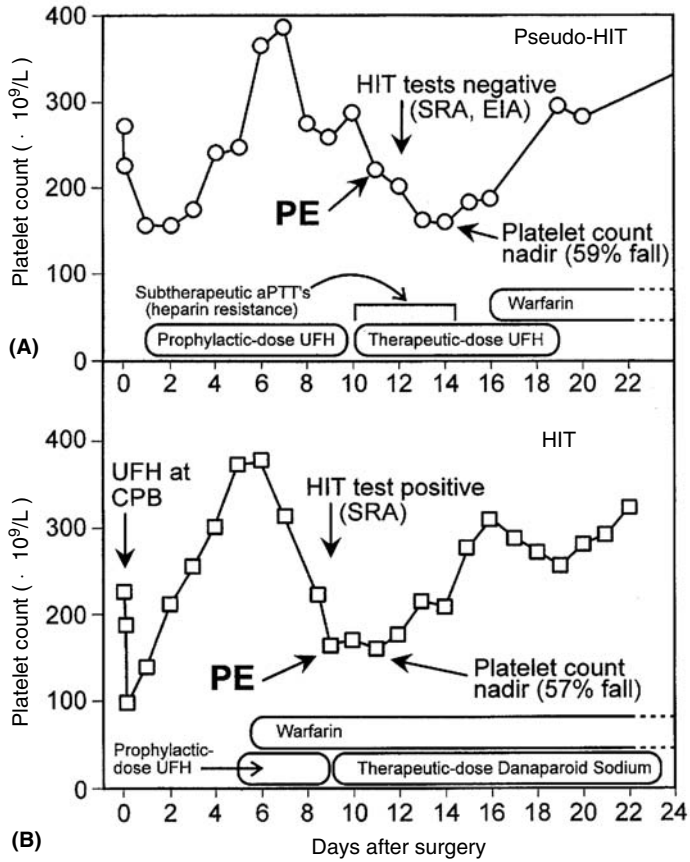

FIGURE 4 Pseudo-HIT associated with PE versus HIT: (**A**) A patient developed a platelet count fall from 387 to 159×10^9/L (59% fall) that began on day 7 of UFH prophylaxis following orthopedic surgery. PE was diagnosed by pulmonary angiography on postoperative day 11. The platelet count fell during initial intravenous heparin therapy, rising only when sufficient UFH was given (2360 U/h) to overcome "heparin resistance" (as shown by subtherapeutic activated partial thromboplastin times, aPTTs). HIT antibodies were not detectable (day 12), either by SRA, (<5% release) or PF4-heparin-EIA (optical density, 0.149; negative, <0.450). (**B**) A platelet count profile similar to that seen in (**A**) also occurred in a patient who developed a platelet count fall from 378 to 161×10^9/L (57% fall) that began on day 7 after cardiac surgery in which UFH was given for CPB. The platelet count recovered on therapeutic-dose danaparoid. Only one clinical clue pointed to the diagnosis of HIT: erythematous skin lesions at the UFH injection sites were also observed on day 7 (not shown on figure). Testing for HIT antibodies was strongly positive in the SRA (98% release at 0.1 U/mL heparin; 0% release at 100 U/mL heparin and at 0.1 U/mL heparin in the presence of Fc receptor-blocking monoclonal antibody). The similar platelet count profiles between these patients illustrate the difficulty in determining on clinical grounds whether postoperative PE is caused by HIT or not. *Abbreviations*: CPB, cardiopulmonary bypass; EIA, enzyme-immunoassay; HIT, heparin-induced thrombocytopenia; PE, pulmonary embolism; SRA, serotonin-release assay; UFH, unfractionated heparin.

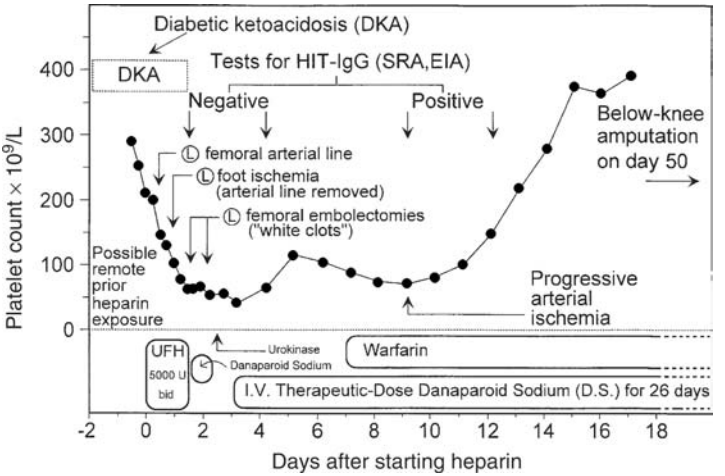

FIGURE 5 Pseudo-HIT during DKA, later complicated by HIT: A 27-yr-old man developed rapid onset of thrombocytopenia and white clots in the left femoral artery (at a femoral artery catheter site) during management of DKA that included prophylactic-dose UFH. HIT was suspected erroneously on the basis of a possible previous remote heparin exposure (gastric surgery 10 yr earlier). The patient underwent two embolectomies as well as treatment with urokinase and i.v. danaparoid. The patient developed a second platelet count fall during danaparoid treatment that began on day 6 in relation to the initial course of UFH. Tests for HIT antibodies changed from negative (SRA: days 1 and 4, serotonin release <5%) to positive (days 9 and 12, serotonin release 92% and 80%, respectively). By PF4-heparin-EIA (set up to detect IgG antibodies), the day 1 sample also was negative (O.D., 0.262; negative, <0.450), the day 4 sample was weakly positive (0.804), and the day 9 and l2 samples were strongly positive (1.863 and 1.002, respectively). Although the possibility of in vivo cross-reactivity of danaparoid with the HIT antibodies is suggested by the thrombocytopenia and progression of limb ischemia, the platelet count subsequently rose during danaparoid treatment, and no additional thromboembolic events occurred. In vitro cross-reactivity was detected on the day 9, but not the day 12, blood sample. *Abbreviations*: HIT, heparin-induced thrombocytopenia; DKA, diabetic ketoacidosis; UFH, unfractionated heparin; i.v., intravenous; SRA, serotonin-release assay; EIA, enzyme-immunoassay.

Antiphospholipid antibody syndrome (APLAS) is characterized by increased risk for thrombosis and recurrent fetal loss; limb or intra-abdominal vein thrombosis, cerebral venous (dural sinus) thrombosis, nonatheromatous arterial thrombosis, cardiac valvulitis, and microvascular thrombosis (e.g., acrocyanosis, "blue toe syndrome," digital ulceration or gangrene, livedo reticularis) are described (Hojnik et al., 1996; Gibson et al., 1997). Many patients have thrombocytopenia (Morgan et al., 1993; Galli et al., 1996), which is typically mild and intermittent. The explanation for thrombocytopenia is uncertain: Some patients have platelet-reactive autoantibodies (Galli et al., 1994; Lipp et al., 1998), but platelet-activating effects of IgG are also suspected (Vermylen et al., 1997).

The explanation for the prothrombotic tendency of APLAS is also elusive. A multifactorial pathogenesis is likely, because the antibodies recognize complexes of negatively charged phospholipids with many different protein cofactors such as β_2-glycoprotein I (β_2GPI), prothrombin, protein C, protein S, and annexin V (Galli, 1996; Triplett, 1996). Indeed, interference with endothelial cell function, impaired fibrinolysis, disturbances in protein C anticoagulant pathway activities, and

TABLE 2 Clinical Parallels Between HIT and APLAS

	HIT	APLAS
Thrombotic paradox	Thrombosis despite thrombocytopenia	Thrombosis despite prolonged coagulation tests (± thrombocytopenia)
Spectrum of thrombotic events	Venous > arterial thrombosis; adrenal infarction, dural sinus thrombosis	Venous > arterial thrombosis; adrenal infarction, dural sinus thrombosis
Severity of thrombocytopenia	Mild to moderate thrombocytopenia	Mild to moderate thrombocytopenia
Laboratory diagnosis by (1) functional or (2) antigen assays	(1) Platelet activation assays (e.g., serotonin-release assay, heparin-induced platelet activation test); (2) platelet factor 4-heparin-EIA	(1) Lupus anticoagulant (i.e., prolonged phospholipid-dependent coagulation assay in presence of patient plasma); (2) β_2GPI-dependent anticardiolipin-EIA
Pathogenesis	Platelet activation by platelet Fc receptors; endothelial activation by immune injury	Uncertain pathogenesis: immune platelet activation and endothelial injury are possible factors

Note: Further laboratory parallels between HIT and APLAS are discussed in Chapter 10.
Abbreviations: β_2GPI, β_2 glycoprotein I; EIA, enzyme-immunoassay; HIT, heparin-induced thrombocytopenia; APLAS, antiphospholipid antibody syndrome.

antibody-mediated platelet activation have all been described (for review see Petri, 1997; Gruel, 2000; Arnout and Vermylen, 2003).

Parallels Between APLAS and HIT

Table 2 lists some common features of APLAS and HIT. Both clinicopathologic disorders are characterized by thrombocytopenia, a paradoxical risk for venous and arterial thrombosis, and associated antibodies that can be detected by either functional or antigen assays (see Chapter 10). Moreover, for both APLAS and HIT, positive functional assays are more strongly associated with thrombosis than positive antigen assays (Ginsberg et al., 1995; Warkentin et al., 2000; Galli et al., 2003). The parallels between these disorders led Arnout (1996) to hypothesize that IgG-mediated platelet activation could explain thrombosis in APLAS. Supportive experimental data include the observations that antiphospholipid antibodies enhance platelet activation induced by other agonists (Martinuzzo et al., 1993). Furthermore, Arvieux et al. (1993) observed murine monoclonal antibodies reactive against β_2GPI induced platelet activation in the presence of subthreshold concentrations of ADP and epinephrine, an effect dependent on binding to platelet FcγIIa receptors. However, other workers were unable to demonstrate enhanced platelet activation in the presence of IgG antiphospholipid antibodies (Shi et al., 1993; Ford et al., 1998) or showed no role for platelet FcγIIa receptors (Lutters et al., 2001; Jankowski et al., 2003).

Thrombocytopenia in Patients with APLAS Receiving Heparin

In retrospective studies, Auger and colleagues (1995) reported that platelet counts typically fell by about 50% in patients with chronic thromboembolic disease and the lupus anticoagulant who were treated with heparin. Neither timing of the

onset of thrombocytopenia nor results of specific antigen or activation assays for HIT antibodies were reported; so it remains uncertain whether these patients had (immune) HIT. It is possible that nonidiosyncratic platelet activation caused by heparin could increase the thrombocytopenic potential of antiphospholipid antibodies in the absence of HIT antibodies. Alternatively, some patients with APLAS may have low levels of circulating HIT antibodies even in the absence of previous heparin exposure (Lasne et al., 1997; Martinuzzo et al., 1999). We observed a young woman with ischemic stroke who developed thrombocytopenia and lower-limb thrombosis when therapeutic-dose heparin was given; pretreatment blood samples contained both antiphospholipid antibodies and platelet-activating anti-PF4/heparin IgG (Lo et al., 2006). Bourhim and colleagues (2003) showed that affinity-purified IgM anti-β_2GPI from a patient with APLAS gave a positive reaction in PF4-dependent enzyme-immunoassays (EIAs). Further, mice actively immunized with the purified IgM anti-β_2GPI generated anti-β_2GPI antibodies (via an idiotype–anti-idiotype mechanism) that also cross-reacted with PF4-heparin.

E. Thrombolytic Therapy

Early-onset thrombocytopenia occurs in about 1% of patients with acute coronary syndrome whether treated by heparin or non-heparin anticoagulants (Eikelboom et al., 2001). The frequency of thrombocytopenia is even higher in patients treated with streptokinase, especially when this thrombolytic agent is combined with heparin (Balduini et al., 1993) (Fig. 6). This could represent a direct, activating stimulus of heparin on platelets that perhaps is exacerbated by procoagulant

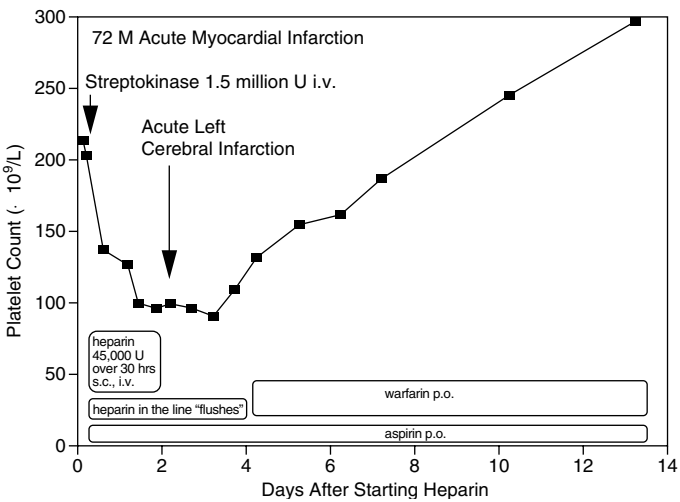

FIGURE 6 Pseudo-HIT associated with thrombolytic therapy. A 72-yr-old man developed moderate thrombocytopenia shortly after receiving streptokinase and heparin, which resolved following discontinuation of heparin. The early onset of thrombocytopenia, as well as the negative testing for HIT-IgG using the platelet serotonin-release assay, was consistent with pseudo-HIT. *Abbreviations*: HIT, heparin-induced thrombocytopenia; i.v., intravenous; p.o., per os; s.c., subcutaneous. *Source*: From Warkentin and Kelton, 1994.

effects of thrombolytic therapy. For example, fibrin degradation products generated by thrombolytic agents bind and protect thrombin from inhibition by heparin (Weitz et al., 1998). Such a mechanism could explain thrombocytopenia after the use of any thrombolytic drug. However, some investigators have reported that plasma containing antistreptokinase antibodies can activate platelets through their Fcγ receptors in the presence of streptokinase (Vaughan et al., 1988; Lebrazi et al., 1995; Regnault et al., 2003). Thus, high-titer antistreptokinase antibodies found in some normal individuals could explain the occasional occurrence of thrombocytopenia and thrombosis following treatment with streptokinase.

F. Septicemia-Associated Purpura Fulminans

Septicemia complicated by DIC occasionally results in progressive ischemia and necrosis of fingers or hands and toes or feet, producing a syndrome of symmetrical peripheral gangrene also known as purpura fulminans (Knight et al., 2000). The association with DIC suggests that increased thrombin generation in vivo, together with severe consumption and depletion of natural anticoagulant factors (e.g., protein C, protein S, antithrombin), leads to dysregulated fibrin deposition in the microvasculature. Other contributing factors can include hypotension or shock, pharmacological vasoconstriction (e.g., dopamine, epinephrine, norepinephrine) (Winkler and Trunkey, 1981; Hayes et al., 1992), vessel injury from invasive catheters, impaired hepatic synthesis of natural anticoagulants (e.g., vitamin K deficiency, postoperative hepatic dysfunction or failure), postsplenectomy status, or congenital deficiency of natural anticoagulants. Rarely, purpura fulminans occurs several weeks after varicella infection, usually because of autoantibodies reactive against protein S (Smith and White, 1999).

Meningococcemia in particular is often complicated by peripheral tissue necrosis that seems to parallel the severity of protein C depletion (Fijnvandraat et al., 1995). Recent trials suggest that protein C replacement therapy improves the natural history of this infection (Smith and White, 1999; White et al., 2000). Other infections that sometimes are complicated by symmetrical peripheral gangrene include septicemia secondary to pneumococcus (Johansen and Hansen Jr., 1993), *Escherichia coli* (Rinaldo and Perez, 1982), *Haemophilus influenzae* type b (Hayes et al., 1992), and *Capnocytophaga canimorsus* (Kullberg et al., 1991), among others. Sometimes severe systemic inflammatory response syndromes, such as ARDS, in the absence of demonstrable infection, can be complicated by limb necrosis (Bone et al., 1976). Acquired antithrombin deficiency in such patients with ARDS could be associated with thrombosis (Owings et al., 1996).

The development of acral tissue ischemia or necrosis in a thrombocytopenic, septic patient receiving heparin may suggest HIT. Although a common therapeutic response to such a diagnostic dilemma might be to stop heparin pending results of diagnostic testing for HIT antibodies, this could result in further ischemic injury, because anticoagulants might help prevent microvascular thrombosis (White et al., 2000). Furthermore, alternative non-heparin anticoagulants could be relatively contraindicated in a patient with significant renal or hepatic dysfunction. Thus, a reasonable treatment approach might well include continued heparin if clinical judgment posited a higher likelihood of septicemia, rather than HIT, as the cause of the microvascular thrombosis.

Only a small minority of septic patients develop acral limb ischemia or necrosis. Many, however, develop thrombocytopenia, with or without laboratory

evidence for DIC. The predominant explanation for increased platelet destruction in sepsis is uncertain, but appears to involve the underlying inflammatory host response (Aird, 2003a,b). Since hospitalized septic patients frequently are exposed to heparin, diagnostic confusion with HIT can result. Low protein C levels correlate with poor outcomes in sepsis (Yan et al., 2001), and recombinant human activated protein C (drotrecogin, Xigris) has been shown to reduce mortality in septic patients (Bernard et al., 2001). It is possible that this therapy might reduce risk of limb ischemia from microvascular thrombosis in this patient population. A potential dilemma is that septic patients with severe thrombocytopenia ($<30 \times 10^9$/L) were excluded in the clinical trials because of the bleeding potential of drotrecogin; however, as relative and absolute efficacy was greatest in the patients with the most severe sepsis, it has been suggested that otherwise eligible patients with such severe thrombocytopenia be considered as candidates for drotrecogin following platelet transfusion (Warkentin et al., 2003b).

G. Infective Endocarditis

Infective endocarditis is frequently complicated by thrombocytopenia. These patients are also at risk for septic emboli manifesting as thrombotic or hemorrhagic stroke, myocardial infarction, renal infarction, or even acute limb ischemia (de Gennes et al., 1990). Thus, the profile of macrovascular thrombosis and thrombocytopenia characteristic of HIT can be mimicked, especially as heparin is often used to anticoagulate patients with septic endocarditis (Delahaye et al., 1990). Microembolization leading to multiple small infarcts or microabscesses, in such organs as muscles, adrenal glands, and spleen, is an additional feature of endocarditis (Ting et al., 1990) that is not seen in HIT. When endocarditis-associated thrombocytopenia is unusually severe, potential explanations include platelet-reactive autoantibodies (Arnold et al., 2004) or procoagulant monocyte-stimulating factors secreted by microorganisms from within large vegetations (Selleng et al., 2006).

H. Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal myeloid disorder characterized by an acquired defect in the X-linked phosphatidylinositol glycan class A (PIG-A) gene, leading to loss of cell surface glycosylphosphatidylinositol-anchored proteins (for review see Rosse, 1997). Loss of the complement-regulating glycosylphosphatidylinositol-linked surface proteins, decay-accelerating factor and membrane attack complex inhibitory factor, causes the red cells to be exquisitely sensitive to complement-mediated hemolysis. Some patients have thrombocytopenia, and an increased risk for unusual, life-threatening venous thrombotic events, such as hepatic vein thrombosis, occurs in some patients. Thus, the clinical profile of HIT potentially can be mimicked. The thrombocytopenia could be related either to decreased platelet production or to complement-mediated formation of procoagulant platelet-derived microparticles (Wiedmer et al., 1993).

I. Post-Surgical Thrombocytopenic Thrombocytopenia Purpura

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder characterized by thrombocytopenia and microangiopathic hemolytic anemia (Coombs-negative hemolysis with prominent red cell fragmentation). Ischemic necrosis of brain, kidneys, heart, pancreas, and other tissues can result from disseminated

arteriolar occlusions by platelet-von Willebrand factor (vWF) microthrombi. In many patients, there is evidence for autoantibodies that inhibit or clear the enzyme, ADAMTS13 (*a d*isintegrin *a*nd *m*etalloprotease with *t*hrombospondin-1-like domains), which is responsible for cleaving ultralarge vWF multimers released from endothelium. Thus, the pathogenesis of idiopathic (primary) TTP likely reflects the formation of arteriolar-occluding complexes of ultralarge vWF multimers and platelets, thereby explaining both the thrombocytopenia and the tissue ischemia. "Secondary" TTP has been reported to occur in association with pregnancy, certain drugs (e.g., ticlopidine, clopidogrel, quinine, cyclosporine, mitomycin), autoimmune disorders (systemic lupus erythematosus), organ transplantation, and infections (human immunodeficiency virus, bacterial endocarditis). TTP clinically resembles a nephrotropic microangiopathic hemolytic anemia known as hemolytic uremic syndrome (HUS); however, there are certain unique triggers of HUS (especially, preceding infection with *E. coli* H0157) and anti-ADAMTS13 autoantibodies are not detected in HUS.

In recent years, an entity known as postoperative TTP has been recognized (Naqvi et al., 2004). The most common clinical setting is post-cardiac surgery, with cases seen beginning 2–19 days (median, day 5–6) after surgery (Chang et al., 1996; Pavlovsky and Weinstein, 1997; Chang and Ikhlaque, 2004; Almehmi et al., 2004). Other preceding events have included vascular surgery (Chang et al., 1996), abdominal surgery (Robson and Abbs, 1997; Chang et al., 2000), and orthopedic surgery (Iosifidis et al., 2006). The authors advocate plasmapheresis (the therapeutic mainstay of primary TTP) when postoperative TTP is diagnosed. Given the frequent formation of anti-PF4/heparin antibodies after surgery in heparin-treated patients, it is possible that coincidental formation of nonpathogenic anti-PF4/heparin antibodies could cause a false diagnosis of HIT in a patient with this rare entity of postoperative TTP. On the other hand, peripheral digit ischemic syndrome leading to amputations has been reported in post-cardiac surgery TTP (Chang and Ikhlaque, 2004), further blurring the distinctions between HIT and TTP. One patient has been reported in whom the authors believed the patient had concomitant TTP and HIT (Benke and Moltzan, 2005); an alternative explanation is that HIT-associated DIC produced thrombi in the renal microvasculature (thus, HIT may have mimicked TTP).

J. Glycoprotein IIb/IIIa Antagonist-Induced Thrombocytopenia

Glycoprotein (GP) IIb/IIIa antagonists (abciximab, tirofiban, eptifibatide) are used during coronary angioplasty to reduce platelet-mediated thrombosis. However, in a few patients (~1%), acute thrombocytopenia begins within hours of GPIIb/IIIa antagonist use (Aster et al., 2006; Warkentin, 2007). The thrombocytopenia is typically severe (usually $<20 \times 10^9$/L) and life-threatening bleeding can sometimes occur. Interestingly, most reported cases have occurred after *first* exposure to one of these drugs, although the frequency may be higher with repeat exposures (especially with abciximab) (Curtis et al., 2002). Platelet counts usually recover in 2–5 days after discontinuing the drug. Thrombocytopenia occurring after first exposure to a GPIIb/IIIa antagonist is explained by naturally occurring antibodies that recognize GPIIb/IIIa in the presence of the provoking drug (Bougie et al., 2002). Delayed onset of thrombocytopenia is explained by persistence of platelet-bound drug for several weeks after treatment, rendering platelets susceptible to destruction by newly formed antibodies (Curtis et al., 2004). A serologic problem

is the distinction of "pathologic" from "benign" antibodies found commonly among normal individuals. The blood film should always be examined when "thrombocytopenia" appears after abciximab use: this is because in some patients this GPIIb/IIIa antagonist can induce platelet clumping ex vivo (when the blood is drawn into a calcium-chelating anticoagulant), resulting in a "pseudothrombocytopenia" that is clinically benign (unless inappropriate platelet transfusions are initiated) (Sane et al., 2000).

What should the clinician suspect when a patient develops abrupt onset of severe thrombocytopenia immediately after cardiac angioplasty in which both heparin and a GPIIb/IIIa antagonist have been used? (Assume also that heparin has been given previously, but a GPIIb/IIIa antagonist has not.) The surprising answer is that this clinical scenario almost always is caused by the GPIIb/IIIa antagonist. Thus, it would be wrong to treat such a patient presumptively as rapid-onset HIT, particularly since further anticoagulation with therapeutic doses of a non-heparin anticoagulant could lead to dangerous bleeding, especially considering the patient's severe thrombocytopenia and GPIIb/IIIa-antagonized platelets.

K. Posttransfusion Purpura

Posttransfusion purpura (PTP) is a rare syndrome characterized by severe thrombocytopenia and mucocutaneous bleeding that begins 5–10 days after blood transfusion, usually red cell concentrates. More than 95% of affected patients are older women, in keeping with its pathogenesis of an anamnestic recurrence of platelet-specific alloantibodies in women previously sensitized by pregnancy. Destruction of autologous platelets is believed to result from the pseudospecificity of the alloimmune response, e.g., the high-titer anti-human platelet antigen-la (anti HPA-la) alloantibodies (the most frequent cause of the syndrome) probably somewhat recognize the autologous HPA-lb alloantigen.

Because both PTP and HIT typically occur about a week after surgery managed with perioperative blood transfusions and postoperative heparin prophylaxis, a diagnostic dilemma can arise (Lubenow et al., 2000). A useful clinical clue is the presence or absence of petechiae: PTP almost invariably is characterized by this hallmark of severe thrombocytopenia, whereas patients with HIT generally do not develop petechiae, even if they have very severe thrombocytopenia. The presence of high titers of platelet-reactive alloantibodies suggests PTP.

III. RECOGNITION AND TREATMENT OF PSEUDO-HIT

Many patients with pseudo-HIT can be distinguished from HIT because of the early onset of thrombocytopenia (Table 1). Unless the patient received heparin within the past 30, and at most 100, days, the early platelet count fall is the strong evidence against HIT (Warkentin and Kelton, 2001; Lubenow et al., 2002) (see Chapter 2).

However, for patients with adenocarcinoma-associated DIC, or postoperative pulmonary embolism, in whom the platelet count fall can occur after 5 days of heparin treatment, the diagnosis initially could be uncertain. As alternative anticoagulants (danaparoid, lepirudin, or argatroban) are available in most countries, treatment with one of these agents before obtaining results of HIT antibody testing may be appropriate. For patients with adenocarcinoma without HIT antibodies, management is more successful with LMWH or UFH than with warfarin (Prandoni, 1997; Lee et al., 2003).

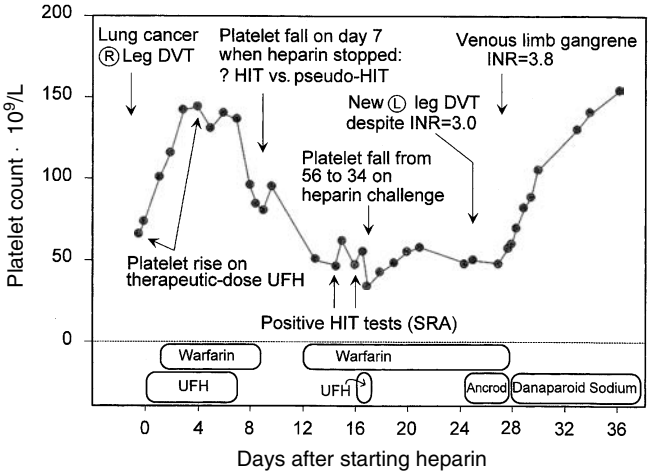

FIGURE 7 Pseudo-HIT complicated by HIT: A 78-yr-old man, with right proximal lower-limb DVT and thrombocytopenia, developed progressive platelet count increase during therapeutic-dose UFH treatment. Recurrent thrombocytopenia developed after UFH was stopped and when the patient was anticoagulated with warfarin. A liver biopsy on day 9 showed metastatic adenocarcinoma (primary lung neoplasm), and adenocarcinoma-associated DIC was diagnosed. However, a heparin challenge produced a further platelet count fall; HIT antibody testing was strongly positive (SRA: 88% serotonin release at 0.1 U/mL heparin; <15% release at 0 and 100 U/mL heparin). Subsequently, the patient developed new left-sided DVT, as well as venous gangrene of the left foot during treatment with warfarin and ancrod (peak INR = 3.8). Although the clinical course was initially identical with pseudo-HIT (rising platelet count on heparin therapy; abrupt platelet count fall after heparin administration was stopped), the subsequent heparin-induced fall in the platelet count, and strong positive HIT test results, indicate the patient also had HIT. *Abbreviations*: DIC, disseminated intravascular coagulation; DVT, deep venous thrombosis; HIT, heparin-induced thrombocytopenia; INR, international normalized ratio; SRA, serotonin-release assay; UFH, unfractionated heparin .

A. Pseudo-HIT Complicated by HIT

HIT is a relatively common complication of heparin therapy. It may be even more common in patients who have baseline platelet activation and PF4 release, as occurs in adenocarcinoma-associated DIC or DKA. Therefore, a patient with early thrombocytopenia attributable to a pseudo-HIT disorder may subsequently develop clinically significant HIT antibodies (Greinacher, 1995) (Fig. 5). Another example is that of a patient with lung cancer and DVT who developed a platelet count rise during intravenous heparin therapy, followed by recurrent thrombocytopenia and, ultimately, venous limb gangrene during anticoagulation with warfarin and ancrod (Fig. 7). In this situation, one might have expected platelet count recovery during a second course of heparin. However, an intravenous heparin challenge resulted in worsening of thrombocytopenia, and the patient had a strong positive assay for HIT antibodies, indicating the concurrence of cancer-associated DIC and HIT.

Opatrny and Warner (2004) observed 11 patients with cancer who also developed evidence for HIT, three of whom required amputations for venous limb gangrene (one case attributable to warfarin treatment). The authors suggested that the risk of thrombotic sequelae—particularly limb ischemia—is especially high

when HIT complicates anticoagulation for cancer. The wider availability of assays to detect HIT antibodies should help clinicians better elucidate when HIT plays a pathogenic role in explaining such unusual thrombotic events.

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12 Treatment of Heparin-Induced Thrombocytopenia: An Overview

Andreas Greinacher
Ernst-Moritz-Arndt-University-Greifswald, Greifswald, Germany

Theodore E. Warkentin
Michael G. DeGroote School of Medicine, McMaster University, and Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario, Canada

I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) presents a unique situation: heparin causes the very complications its use was intended to prevent, e.g., pulmonary embolism, stroke, and limb gangrene. Furthermore, several treatment paradoxes pose serious management pitfalls (Table 1). This chapter summarizes our treatment approaches, with emphasis on practical management issues. We wish to highlight two important issues. First, HIT is a syndrome of increased thrombin generation ("hypercoagulability state"). Accordingly, we emphasize the use of rapidly acting anticoagulant drugs that control thrombin generation in HIT. Second, there is increasing evidence that in most patients in whom testing for HIT antibodies is requested, a non-HIT diagnosis ultimately is made (Juhl et al., 2006; Lo et al., 2006). Thus, the risk of failing to prevent a HIT-associated thrombosis (through timely use of a non-heparin anticoagulant) must be balanced against the risk of inducing adverse effects from using another anticoagulant, e.g., bleeding complications for which no antidote exists.

This chapter is not the outcome of a formal consensus conference, as defined elsewhere (Mclntyre, 2001). Nevertheless, we have used an evidence-based approach to frame our recommendations, modeled after the Seventh American College of Chest Physicians (ACCP) Consensus Conference on Antithrombotic Therapy (Warkentin and Greinacher, 2004). According to these guidelines, the recommendation to use (or not to use) a particular treatment is based on the trade-off between the expected benefits on the one hand and the risks on the other. Thus, based upon the evidence, as well as our own experience, when we concluded that benefits of a particular treatment generally outweighed the risks, we recommended the treatment. If we were quite certain the evidence favored the recommendation, a level 1 recommendation was made. If we were less certain of the trade-off between benefits and risks, a weaker recommendation (level 2) was made.

We also assessed the methodological quality of the studies supporting the recommendations, also using the ACCP guidelines: *grade A:* randomized controlled trials (RCTs) without important limitations; *grade B:* RCTs with important limitations; and *grade C:* observational studies.

Regarding studies of HIT, there is only one small RCT (Chong et al., 2001), and this study had methodological flaws such as non-blinded assessment of

TABLE 1 Treatment Paradoxes of HIT Management

Treatment for HIT	Paradoxical effect of treatment	Comments
Discontinue heparin	Potential for increase in thrombin generation; high frequency of thrombosis despite stopping heparin	Use an alternative, rapid-acting anticoagulant[a] when heparin is stopped because of strongly suspected HIT
Coumarin (e.g., warfarin, phenprocoumon)	High frequency of thrombosis; potential for coumarin necrosis (venous limb gangrene and skin necrosis syndromes)	Control thrombin generation with an alternative anticoagulant[a]; postpone coumarin pending substantial platelet count recovery
LMWH	High frequency of exacerbating thrombocytopenia when given to patients with acute HIT	Although LMWH is less likely than UFH to cause HIT, LMWH is likely to maintain or worsen acute HIT caused by UFH
Low-dose danaparoid[b]	High frequency of thrombosis if low-dose danaparoid is given to patients with "isolated HIT"	High (therapeutic)-dose danaparoid recommended for patients strongly suspected (or confirmed) to have isolated HIT or HIT-thrombosis
Platelet transfusions	May increase risk for platelet-mediated thrombosis	Spontaneous bleeding is uncommon in HIT even with severe thrombocytopenia; thus, prophylactic platelet transfusions are relatively contraindicated
IVC filters	May increase risk for IVC thrombosis, pulmonary embolism, limb ischemia/ necrosis	IVC filters should be avoided in acute HIT; if used, concomitant anticoagulation in therapeutic doses should be given
Use low-dose alternative anticoagulation or heparin in patients with low probability for HIT	Risk of bleeding outweighs risk of thrombosis in patients with thrombocytopenia due to other reasons than HIT	The risk-benefit ratio of therapeutic-dose non-heparin anticoagulation is not favorable for non-HIT thrombocytopenia, given the high risk of bleeding and lower risk of thrombosis (overall, only 5–10% of patients evaluated serologically for HIT are shown to have heparin-dependent, platelet-activating antibodies)
Use heparin for cardiovascular surgery despite previous history of HIT	In patients with a history of HIT who subsequently test negative for HIT antibodies, heparin is safer for intravascular anticoagulation than alternative anticoagulants	HIT antibodies usually disappear quickly (within a few weeks or months), and are not regenerated within 5 days following reexposure to heparin, thus allowing heparin use during surgery

[a]Rapidly acting alternative anticoagulants include danaparoid, lepirudin, and argatroban.
[b]Low-dose danaparoid (750 U two or three times a day) is approved for prevention of thrombosis in acute HIT in some jurisdictions.
Abbreviations: HIT, heparin-induced thrombocytopenia; IVC, inferior vena cava; LMWH, low molecular weight heparin; UFH, unfractionated heparin.

treatment outcomes. Hence, we have no grade A and only one grade B recommendation. Grade C recommendations are based upon observational studies. Regarding HIT, this includes prospective cohort treatment studies with historical controls (Greinacher et al., 1999a,b, 2000; Lubenow et al., 2005; Lewis et al., 2001, 2003, 2006); case-control series (Warkentin et al., 1997; Lubenow et al., 2006); and large case series (e.g., Magnani, 1993, 1997; Magnani and Gallus, 2006; Warkentin and Kelton, 1996; Wallis et al., 1999). Thus, our recommendations are graded as follows, with the implications of the recommendation shown:

> *Grade 1B and Grade 1C*: strong recommendations, which apply to most patients in most circumstances; and
> *Grade 2C*: weak recommendations; other alternatives may be equally reasonable.

As no studies have directly compared the three major treatment options for HIT (danaparoid, lepirudin, and argatroban), any recommendation for use of one of these drugs does not imply any proven or consistent advantage over any of the others. However, there are important pharmacokinetic differences, which might well favor use of one in the particular circumstances of an individual patient situation (see Chapters 13–20).

A. Disclaimer

There are several challenging aspects to treating patients with HIT. These patients are not clinically homogeneous; they represent a complex mix of varying initial indications for heparin, location, and severity of HIT-associated thrombosis, and, not infrequently, dysfunction of one or more vital organs. This presents difficulties both for performing clinical studies as well as in the application of treatment recommendations for individual patients. Furthermore, there are important differences among countries in the approval or availability status of certain recommended treatment approaches. *Thus, the treatment recommendations we make cannot be indiscriminately applied to all patients with suspected or confirmed HIT.*

A further practical problem is that the major treatment options for HIT include relatively new and, for some physicians, unfamiliar or even unapproved anticoagulant agents. This presents extra challenges to physicians and also to laboratories asked to monitor anticoagulant treatment effects, as the treatment "learning curve" may occur in emergency situations. Also, immediate results of reliable laboratory tests for HIT are usually unavailable. Difficult management decisions may be needed amid diagnostic uncertainty: A diagnosis of HIT that seems obvious in retrospect may not have been so clear during its early evolution.

As an iatrogenic illness that occurs unpredictably and unexpectedly, often in a setting of antithrombotic prophylaxis, medicolegal aspects must also be considered (Mclntyre and Warkentin, 2004; Ulsenheimer, 2004). Thus, once HIT is entertained as part of a differential diagnosis, we suggest that physicians document carefully the various diagnostic and treatment considerations as events unfold.

As a *common, rare disease* (we acknowledge Prof. R. Hull [Calgary, Canada] for this description of HIT) that physicians only occasionally manage and that only rarely enters into clinical studies, we need to acknowledge that no final answer for treatment is likely to emerge. Therefore, even in this fourth edition, this chapter should be viewed as a basis for further discussion and study of the treatment of HIT patients.

II. NONIMMUNE HEPARIN-ASSOCIATED THROMBOCYTOPENIA

In some patients, especially those with comorbid conditions associated with platelet activation (burns and anorexia nervosa), heparin treatment can result in a transient decrease in platelet count (Burgess and Chong, 1997; Reininger et al., 1996) (see Chapter 4). Unfractionated heparin (UFH) activates platelets directly (Salzman et al., 1980), an effect observed less frequently with low molecular weight heparin (LMWH) (Brace and Fareed, 1990). Known as nonimmune heparin-associated thrombocytopenia (nonimmune HAT), this direct proaggregatory effect of heparin occurs predominantly in patients receiving high-dose, intravenous (iv) UFH therapy. Typically, platelet counts decrease within the first 1–2 days of treatment and then recover over the next 3–4 days. There are no data indicating that these patients are at increased risk for adverse outcomes, including thrombosis. Indeed, it is possible that inappropriate discontinuation of heparin for nonimmune HAT could *increase* the risk for thrombosis, owing to the underlying clinical condition for which the heparin is being given.

Management of patients in whom HIT is a potential reason for the decrease in platelet count, but judged nevertheless to be at low probability of having HIT, is discussed in section III.F (p. 298).

III. THERAPY OF (IMMUNE) HIT

A. Pathogenesis of HIT: Treatment Implications

HIT is caused by antibodies that usually recognize multimolecular complexes of platelet factor 4 (PF4) and heparin. HIT can be viewed as a syndrome of in vivo thrombin generation that results from the activation of platelets, endothelium, monocytes, and coagulation pathways (Warkentin and Kelton, 1994; Greinacher, 1995; Warkentin, 2003; Warkentin et al., 1998) (Fig. 1) (see Chapters 4–9). Given this model of pathogenesis, therapy for acute HIT should focus on the following issues: (1) rapid reduction of increased thrombin generation; (2) treatment of HIT-associated thrombosis, and (3) interruption of the immune response and heparin-dependent platelet activation (i.e., discontinuation of heparin). In most patients with HIT, effective pharmacological therapy for thrombosis will involve an agent that rapidly controls thrombin generation, although in some situations, additional adjunctive treatments may be necessary (e.g., surgical thromboembolectomy).

An increasingly recognized treatment issue involves patients with detectable anti-PF4/heparin antibodies but no platelet count reduction or other clinical evidence of HIT. With increased testing for HIT antibodies, it is now clear that many patients develop anti-PF4/heparin antibodies without developing clinical HIT (see Chapters 3 and 10). In these patients, it seems acceptable to continue heparin treatment but to monitor the platelet counts carefully ("watch-and-wait" strategy).

Several studies of heparin administration in the contexts of acute coronary syndrome, cardiac surgery, and hemodialysis have suggested that presence of anti-PF4/heparin antibodies confer adverse prognosis (increased cardiovascular events) even in the absence of clinically evident HIT (Mattioli et al., 2000; Williams et al., 2003; Mascelli et al., 2004a,b; Bennett-Guerrero et al., 2005; Pena de la Vega et al., 2005). However, whether this reflects true pathogenicity of these antibodies in the presence of heparin ("forme fruste" HIT) or whether the presence of antibodies represents simply a surrogate marker for other adverse prognostic markers

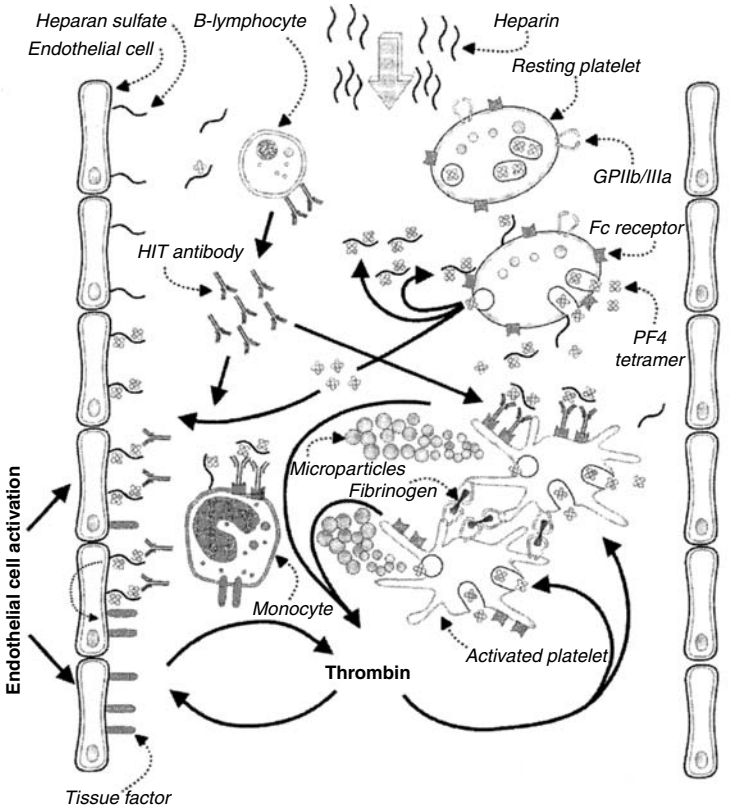

FIGURE 1 Pathogenesis of HIT; a central role for thrombin generation: HIT-IgG antibodies bind to several identical epitopes on the same antigen complex, thus forming immune complexes that become localized to the platelet surface. The IgG immune complexes can cross-link the platelet FcγIIa receptors, resulting in FcγIIa receptor-dependent platelet activation (Kelton et al., 1988). The GP IIb/IIIa complex is not required for platelet activation (Greinacher et al., 1994a). The activated platelets trigger a cascade of events that ultimately lead to activation of the coagulation pathways, resulting in thrombin generation. Activated platelets release their α-granule proteins (Chong et al., 1994), including PF4, leading to formation of more multimolecular PF4-heparin complexes, setting up a vicious cycle of platelet activation, triggering even more platelet activation (Greinacher, 1995). The activated platelets bind fibrinogen, recruit other platelets, and begin to form a primary clot. During shape change, procoagulant, platelet-derived microparticles are released, providing a phospholipid surface for amplifying thrombin generation (Warkentin et al., 1994). The released PF4 also binds to endothelial cell heparan sulfate, forming local antigen complexes to which HIT antibodies bind (Cines et al., 1987; Visentin et al., 1994; Greinacher et al., 1994b). Tissue factor expression on activated endothelial cells and monocytes (Arepally and Mayer, 2001; Pouplard et al., 2001) further enhances thrombin generation. *Abbreviations*: GP, glycoprotein; HIT, heparin-induced thrombocytopenia; PF4, platelet factor 4.

(e.g., inflammation) is an important unresolved question (Warkentin and Sheppard, 2006).

B. Discontinuation of Heparin for Clinically Suspected HIT

Numerous case reports describe the occurrence of new, progressive, or recurrent thromboembolic events during continued or repeated use of heparin in patients with acute HIT. Moreover, the thrombocytopenia usually persists if the administration of heparin is not stopped. Thus, all heparin treatment should be discontinued in patients strongly suspected of having HIT and usually substituted by another anticoagulant (discussed subsequently), while awaiting results of HIT antibody testing. The rationale behind substituting heparin with another anticoagulant is that the potential benefit of stopping heparin (e.g., less antibody-induced heparin-dependent platelet activation) might be outweighed in some patients by a "rebound" in thrombin generation following loss of heparin's anticoagulant action. Moreover, as discussed later, HIT antibodies sometimes can cause platelet activation even in the absence of pharmacologic heparin.

> *Recommendation.* All heparin administration should be discontinued in patients clinically suspected of having (immune) HIT (grade 1C).

The routine use of heparin (e.g., line flushing) is pervasive in hospitals. Thus, based on our experience, it can be helpful to institute methods to reduce the risk for inadvertent heparin use in hospitalized patients with HIT.

> *Recommendation.* A clearly visible note should be placed above the patient's bed stating "NO HEPARIN: HIT" (grade 2C).

Not infrequently, patients in whom heparin administration has been stopped because of clinically suspected HIT subsequently are found to have negative laboratory tests for HIT antibodies. In our experience, it is reasonable and safe to restart heparin therapy in these patients, provided the intervening clinical events are consistent with an alternative explanation for thrombocytopenia (see Chapters 2 and 11) and provided the laboratory has adequately excluded the presence of HIT antibodies (see Chapter 10).

> *Recommendation.* Heparin can be restarted in patients proved not to have HIT antibodies by a sensitive platelet activation assay or a PF4-dependent antigen assay (grade 1C).

C. Anticoagulation of the HIT Patient with Thrombosis

The Need for Anticoagulation of HIT-Associated Thrombosis

HIT is a strong, independent risk factor for venous and arterial thrombosis (Warkentin et al., 1995, 2003). HIT can be complicated by thrombosis in several ways: (1) a preceding thrombosis, leading to the heparin treatment that caused HIT (this is usually *not* considered to be HIT-associated thrombosis); (2) new, progressive, or recurrent thrombosis resulting from HIT itself; or (3) both reasons. The relationship between thrombocytopenia and thrombosis in HIT is variable: thrombosis can both precede (or coincide with) the onset of thrombocytopenia or thrombosis can occur several days (or even a few weeks) later (Warkentin and Kelton, 1996; Greinacher et al., 2005).

For a HIT patient with thrombosis in whom heparin administration has been discontinued, there is a high risk for subsequent thrombosis. It is increasingly clear

that many—perhaps most—HIT patients' sera contain antibodies with heparin-*in*dependent platelet-activating properties (Prechel et al., 2005; Warkentin and Kelton, 2001a); thus, ongoing antibody-induced platelet activation will continue for a time, even if heparin is stopped. That this phenomenon may be biologically relevant is suggested by three prospective treatment cohort studies (Greinacher et al., 1999a,b; Lubenow et al., 2005), in which the initial incidence of thrombotic events ranged from 5% to 10% per patient day (see Chapter 14). This high event rate (5.1% per day in the meta-analysis) occurred after stopping heparin therapy and after laboratory confirmation of HIT but before institution of alternative anticoagulation with lepirudin (mean period of treatment delay; 1.3 days) (Greinacher et al., 2000; Lubenow et al., 2005). This experience suggests that alternative anticoagulant therapy should not be delayed for results of HIT antibody testing in patients strongly suspected of having HIT.

Anticoagulants Evaluated for Treatment of HIT

Current treatment of HIT focuses on agents that rapidly control thrombin generation (Fig. 2) (see Chapters 13–17). Table 2 lists the available evidence on efficacy for three such agents: danaparoid, lepirudin, and argatroban (listed in the order the drugs became available), which are approved for use in HIT in various countries. Danaparoid is an *indirect* (antithrombin-dependent) inhibitor of factor Xa and, to a lesser extent, thrombin, whereas lepirudin and argatroban inhibit thrombin directly and without the need for a cofactor, i.e., they are classified as *direct* thrombin inhibitors (DTIs). Only one RCT for the management of HIT has been performed. This study compared danaparoid with dextran 70 for the treatment of HIT-associated thrombosis (Chong et al., 2001) (see Chapter 13). As the study was small and open-label, we have listed it as supporting a level 1B recommendation.

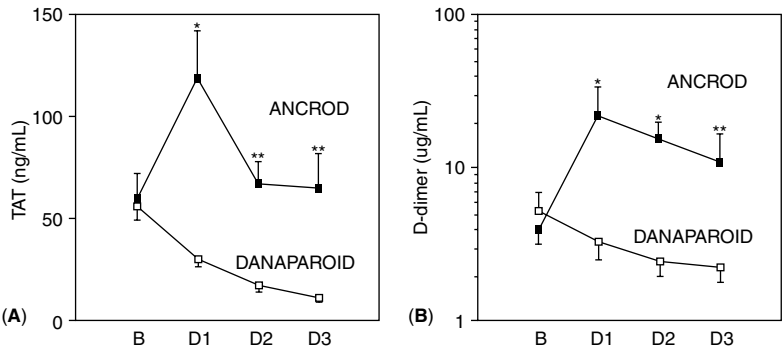

FIGURE 2 Thrombin generation and fibrin formation in acute HIT. (**A**) Thrombin generation, as assessed by TAT complexes, is markedly increased in acute HIT (mean, 55 ng/mL; normal, <4.1 ng/mL). Whereas danaparoid reduces thrombin generation in these patients, the defibrinogenating snake venom, ancrod, does not. (**B**) Levels of cross-linked fibrin degradation products (D-dimer) are increased in patients with acute HIT (mean, 4–5 μg/mL; normal, <0.5 μg/mL). Whereas danaparoid reduces D-dimer levels, ancrod increases their levels. Baseline (B) samples were obtained at diagnosis of HIT and before treatment with danaparoid or ancrod; subsequent values are shown for day 1 (D1, 1–24 h postinitiation of treatment), day 2 (D2, 25–48 h), and day 3 (D3, 49–72 h). *$p < 0.001$, **$p < 0.002$. *Abbreviations*: HIT, heparin-induced thrombocytopenia; TAT, thrombin–antithrombin. *Source*: From Warkentin, 1998.

TABLE 2 Rapidly Acting Anticoagulants for the Treatment of HIT: Main Studies Supporting Efficacy

Drug	Studies
Danaparoid	Randomized controlled trial comparing danaparoid + warfarin vs. dextran 70 + warfarin (Chong et al., 2001)
	Retrospective comparison of danaparoid vs. lepirudin (Farner et al., 2001)
	Retrospective comparison of danaparoid vs. ancrod and/or coumarin (Lubenow et al., 2006)
	Compassionate release program (Magnani, 1993, 1997; Magnani and Gallus, 2006)
Lepirudin	HAT-1: prospective cohort study with historical controls (Greinacher et al., 1999a)
	HAT-2: prospective cohort study with historical controls (Greinacher et al., 1999b)
	Meta-analysis of HAT-1 and -2 studies (Greinacher et al., 2000)
	HAT-3: prospective cohort study with historical controls (Lubenow et al., 2005)
	Meta-analysis of HAT-1, -2, and -3 studies (Lubenow et al., 2005)
	Retrospective comparison of lepirudin (prospective cohort) and danaparoid (contemporaneous controls) (Farner et al., 2001)
	Post-marketing surveillance (Lubenow et al., 2002a)
Argatroban	Arg-911: prospective cohort study with historical controls (Lewis et al., 2001)
	Arg-915: prospective cohort study with historical controls (Lewis et al., 2003)
	Meta-analysis of Arg-911, -915 and -915X studies, including use of "thrombotic endpoint" (Lewis et al., 2006)
	Argatroban substudies (data from Arg-911, -915, and -915X studies)
	Argatroban dose adjustments (Verme-Gibboney and Hursting, 2003)
	Patients with thrombotic stroke (LaMonte et al., 2004)
	Patients with a history of HIT (Matthai et al., 2005)
	Patients with venous thromboembolism (Begelman et al., 2005)
	Patients undergoing argatroban/warfarin overlap (Bartholomew and Hursting, 2005; Hursting et al., 2005)
	Patients undergoing renal replacement therapy (Reddy et al., 2005)
	Patients with renal dysfunction (Guzzi et al., 2006)
	Patients with hepatic dysfunction (Levine et al., 2006)
	Patients who are critically ill (Gray et al., 2007)
	Patients with coronary artery disease (Jang et al., 2007)
	Cost-effectiveness analysis (Arnold et al., 2006)

Abbreviations: HAT, heparin-associated thrombocytopenia; HIT, heparin-induced thrombocytopenia.

> *Recommendation.* Therapeutic-dose anticoagulation with a rapidly acting anticoagulant, e.g., danaparoid (grade 1B), lepirudin (grade 1C), or argatroban (grade 1C), should be given to a patient with thrombosis complicating acute HIT. Treatment should not be delayed pending laboratory confirmation in a patient strongly suspected (or confirmed) to have HIT.

Availability of anticoagulants varies in different countries. Although currently not available in the U.S., danaparoid is the alternative anticoagulant agent available in most countries worldwide. Bivalirudin and fondaparinux are also potential options for managing some patients with HIT, but current information is still too preliminary to allow for broad recommendations (see Chapters 16 and 17).

Pharmacologic and Pharmacokinetic Considerations in Anticoagulant Selection

The lack of prospective comparative studies between danaparoid, lepirudin, and argatroban precludes definitive conclusions about relative efficacy and safety. However, there are several pharmacological and pharmacokinetic differences that physicians should consider when determining which drug might be preferred in

TABLE 3 Main Characteristics of Danaparoid Sodium

Mechanism of action, pharmacokinetics	Monitoring	Undesirable effects	Comments
Catalyzes the inactivation of factor Xa by AT, and of thrombin (IIa) by AT and HCII Bioavailability after sc injection ~100%; peak anti-Xa levels, 4–5 h after injection (Danhof et al., 1992) Mean plasma distribution time following iv bolus, ~2.3 h Plasma $t_{1/2}$ of anti-Xa activity, 17–28 h (mean, 25 h); $t_{1/2}$ of anti-IIa activity, 2–4 h (Danhof et al., 1992)	Anti-Xa levels during treatment by an amidolytic assay using danaparoid reference curve Monitoring recommended in patients with: (1) significant renal impairment; (2) body weight < 45 kg or >110 kg; (3) life- or limb-threatening thrombosis; (4) unexpected bleeding; (5) critically ill or unstable patient	XR with HIT antibodies: in vitro XR usually not associated with adverse effects; patients should be monitored for in vivo XR (unexplained platelet count fall, progressive new TECs); in vivo XR is estimated to occur in ~3% of patients (Magnani and Gallus, 2006) Bleeding complications in compassionate-release study (Ortel and Chong, 1998): fatal (0.9%), major nonfatal bleeding (6.5%); no major bleeds in RCT (Chong et al., 2001) Skin hypersensitivity: rare	Anticoagulant effect depends on adequate AT levels Does not significantly prolong the aPTT, ACT, PT/INR (does not interfere with monitoring of overlapping oral anticoagulants) Reduce dosage if serum creatinine >265 μmol/L *No antidote*: in case of overdosage, stop the drug and treat bleeding with blood products as indicated

Abbreviations: ACT, activated clotting time; aPTT, activated partial thromboplastin time; AT, antithrombin III; HCII, heparin cofactor II; HIT, heparin-induced thrombocytopenia; iv, intravenous; PT/INR, prothrombin time/international normalized ratio; RCT, randomized controlled trial; sc, subcutaneous; TEC, thromboembolic complication; $t_{1/2}$, drug half-life; XR, cross-reactivity .

an individual patient (Tables 3–5). For example, in a patient with vital organ or limb ischemia or infarction, who might need urgent surgical intervention, an agent with a short half-life may be desirable. But, in a patient with venous thromboembolism in whom an uncomplicated overlap with (longer-term) warfarin anticoagulation is anticipated or who requires outpatient treatment by subcutaneous (sc) injections, danaparoid use is advantageous. Argatroban (which undergoes hepatobiliary clearance) is suited for patients with renal insufficiency, as dose reduction is generally not required (cf. lepirudin). Conversely, lepirudin may be more suitable than argatroban for patients with hepatobiliary dysfunction, including reduced liver perfusion due to cardiac insufficiency.

As the key therapeutic goal of anticoagulation during acute HIT is to achieve effective inhibition of thrombin or its generation, the ability to determine accurately the drug anticoagulant levels is an important issue. In the case of danaparoid, drug levels can be measured directly (via antifactor Xa levels), whereas in most medical centers, the anticoagulant levels of the DTIs are measured *indirectly*, using the activated partial thromboplastin time (aPTT). This can cause underdosing in patients with prothrombin deficiency, as a "therapeutic" aPTT may not necessarily indicate adequate dosing of the DTI (see Chapter 14). Other factors to consider include drug availability to, and prior experience of, the physician and availability and turnaround time of laboratory monitoring.

TABLE 4 Main Characteristics of the r-Hirudin, Lepirudin

Mechanism of action, pharmacokinetics	Monitoring	Undesirable effects	Comments
Direct, noncovalent, irreversible inhibitor of free and clot-bound thrombin Bioavailability after sc injection, ~100%; peak effect, 2–3 h Mean plasma distribution time after iv bolus, ~2 h Mean plasma $t_{1/2}$, 1.3 h; $t_{1/2}$ greatly prolonged in renal failure (~200 h in nephrectomized patients)	aPTT during treatment; a more precise monitoring is possible by the ECT (see Chapters 14, 16, and 19) Daily aPTT monitoring is recommended in all patients (see comments re: antihirudin antibodies) Monitoring by ECT recommended: 1. During CPB 2. Unexpected bleeding Monitoring by quantitative hirudin EIA or ECA recommended: when prothrombin levels are decreased (Lindhoff-Last et al., 2000; see Chapter 14)	Development of antihirudin antibodies in ~40% of patients. In about 3% of patients, these antibodies enhance the anticoagulant effect of hirudin, and require a substantial dose reduction Anaphylactic reactions: ~0.015% (first exposure) ~0.15% (reexposure) associated with iv bolus injection (Greinacher et al., 2003) Reduce dosage if serum creatinine >90 μmol/L (see Chapter 14) Allergic reactions: very rare Skin hypersensitivity: very rare Bleeding complications in HIT patients in prospective studies: major bleeding in two prospective studies, 13.4, 17% (see Chapter 14)	~40% of patients develop antihirudin antibodies on day 5 or later of treatment; in only ~5% of these patients is a dose reduction or increase needed; risk of anaphylactic reactions post-iv bolus No major effect on PT/INR (Greinacher et al., 2000) *No antidote:* In case of overdosage, stop the drug and treat bleeding with blood products as indicated (hemofiltration with a high-flux membrane is a possible treatment for life-threatening bleeding)

Abbreviations: aPTT, activated partial thromboplastin time; CPB, cardiopulmonary bypass; ECA, ecarin chromogenic assay; ECT, ecarin-clotting time; EIA, enzyme-immunoassay; HIT, heparin-induced thrombocytopenia; iv, intravenous; PT/INR, prothrombin time/international normalized ratio; sc, subcutaneous; $t_{1/2}$, drug half-life.

DTI Dosing

An emerging issue is the growing recognition that the approved dosing regimens of the DTIs—as presented within the product monographs—is often too high, especially with lepirudin (Lubenow et al., 2005; Tardy et al., 2006; see also Chapter 14). Accordingly, it is recommended that for most situations, the initial iv bolus be omitted and the initial iv infusion rate be reduced by about 50% (from 0.15 mg/kg/h to 0.05–0.10 mg/kg/h), even in patients with normal renal function (the dose is reduced substantially more in the presence of renal dysfunction) (Warkentin et al., 2007). Moreover, aPTT monitoring should be performed at 4-h intervals until stable anticoagulation within the therapeutic range is observed (whereupon once-daily monitoring is appropriate). For argatroban, post-marketing studies indicate that it is increasingly common to reduce the initial infusion rate from the approved dose (2 μg/kg/min) to 0.5–1.2 μg/kg/min, especially in

TABLE 5 Main Characteristics of the Direct Thrombin Inhibitor, Argatroban

Mechanism of action, pharmacokinetics	Monitoring	Undesirable effects	Comments
Direct, noncovalent, reversible inhibitor of free and clot-bound thrombin ~50% of the drug is plasma protein bound Steady state is reached 1–3 h after starting iv infusion Mean plasma $t_{1/2}$ is 40–50 min; $t_{1/2}$ is prolonged 4- to 5-fold in moderate liver impairment	aPTT during treatment; no data exist as to whether more precise monitoring at higher doses would be achieved using other methods, such as ECT Target INR is >4.0 when warfarin is overlapped with argatroban (however, following discontinuation of argatroban, the usual target INR of 2.0–3.0 applies during further warfarin treatment) Note: in case of prothrombin deficiency, aPTT gives falsely high values	No major side effects besides bleeding complications Argatroban makes all functional clotting assays unreliable	Only iv use of argatroban has been tested in HIT Reduce dosage by 75% in case of liver impairment No dose reduction in renal failure *No antidote:* in case of overdosage or severe bleeding, stop the drug and treat bleeding with blood products as indicated Argatroban prolongs the INR and requires a strategy adopted to the INR reagent used for overlapping treatment with warfarin (see Chapter 15)

Abbreviations: aPTT, activated partial thromboplastin time; ECT, ecarin-clotting time; HIT, heparin-induced thrombocytopenia; INR, international normalized ratio; iv, intravenous; $t_{1/2}$, drug half-life.

critically ill patients or in patients with cardiac dysfunction, even when hepatic dysfunction is not clinically apparent (see Chapter 15).

Danaparoid Cross-Reactivity

Danaparoid is a mixture of non-heparin anticoagulant glycosaminoglycans, predominantly (low-sulfated) heparan sulfate, dermatan sulfate, and chondroitin sulfate (see Chapter 13). About 10–40% of HIT patient sera "cross-react" in vitro with danaparoid, depending on the assay used (lower cross-reactivity rates by platelet aggregometry, higher rates with fluid-phase PF4/heparin immunoassays) (Vun et al., 1996; Warkentin et al., 2005; Magnani and Gallus, 2006). The question arises as to the in vivo relevance (if any) of this phenomenon. In our experience, the majority of patients with detectable in vitro cross-reactivity have favorable clinical courses that do not differ significantly (either in clinical outcomes or in time to platelet count recovery) from patients without cross-reactivity (Warkentin, 1996). Furthermore, it is not possible to distinguish in vivo cross-reactivity from a severe natural history of HIT. For example, the authors are aware of HIT patients with strong antibodies, whose thrombocytopenia persisted for several weeks despite cessation of all heparin and during treatment with a DTI; had such a patient received danaparoid, the physicians inevitably would have judged the patient to have had in vivo cross-reactivity. Furthermore, in many patients, therapeutic concentrations of danaparoid *disrupt* PF4/heparin/IgG immune complexes, thereby inhibiting HIT antibody-induced platelet activation (Chong et al., 1989); this is a unique pharmacologic attribute not shared by any other

anticoagulant. For all of these reasons, we do not advise testing for in vitro cross-reactivity in patients in whom danaparoid treatment is planned.

> *Recommendation.* In vitro cross-reactivity testing for danaparoid using HIT patient serum or plasma is not recommended prior to danaparoid administration (grade 1C).

Other Drugs that Reduce Thrombin Generation in HIT

Other drugs with antithrombotic activity described anecdotally as treatment for HIT include bivalirudin (see Chapter 16), dermatan sulfate (Agnelli et al., 1994; Taliani et al., 1999; Imberti et al., 2003), and the antithrombin-binding pentasaccharide, fondaparinux (see Chapter 17). The last agent is a particularly attractive option for thromboprophylaxis or thrombosis treatment in a patient with a history of HIT, as the risk of inducing HIT with this agent is believed to be negligible (Warkentin et al., 2005). However, the use of fondaparinux for management of a patient with acute HIT is debated (Table 6). The available evidence is too limited to draw definite conclusions about its efficacy and safety in this patient population.

TABLE 6 Fondaparinux as a Potential Treatment for HIT: Pros and Cons

Pro	Con
Fondaparinux does not promote platelet activation by HIT antibodies (negligible in vitro cross-reactivity by platelet activation assays) (Savi et al., 2005)	Fondaparinux in therapeutic concentrations does not inhibit platelet activation by HIT antibodies (cf. danaparoid[a])
Fondaparinux does not promote binding of HIT antibodies in vitro to PF4 (negligible in vitro cross-reactivity by EIA)	Fondaparinux treatment is associated with formation of anti-PF4/heparin antibodies (Warkentin et al., 2005)
To date, only one case of HIT reported with fondaparinux, despite many thousands of patients treated since approval (Warkentin et al., 2007)	Duration of fondaparinux treatment often limited to <1 wk in approval trials (thus limiting ability to detect immune HIT)
Several case reports suggest that fondaparinux may be a treatment option for HIT	Many of the case reports do not include convincing clinical and serologic evidence for acute HIT
Effective anticoagulant despite lack of anti-thrombin (anti-IIa) activity, based on studies of several non-HIT patient groups	Perhaps anti-IIa activity is important in HIT management (note: DTIs have anti-IIa activity; and danaparoid has some anti-IIa activity)
Once-daily sc injection ($t_{1/2} \sim 17$ h)—this provides the option for outpatient administration, which is not readily practical for lepirudin and argatroban	Steady-state levels take several days to achieve, whereas maximal therapeutic drug effect in HIT is required initially when thrombin generation is maximal; also, absorption of sc injection may be suboptimal in certain patient populations
No anticoagulant monitoring is necessary	Fondaparinux can accumulate due to its long $t_{1/2}$, especially in patients with renal dysfunction
Both prophylactic- and therapeutic-dose regimens exist	Lack of established dosing regimen for management of acute HIT (with or without thrombosis)
Costs of therapeutic-dose fondaparinux less than that of DTI or danaparoid therapy	Lack of approval status for HIT may cause medicolegal difficulties; informed consent for use in treatment of HIT may be required

[a]In some patients, danaparoid in therapeutic concentrations inhibits HIT antibody-induced platelet activation (Chong et al., 1989).

Abbreviations: DTI, direct thrombin inhibitor; EIA, enzyme-immunoassay; HIT, heparin-induced thrombocytopenia; PF4, platelet factor 4; sc, subcutaneous; $t_{1/2}$, drug half-life.

D. Anticoagulation of the HIT Patient Without Thrombosis

Approximately 50% of patients with HIT do not have a new HIT-associated thrombosis at the time HIT is first clinically suspected on the basis of thrombocytopenia alone (Warkentin and Kelton, 1996; Greinacher et al., 1999a,b, 2005). In a retrospective cohort study of 62 such patients with "isolated HIT," the subsequent 30-day cumulative thrombotic event rate was high (52.8%) (see Fig. 2 in Chapter 3). The rate of thrombosis was similar in the two largest patient subgroups: patients treated with discontinuation of heparin therapy alone (20/36, 56%) and patients treated with substitution of warfarin for heparin (10/21, 48%). The majority of events involved the venous circulation (4 : 1 ratio), with six of the 62 patients developing pulmonary embolism (two fatal); another patient who died suddenly may also have had a fatal pulmonary embolism.

In a subsequent large retrospective cohort study of serologically confirmed HIT performed by Wallis and coworkers (1999), a 38% thrombotic event rate was observed in patients with isolated HIT managed by cessation of heparin. Further, early cessation of heparin was not associated with a reduction in the rate of thrombosis. In another study, Zwicker and colleagues (2004) observed that five (36%) of 14 patients with clinically suspected HIT who tested strongly positive (>1.00 units of optical density) for anti-PF4/heparin antibodies by anti-PF4/heparin enzyme-immunoassay (EIA) developed symptomatic thrombosis. The high symptomatic thrombotic event rates observed in these three retrospective cohort studies are consistent with prospective treatment cohort studies that also observed a high rate of thrombosis (5–10%/day over the first 1–2 days) soon after the diagnosis of HIT (Greinacher et al., 1999a,b, 2000).

These high thrombotic event rates among patients with isolated HIT suggest that many patients may have had subclinical deep-vein thrombosis (DVT) at the time that HIT was first suspected. Indeed, Tardy and colleagues (1999) found that eight of 16 patients identified as having isolated HIT had subclinical DVT identified by systematic duplex ultrasound investigations.

> *Recommendation.* Patients suspected to have acute HIT should undergo imaging studies for lower limb DVT, especially those at highest risk for venous thromboembolism, such as postoperative patients (grade 1C).

There is evidence that therapeutic-dose anticoagulant therapy of isolated HIT is effective. In a retrospective analysis of patients with isolated HIT comparing treatment with danaparoid and lepirudin, it was observed that patients who received *prophylactic*-dose danaparoid (750 U sc b.i.d. or t.i.d.) had a trend to a higher rate of thrombosis than patients treated with lepirudin (0.1 mg/kg b.w./h, aPTT-adjusted) (Farner et al., 2001). In contrast, patients with HIT-associated thrombosis had similar outcomes when treated with therapeutic doses of either drug. This indicates that *therapeutic*, rather than prophylactic, doses of danaparoid may be more effective for patients with isolated HIT (Farner et al., 2001; Warkentin, 2001). Further evidence supporting the use of therapeutic-dose anticoagulation for isolated HIT includes the results of (aPTT-adjusted) therapy with the DTIs, lepirudin, and argatroban (Lubenow et al., 2004; Lewis et al., 2001, 2003, 2006).

We usually prescribe an alternative anticoagulant in *therapeutic* doses in this situation of strongly suspected (or confirmed) HIT. However, prophylactic-dose anticoagulation is a reasonable option in patients with a low or intermediate likelihood of having HIT (see Table 7 in Chapter 2) or in HIT patients judged to be at high risk for bleeding complications. Another option could be regular screening

for venous thrombosis without anticoagulation in a patient at very high bleeding risk. Thrombocytopenia itself should not be considered a contraindication to anticoagulation in patients with HIT, as petechiae and other spontaneous hemorrhagic manifestations are not usually seen in these patients (see Chapter 2). However, if the platelet count is less than 20×10^9/L and bleeding signs, but not thrombosis, are observed, then alternative diagnoses such as posttransfusion purpura or other drug-dependent immune thrombocytopenic disorders should be considered (Kiefel, 2004).

> *Recommendation.* Alternative therapeutic-dose anticoagulation with an appropriate anticoagulant, such as danaparoid, lepirudin, or argatroban, should be considered in patients strongly suspected (or confirmed) to have HIT even in the absence of symptomatic thrombosis. Anticoagulation should be continued at least until recovery of the platelet counts to a stable plateau (grade 1C).

It is uncertain whether anticoagulation of isolated HIT beyond the time to platelet count recovery (to a stable plateau) is required, if there are no ongoing risk factors for thrombosis, such as atrial fibrillation or prolonged immobility. In the prospective lepirudin studies (Lubenow et al., 2004), the risk of subsequent thrombosis (35 day follow-up) among patients with isolated HIT treated until full platelet count recovery was low. We therefore do not usually give prolonged anticoagulation in our own clinical practice. (See also sections III.E and VI.B regarding *contraindication* to the use of vitamin K antagonists during the acute thrombocytopenic phase of HIT). It is reasonable to repeat a duplex ultrasound of the lower extremities prior to discharging a patient to home when ongoing anticoagulation will not be given.

E. Longer-Term Anticoagulant Management of the HIT Patient with Thrombosis

Acute HIT by itself is not an indication for longer-term anticoagulation (i.e., 3–6 mo). However, HIT-associated thrombosis, or the underlying disease itself, often is. For longer-term control of thrombosis, oral anticoagulants of the coumarin class (e.g., warfarin or phenprocoumon) are the treatment of choice. However, as discussed subsequently, it is important that coumarin therapy be delayed until there has been substantial recovery in the platelet count. Another option is to avoid coumarin therapy completely, e.g., a patient can be anticoagulated with danaparoid (e.g. 1500 U sc t.i.d. for 4 wk, followed by 1500 U sc b.i.d.). Some physicians have transitioned patients from DTI therapy to sc fondaparinux following platelet count recovery.

Transition to Vitamin K Antagonist (Coumarin) Therapy

Generally, it takes at least 5 days of oral anticoagulant therapy before therapeutic functional hypoprothrombinemia is achieved (Harrison et al., 1997). It is important that thrombin generation be controlled in patients with acute HIT before and during initiation of coumarin treatment, particularly in patients with severe HIT-associated DVT, because otherwise coumarin-induced necrosis (venous limb gangrene and skin necrosis syndromes) can be induced (Warkentin et al., 1997; Srinivasan et al., 2004) (see Chapter 2). This proscription against coumarin use applies particularly during the acute thrombocytopenic phase of HIT, as coumarins fail to inhibit the marked hypercoagulability state of HIT, while at the same time they can cause severe depletion of the vitamin K-dependent natural anticoagulant,

protein C. These are the circumstances predisposing to the disturbed procoagulant-anticoagulant balance characteristic of the coumarin necrosis syndromes in HIT. Thus, it is important to *postpone* starting administration of coumarin anticoagulants until therapeutic anticoagulation is achieved with danaparoid, lepirudin, or argatroban *and* until there has been substantial platelet count recovery (usually to at least 150×10^9/L, indicating that the platelet-activating effects of the HIT antibodies have largely resolved).

> *Recommendation.* To minimize the risk of coumarin necrosis in a patient with acute HIT, vitamin K antagonist (coumarin) therapy should be delayed until the patient is adequately anticoagulated with a rapidly acting parenteral anticoagulant, and not until there has been substantial platelet count recovery (at least $>150 \times 10^9$/L). The vitamin K antagonist should be started in low maintenance doses (e.g., $\leq$5 mg warfarin), with at least 5 days of overlap with the parenteral anticoagulant (including at least 2 days in the target-therapeutic range), and the parenteral anticoagulant should not be stopped until the platelet count has reached a stable plateau (Grade 1C).

Besides minimizing the risk of coumarin-induced microthrombosis/necrosis, there are two other important reasons for postponing coumarin anticoagulation in a patient with acute HIT. First, since coumarins increase the aPTT, and since the aPTT is usually used to monitor the anticoagulant effect of the DTIs, the patient is at risk of receiving insufficient dosing of the DTI if coumarin has already been given. This phenomenon has been implicated in some patients who have developed venous limb gangrene during overlapping DTI—coumarin therapy (Warkentin, 2006). Second, the DTIs have varying effects upon the global clotting assays, in particular, the prothrombin time/international normalized ratio (PT/INR), as follows: argatroban > bivalirudin > lepirudin (Gosselin et al., 2004; Warkentin et al., 2005; see Fig. 3 in Chapter 16). Since the INR is used to guide coumarin therapy, a special issue during management of DTI—coumarin overlap is the effect of the DTI upon the INR, especially with argatroban. Once the anticoagulant effect of the DTI has dissipated (usually within a few hours of stopping the DTI), the circumstances favoring microvascular thrombosis—and, hence, coumarin-induced necrosis—might well be present, i.e., ongoing thrombin generation from acute HIT, warfarin-induced protein C depletion, and active DVT (Warkentin et al., 1997; Smythe et al., 2002; Srinivasan et al., 2004). Thus, postponing coumarin therapy in a patient with acute HIT until the platelet count has normalized will reduce the risk that premature discontinuation of the DTI could occur at a time when HIT antibodies are still causing substantial activation of platelets and the clotting system.

A corollary to the above considerations is that it is important to reverse with vitamin K the effects of coumarin, if HIT is recognized *after* coumarin therapy has already been begun (e.g., 10 mg vitamin K by slow iv infusion over 30–60 min) (Warkentin and Greinacher, 2004; Warkentin, 2006). This is particularly important if a DTI will be used to manage anticoagulation (in contrast to the DTIs, danaparoid prolongs neither the aPTT nor INR to any significant extent).

> *Recommendation.* Oral or iv vitamin K should be given to reverse coumarin anticoagulation in a patient recognized as having acute HIT after coumarin has been commenced (grade 1C).

In case of coumarin overdose and severe bleeding during the first 3 mo after an episode of HIT, prothrombin complex concentrates should only be used with extreme caution to "reverse" coumarin anticoagulation. This is because these

concentrates contain heparin and have caused recurrent thrombocytopenia and thrombosis in patients with circulating HIT antibodies (Greinacher et al., 1992).

Recommendation. Prothrombin complex concentrates should not be used to reverse coumarin anticoagulation in a patient with acute or recent HIT unless bleeding is otherwise unmanageable (grade 2C).

F. Management of the Patient with a Low or Intermediate Probability of HIT (Pending Results of HIT Antibody Testing)

In patients with HIT without thrombosis, the risk of major bleeding with therapeutic-dose DTI therapy per patient day has been reported to be as high as 1.0% for lepirudin (i.e., 14.3% major bleeding over a mean treatment period of 13.9 days) (Lubenow et al., 2004) and for argatroban it was 0.6% and 1.0% (3.1% and 5.3% major bleeding over a mean treatment period of 5.3 and 5.1 days, respectively) (Lewis et al., 2001, 2003). In the large case series of HIT patients treated with danaparoid (Magnani and Gallus, 2006), the risk for major bleeding was 0.4% for prophylactic-dose therapy (3.2% major bleeding over a median treatment duration of 6 days) (personal communication, Dr H. Magnani).

These relatively high risks for bleeding (especially with DTIs) should be contrasted with the much lower expected rate of thrombosis (~0.3–0.5% per patient day) among all patients investigated for HIT by ordering laboratory testing for HIT antibodies. This calculation is based upon the estimated initial thrombotic event-rate per day (~5%) multiplied by the relatively low overall risk of obtaining a positive test result using a functional test for platelet-activating heparin-dependent antibodies (~6–10%) (Warkentin and Sheppard, 2006; Greinacher et al., 2007). Thus, the high risk of bleeding with therapeutic-dose anticoagulation suggests that such therapy is justified only if the clinical likelihood is judged to be at least intermediate, if not high, based upon the clinical picture, prior to obtaining the results of laboratory testing for HIT antibodies. Unless otherwise dictated by the patient's clinical condition, the use of an alternative anticoagulant in *prophylactic doses* might be safer in this situation. This could be achieved in several ways: e.g., danaparoid 750 U t.i.d. sc, a dosing regimen that is approved for prophylaxis of new thrombosis in several jurisdictions. Another potential option is prophylactic-dose fondaparinux (2.5 mg once-daily sc), although there is less experience in this setting with fondaparinux than with danaparoid.

Recommendation. In a patient with a low probability for HIT (e.g., 4T's score ≤ 3) pending the results of laboratory testing for HIT antibodies, we suggest either continuing the use of heparin or using alternative, non-heparin anticoagulation in prophylactic, rather than in therapeutic, doses (assuming there is no other reason for therapeutic-dose anticoagulation) (Grade 1C).

Recommendation. In a patient with an intermediate probability for HIT (e.g., 4T's score of 4 or 5), who has an alternative explanation for thrombocytopenia and who does not require therapeutic-dose anticoagulation for other reasons, we suggest alternative anticoagulation in prophylactic, rather than in therapeutic, doses (Grade 2C).

G. Reexposure of the HIT Patient to Heparin

Heparin Reexposure of the Patient with Acute or Recent HIT

Deliberate or accidental readministration of heparin to a patient with acute or recent HIT can cause an abrupt platelet count fall, sometimes complicated by thrombosis or acute systemic reactions (see Chapter 2). Accordingly, deliberate

heparin rechallenge for diagnostic purposes is not recommended, especially because sensitive assays for HIT antibodies are available. This is a strong recommendation because the diagnostic usefulness of laboratory assays for HIT has been established in controlled studies (see Chapter 10).

> *Recommendation.* Deliberate reexposure to heparin of a patient with acute or recent HIT for diagnostic purposes is not recommended. Rather, the diagnosis should first be excluded or confirmed in most situations by testing acute patient serum or plasma for HIT antibodies using a sensitive activation or antigen assay (grade 1C).

Heparin Reexposure of the Patient with a History of Remote HIT

HIT antibodies are usually not detectable 3 mo after an episode of HIT (Warkentin and Kelton, 2001b). There are few data describing the clinical and serological outcomes of patients reexposed with heparin with previously documented HIT in the remote past (arbitrarily, >3 mo ago, or sooner, if HIT antibodies have disappeared). One patient who developed fatal HIT on day 15 of UFH treatment had a history of HIT complicated by thrombosis 6 yr earlier (Gruel et al., 1990). However, several patients with previous remote HIT have been observed in whom repeat heparin use caused neither HIT nor HIT antibody formation (Pötzsch et al., 2000; Warkentin and Kelton, 2001b).

Because there are acceptable alternative anticoagulant options for most prophylactic and therapeutic indications, both UFH and LMWH usually should be avoided in patients with a previous history of HIT. As discussed in the following section, however, there are special circumstances, such as cardiac or vascular surgery, during which it is reasonable to use heparin for a patient with a previous history of HIT, provided certain precautions are taken.

> *Recommendation.* Heparin should not be used for antithrombotic prophylaxis or therapy in a patient with a previous history of HIT, except under special circumstances (e.g., cardiac or vascular surgery) (grade 2C).

IV. HIT IN SPECIAL CLINICAL SITUATIONS

A. Cardiac or Vascular Surgery

Management of the Patient with Acute or Recent HIT

For patients with acute HIT who require heart surgery, or with recent HIT and persistence of circulating HIT antibodies, it is possible to use alternative anticoagulants during cardiopulmonary bypass (CPB) (see Chapter 19). Options for alternative anticoagulation for such patients include bivalirudin and lepirudin (minimal data regarding use of argatroban for this indication precludes recommendations). Unfortunately, the lack of a specific antidote, the need for special intraoperative monitoring, and other considerations mean that none is ideal for managing CPB.

Another approach is to administer heparin together with a potent antiplatelet agent, e.g., tirofiban (GPIIb/IIIa antagonist) or epoprostenol (prostacyclin analogue). Danaparoid is not recommended for anticoagulation during CPB due to its long half-life. This special topic of managing cardiac surgery patients with acute or previous HIT is discussed in detail in Chapter 19, as well as in relation to specific anticoagulant agents in Chapters 13–16.

Danaparoid and lepirudin have also been used to provide intraoperative anticoagulation, as well as to "flush" blood vessels during vascular surgery in

patients with acute HIT (for review, see Warkentin, 2004). There is some experience using argatroban for vascular surgery.

> *Recommendation.* Alternative anticoagulation should be used for heart or vascular surgery in a patient with acute or recent HIT with detectable heparin-dependent, platelet-activating antibodies. Either bivalirudin or lepirudin are appropriate alternatives for intraoperative anticoagulation, provided that appropriate, rapid-turnaround laboratory monitoring and blood product support to manage potentially severe bleeding complications are available. Another approach is to give heparin together with a potent antiplatelet agent (grade 2C).

Management of the Patient Following Disappearance of HIT Antibodies

The drawbacks of alternative anticoagulants for CPB provide a rationale for the use of heparin in two groups of patients with a previous history of HIT: (1) a patient with a history of HIT, but who no longer has circulating HIT antibodies detected by a sensitive (washed platelet) activation assay; and (2) a patient with acute or recent HIT who requires elective heart surgery. In the latter situation, it is reasonable to delay cardiac surgery until HIT antibodies become undetectable, which usually occurs in a few weeks or months (Warkentin and Kelton, 2001b; Warkentin and Greinacher, 2003).

It is feasible to give UFH for cardiac or vascular surgery in a patient with a previous history of HIT, provided that HIT antibodies are not detectable at the time of surgery (Olinger et al., 1984; Smith et al., 1985; Makhoul et al., 1987; Pötzsch et al., 2000; Warkentin and Kelton, 2001b; Warkentin and Greinacher, 2003). We recommend that heparin be avoided completely both before surgery (to prevent potential for restimulation of HIT antibodies) and after surgery (thus making HIT unlikely even if HIT antibodies are reformed). Current evidence suggests that there is a minimum time to formation of clinically significant HIT antibodies of 5 days even in patients who have a previous history of HIT (Cadroy et al., 1994; Warkentin and Kelton, 2001b; Lubenow et al., 2002b). The patient should receive routine doses of UFH for the surgical procedure itself. Preoperative anticoagulation (e.g., for heart catheterization) and postoperative antithrombotic prophylaxis can be achieved with a non-heparin agent such as danaparoid (750 U b.i.d. or t.i.d.) or r-hirudin (15 mg b.i.d. sc) (Eriksson et al., 1997) (see Chapters 13 and 14).

> *Recommendation.* In a patient with a previous history of HIT, heart or vascular surgery can be performed using heparin, provided that HIT antibodies are absent (by sensitive assay) and heparin use is restricted to the surgical procedure itself (grade 1C).

B. HIT During Pregnancy

There are a few reports describing HIT during pregnancy (Meytes et al., 1986; Henny et al., 1986; Copplestone and Oscier, 1987; Calhoun and Hesser, 1987; van Besien et al., 1991; Greinacher et al., 1993a). Danaparoid has been used in at least 32 pregnant women using dosing schedules similar to those in nonpregnant patients (Lindhoff-Last et al., 2005). Danaparoid does not cross the placenta, based on cord blood assessment (see Chapter 13).

Lepirudin, bivalirudin, argatroban, danaparoid, and fondaparinux are category B drugs, i.e., indicating absence of fetal damage in certain high-dose animal studies, but limited (if any) human data. Few reports describe use of lepirudin during pregnancy (Huhle et al., 2000; Furlan et al., 2006). Danaparoid does not

appear to cross the placenta (Lagrange et al., 2002; Magnani and Gallus, 2006), while about 10% of the maternal blood concentration of fondaparinux were found in the cord blood of a newborn (Harenberg, 2007). Hirudin can cross the placenta in low doses (Markwardt et al., 1988) and has caused embryopathy in rabbits given high doses of hirudin (Lubenow and Greinacher, 2000). Further, a zebrafish model reveals that thrombin plays a role in embryogenesis (Jagadeeswaran et al., 1997). Thus, danaparoid and fondaparinux may be preferable for treatment of HIT during (early) pregnancy.

> *Recommendation.* If available, danaparoid (and possibly fondaparinux) is preferred for parenteral anticoagulation of pregnant patients with HIT, or in those who have a previous history of HIT (grade 2C).

C. Treatment of HIT in Children

There are only a few reports describing the management of HIT in children (for review, see Klenner et al., 2004) (see Chapter 20); therefore, no clear treatment recommendations can be made. Experience from small case series suggest that lepirudin, argatroban, and danaparoid can be used successfully in children. The dosing schedules for adults (appropriately weight-adjusted for the child) can be used as a guideline, but careful monitoring is recommended.

V. ADJUNCTIVE THERAPIES

A. Medical Thrombolysis

Thrombocytopenia is not a contraindication to thrombolytic therapy in patients with HIT. Streptokinase (Fiessinger et al., 1984; Cohen et al., 1985; Bounameaux et al., 1986; Cummings et al., 1986; Mehta et al., 1991), urokinase (Leroy et al., 1985; Krueger et al., 1985; Clifton and Smith, 1986), and tissue plasminogen activator (t-PA) (Dieck et al., 1990; Schiffman et al., 1997) have been used both systemically and by local infusion (Quinones-Baldrich et al., 1989). In patients at high bleeding risk, an ultra-low-dose t-PA (2 mg/h over 12 h) was successfully applied without bleeding complications (Olbrich et al., 1998). As thrombin generation is not inhibited by thrombolysis, concomitant non-heparin anticoagulation should be given, in reduced dose, until the fibrinolytic effects have waned.

> *Recommendation.* Regional or systemic pharmacological thrombolysis should be considered as a treatment adjunct in selected patients with limb-threatening thrombosis or pulmonary embolism with severe cardiovascular compromise (grade 2C).

B. Surgical Thromboembolectomy and Fasciotomies

Vascular surgery is often needed to salvage an ischemic limb threatened by HIT-associated acute arterial thromboembolism involving large arteries (Sobel et al., 1988). When performing vascular surgery during acute HIT, it is appropriate to maintain anticoagulation at least in the lower therapeutic range, if possible, before, during, and after surgery, until platelet count recovery. In patients with latent HIT (i.e., no longer thrombocytopenic, but with clinically significant levels of HIT antibodies still present), the intensity of anticoagulation depends on the perceived risk of vessel (or graft) occlusion. In patients at high risk of occlusion (e.g., surgery involving below-knee vessels), the patient should be therapeutically anticoagulated before vessel clamping (in addition to receiving intraoperative flushes with anticoagulant), with therapeutic anticoagulation maintained for several days after

surgery. In surgery involving larger vessels, the use of intraoperative flushes alone, followed by postoperative prophylactic-dose anticoagulation, might be sufficient.

Either danaparoid or lepirudin can provide intraoperative anticoagulation. One author (AG) uses one of the following solutions to flush the vessel postembolectomy: (1) lepirudin, 0.1 mg/mL saline (one 20 mg ampule in 200 mL saline), using up to 250 mL in a normal-weight patient, and assessing the aPTT before giving more lepirudin to avoid overdosage (the lepirudin flushes thus can achieve therapeutic intraoperative anticoagulation; see Chapter 14); (2) danaparoid, 3 anti-Xa U/mL (one 750 U ampule in 250 mL saline), using up to 50 mL in a normal-weight patient (this small flush dose is used because systemic anticoagulation is achieved by giving a 2250 U bolus of danaparoid preoperatively (see Chapter 13).

> *Recommendation.* Surgical thromboembolectomy is an appropriate adjunctive treatment for selected patients with limb-threatening large-vessel arterial thromboembolism. Thrombocytopenia is not a contraindication to surgery. An alternative anticoagulant to heparin should be used for intraoperative anticoagulation (grade 1C).

In contrast to large artery thrombosis, a surgical role for severe venous or microvascular limb ischemia is less certain (Warkentin, 2007). Fasciotomy is sometimes performed in patients with severe venous limb ischemia and suspected compartment syndrome, but this procedure may delay or interrupt much-needed anticoagulation. Further, it is uncertain to what extent compartment syndromes contribute to limb ischemia/necrosis in patients with HIT-associated DVT and associated microvascular thrombosis, including those related to severe disseminated intravascular coagulation (DIC) and/or coumarin-induced protein C depletion. In our view, therapy should focus on intensive medical therapy, including aggressive anticoagulation and (when appropriate) reversal of coumarin anticoagulation with iv vitamin K.

C. Intravenous Gammaglobulin

In vitro, both intact IgG as well as its Fc fragments inhibit HIT antibody-induced platelet activation, an effect that depends somewhat on the method of immunoglobulin preparation (Greinacher et al., 1994a) (see Chapter 8). Case reports describe rapid increase in the platelet counts after high-dose ivIgG (Vender et al., 1986; Frame et al., 1989; Nurden et al., 1991; Grau et al., 1992; Prull et al., 1992; Warkentin and Kelton, 1994). The possibility that ivIgG treatment interrupts platelet activation by HIT antibodies provides a rationale for its use as an adjunct to anticoagulant therapy in certain life- or limb-threatening situations. The dose should be 1 g/kg body weight per day for two consecutive days.

> *Recommendation.* ivIgG is a possible adjunctive treatment in selected patients requiring rapid blockade of the Fc receptor-dependent platelet-activating effects of HIT antibodies (e.g., management of patients with cerebral venous thrombosis, severe limb ischemia, or very severe thrombocytopenia) (grade 2C).

D. Plasmapheresis

Plasmapheresis has been associated with successful treatment outcomes in uncontrolled studies of patients with severe HIT (Vender et al., 1986; Bouvier et al., 1988; Nand and Robinson, 1988; Manzano et al., 1990; Thorp et al., 1990; Brady et al., 1991; Poullin et al., 1998). Whether this is due to removal of HIT antibodies or pathogenic immune complexes, or even correction of acquired natural anticoagulant

deficiencies by normal plasma replacement, is unresolved. For example, a patient with warfarin-induced acquired protein C deficiency and severe venous limb ischemia may have benefited from correction of the protein C deficiency with apheresis using plasma replacement (Warkentin et al., 1997).

> *Recommendation.* Plasmapheresis, using plasma as replacement fluid, may be a useful adjunctive therapy in selected patients with acute HIT and life- or limb-threatening thrombosis who are suspected or proved to have acquired deficiency of one or more natural anticoagulant proteins (grade 2C).

E. Antiplatelet Agents

Dextran

High molecular weight dextran in high concentrations inhibit platelet function and fibrinogen polymerization; they also inhibit HIT antibody-mediated platelet aggregation (Sobel et al., 1986). However, an RCT (Chong et al., 2001) (see Chapter 13) showed that in patients with severe HIT-associated thrombosis, dextran 70 was less effective therapy than danaparoid. It is unknown whether dextran would provide additional clinical benefit if combined with another anticoagulant. We do not advocate dextran for the management of HIT.

> *Recommendation.* Dextran should not be used as primary therapy for acute HIT complicated by thrombosis (grade 1B).

Acetylsalicylic Acid, Dipyridamole, and Clopidogrel

Both acetylsalicylic acid (aspirin, ASA) and dipyridamole have been used in HIT patients with variable success (Janson et al., 1983; Makhoul et al., 1986; Kappa et al., 1987, 1989; Laster et al., 1989; Gruel et al., 1991; Hall et al., 1992; Almeida et al., 1998). Sometimes the platelet count appeared to rise promptly with the application of antiplatelet therapy (Warkentin, 1997). However, HIT antibodies are potent platelet activators, and their effect cannot always be blocked in vitro by ASA or dipyridamole—indeed, HIT has occurred in patients who receive dual antiplatelet therapy with ASA and clopidogrel) (Selleng et al., 2005). These antiplatelet agents may be used as *adjunctive* therapy (to anticoagulant therapy), particularly in patients with arteriopathy. A potential drawback is increased bleeding (especially when combined with other antithrombotic agents).

> *Recommendation.* Antiplatelet agents, such as aspirin or clopidogrel, may be used as adjuncts to anticoagulant therapy of HIT, particularly in selected (arteriopathic) patients at high risk for arterial thromboembolism. The possible benefit in preventing arterial thrombosis should be weighed against the potential for increased bleeding (grade 2C).

Platelet Glycoprotein IIb/IIIa Inhibitors

Several platelet glycoprotein (GP) IIb/IIIa inhibitors are now available that potently block fibrinogen binding to platelets. They also can reduce thrombin generation by inhibiting the exposure of procoagulant phospholipid surfaces on platelets (Pedicord et al., 1998; Keularts et al., 1998; Hérault et al., 1998). In vitro, GPIIb/IIIa antagonists inhibit platelet aggregation (Hérault et al., 1997), endothelial cell activation (Herbert et al., 1998), and platelet microparticle generation (Mak et al., 1998) by HIT antibodies. However, Fc receptor-dependent platelet activation by HIT antibodies is independent of the GPIIb/IIIa complex (Greinacher et al., 1994a); therefore, GPIIb/IIIa inhibitors do not inhibit platelet granule release (Tsao et al., 1997;

Polgár et al., 1998). As these agents do not have a direct anticoagulant effect, they probably need to be combined with an anticoagulant (danaparoid, lepirudin, or argatroban) to treat HIT. Because there are no data available on the interaction of these newer anticoagulants with the GPIIb/IIIa inhibitors, and because a synergistic effect on bleeding is likely, combined use for the management of HIT should be considered experimental. Theoretically, synthetic GPIIb/IIIa inhibitors with a short half-life could be safer than agents with a long half-life (e.g., abciximab)

Recommendation. GPIIb/IIIa inhibitors should be considered as experimental treatment in HIT and used with caution if combined with anticoagulant drugs (grade 2C).

VI. CAVEATS FOR THE TREATMENT OF HIT

A. Low Molecular Weight Heparin

LMWH is less likely than UFH to cause HIT antibody formation as well as clinical HIT (Warkentin et al., 1995, 2003). Furthermore, LMWH binds less avidly to platelets than does UFH (Greinacher et al., 1993b). With functional assays employing platelet-rich plasma, several investigators reported a reduced cross-reactivity of HIT antibodies with LMWH compared with UFH (Ramakrishna et al., 1995; Slocum et al., 1996; Vun et al., 1996); however, with sensitive washed platelet functional assays, the cross-reactivity rate of LMWH is nearly 100% (Greinacher et al., 1992; Warkentin et al., 1995, 2005) (see Chapter 10).

Owing to the unavailability of other anticoagulant options during the 1980s, LMWH preparations were often used in Europe for further parenteral anticoagulation of HIT patients. No prospective cohort studies are available, but case reports (Roussi et al., 1984; Leroy et al., 1985; Vitoux et al., 1986; Gouault-Heilmann et al., 1987; Bauriedel et al., 1988; Kirchmaier and Bender, 1988) and a review (Reuter, 1987) suggest that LMWH may benefit some patients. Other case series, however, clearly show that LMWH is associated with disastrous complications in HIT patients (Horellou et al., 1984; Leroy et al., 1985; Gouault-Heilmann et al., 1987; Greinacher et al., 1992; Kleinschmidt et al., 1993). Unfortunately, no laboratory assay reliably predicts these differing treatment responses.

Treatment of HIT with LMWH is frequently unsuccessful. Of eight consecutive HIT patients who received LMWH, thrombocytopenia persisted in all, and new thromboembolic events occurred in two patients (Greinacher et al., 1992). After LMWH became available in North America, a similar experience was observed in seven HIT patients treated with LMWH (Warkentin, 1997). Another study has also shown a relatively high risk of adverse outcomes of treating HIT with LMWH (Ranze et al., 2000).

Recommendation. LMWH should not be used to treat patients with acute HIT (grade 1C).

B. Vitamin K Antagonists

Although vitamin K antagonists, such as warfarin, phenprocoumon, and other coumarin agents, are an important part of the longer-term management of patients with HIT-associated thrombosis, they are ineffective, and potentially dangerous, when given to patients with acute HIT as single therapy, or in combination with ancrod (a defibrinogenating snake venom that is no longer used as therapy for HIT) (Warkentin et al., 1997; Smythe et al., 2002; Srinivasan et al., 2004) (see Chapter 2). In patients with active DVT, oral anticoagulants may cause thrombosis

to progress to involve even the microvasculature, leading to coumarin-induced venous limb gangrene. This syndrome appears to result from a transient disturbance in procoagulant-anticoagulant balance: increased thrombin generation associated with HIT remains high during early warfarin treatment, while simultaneously there is severe, acquired deficiency in the natural anticoagulant protein C. Although high doses of oral anticoagulants may be more likely to cause this syndrome, even relatively low doses that produce a rise in the INR (especially to >4.0) can cause limb gangrene in some patients, particularly in patients with severe HIT-associated hypercoagulability and overt (decompensated) DIC. Thus, warfarin and phenprocoumon should always be given in combination with an agent that reduces thrombin generation in patients with acute HIT, and must only be started once the acute HIT has largely subsided, as judged by substantial recovery of the platelet count (in general, $>150 \times 10^9$/L). Furthermore, anticoagulant—coumarin overlap should occur over at least 5 days, and the alternative anticoagulant should not be stopped until the platelet count has reached a stable plateau (see also section III.E, Longer-term anticoagulant management of the HIT patient with thrombosis).

> *Recommendation.* Vitamin K antagonist (coumarin) therapy is *contraindicated* during the acute (thrombocytopenic) phase of HIT. In patients who have already received coumarin when HIT is diagnosed, reversal with vitamin K is recommended. (See section III.E for specific details of managing coumarin therapy in HIT, including recommendation grades.)

C. Ancrod

Ancrod, a defibrinogenating thrombin-like enzyme (Malayan pit viper venom), cleaves fibrinopeptide A but not fibrinopeptide B from fibrinogen (Bell, 1997). Ancrod was previously used to treat HIT, especially in Canada (Teasdale et al., 1989; Cole et al., 1990; Demers et al., 1991). However, ancrod does not inhibit—and may even increase—thrombin generation in HIT (Fig. 2) (Warkentin, 1998). Further, its use might predispose to coumarin-induced venous limb gangrene (Warkentin et al., 1997; Gupta et al., 1998). Also, ancrod was less effective than danaparoid in a historically controlled study (Lubenow et al., 2006).

The manufacturer discontinued ancrod in 2002.

D. Platelet Transfusions

Usually there is no need to treat thrombocytopenia with platelet transfusions, as patients with HIT rarely bleed spontaneously. Indeed, platelet transfusions should be avoided because the transfused platelets can be activated by the same immune mechanisms as the patient's own platelets. Anecdotal experience describes thrombotic events soon after platelet transfusions given to patients with acute HIT (Babcock et al., 1976; Cimo et al., 1979). Several consensus conferences (Contreras, 1998; Hirsh et al., 2001; British Committee for Standards in Haematology, 2003; Warkentin and Greinacher, 2004) stated that thrombotic thrombocytopenic purpura (TTP) and HIT are two disorders in which prophylactic platelet transfusions are not recommended because of the risk of precipitating thrombosis.

> *Recommendation.* Prophylactic platelet transfusions are relatively contraindicated in patients with acute HIT (grade 2C).

Therapeutic platelet transfusions are appropriate for patients with HIT who develop severe hemorrhage, particularly if the heparin administration has been discontinued for more than a day.

E. Vena Cava Filters

Vena cava (Greenfield) filters are sometimes used to manage patients judged to be at high risk for life-threatening pulmonary embolism. However, their use can be complicated by massive vena cava thrombosis, including the renal veins, and other serious progression of venous thromboembolism, especially if pharmacological anticoagulation is not given (Sobel et al., 1988; Jouanny et al., 1993). In our opinion, these devices are risky in the setting of acute HIT, and we do not advocate their use.

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13 Danaparoid for the Treatment of Heparin-Induced Thrombocytopenia

Beng Hock Chong
St. George Hospital and University of New South Wales, Kogarah, New South Wales, Australia

Harry N. Magnani
Medical Consultant, Oss, The Netherlands

I. INTRODUCTION

In patients with heparin-induced thrombocytopenia (HIT), cessation of heparin is mandatory. Thereafter, an alternative anticoagulant is usually needed for the treatment of HIT-associated venous or arterial thrombosis, for the prevention of thrombosis in isolated HIT, or for other indications (Chong, 1995; Warkentin and Greinacher, 2004) (see Chapter 12). Danaparoid (Orgaran®, NV Organon, The Netherlands; formerly known as Org 10172 and Lomoparan®) is the most widely used alternative antithrombotic for treatment of HIT outside of the U.S. (where it is not marketed). Worldwide, over 150,000 patients with HIT have been treated with danaparoid.

A. Chemistry, Pharmacology, Pharmacodynamics, and Pharmacokinetics

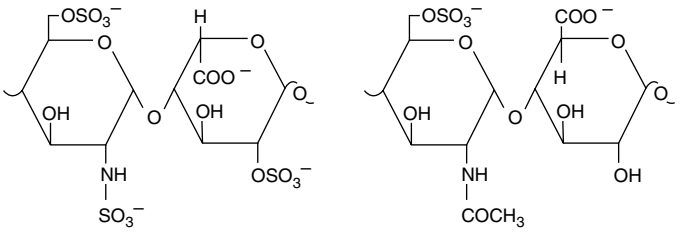
Chemistry

Although danaparoid is often referred to as a low molecular weight (LMW) "heparinoid" (implying that it has heparin-like activity), there are substantial differences in the chemistry, pharmacology, and pharmacokinetics between danaparoid and both unfractionated heparin (UFH) and low molecular weight heparin (LMWH).

Danaparoid consists of a mixture of LMW glycosaminoglycans (GAGs): heparan sulfate (84%), dermatan sulfate (12%), and chondroitin sulfate (4%) (Meuleman, 1992). A small proportion of the heparan sulfate molecules have high affinity for antithrombin (AT) (Meuleman, 1992; Ofosu, 1992). Danaparoid has an average molecular mass of approximately 6000 Da. It does not contain heparin or heparin fragments, and differs in chemical composition from heparin in that the repeating disaccharide subunits in heparan sulfate, its principal constituent, are predominantly glucuronic acid and *N*-acetyl-glucosamine, whereas in heparin, they are mostly iduronic acid and glucosamine-*N*-sulfate (Gordon et al., 1990) (Fig. 1). Compared with LMWH, this difference in chemistry plus a lower degree of sulfation and a lower charge density play an important role in the lack of binding of danaparoid to plasma proteins and platelets and are particularly relevant for its pharmacological profile (Casu, 1991).

Pharmacology

Danaparoid exerts its antithrombotic effects predominantly by indirect inhibition of factor Xa; it has only minimal anti-factor IIa (antithrombin) activity. Hence,



(LMW) Heparin Danaparoid

FIGURE 1 Comparison of predominant disaccharide structure of heparin with danaparoid. The LMW heparin disaccharide is mostly (*left*) glucosamine-*N*-sulfate and (*right*) iduronic acid, whereas danaparoid's principal constituent, heparan sulfate, is predominantly (*left*) *N*-acetyl-glucosamine and (*right*) glucuronic acid. The degrees of sulfation (sulfate groups per disaccharide unit) for heparin and danaparoid are approximately 2.0–2.5 and 1.0–1.5, respectively (see Chapter 7). *Abbreviation*: LMW, low molecular weight.

the ratio of anti-factor Xa (anti-Xa) to antithrombin (anti-IIa) is $\geq$22:1, which is considerably greater than that of LMWH preparations (2:1–4:1) and UFH (1:1) (Meuleman et al., 1982; Gordon et al., 1990; Meuleman, 1992). Its inhibition of factor Xa is mediated by AT and its minor inhibitory effect on thrombin by both AT and heparin cofactor II. Danaparoid also inhibits factor IX activation by IIa, thereby dampening a major feedback loop for thrombin generation (Ofosu, 1992). The predominant anticoagulant effect of danaparoid can be categorized as thrombin generation inhibition (TGI).

Danaparoid does not interfere with platelet function (Meuleman et al., 1982; Mikhailidis et al., 1984, 1987; Meuleman, 1987) and so, unlike UFH, it has minimal effect on formation of the platelet-dependent hemostatic plug (Meuleman, 1992). These characteristics of danaparoid contribute to its high therapeutic index (i.e., favorable benefit/risk ratio).

Danaparoid binds poorly to platelet factor 4 (PF4), the main heparin-binding protein responsible for HIT antibody induction, but it can interfere with the interaction between HIT antibodies and platelets (Chong et al., 1989).

Pharmacokinetics

Danaparoid has a pharmacokinetic profile different from that of UFH or LMWH. It is well absorbed after subcutaneous (sc) administration, with its bioavailability approaching 100% (Stiekema et al., 1989; Danhof et al., 1992). In comparison, the bioavailability of LMWH is 87–92%, and that of UFH only 15–20% (Skoutakis, 1997). Danaparoid's plasma anti-Xa levels peak 4–5 h following sc injection (Danhof et al., 1992). Unlike heparins, it is not neutralized by plasma proteins, such as PF4 and histidine-rich glycoprotein, accounting for its high bioavailability after sc or intravenous (iv) administration. Danaparoid exhibits linear pharmacokinetics and has relatively predictable plasma levels.

Danaparoid is eliminated mainly by the kidneys. It has a relatively long plasma anti-Xa half-life ($t_{1/2}$) of about 25 h. Plasma $t_{1/2}$ values of anti-IIa activity and TGI activity are much shorter, ranging from 2 to 4 h and 3 to 7 h, respectively (Bradbrook et al., 1987; Stiekema et al., 1989; Danhof et al., 1992). In patients with moderate to severe impairment of renal function the drug tends to accumulate, and the dose should be reduced in accordance with monitoring of plasma anti-Xa levels.

Danaparoid's metabolism is not affected by hepatic cytochrome P-450, nor does it affect hepatic or renal handling of other drugs. It has no significant effect on the pharmacodynamics and pharmacokinetics of coumarins. Its pharmacokinetics are not modified by age or body weight (Stiekema et al., 1989; Danhof et al., 1992).

There is no antidote for danaparoid (Stiekema et al., 1989). Protamine chloride only minimally neutralizes its anticoagulant activity. If severe bleeding occurs, the drug should be stopped and blood product replacement given, as indicated clinically. There is limited evidence that plasmapheresis can accelerate drug elimination (Schmahl et al., 1997), but this option is seldom practical.

II. CLINICAL USE OF DANAPAROID

A. Clinical Use of Danaparoid in Disorders Other Than HIT

Controlled clinical trials of danaparoid for the routine prophylaxis and treatment of venous thromboembolism in non-HIT patients have confirmed its efficacy as an antithrombotic agent. In eight prospective, randomized, controlled, and assessor-blind studies, danaparoid was more effective than other standard antithrombotic agents (e.g., warfarin, dextran, low-dose UFH plus dihydroergotamine) in preventing deep vein thrombosis (DVT) after total hip replacement (Hoek et al., 1992; Leyvraz et al., 1992; Org 10172 Report, 1994; Gent et al., 1996; Comp et al., 1998) or hip fracture surgery (Bergqvist et al., 1991; Gerhart et al., 1991). Danaparoid also compared favorably with LMWH in patients undergoing fractured hip surgery (TIFDED Study Group, 1999). In addition, prospective controlled studies have demonstrated the efficacy of danaparoid for DVT thromboprophylaxis after major thoracic and abdominal surgery for cancer (Cade et al., 1987; Gallus et al., 1993) and after spinal cord injury (Merli et al., 1991). Danaparoid (2000 U initial dose iv, then 2000 U twice daily by sc injection) was more effective than UFH in the treatment of DVT (de Valk et al., 1995). Case series also suggest efficacy in patients with disseminated intravascular coagulation (DIC) complicating promyelocytic leukemia (Nieuwenhuis and Sixma, 1986) (danaparoid is approved for treatment of DIC in Japan), as well as in the prevention of fibrin deposition on the dialysis membrane during hemodialysis (Henny et al., 1983; von Bonsdorff et al., 1990).

B. Clinical Use of Danaparoid in Patients with HIT

Danaparoid has been used extensively to treat patients with HIT (Chong and Magnani, 1992; Magnani, 1993, 1997; Magnani and Gallus, 2006). After the diagnosis of HIT and discontinuation of heparin administration, patients often require an alternative anticoagulant for any one of the following indications: (1) treatment of a recent or new thrombosis; (2) prophylaxis of venous thromboembolism; (3) anticoagulation for cardiopulmonary bypass (CPB) surgery or peripheral arterial surgery; (4) anticoagulation for intermittent or continuous hemodialysis or continuous renal replacement therapy (CRRT); (5) cardiac catheterization or coronary angioplasty; or (6) maintenance of intravascular catheter patency. The rationale for the use of danaparoid in these various situations includes: its non-heparin structure, its low degree of cross-reactivity with HIT antibodies compared with LMWH (Makhoul et al., 1986; Chong et al., 1989; Greinacher et al., 1992; Kikta et al., 1993; Vun et al., 1996), its ability to inhibit HIT antibody-induced platelet activation (Chong et al., 1989), and its overall favorable efficacy and safety profile.

The largest clinical experience with the use of danaparoid in the treatment of patients with HIT is in the compassionate-use (named patient) program organized

by the manufacturer (Magnani, 1993, 1997). From 1981 to 1997, over 750 patients were treated under this program for the various indications listed earlier (Ortel and Chong, 1998). The duration of treatment ranged from 1 day to 3.5 yr, and the post-treatment follow-up was 3 mo. Interim, updated reports of this program have been published (Chong and Magnani, 1992; Magnani, 1993, 1997). The overall success rate, defined as platelet count recovery without new, progressive, or recurrent thrombosis during the danaparoid treatment period, or thrombotic death during 3 mo follow-up, and the absence of any adverse effect necessitating treatment cessation, has been over 90%, as judged by the local physician-investigators. However, as this definition does not include non-thrombotic death, the overall mortality observed in the program was 18%, including deaths during the post-treatment follow-up. Most patients in this program received danaparoid for the treatment of acute thromboembolism, often in the setting of severe illness such as renal or multisystem organ failure.

Besides this compassionate-use program, other studies supporting the efficacy of danaparoid therapy for acute HIT include a randomized controlled trial comparing danaparoid with dextran (Chong et al., 2001), a retrospective analysis comparing danaparoid with lepirudin (Farner et al., 2001), and a historically controlled retrospective cohort study that compared danaparoid with ancrod (Lubenow et al., 2006).

Treatment of Venous and Arterial Thromboembolism

Patients with HIT frequently have one or more acute thromboses, which may have occurred before the development of HIT, as a complication of HIT itself, or both (Warkentin and Kelton, 1996). Venous thrombosis complicates HIT more often than does arterial occlusion. Indeed, in the compassionate-use program, the ratio of venous to arterial thrombosis was 2 : 1 (Ortel and Chong, 1998). Even without thrombosis, HIT patients require continuation of antithrombotic therapy after heparin cessation (Warkentin and Greinacher, 2004). Currently, danaparoid, lepirudin, and argatroban are believed to be effective in this situation (Warkentin et al., 1998). These agents have in common the capacity to inhibit thrombin directly (lepirudin, argatroban) or its generation (danaparoid).

Initially in the compassionate-use program, it was recommended that HIT patients with acute thrombosis receive iv danaparoid administered as a bolus of 2500 U, followed by an infusion of danaparoid at 400 U/h for 4 h, followed by 300 U/h for 4 h, and then 150–200 U/h for at least 5 days, aiming for a plasma anti-Xa level of 0.5–0.8 anti-Xa U/mL. Table 1 describes the current protocol that takes into account the current amount of danaparoid per marketed ampule (750 anti-Xa U/ampule), as well as certain initial bolus dose adjustments based on body weight. Danaparoid is also effective when administered sc (de Valk et al., 1995); in this situation, the equivalent 24-h actual or estimated iv dose is given in two to three divided doses by sc injection over a 24-h period. For example, 2250 U (three ampules) every 12 h by sc injection is approximately equal to 190 U/h by iv infusion given over 24 h. In the compassionate-use program, 464 patients with acute thromboembolism were treated with danaparoid, with efficacy judged to be over 90% (Ortel and Chong, 1998).

Danaparoid also proved efficacious for HIT-associated thrombosis in a prospective, randomized, controlled study (Chong et al., 2001). HIT patients with an acute thrombosis (venous, arterial, or both) were randomized to receive either danaparoid plus warfarin or dextran 70 plus warfarin. Dextran 70 is a glucose polymer with an average molecular mass of 70,000 Da. It is a weak antithrombotic

TABLE 1 Danaparoid Dosing Schedules in HIT Patients

Clinical indication	Danaparoid dosing schedule
Prophylaxis of VTE	750 U sc b.i.d. or t.i.d. for patients with history of HIT or who have low suspicion for HIT. For patients with (confirmed or strongly suspected) acute HIT with or without thrombosis, use treatment doses (see below)
Treatment of VTE or arterial thromboembolism	2250 U iv bolus[a] followed by 400 U/h for 4 h, 300 U/h for 4 h, then 150–200 U/h for $\geq$ 5 days, aiming for a plasma anti-Xa level of 0.5–0.8 U/mL Subcutaneous administration[b]: 1500–2250 U sc b.i.d.
Embolectomy or other peripheral vascular surgery	Preoperative: 2250 U iv bolus[a]; intraoperative flushes: 750 U in 250 mL saline, using up to 50 mL; postoperative: 750 U sc t.i.d. (low-risk patients) or 150–200 U/h (high-risk patients) beginning at least 6 h after surgery
Intermittent hemodialysis (on alternate days)	3750 U iv before first and second dialyses; 3000 U for third dialysis; then 2250 U for subsequent dialyses, aiming for plasma anti-Xa level of $<$ 0.3 U/mL predialysis, and 0.5–0.8 U/mL during dialysis.
CRRT	2250 U iv bolus, followed by 400 U/h for 4 h, then 300 U/h for 4 h, then 150–400[c] U/h aiming for a plasma anti-Xa level of 0.5–0.8 U/mL
CPB	125 U/kg iv bolus after thoracotomy; 3 U/mL in priming fluid of apparatus; 7 U/kg/h iv infusion commencing after CPB hookup, and continued until 45 min before expectation of stopping CPB
Cardiac catheterization	Preprocedure: 2250 U iv bolus (3000 U if 75–90 kg and 3750 U if $>$ 90 kg)
PCI or intra-aortic balloon pump	Preprocedure: bolus as per foregoing Postprocedure: 150–200 U/h for 1–2 days post-PCI (or until removal of balloon pump)
Catheter patency	750 U in 50 mL saline, then 5–10 mL per port, or as required
Pediatric dosage considerations	Refer to Bidlingmaier et al., 2006; see also Chapter 20

Note: Compatibility with intravenous solutions: Danaparoid is compatible for dilution with the following solutions: saline, dextrose, dextrose—saline, Ringer's, lactated Ringer's, 10% mannitol. Preparation of solution for infusion: One option is to add four ampules containing 3000 U (i.e., 750 anti-Xa U/0.6 mL ampule) of danaparoid to 300 mL of intravenous solution, i.e., a solution that comprises 10 U danaparoid per milliliter of intravenous solution: thus, an infusion rate of 40 mL/h corresponds to a dose of 400 U/h: 20 mL/h to a dose of 200 U/h, and so on.

[a]Adjust iv danaparoid bolus for body weight: $<$60 kg, 1500 U; 60–75 kg, 2250 U; 75–90 kg, 3000 U; $>$90 kg, 3750 U.

[b]Danaparoid should be given iv during the acute (thrombocytopenic) phase of HIT (see above).

[c]Initially up to 600 U/h may be required if the filter has recently shown excessive clotting. Once filter life is restored to normal, the rate can be lowered.

Abbreviations: b.i.d., twice daily; b.w., body weight; CPB, cardiopulmonary bypass surgery; CRRT, continuous renal replacement therapy; HIT, heparin-induced thrombocytopenia; iv, intravenous; PCI, Percutaneous coronary intervention; t.i.d., three times daily; VTE, venous thromboembolism.

agent that has been used to prevent DVT in postoperative patients (Aberg and Rausing, 1978; Bergqvist, 1980). Known to block HIT antibody-induced platelet aggregation in vitro (Sobel et al., 1986), it was regarded as a potentially useful drug for the treatment of HIT. Dextran 70 was also the only other rapid-acting non-heparin antithrombotic drug available in Australia at study commencement in 1988.

The danaparoid treatment regimen for this early study differed slightly from that of the compassionate-use program because ampules at that time contained 800 U. Thus, danaparoid was given as a bolus of 2400 U, followed by an infusion of 400 U/h for 2 h, 300 U/h for 2 h, and then 200 U/h for 5 days. In the dextran 70 arm, patients received dextran, 1 L on day 1, and then 500 mL/day from days 2 to 5. In both treatment arms, the patients also received warfarin, with doses

adjusted to an international normalized ratio (INR) of 2–4; the warfarin was continued for 3 mo. Patients were also stratified at randomization, depending on the severity of their thrombosis, using predefined criteria.

Resolution of thrombocytopenia showed a non-significant trend in favor of danaparoid over dextran 70. Among the patients stratified as having "mild" thrombosis, a slightly higher percentage of patients treated with danaparoid (83%) improved compared with those who received dextran 70 (73%). In contrast, a substantial and significant difference in treatment outcome occurred in patients with "serious" thrombosis: 88% of danaparoid-treated thromboembolic events recovered, compared with only 44% of those treated with dextran 70. No serious bleeding events were observed. These data suggest that the use of an effective anticoagulant to treat HIT-associated thromboembolism is particularly important in those with more severe disease.

In another study, patients treated prospectively with lepirudin were compared with patients treated with danaparoid (Farner et al., 2001). Although not a randomized trial, this study had important strengths. First, all patients had serologically confirmed HIT (about 70% had thrombosis at study entry). Second, all patients met identical inclusion and exclusion criteria, had similar baseline characteristics, and were treated during the same time period (25 mo ending April 1996). Third, many patients were studied (danaparoid, $n = 126$; lepirudin, $n = 175$). Furthermore, patients were subdivided into those treated with prophylactic or therapeutic doses. The results of this study suggest that both danaparoid and lepirudin have similar efficacy for treatment of HIT-associated thrombosis when given in *therapeutic* doses: the day 42 success rate was about 80% for either agent, when failure was defined as having a composite endpoint of new thrombosis, death, and/or limb loss (Fig. 2a). When evaluating the single endpoint of new thrombosis in patients who received therapeutic doses of study drug, danaparoid and lepirudin also showed similar efficacy (90.6% vs. 92.1%; $p = 0.74$). Moreover, safety analysis of all patients (regardless of dose received) showed significantly fewer major bleeds with danaparoid (2.5% vs. 10.4%; $p = 0.009$) (Fig. 2b). These data suggest that the favorable therapeutic index of danaparoid extends also to HIT complicated by thrombosis.

More recently, a retrospective evaluation of treatment outcomes was made between a period when HIT patients were often treated with the defibrinogenating snake venom, ancrod (usually with coumarin) or coumarin alone, and a later period when treatment of new HIT patients was mainly performed using danaparoid (usually with overlapping coumarin) (Lubenow et al., 2006). This historical "switch" in choice of HIT treatment (which occurred in Canada) was associated with a highly significant reduction in the composite endpoint of new/progressive/recurrent thrombosis, thrombotic death, and limb amputation among patients treated with danaparoid (day 7 outcomes: 12.9% vs. 39.3%; $p = 0.0014$) (Fig. 3). In addition, significantly less major bleeding was observed with danaparoid (11.3% vs. 28.6%; $p = 0.0211$). These investigators further compared the efficacy and safety outcomes observed using danaparoid treatment against those of published trials of lepirudin and argatroban (Table 2). The results suggest that the efficacy : safety relationship of danaparoid is especially favorable, although the lack of head-to-head randomized comparisons between danaparoid and either direct thrombin inhibitors (DTIs) prevents any definitive conclusions.

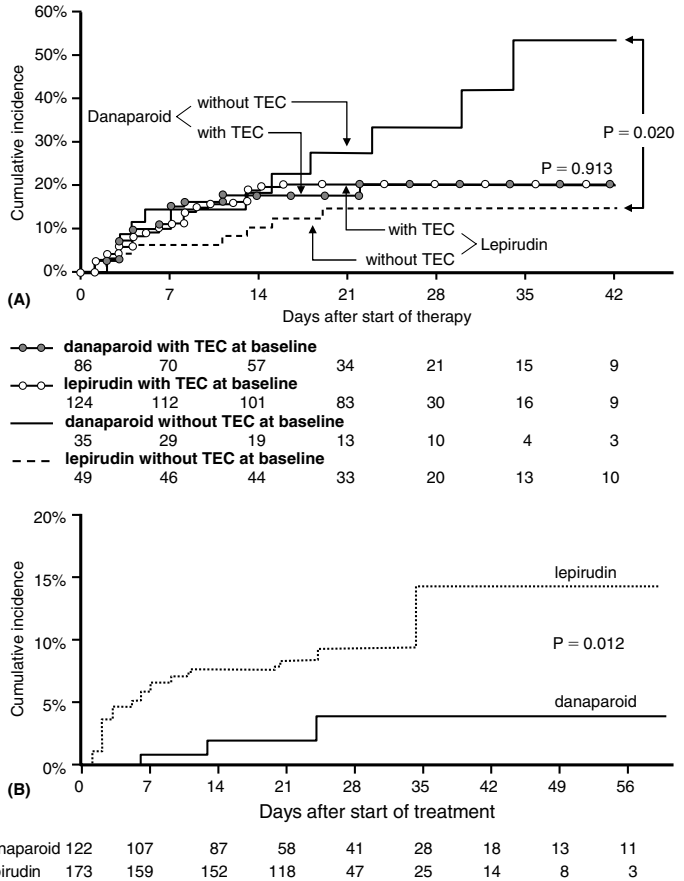


FIGURE 2 Efficacy and safety outcomes of HIT patients, with and without TEC: comparison of danaparoid with lepirudin. Patients in the prospective lepirudin-treatment studies were compared with contemporaneous patients treated with danaparoid who met the identical inclusion and exclusion criteria as in the lepirudin trials. Also shown is the number of patients at risk on the starting day and at subsequent 7-day intervals. (**A**) Efficacy: time-to-event analysis of the incidences of a combined endpoint (new TECs, limb amputation, death; maximum, one endpoint per patient) up to day 42. Among patients without TEC at baseline (most of whom were treated with a *prophylactic*-dose regimen), there was a significantly higher incidence of the combined endpoint among patients treated with danaparoid, compared with lepirudin ($p = 0.02$, log-rank test). This suggests that a prophylactic-dose regimen for danaparoid (750 U b.i.d. or t.i.d. by sc injection, without anticoagulant monitoring) may be relatively less effective for managing patients with isolated HIT compared with a "prophylactic" regimen of lepirudin in which aPTT-adjusted monitoring occurs (Warkentin, 2001). In marked contrast, the combined endpoint did not differ significantly between danaparoid and lepirudin for patients with TEC at baseline (most of whom received *therapeutic*-dose danaparoid), suggesting that therapeutic (treatment) doses of danaparoid (Table 1) has similar efficacy as does therapeutic-dose lepirudin. (**B**) Safety: Time-to-event analysis of the incidences of major bleeding. Major bleeding was defined as overt bleeding requiring transfusion of two or more red blood cell concentrates or intracerebral bleeding. The bleeding rate was significantly lower in patients treated with danaparoid ($p = 0.012$, log-rank test). This indicates that the therapeutic window of lepirudin is rather narrow. *Abbreviations*: aPTT, activated partial thromboplastin time; HIT, heparin-induced thrombocytopenia; TEC, thromboembolic complications. *Source*: From Farner et al., 2001.

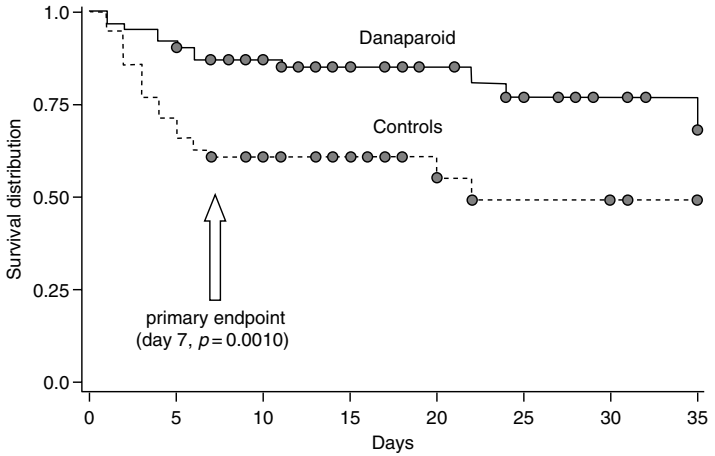


FIGURE 3 Efficacy outcomes: comparison of danaparoid (with or without coumarin) with ancrod (with or without coumarin) or coumarin alone. Time-to-event analysis of composite endpoint (new thrombosis, thrombotic death, amputation; maximum, one event per patient). The primary efficacy endpoint (at day 7) was reduced in the danaparoid-treated patients ($p = 0.001$, log-rank test); at day 35 (secondary endpoint), the composite endpoint remained reduced ($p = 0.0022$, log-rank test). When a different composite endpoint that included all-cause mortality was analyzed (i.e., new thrombosis, all-cause mortality, amputation; maximum, one event per patient), the differences remained significant (day 35; $p = 0.0013$ by log-rank test; data not shown). *Source*: From Lubenow et al., 2006.

Finally, a review of 1478 danaparoid-treated patients available from the company files (including the compassionate-use program and spontaneous adverse event reports) and independent published literature has been made (Magnani and Gallus 2006). A wide variety of clinical uses were described, ranging from treatment of thromboembolism to extracorporeal support for renal failure, from routine medical use to major general and cardiovascular surgery, and including elderly and pediatric patients, and pregnancy. In all these settings, danaparoid appeared to be safe and effective in treating HIT and associated

TABLE 2 Comparison of Efficacy and Major Bleeding Endpoints of Danaparoid, Lepirudin, and Argatroban vs. Control

		Composite efficacy[a]		Major bleeding[a]	
Study	Drug studied	Study drug	Control	Study drug	Control
Lubenow et al., 2006	Danaparoid	24.2%[b]	50.0%	12.9%[b]	33.9%
HAT-1 and HAT-2	Lepirudin	22.1%[b]	40.0%	19.5%	12.0%
Arg-911	Argatroban	43.8%	56.5%	11.1%	2.2%
Arg-915	Argatroban	41.5%	56.5%	6.1%	2.2%

[a]Up to day 35 for danaparoid and lepirudin: day 37 for argatroban.
[b]Statistically significant ($p < 0.01$) compared with control (by categorical analysis).
Abbreviation: HAT, heparin-associated thrombocytopenia.
Source: From Lubenow et al., 2006.

thromboses. The frequency of a composite of adverse outcomes (new/extended thrombosis, new/persistent platelet count reduction, unplanned amputation) during the treatment period (range, 1 day to 3.5 yr), plus a follow-up period (up to 3 mo), was 16.4%. Thrombotic events occurred in 11.0% of the patients, whereas major bleeding was reported in 8.1%.

Overlapping Oral Anticoagulants with Danaparoid

Many danaparoid-treated HIT patients also receive overlapping warfarin treatment, since oral anticoagulants are usually preferred when at least 3–6 mo of further anticoagulation is indicated because of venous or arterial thromboembolism. However, it generally takes at least 5 days for warfarin to achieve a therapeutic effect (Harrison et al., 1997). Although warfarin likely can be started safely at the beginning of danaparoid treatment in most patients with HIT-associated thrombosis, it is prudent to delay start of warfarin until the thrombotic process is controlled and substantial resolution of the thrombocytopenia has occurred (usually, to a platelet count $>150 \times 10^9$/L). This caveat is based on the observation that warfarin can aggravate the thrombotic process during the first few days of its administration by reducing levels of the natural anticoagulant protein C, particularly when thrombin generation is high (Warkentin, 1996a; Warkentin et al., 1997; Pötzsch et al., 1996) (see Chapters 2 and 12). Warfarin does not neutralize activated coagulation factors (which are increased in acute HIT), and even when sufficient time has passed for its antithrombotic effects to be achieved through reduction in the vitamin K-dependent procoagulant factors (particularly prothrombin), discontinuation of danaparoid prior to resolution of HIT theoretically could result in warfarin-induced microthrombosis. Thus, during overlapping danaparoid-coumarin therapy, danaparoid usually is discontinued only when: the acute thrombosis appears controlled on clinical grounds, at least 5 days of overlapping danaparoid-coumarin therapy have been given, and at least two INR measurements (at least 24 h apart) are within the target therapeutic range (2.0–3.0). Unlike lepirudin and argatroban, danaparoid does not interfere with INR or activated partial thromboplastin time (aPTT) measurements during oral anticoagulant therapy; thus, the potential for underdosing of DTI therapy during concomitant coumarin therapy (Warkentin, 2006) does not apply to danaparoid.

Prophylaxis During Acute HIT

In contrast to the comparable efficacy of danaparoid and lepirudin when used in therapeutic doses to treat HIT-associated thrombosis (discussed previously), lepirudin appeared somewhat more effective than danaparoid when prophylactic-dose regimens were compared for preventing the single endpoint of new thrombosis (91.4% vs. 81.4%; $p = 0.138$); this difference was larger, and reached statistical significance, when the composite endpoint (new thrombosis, limb amputation, or death) was examined (Fig. 2a). However, superior efficacy of lepirudin came at a price: for patients without thrombosis at baseline (most of whom thus received prophylactic-dose therapy), lepirudin was associated with a trend to more major bleeding events than danaparoid (16.3% vs. 2.9%; $p = 0.075$) (Farner et al., 2001). Kodityal and colleagues (2003) also reported five patients who developed new thromboses while receiving relatively low doses of danaparoid (usually, 1250 U every 12 h by sc injection).

These data, and other observations describing early new thrombotic events in patients on prophylactic-dose danaparoid and which respond favorably to increases in danaparoid dosing intensity (Lindhoff-Last et al., 2005; Magnani and

Gallus, 2006), support the current treatment recommendation that *therapeutic* doses of danaparoid are appropriate for most patients with HIT, whether they have HIT-associated thrombosis or just isolated HIT (Warkentin, 2001; Warkentin and Greinacher, 2004) (see Chapters 1 and 12). A comparable situation exists with the two DTIs available to treat HIT: argatroban is approved in the United States in identical therapeutic-dose regimen both for HIT-associated thrombosis and isolated HIT (see Chapter 15) and although there exists a lower-dose (prophylactic) regimen for lepirudin to manage isolated HIT (see Chapter 14), the use of anticoagulant monitoring to adjust the infusion rate means that most patients eventually receive doses that approach the therapeutic regimen (Warkentin, 2001).

Prophylaxis of Venous Thromboembolism

Patients with a previous history of HIT may require an alternative anticoagulant to prevent venous thromboembolism if they require prolonged bed rest and/or surgery or become pregnant. UFH cannot be used, particularly during the first 1 or 2 mo after the onset of HIT when HIT antibodies still circulate. Thereafter, although HIT antibodies are usually undetectable, and the risk of recurrent HIT is possibly relatively low (Warkentin and Kelton, 2001), most physicians are understandably reluctant to re-administer heparin in this situation.

Danaparoid is an effective and convenient drug for the prevention of venous thromboembolism in patients with prior HIT. In the compassionate-use program, 390 patients received danaparoid, 750 U by sc injection, usually twice daily for DVT prophylaxis for many postoperative settings, including general, gynecological, neurological, cancer, and organ transplant surgery. A high rate of success was observed (Magnani, 1997; Ortel and Chong, 1998).

Prophylaxis of Arterial Thromboembolism

Danaparoid has been used to prevent arterial thromboembolism in patients undergoing various vascular operations, including peripheral artery bypass graft surgery, embolectomy, and endarterectomy. In these patients, it was given as a preoperative iv bolus of 2500 or 2250 U, and in some it was also administered postoperatively. Given as an iv bolus of 2500 or 2250 U immediately before the procedure, danaparoid has also been used to provide antithrombotic cover during percutaneous coronary intervention (PCI), with or without stenting, and for insertion of intra-aortic balloon devices.

Anticoagulation for Cardiac Surgery

Patients with acute HIT, or recent previous HIT with persisting HIT antibodies, may need to undergo cardiac surgery. UFH is contraindicated during acute HIT, necessitating an alternative anticoagulant for use during CPB. After successful experiments in dogs (Henny et al., 1985a), danaparoid underwent use for CPB anticoagulation in such situations (Magnani, 1993; Wilhelm et al., 1996; Westphal et al., 1997; Christiansen et al., 1998; Fernandes et al., 2000; Olin et al., 2000). Magnani et al. (1997) reported the experience of 47 such evaluable patients. The initial recommended dosing schedule, which consisted of an iv bolus to the patient both before and after thoracotomy plus addition of danaparoid to the priming fluid (and if necessary further iv booster doses during surgery if fibrin formation became a problem), often led to the patient receiving over 16,250 U danaparoid in total. In some patients, attempts were made to prolong the activated clotting time

(ACT) prior to surgery, but since this test is insensitive to danaparoid (Gitlin et al., 1998), it led to serious overdosing. Although two of the 47 operations had to be abandoned because of intra-operative clotting, the biggest problem (22%) was serious postoperative bleeding (Magnani et al., 1997). It seemed that a total dose of >16,250 U (>250 U/Kg) danaparoid was more likely to increase postoperative blood loss significantly. Therefore, a new dosing regimen (Table 1) was developed that delivers no more than 232 U/kg of the drug. Continuous intraoperative danaparoid infusion is also recommended, which might reduce the need for a further drug bolus shortly before wound closure, as well as provide therapeutic drug levels throughout CPB.

This new regimen and its modifications were used by Olin and coworkers (2000) and Fernandes et al. (2000). Disappointingly, the new regimen did not reduce postoperative bleeding. Consequently, danaparoid is not recommended for CPB (Buys et al., 2003; see also Chapter 19), unless no other suitable alternative is available.

Advances in surgical method may permit other treatment approaches in selected patients. For example, the off-pump ("beating heart") technique does not utilize CPB, and thus a far lower dose of danaparoid may be feasible for intraoperative anticoagulation. This approach was used successfully to perform multiple coronary artery bypass grafting in a patient with acute HIT and unstable angina (Warkentin et al., 2001). A relatively low target plasma anti-factor Xa level (0.6 U/mL) was used, rather than the levels (> 1.5 U/mL) sought during CPB (see Chapter 19).

A randomized, double-blind comparison of danaparoid ($n = 34$) with UFH ($n = 37$) for off-pump coronary artery bypass grafting in non-HIT patients showed a non-significant trend to greater postoperative blood loss (mean, 264 mL) but a significant increase in patients exposed to homologous blood (53% vs. 27%) with danaparoid. Clinical outcomes appeared similar, and the authors concluded that danaparoid could be a valuable option in patients undergoing off-pump surgery when UFH is contraindicated (Carrier et al., 2003).

Hemodialysis, Hemofiltration, and Intensive Care Use

Danaparoid was first used to anticoagulate non-HIT patients requiring hemodialysis in one of several clinical settings: stable chronic renal failure (CRF) (ten Cate et al., 1985; Henny et al., 1990, von Bonsdorff et al., 1990) or intensive care unit (ICU) patients who developed postoperative acute renal failure (ARF) (Henny et al., 1983). Danaparoid was then used to treat very ill patients in intensive care settings who developed HIT during CRRT for ARF (Wester et al., 2000; Lindhoff-Last et al., 2001). Switching from UFH to danaparoid overcame the repeated deposition of fibrin on the hemodialysis/filtration membranes, thus restoring the lifespan of the filters and allowing continuation of extracorporeal circuit use without further incident (Burgess and Chong, 1997; van Eps et al., 2000; Lindhoff-Last et al., 2001). Such fibrin deposition may also be secondary to UFH-induced platelet aggregation and microthrombus formation and, because HIT antibodies are often absent, this may be a manifestation of non-immune heparin-associated thrombocytopenia (HAT) since significant thrombocytopenia usually does not occur (Burgess and Chong, 1997).

Danaparoid may accumulate in the blood of patients with moderate to severe renal dysfunction (creatinine clearance <30 mL/min) because it is cleared renally. During hemodialysis or hemofiltration, it is not cleared by the artificial

kidney. Drug accumulation and the potential risk for bleeding can be minimized by suitable danaparoid dose reduction:

1. For intermittent hemodialysis, this is achieved by using a pre-dialysis iv bolus of 3750 U (2250 U if the body weight is < 55 kg) for the first two procedures and then reducing the pre-dialysis bolus (usually to 2250 or 3000 U) according to the previous pre-dialysis plasma anti-Xa levels. Thus, a plasma anti-Xa level should be performed before the second and subsequent dialyses and each level is used to adjust the danaparoid dose for the dialysis. The aim is to maintain the plasma anti-Xa level between 0.5 and 0.8 U/mL during dialysis. Usually by hemodialysis number 4 or 5, a "steady-state" pre-dialysis bolus dose has been found, which will allow effective anticoagulation during dialysis. Following this regimen, danaparoid has been used for up to 4 yr for intermittent hemodialysis (three times per week).
2. For CRRT, the dosing regimen is similar to the iv infusion regimen used for the treatment of venous thrombosis (Table 1). However, if severe hemofilter clotting had occurred with UFH or LMWH, then an initial higher maintenance infusion rate (up to 600 U/h if bleeding is not observed) may be needed until filter life has been restored to a reasonable duration. Danaparoid has been used for up to 39 days for continuous hemofiltration or hemodialysis in ICU patients.

A recent review (Magnani and Gallus, 2006) of 291 HIT and non-HIT ICU patients with or without renal failure showed that danaparoid can provide efficacious and safe anticoagulation for this patient population at high risk for thrombosis and bleeding. The incidence of major bleeding was 8.9% and thrombotic events (mainly circuit clotting) was 7.6%; the all-cause mortality rate was 24.4%. Many of the bleeding events and circuit clotting occurred during the danaparoid dose-adjustment phase and ceased after dose optimization (Lindhoff-Last et al., 2001). Most deaths appeared to be due to sepsis and/or multiple organ failure. A generally favorable experience in the use of danaparoid in 42 consecutive ICU patients with HIT was also reported by Tardy-Poncet and colleagues (1999).

Use in Children and Pregnant Women

Danaparoid has been used in a small number of pediatric patients (Saxon et al., 1999; Bidlingmaier et al., 2005; see Chapter 20). For 33 of 34 children aged between 2 wk and 17 yr, danaparoid was used for the treatment of HIT. Sixteen children were treated with danaparoid for various indications, including maintenance of catheter patency, renal failure, cardiac surgery, and thrombosis. In general, it was noted that children, particularly infants, often required higher doses of danaparoid than adults on a weight-adjusted basis. Twenty-six children survived (78.8%), five died, and for two, there is no outcome information. The causes of death were thrombotic (one of three patients with a thrombotic event), bleeding (two of four patients with a major bleeding event), treatment withdrawn (one), and septicemia-induced multiple organ failure (one). Overall, danaparoid appeared safe and effective in children, except in cases requiring CPB, since this was associated with three of the four major bleeds.

Danaparoid is reported to have been used in at least 32 pregnancies complicated by HIT (Lindhoff-Last et al., 2005). All had a history of thrombosis, either acute or during a previous pregnancy. Danaparoid therapy was initiated in the first trimester in 18/32 (56.3%) and continued for up to 34 wk. The doses used

were the same as those in non-pregnant women for the same indications. In 24 pregnancies (75%) carried to term, normal babies were delivered either vaginally or by caesarian section, although in some a maternal adverse event (usually associated with a placental abnormality) occurred. In eight pregnancies, danaparoid was stopped prematurely. In two patients, this was because of successful short-term use (no further follow-up). Early fetal death occurred in three pregnancies, two in association with the maternal antiphospholipid syndrome, the other a therapeutic abortion necessitated by a maternal amputation due to thrombosis extension in the second trimester. In two pregnancies, major bleeds occurred, one due to placental abruption (fatal bleeding followed caesarian section in a Jehovah's Witness) after 24 wk of problem-free danaparoid use, and one due to placenta previa with fatal cardiopulmonary complications after caesarian section. One pregnancy was complicated by the onset of hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome, which manifested 6 wk into treatment with danaparoid. Danaparoid was replaced by warfarin and aspirin, but 2 wk later an emergency caesarian was needed because of impaired fetal growth and the premature, 24 wk, neonate died 2 days later of pulmonary hemorrhage.

In five infants' cord plasma no anti-Xa activity could be detected and four breast milk samples obtained from mothers continuing danaparoid postpartum had virtually undetectable anti-Xa activity. Thus, at least the main antithrombotic subfraction of danaparoid does not cross the placenta, and the tiny amounts that appear in the breast milk are probably hydrolyzed in the infant's stomach (Lindhoff-Last and Bauersachs, 2002). No increase in postpartum bleeding in 22 danaparoid-treated pregnant mothers was reported. The 19 treatment outcomes in non-HIT pregnancies were similar (Lindhoff-Last et al., 2005).

C. Laboratory Monitoring

Laboratory monitoring of the plasma anti-Xa activity response is not required for routine use of danaparoid for thrombosis prophylaxis or treatment. However, it is recommended in the following clinical settings: (1) patients with substantial renal impairment; (2) children or adults with unusually low or high body weight; (3) patients with life- or limb-threatening thrombosis; (4) patients with high bleeding risk; and (5) critically ill or unstable patients.

All pharmacokinetics and recommended plasma anti-Xa activity responses are based upon an amidolytic assay. Since danaparoid, unlike UFH and the LMWHs, does not significantly prolong the aPTT, prothrombin time/INR, or ACT, except at very high doses, these assays cannot be used for monitoring. The lack of binding to plasma proteins means that danaparoid anti-Xa levels will be overestimated if a LMWH standard curve is used. Further, there are differences in the stated therapeutic range among these various anticoagulants (UFH, 0.2–0.4 U/mL by protamine titration; UFH, 0.3–0.7 anti-Xa U/mL; LMWH, 0.6–1.0 U/mL; danaparoid, 0.5–0.8 anti-Xa U/mL) (Hirsh et al., 1998; Laposata et al., 1998; Warkentin et al., 1998). This means that for any assay of danaparoid plasma anti-Xa levels, the standard calibration curve must be constructed using danaparoid, and not UFH or even LMWH (Laposata et al., 1998).

The 100% bioavailability of danaparoid also allows predictable plasma levels after both sc or iv injection, but in some clinical treatment settings, it might be advisable to aim for a lower anti-Xa level (e.g., about 0.3 U/mL for a patient judged to have a high risk of bleeding); sometimes, a higher target anti-Xa level

should be sought (e.g., about 1.0 U/mL for a patient with life- or limb-threatening venous or arterial thrombosis or clotting during CRRT).

The amidolytic anti-Xa assay uses a chromogenic substrate (i.e., a method similar to that performed for monitoring LMWH treatment). A standard reference curve must be constructed using various dilutions of danaparoid (e.g., 1.6, 1.0, 0.5, 0.3, and 0 U/mL danaparoid, diluted in pooled normal platelet-poor plasma). Control plasma samples are prepared by adding known quantities of danaparoid to normal pooled plasma aliquots (assuming 100% recovery of the known quantity of danaparoid added) in three different concentrations approximating treatment situations (e.g., 0.2, 0.7, and 1.25 U/mL, corresponding to low-, mid-, and high-control danaparoid levels). Aliquots stored at −70°C are stable for several years if used only once, without re-freezing and re-thawing.

D. Cross-Reactivity of HIT Antibodies with Danaparoid

As danaparoid consists of a mixture of GAGs (mainly heparan sulfate), it is not surprising that a small percentage of antibodies from HIT patients cross-react with the drug. The mean overall reported frequency of in vitro danaparoid cross-reactivity rate in over 2200 plasma samples from HIT patients is 7.6% (Magnani and Gallus, 2006). If aggregation studies using citrated platelet-rich plasma are performed (Makhoul et al., 1986; Kikta et al., 1993; Ramakrishna et al., 1995; Vun et al., 1996), the cross-reactivity is less compared with the more sensitive washed platelet activation assays (Warkentin, 1996b; Koster et al., 2000) or a fluid-phase enzyme immunoassay (EIA) (Newman et al., 1998; Warkentin et al., 2006) (see Chapter 10). Because activation assays are dependent on the donor platelets used for testing, highly reactive donor platelets under standardized conditions have been used for the platelet aggregation test to investigate the cross-reactivity of HIT antibodies for danaparoid and LMWH. Using this modification, cross-reactivity rates of 7% with danaparoid and 83–89% with LMWH (Vun et al., 1996) were found. However, with the sensitive fluid-phase EIA, a higher cross-reactivity rate of 50% with danaparoid and 88% with LMWH (Newman et al., 1998) was observed (Fig. 4). Importantly, even when in vitro reactivity with danaparoid is observed, it is generally weak and quantitatively much less than seen with LMWH.

The in vitro cross-reactivity of the HIT antibodies with danaparoid does not appear to be usually clinically significant. Newman et al. (1998) investigated the clinical significance of in vitro cross-reactivity in 21 patients treated with danaparoid. The eight patients who tested positive for cross-reactivity by the fluid-phase EIA, but negative by the [^{14}C]serotonin-release washed platelet assay, recovered with resolution of their thrombocytopenia and thrombosis, in a fashion similar to the 11 patients who did not manifest in vitro danaparoid cross-reactivity in either assay. Two patients tested positive in both assays: in one patient, both thrombocytopenia and pulmonary embolism resolved during danaparoid treatment. However, in the other patient, thrombocytopenia and extensive thrombosis persisted despite danaparoid therapy, although it was unclear whether this unusual patient course represented a specific danaparoid treatment failure (the patient's subsequent clinical course was characterized by consistent failure of all antithrombotic therapies used) (Fig. 5).

Warkentin (1996b) also evaluated the clinical significance of in vitro cross-reactivity with danaparoid in 29 HIT patients treated with danaparoid. This investigator found no difference in clinical outcomes, or in the time to platelet count recovery, between the two patient groups.

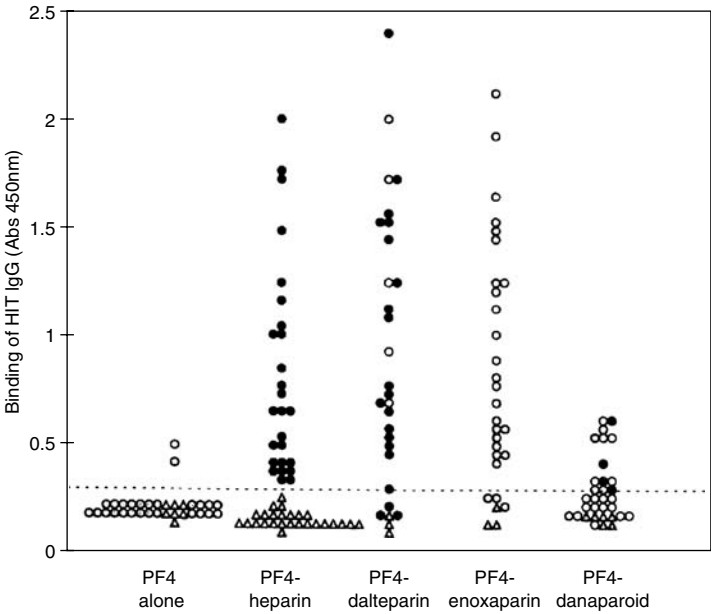


FIGURE 4 Cross-reactivity of HIT-IgG antibodies with PF4 complexed to heparin-like anticoagulants: The fluid-phase EIA was used to assess the degree to which IgG present in HIT sera or plasma bound to PF4 alone, PF4-heparin, PF4-dalteparin (Fragmin), PF4-enoxaparin (Clexane), or PF4-danaparoid (Orgaran). The positive cutoff (*dashed line*) is three standard deviations above the mean (log transformed) absorbance of the normal samples (*triangles*) using PF4-heparin. The binding of normal antibodies is indicated by triangles. Circles indicate HIT samples that have been positive (*closed circle*), negative (*open circle*), or not tested (*speckled circle*) in a functional assay with the corresponding drug. *Abbreviations*: EIA, enzyme immunoassay; HIT-IgG, heparin-induced thrombocytopenia immunoglobulin G; PF4, platelet factor 4. *Source*: From Newman et al., 1998.

Isolated anecdotal reports of unfavorable clinical outcomes in HIT patients treated with danaparoid exist (Tandy-Poncet et al., 1995; Insler et al., 1997; Muhm et al., 1997). Cross-reactive antibodies were not always investigated, and their potential role vis-á-vis other clinical factors remains uncertain.

Magnani and Gallus (2006) found in a review of 1418 HIT patients treated with danaparoid that among 36 patients with apparent pre-treatment danaparoid cross-reactivity, 23 had clinical events (platelet count fall, thrombotic event) possibly indicating in vivo cross-reactivity. The remaining 13 patients were treated for a median of 11 days without a problem. A more recent update (Magnani, unpublished), which includes patients reported by Warkentin (1996b) and Newman et al. (1998), shows that of 58 patients treated with danaparoid despite a positive danaparoid pre-treatment cross-reactivity test, 31 (53%) were treated for up to 42 days with full recovery of platelet counts and no further thromboembolic events. In 14 (24.2%) of the remaining patients, the platelet count did not recover (half of these developed a thromboembolic event).

A further 22 patients developed apparent cross-reactivity seroconversion (i.e., negative pre-danaparoid cross-reactivity test became positive 2–14 days after

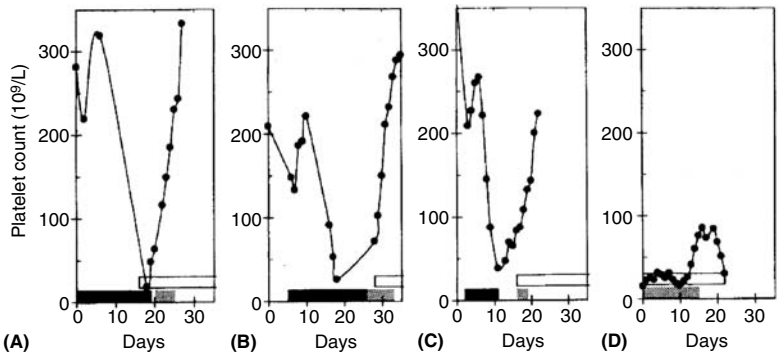


FIGURE 5 Serial platelet counts of representative HIT patients treated with danaparoid: Solid black bar shows duration of heparin administration, striped bar indicates danaparoid therapy, open bar shows warfarin therapy: (**A**) Typical profile of the 11 patients who were negative in both fluid-phase EIA and functional assay, (**B**) Typical profile of the eight patients who were positive in the fluid-phase, but negative in a functional assay, (**C**) Profile of patient A, one of the two patients who were positive in both types of assay. She recovered during a short course of danaparoid. (**D**) Profile of patient B, the other patient positive in both assays. The profile indicates the course of HIT following transfer to a major hospital. Despite treatment with many antithrombotic agents, he eventually died following major thrombosis. *Abbreviations*: HIT, heparin-induced thrombocytopenia; EIA, enzyme immunoassay. *Source*: From Newman et al., 1998.

initiating danaparoid treatment); a thromboembolic event occurred in seven (two fatal). Thus, in total, 3.2% of the 1418 patients reviewed by Magnani and Gallus (2006) were reported to have had serologically confirmed clinical cross-reactivity with danaparoid (23 associated with pre-treatment cross-reactivity, 22 associated with cross-reactivity seroconversion). However, the development of platelet count reduction and/or a thromboembolic event during danaparoid treatment is not necessarily a marker of cross-reactivity, since 10 patients re-tested at the time of suspected clinical cross-reactivity remained seronegative (Magnani and Gallus, 2006).

There are several important considerations regarding danaparoid cross-reactivity. First, it is clear that a positive test for in vitro cross-reactivity does not necessarily mean that adverse effects related to danaparoid therapy will occur (at least half the patients will do well). Second, in vitro cross-reactivity itself might indicate more severe HIT, with the potential for greater risk of complications irrespective of which anticoagulant is given. Thus, it might not be possible to distinguish clinical cross-reactivity with the natural course of a severe episode of HIT (Warkentin, 1998; Newman et al., 1998; Baumgartel et al., 2000). Third, no standardized, validated testing method for cross-reactivity exists. Indeed, apparent in vitro cross-reactivity reported in some studies could even reflect the phenomenon of heparin-*in*dependent platelet activation caused by some HIT patient serum (see Chapter 10). Besides mimicking in vitro cross-reactivity (as some serum-induced platelet activation will occur whether or not danaparoid is added), this serological feature itself could portend a poor prognosis. Fourth, prospective and retrospective studies (Chong et al., 2001; Farner et al., 2001; Lubenow et al., 2006) showed favorable clinical outcomes when danaparoid was given without delay for performing in vitro cross-reactivity. This indicates that a high likelihood of satisfactory outcome can be obtained by using a strategy of prompt

anticoagulation with danaparoid without performing in vitro cross-reactivity studies. Indeed, despite the issue of in vitro cross-reactivity with danaparoid, it is noteworthy that danaparoid-induced immune-mediated thrombocytopenia has never been reported. Based upon these considerations, in vitro cross-reactivity testing is not recommended prior to commencing therapy with danaparoid for suspected HIT (Warkentin and Greinacher, 2004). However, cross-reactivity testing may be appropriate in patients who develop new, progressive, or recurrent

TABLE 3 Countries in Which Danaparoid Is Approved for Clinical Use[a]

	DVT prophylaxis		Heparin-induced thrombocytopenia	
Country	Perioperative[b]	Poststroke	Prophylaxis	Treatment
North America				
Canada	X	X	X	X
United States	X[c]			
Europe				
Austria	X	X	X	X
Belgium	X		X	X
Czech Republic			X	X
Denmark	X			
Finland			X	X
France	X[d]		X	X
Germany	X[e]		X	X
Great Britain	X			X
Greece	X		X	X
Ireland	X			X
Italy	X		Pending	
Luxembourg	X		X	X
The Netherlands	X[e]	X[e]	X	X
Norway	X	X		
Portugal	X		X	X
Sweden	X	X		X
Switzerland	X		X	X
Australasia and Africa				
Australia	X		X	
Japan[f]				
Korea	X			
New Zealand	X	X	X	X
South Africa			X	X

[a]Danaparoid is no longer marketed in some of these countries, e.g., United States, Norway, and Denmark.
[b]Orthopedic and general surgery only (unless otherwise indicated); approval includes starting danaparoid 1–4 h preoperatively (except for United States).
[c]Elective hip surgery only.
[d]Orthopedic and cancer surgery only.
[e]Approval modified to facilitate approval for HIT in Finland and Germany.
[f]Approved for the treatment of DIC.
Abbreviations: DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis; HIT, heparin-induced thrombocytopenia.

thrombocytopenia or thrombosis during treatment with danaparoid, although the implications of a positive result remain uncertain.

E. Adverse Effects

Bleeding is the most serious adverse effect of danaparoid. However, serious bleeding is uncommon except in patients who are treated with very high doses of the drug (e.g., cardiac surgery using CPB), or in those who develop drug accumulation (renal failure), or who have additional hemostatic or vascular defects. In contrast, bleeding was not seen in the randomized trial in which HIT patients with venous or arterial thromboses received danaparoid plus warfarin (Chong et al., 2001). When CPB patients are excluded, the overview of major bleeding episodes in relation to danaparoid use shows a drop in frequency from 8.1% to 4.6% (Magnani and Gallus, 2006).

Recurrence of skin hypersensitivity reactions in patients with reactions to UFH or LMWHs have been reported with danaparoid, but these are rare (Magnani, 1993, Magnani and Gallus 2006) and there are reports of successful use of danaparoid in such patients (Grassegger et al., 2001; Harrison et al., 2001; de Saint-Blanquat et al., 2000; Taylor, 2001). Pre-treatment skin cross-reactivity testing often reveals positive results (Figarella et al., 2001; Grassegger et al., 2001; Harenberg et al., 1999), but this does not necessarily translate into clinical problems, since in some patients, the reactions have been observed to diminish with each danaparoid injection and disappear after a few days. In addition, iv treatment with danaparoid of patients with positive skin tests has also proved successful (Boehnke et al., 1996; Jappe et al., 2002; Bircher et al., 2006).

Osteoporosis (a significant complication of prolonged UFH treatment) was not reported in any danaparoid-treated patients in the compassionate-use program, including pregnant patients treated for more than 3 mo.

F. Availability of Danaparoid

Table 3 lists the countries where danaparoid has been approved for the treatment of HIT, either with or without associated thrombosis. In countries in which danaparoid is approved for DVT prophylaxis, physicians generally have the legal option to prescribe danaparoid for HIT (i.e., for "off-label" use in a non-approved indication). Danaparoid is no longer marketed in some countries (e.g., United States [since April 2002], Norway, and Denmark).

III. CONCLUSION

Danaparoid is a safe and effective anticoagulant for the prevention or treatment of venous or arterial thrombosis in HIT patients. It can be administered by sc and iv routes of administration, and appears to have a favorable benefit:risk ratio. In vivo cross-reactivity of danaparoid for HIT antibodies is an infrequent complication.

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14 Lepirudin for the Treatment of Heparin-Induced Thrombocytopenia

Andreas Greinacher
Ernst-Moritz-Arndt-University-Greifswald, Greifswald, Germany

I. INTRODUCTION

With its approval by the European Medical Evaluation Agency (EMEA) in 1997 and by the U.S. Food and Drug Administration (FDA) in 1998 for the treatment of heparin-induced thrombocytopenia (HIT) complicated by thrombosis, lepirudin (Refludan®, a registered trademark of the Bayer Group) became the first direct thrombin inhibitor (DTI) available for treating HIT.

II. HIRUDIN AND ITS DERIVATIVES

A. Chemistry

Hirudin, the most potent natural thrombin inhibitor identified to date, is a 65-amino-acid polypeptide (molecular mass, approximately 7 kDa) produced by the parapharyngeal glands of the medicinal leech, *Hirudo medicinalis.* The NH_2-terminal part of the molecule (residues 1–39) is stabilized by three disulfide bridges integral to its function. The COOH-terminal moiety (residues 40–65) is highly acidic. In the 3D structure of hirudin (Clore et al., 1987; Sukumaran et al., 1987), three areas are distinguished: a central core (residues 3–30, 37–46, 56–57), a "finger" (residues 31–36), and a loop (residues 47–55). Hirudin is very stable at extremes of pH (1.5–13.0) and at high temperatures (up to 90°C). It is soluble in water but insoluble in alcohol or acetone. The isoelectric point of hirudin is approximately 4.

Hirudins for therapeutic use are now produced by recombinant biotechnology, using the yeast *Saccharomyces cerevisiae,* yielding recombinant hirudin (r-hirudin). Lepirudin, a desulfatohirudin, differs from natural hirudin by lacking the sulfate group at Tyr-63 and also has an NH_2-terminal leucine residue in place of the isoleucine. Although such structural differences result in a 10-fold reduction in the dissociation constant of r-hirudin, as compared with natural hirudin, r-hirudins remain highly selective inhibitors of thrombin, with an inhibition constant for thrombin in the picomolar range (Stone and Hofsteenge, 1986).

B. Pharmacology

Lepirudin acts independently of the cofactors antithrombin and heparin cofactor II (Markwardt, 1992) and forms tight, noncovalent 1 : 1 complexes with thrombin. Interacting with both binding sites, lepirudin is a *bivalent* inhibitor of thrombin (Fig. 1). Lepirudin inhibits all the biological activities of thrombin.

Three amino acids (residues 46–48) near the NH_2-terminus of hirudin bind to the active site cleft on thrombin, while the core of the hirudin molecule closes off the active site pocket of thrombin. The COOH-terminal tail of hirudin interacts with the fibrinogen anion-binding site, helping to block thrombin-catalyzed fibrinogen

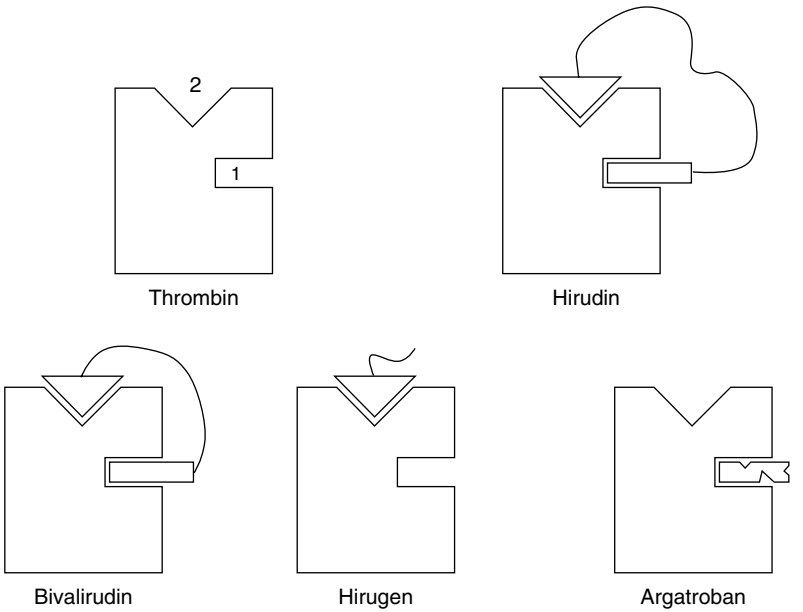

FIGURE 1 Schematic representation of the thrombin molecule and its inhibition by hirudin, bivalirudin (formerly, Hirulog), hirugen, and argatroban: (1) active-site pocket; (2) fibrinogen-binding site. The active-site pocket catalyzes most of the functions of the thrombin molecule, whereas the fibrinogen-binding exosite mediates the binding of thrombin to fibrinogen. Hirudin is a 7000 Da (7 kDa) protein composed of 65 amino acids, which binds to the active-site pocket and the fibrinogen-binding exosite of thrombin, i.e., it is a *bivalent* direct thrombin inhibitor. Bivalirudin is a small synthetic peptide (20 amino acids) designed also to block both of these sites on thrombin. Hirugen, a synthetic peptide, mimics the binding site of fibrinogen to thrombin, thereby inhibiting binding of thrombin to fibrinogen and, therefore, fibrinogen cleavage by thrombin. The arginine derivative argatroban binds competitively to only the active binding site pocket of thrombin. Hirugen and argatroban are *univalent* direct thrombin inhibitors. *Source*: Adapted from Hermann et al., 1997.

cleavage. Hirudin inhibits the feedback loop whereby thrombin enhances its own generation via activation of factors Va and VIIIa (Kaiser and Markwardt, 1986; Pieters et al., 1989). In addition to inhibiting free thrombin, hirudin inhibits clot-bound thrombin (Hogg and Jackson, 1989; Weitz et al., 1990) and thrombin bound to fibrin split products (Weitz et al., 1998). In contrast, heparin–antithrombin complexes are unable to access and inactivate clot-bound thrombin. This important difference between hirudin and heparin might explain why hirudin is more effective than heparin in promoting dissolution of mural thrombi in experimental models (Meyer et al., 1998). Hirudin shows virtually no interaction with plasma proteins (Glusa and Markwardt, 1990), and its activity is standardized in thrombin inhibitory units (TIU): 1 TIU is the amount of hirudin inhibiting 1 U of thrombin at 37°C. The specific activity of lepirudin is 16,000 TIU/mg.

C. Pharmacokinetics

Lepirudin is administered parenterally. Studies of plasma pharmacokinetics in healthy subjects reveal a two-compartment model. The initial plasma half-life ($t_{1/2}\alpha$) of lepirudin is 8–12 min, after which it is distributed in the extracellular space.

Only 20% of lepirudin is found in the plasma, while the remaining 80% is in the extravascular compartment (Glusa, 1998). Lepirudin is not transported into the cerebrospinal fluid or breast milk (Refludan Package Insert, 2002; Lindhoff-Last et al., 2000a).

The terminal plasma elimination half-life ($t_{1/2}\beta$) ranges from 0.8 to 1.7 h (mean, ~1.3 h or 80 min) following intravenous (iv) injection of bolus doses of 0.01–0.5 mg/kg and 1.1–2.0 h following continuous iv infusions over 6 h. Maximum activated partial thromboplastin time (aPTT) ratios occur about 10 min after iv bolus, 3–6 h following start of 6-h continuous iv infusion, and 2–3 h following subcutaneous (sc) administration (in patients with normal renal function). During iv infusion, therapeutic levels are usually reached within 30–60 min. This correlates well with the peak plasma lepirudin concentrations achieved with these different modes of application. The approved dose for lepirudin in patients with HIT and acute thrombosis (with normal renal function) is an iv bolus of 0.40 mg/kg, followed by an iv infusion of 0.15 mg/kg/h (Table 1). However, there is consistent experience that this dose is too high in most HIT patients (Lubenow et al., 2004, 2005; Hacquard et al., 2005; Tardy et al., 2006). Especially in elderly patients the bolus should be omitted and an initial infusion rate of 0.05–0.10 mg/kg/h commenced so as to avoid overdosage in case of unrecognized renal insufficiency. The infusion rate should then be adjusted according to aPTT every 4 h until a steady state is reached (Lubenow et al., 2004) (Fig. 2). The main indication for application of the bolus is acute life- or limb-threatening thrombosis.

Renal clearance (160–200 mL/min for an adult with normal body surface area of 1.73 m^2) and degradation account for approximately 90% of the systemic clearance of lepirudin. The $t_{1/2}\beta$ of r-hirudin lengthens with deterioration of renal function (Markwardt, 1989; Nowak et al., 1991, 1992, 1997; Vanholder et al., 1994, 1997); in nephrectomized patients, it can be up to 120 h (Wittkowsky and Kondo, 2000; Dager and White, 2001; Fischer, 2002; Shepherd, 2002).

A clinically important observation is that renal blood flow decreases during anesthesia, so that the elimination half-life is prolonged to 3–5 h. If lepirudin is used intraoperatively, the dose should be reduced by 30–50%, and close monitoring is mandatory.

With sc administration, bioavailability is nearly 100%. Dose-ranging studies have shown that its concentration in the blood reaches 0.3–0.5 μg/mL after an sc dose of lepirudin of 0.5 mg/kg and about 0.7 μg/mL after an sc dose of 0.75 mg/kg, making twice-daily injections effective (Schiele et al., 1994; Huhle et al., 2000a; Nowak, 2001). When administered subcutaneously, this drug is usually injected into an abdominal skin fold and reaches its peak concentration after 2–3 h. Lepirudin has been administered subcutaneously for long-term prophylaxis in HIT after the acute disease has been controlled (Huhle et al., 2000a). In one patient the drug was safely administered sc twice daily for 8 mo for antithrombotic therapy in the setting of malignant disease. Lepirudin has been administered sc as an adjunct to streptokinase in patients with acute myocardial infarction (MI) (Neuhaus et al., 1999) and in the outpatient management of acute MI (Begelman and Deitcher, 2002).

D. Tests for Monitoring Anticoagulation

Numerous tests have been evaluated for monitoring anticoagulation by DTIs, ranging from the ubiquitous aPTT to the newer ecarin clotting time (ECT), enzyme-immunoassay (EIA) techniques for directly measuring the hirudin

TABLE 1 Dosing Schedules for Lepirudin Treatment of Patients with HIT

	Bolus[a,b]	IV infusion[a,b]	Target aPTT ratio[c]
Dose recommended in all HIT patients without renal impairment	None[d]	0.05–0.10 mg/kg b.w./h[d]	1.5–2.5 (0.6–1.0 μg/mL)
HIT with isolated thrombocytopenia (dose regimen B in HAT trials)	None[e]	0.10 mg/kg b.w./h[e]	1.5–2.5 (0.6–1.0 μg/mL)
HIT and thrombosis (dose regimen A1 in HAT trials)	(0.40 mg/kg[e] b.w. iv)	0.15 mg/kg b.w./h[e]	1.5–2.5 (0.6–1.0 μg/mL)
Thrombosis prophylaxis in patients with a history of HIT	15 mg sc b.i.d.[f]	–	–
HIT with thrombosis and concomitant thrombolysis (dose regimen A2 in HAT trials)	(0.20 mg/kg b.w. iv[e])	0.10 mg/kg b.w./h[e]	1.5–2.5
Renal dialysis every alternate day	0.10 mg/kg b.w. iv predialysis	–	2.0–2.5
CVVH	–	0.005 mg/kg b.w./h (initial rate)	1.5–2.5
PCI (Mehta et al., 2002); UA or acute MI without ST elevation (OASIS-2, 1999)	0.40 mg/kg b.w. iv	0.15 mg/kg b.w./h	1.5–2.5
Vascular surgery (Hach-Wunderle, 2001)	0.40 mg/kg b.w. iv	0.10 mg/kg/h	1.5–2.5
Vascular surgery (intraoperative vessel flushes)	Use up to 250 mL (0.1 mg/mL solution)	–	–
Postoperative anticoagulation	–	0.10 mg/kg b.w/h	1.5–2.5
Cardiac surgery using CPB (dose regimen C in HAT trials) (see also Chapter 19)	0.25 mg/kg b.w. iv[e] 0.20 mg/kg b.w in the priming fluid	0.50 mg/min[a,g]	Monitored by ECT: >2.5 μg/mL before start of CPB; 3.5–4.5 μg/mL during CPB[h]

Note: Repeat aPTT determinations should be made 4–6 h after any dose adjustment.
[a]A maximum body weight of 100 kg should be used for dose calculations.
[b]Adjust for renal insufficiency.
[c]The ratio is based on comparison with the normal laboratory mean aPTT. If Actin FS or Neothromtin reagents are used, the aPTT target range is usually 1.5–3.0.
[d]This is the author's recommended starting dose in all HIT patients, unless life-or limb-threatening thrombosis is present
[e]Used in the HAT-1, -2, and -3 trials.
[f]Tested in a prospective, randomized trial after orthopedic surgery with desirudin (Eriksson et al.,1996, 1997).
[g]Stop 15 min before and of CPB; put 5 mg into CPB after disconnection to avoid clotting of pump.
[h]The target lepirudin level pre-CPB (>2.5 μg/mL) is lower than the level sought during CPB (3.4–4.5 μg/mL) because of the addition of lepirudin to the pump priming fluid (0.2 mg/kg b.w.).
Abbreviations: aPTT, activated partial thromboplastin time; b.w., body weight; CPB, cardiopulmonary bypass; CVVH, continuous venovenous hemofiltration; ECT, ecarin clotting time; iv, intravenous; MI, myocardial infarction; PCI, percutaneous coronary intervention; UA, unstable angina.

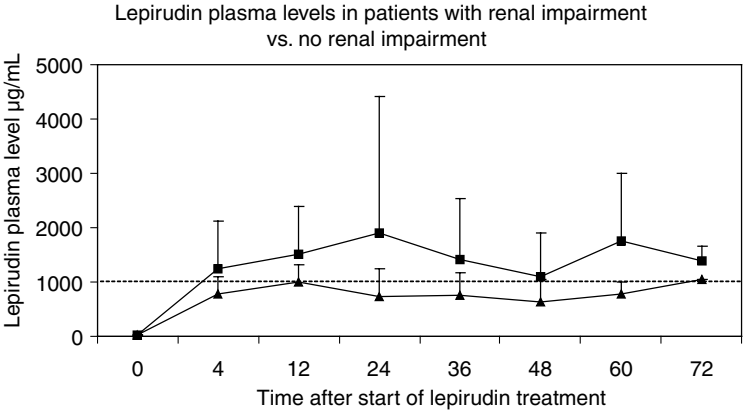

FIGURE 2 Time course of lepirudin plasma levels in patients with normal creatinine levels (*lower line*) and patients with increased creatinine levels (*upper line*). The dotted line indicates the upper therapeutic level. Both groups showed similar plasma concentrations after 4 h, which further increased in patients with renal impairment. Therefore aPTT should be also assessed in all patients 8 h after start of treatment to identify those with drug accumulation. *Abbreviation*: aPTT, activated partial thromboplastin time. *Source*: Lubenow et al., 2004.

concentration (Hafner et al., 2002), and the ecarin chromogenic assay (ECA) (Lange et al., 2003, 2005).

The aPTT is a global coagulation assay and is the current method of choice for monitoring lepirudin therapy in most situations. In patients who require higher levels of plasma hirudin and aPTT values above ~70 s (depending on the reagent), the hirudin concentration–aPTT curve flattens, and even major changes in plasma levels cause only a minor change in the aPTT. Because the sensitivities of different aPTT reagents vary (Gosselin et al., 2004), it is strongly recommended that each laboratory involved in monitoring of DTIs should generate its own standard dose–response curve for their aPTT reagent using "spiked" normal pooled plasma samples, e.g., with 0.25, 0.50, 0.75, 1.0, 1.25, 1.5, and 2.0 µg/mL lepirudin (Fig. 3). This will define the expected range over which the aPTT reliably reflects changes in the DTI plasma concentration. At concentrations above this range, the ECT is more reliable for DTI monitoring. This is especially true for very high doses, such as those used during cardiopulmonary bypass (CPB) surgery.

Unlike global coagulation tests, the ECT monitors prolongation of clotting time caused by thrombin inhibition alone (Callas et al., 1995; Nowak and Bucha, 1996; Pötzsch et al., 1997a,b; Koster et al., 2000a; Fabrizio, 2001; de Denus and Spinier, 2002; Liu et al., 2002). Ecarin, which is obtained from snake venom, catalyzes the cleavage of prothrombin to meizothrombin (Kornalik and Blombäck, 1975; Novoa and Seegers, 1980; Nishida et al., 1995). Meizothrombin is biologically similar to thrombin, except that it cleaves fibrinogen much more slowly than thrombin. The interaction of meizothrombin with hirudin, however, is similar to that of thrombin. Thus, when all the hirudin present in a blood sample has been neutralized by meizothrombin, thrombin will no longer be inhibited, and clotting will occur.

The ECT shows a linear correlation to lepirudin plasma levels over a wide range. At present, this assay is recommended for monitoring anticoagulation when higher concentrations of lepirudin are used. It is mandatory for monitoring of lepirudin during CPB.

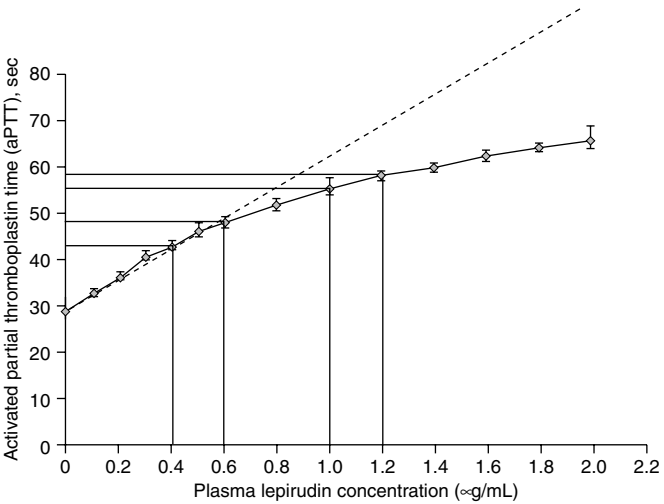

FIGURE 3 Lepirudin standard curve. This curve was generated using seven normal plasmas spiked with various concentrations of lepirudin (μg/mL) using reagent Actin FS and the BCS analyzer (Dade-Behring, Germany). Note that incremental changes in aPTT are much smaller as the dose–response curve flattens at greater plasma lepirudin concentrations. *Abbreviation*: aPTT, activated partial thromboplastin time.

Recently, an automated assay, the ECA, has been introduced that provides a linear dose–response curve for all DTIs independently of the patient's prothrombin and fibrinogen levels (Lange et al., 2003, 2005).

In 2005, a workshop compared several methods for monitoring DTIs: aPTT using local reagents and methods (Actin FS, Thrombosil I, Pathromtin SL, Synthasil aPTT, Automated aPTT, STA-PTT, STA—CK Prest 5); aPTT using a common reagent (C-aPTT; Actin FS; Aventis Pharma, Marburg, Germany); anti-IIa chromogenic assay with the S2238 chromogenic substrate from Instrumentation Laboratory/Haemochrom Diagnostica (Essen, Germany); ECT—wet chemistry (Wet ECT) reagents (University Jena, Germany); ECT—dry chemistry reagent (Cardiovascular Diagnostics Inc., Raleigh, NC, USA) with two ecarin concentrations (low ecarin reagent card: dry ECT; higher concentration ecarin card: TIM); EIA kit (Immuno Bind, Hirudin Elisa kit, American Diagnostica Inc., Greenwich, CT, USA). The interlaboratory variations for measurement of lepirudin were (from lowest to highest): TIM < C-aPTT < Dry ECT < L-aPTT < wet ECT < anti-IIa < EIA (Gray et al., 2005).

Limitations of Functional Monitoring Tests

Results obtained with the aPTT or ECT may be inaccurate in patients whose plasma has a reduced concentration of prothrombin (e.g., severe liver disease, disseminated intravascular coagulation [DIC], treatment with vitamin K antagonists) or in patients with fibrinogen depletion (e.g., post-thrombolysis, hemodilution during CPB) (Lindhoff-Last et al., 2000b; de Denus and Spinier, 2002). This is especially problematic during CPB. In the ECT, this can be overcome by addition of normal plasma 1 : 1 to the assay (Koster et al., 2000a).

EIAs measure the plasma concentration of lepirudin independent of prothrombin concentration. Plasma concentrations for lepirudin are 0.2–0.4 μg/mL

for thrombosis prophylaxis, and 0.6–1.0 μg/mL for treatment of isolated HIT and for treatment of thrombosis complicating HIT.

The ECA also overcomes the problems associated with monitoring of DTIs by aPTT and ECT, with faster turnaround time than the EIA.

E. Dose Adjustments

In lepirudin-treated patients, laboratory values to monitor the anticoagulant effect should be obtained prior to treatment, 4 h after the start of iv infusion, and then every 4 h until a steady state is reached (Lubenow et al., 2004, 2005) (Fig. 2). Also, 4 h after every change in dose, monitoring should be repeated. For most patients, the primary laboratory parameter used is the aPTT, with testing performed at least once daily during treatment with lepirudin. If the target range is exceeded, the infusion should be stopped for 2 h and restarted at a 50% lower dose once the therapeutic range has been reached (Greinacher et al., 1999a,b). When the dose is subtherapeutic, the infusion rate should be increased by 20%.

Renal Impairment

Lepirudin has been studied in patients with varying degrees of renal impairment. It can be used safely and effectively if started at a very low dose of 0.005–0.01 mg/kg/h, if there is evidence for renal compromise. If renal function is normal, the starting dose should be 0.05–0.10 mg/kg/h. In both situations the initial lepirudin bolus should be omitted (Table 2). In case of transient renal failure close monitoring of aPTT is mandatory. Even when renal function appears normal, the potential for unrecognized compensated renal dysfunction exists, so the initial lepirudin bolus should be avoided (unless severe thrombosis is present) and a lower infusion rate of 0.05–0.10 mg/kg/h iv started, with subsequent adjustments by aPTT.

Lepirudin and Vitamin K Antagonists

Long-term treatment of HIT patients often involves a transition from DTI to oral anticoagulation. Initiation of the transition to vitamin K antagonist (coumarin) therapy should begin only after the platelet count has substantially recovered (preferably, $>150 \times 10^9$/L), with a minimum of 5 days of overlapping therapy with an alternative anticoagulant, and with the last 2 days stably within the target therapeutic range (Warkentin and Greinacher, 2004). In patients with deep vein thrombosis (DVT) associated with acute HIT, the use of vitamin K antagonist can be associated with venous limb gangrene, which typically occurs when this anticoagulant is used alone for treatment of HIT, or when overlapping therapy with DTIs is not managed appropriately, e.g., early initiation of coumarin and premature discontinuation of the DTI.

TABLE 2 Initial Lepirudin Dosing in Renal Dysfunction

Serum creatinine mg/dL (μmol/L)	Initial iv infusion rate (subsequently adjusted to aPTT) (mg/kg/h)
1–1.58 (90–140)	0.05
1.58–4.52 (140–400)	0.01
>4.52 (>400)	0.005

Abbreviations: aPTT, activated partial thromboplastin time; iv, intravenous.

Patients in whom venous limb gangrene occurs typically have an elevated international normalized ratio (INR)—which represents a "surrogate marker" for greatly reduced protein C levels—and thrombocytopenia (surrogate marker for persistent thrombin generation in HIT). Prevention of limb gangrene can be accomplished through careful management of the DTI—warfarin transition, particularly the postponement of coumarin therapy pending substantial platelet count recovery. Patients with suspected coumarin-associated venous limb gangrene should immediately receive vitamin K (e.g., 10 mg iv over 30–60 min or 20 mg per os).

In patients who receive vitamin K antagonists before or concomitant with commencement of lepirudin, this can cause elevated aPTT values (due to coumarin-induced prothrombin level reduction) with the potential for inappropriate lepirudin dose reductions (Warkentin, 2006). Therefore, vitamin K should be given to HIT patients who have received vitamin K antagonists when lepirudin is started (Warkentin and Greinacher, 2004; Greinacher and Warkentin, 2006) (see Chapter 12).

In prospective trials, lepirudin caused minimal prolongation of the prothrombin time (PT) (or INR) once the therapeutic range had been reached. However, Stephens et al. (2005) reported that lepirudin elevates the INR in the absence of warfarin if a thromboplastin with a relatively high international sensitivity index (ISI) of $\geq$2 is used. A systematic laboratory study on the effects of different DTIs on the INR (Warkentin et al., 2005) revealed that the differing effects of the DTIs on PT prolongation are primarily driven by their respective molar plasma concentrations required for clinical effect. DTIs with a relatively low affinity for thrombin (e.g., argatroban) require high plasma concentrations to double the aPTT compared with those with a higher affinity for thrombin (e.g., lepirudin). These higher plasma concentrations, in turn, quench more of the thrombin generated in the PT, thereby prolonging the PT to a greater extent.

In general, the transition from lepirudin to warfarin therapy is usually less complicated than the transition from argatroban (Greinacher et al., 2000) (see Chapter 15). Notably, following start of vitamin K antagonist therapy in the prospective lepirudin studies, not a single case of venous limb gangrene occurred.

F. Reversal/Removal of Lepirudin

Bleeding is an important and potentially severe consequence of hirudin treatment (Antman, 1994; Neuhaus et al., 1994; Frank et al., 1999; Lubenow et al., 2005). As with all DTIs, no specific antidote is available. In a patient with minor bleeding and normal renal function, stopping the drug may be sufficient, since the drug concentration drops quickly. However, when bleeding is life-threatening or the patient has renal failure, cessation alone may not be adequate.

Hemodialysis or hemofiltration can reduce plasma levels of lepirudin (Riess et al., 1995). However, only some filters are effective, e.g., polysulfone F80 (Fresenius, Germany) (Frank et al., 1999; Bucha et al., 1999). Variable efficacy of filters in removing lepirudin could explain conflicting results (Vanholder et al., 1997). Clinical data are limited, and hemofiltration is not always a practical option in emergency situations.

Hirudin overdosage may also be treated pharmacologically by administration of desmopressin (Ibbotson et al., 1991; Butler et al., 1993; Bove et al., 1996), or von Willebrand factor (vWF), or vWF-containing factor VIII concentrates (Dickneite et al., 1996, 1998). Irami and coworkers (1995) described a patient in whom r-hirudin induced bleeding was treated by administration of prothrombin complex concentrates, a method previously used in animal models (Diehl et al., 1995). However,

since these concentrates can contain heparin, they could be dangerous for a patient with acute HIT. Recombinant factor VIIa is another "panhemostatic" treatment option (Oh et al., 2006). Meizothrombin could also be a potential antidote, but it is not available for use in humans (Nowak and Bucha, 1995).

G. Clinical Use of Lepirudin

Besides its use in patients with HIT, lepirudin has been investigated extensively in controlled clinical trials for acute coronary syndrome (ACS) ($n > 14{,}000$), including MI (Antman, 1994; Neuhaus et al., 1994) and unstable angina pectoris (Rupprecht et al., 1995; Organization to Assess Strategies for Ischemic Syndromes [OASIS-2], 1999); and in pilot studies for prophylaxis and treatment of DVT (Parent et al., 1993; Schiele et al., 1997). In patients undergoing hemodialysis (see Chapter 18) or cardiac surgery (see Chapter 19), there is observational evidence indicating safe and effective use of lepirudin. Results of three prospective clinical trials with lepirudin and an extensive postmarketing drug monitoring study in HIT patients treated in the "real-world" setting are described in the next section.

III. CLINICAL STUDIES WITH LEPIRUDIN IN HIT

A. Three Prospective Clinical Trials: Heparin-Associated Thrombocytopenia-1, -2, and -3

Three prospective studies with lepirudin for HIT were designated heparin-associated thrombocytopenia (HAT)-l, -2, and -3 (Greinacher et al., 1999a,b, 2000; Lubenow and Greinacher, 2002; Lubenow et al., 2005). There was no approved non-heparin alternative anticoagulant during the 3 yr in which the HAT studies were conducted (March 1994–April 1996), and thus for ethical reasons, a placebo control was not appropriate. The HAT studies therefore included comparisons of clinical outcomes with a historical control group treated before lepirudin became available.

A meta-analysis of HAT-1 and -2 was performed to evaluate patients given lepirudin for treatment of HIT with thrombosis (Greinacher et al., 2000). A second meta-analysis of the HAT-1, -2, and -3 studies was performed to evaluate the effects of lepirudin in patients with HIT and isolated thrombocytopenia ("isolated HIT") (Lubenow et al., 2004). In addition, an observational study termed the drug-monitoring program (DMP) was carried out to determine the effects of lepirudin in a large cohort of patients treated in routine clinical settings (Lubenow et al., 2000).

Objectives

The three HAT trials examined whether lepirudin administered iv to patients with serologically confirmed HIT would safely reduce the risk of new arterial or venous thrombosis, limb amputations, and death (composite endpoint). The laboratory objective was to determine whether the drug would allow an increase in the platelet count in thrombocytopenic patients or maintain the baseline platelet values (in nonthrombocytopenic patients), while providing effective anticoagulation. The latter was defined as a prolongation of the aPTT by 1.5- to 2.5-fold over baseline values with no more than two dose increases. (*Note:* If Actin FS or Neothromtin reagents were used, the aPTT target range was a 1.5- to 3.0-fold prolongation.)

Patients

Patients were eligible for study if their platelet count fell by more than 50% or to fewer than 100×10^9/L or if they exhibited new thrombosis while receiving heparin.

A strict criterion for study entry was laboratory confirmation of the clinical diagnosis of HIT by the heparin-induced platelet activation (HIPA) test (Greinacher et al., 1991; Eichler et al., 1999) (see Chapter 10).

Clinical outcomes included a composite endpoint (new thrombosis, limb amputation, death) as well as each individual endpoint. Clinical events that occurred between diagnosis and start of treatment with lepirudin were included, as were all clinical events that occurred up to day 14 after stopping lepirudin treatment. Clinical outcomes for lepirudin were compared by Kaplan-Meier time-to-event analysis with a historical control group treated conventionally, beginning at laboratory confirmation of HIT for lepirudin-treated patients and one day after laboratory confirmation for controls.

Laboratory response was defined as (1) the maintenance of an on-treatment aPTT ratio higher than 1.5 in at least 80% of measurements and requiring no more than two dose increases and (2) an increase in the platelet count to more than 30% from the nadir and to more than 100×10^9/L by day 10 of lepirudin treatment (thrombocytopenic patients), or maintenance of normal platelet counts on days 3 and 10 (nonthrombocytopenic patients). Patient characteristics are given in Table 3.

Historical Control Group

The historical control patients ($n = 120$; Table 3) also had a diagnosis of HIT confirmed by a positive HIPA test in our laboratory (Greinacher et al., 1999b). They were treated according to hospital protocol with danaparoid ($n = 36$), oral

TABLE 3 Patient Characteristics in the HAT Studies

Patient characteristics	All HAT studies $n = 403$	Historical control ($n = 120$)[a]	*p*-value
Age, yr; median (range)	62 (11–90)	67 (19–90)	<0.0001
Male/Female, n/n	181/222	41/79	0.037
Field of underlying disease, *n* (%)			<0.0001
Internal medicine	168 (41.7)	41 (34.2)	
Orthopedic surgery	64 (15.9)	38 (31.7)	
Traumatology	30 (7.4)	23 (19.2)	
Cardiovascular surgery	43 (10.7)	9 (7.5)	
Other	98 (24.3)	9 (7.5)	
Median baseline platelet count	79×10^9/L	60×10^9/L	0.030
Patients with TEC during heparin treatment *n* (%)	233/403 (57.8)	80/119 (67.2)	0.066
Type of TEC[b], *n* (%)			0.0821
Venous-distal	127 (54.5)	53 (66.3)	
Venous-proximal	113 (48.5)	23 (28.8)	
Pulmonary embolism	110 (47.2)	35 (43.8)	
Arterial-peripheral	56 (24.0)	17 (21.3)	
Venous other[c]	34 (14.6)	–	

[a]Used for comparison in all HAT-studies.
[b]A patient could have suffered multiple TECs of different types.
[c]Pelvic veins, 11; vena cava, 8; iliac vein, 6; subclavian vein, 3; portal vein, 2; brachiocephalic vein, 1; thrombosis leg (unspecified), 3.
Abbreviations: HAT, heparin-associated thrombocytopenia; TEC, thromboembolic complication.

anticoagulants (e.g., phenprocoumon [$n = 27$]), no anticoagulation ($n = 23$), or miscellaneous treatments (e.g., aspirin [$n = 5$], low molecular weight heparin [$n = 8$], or thrombolytics [$n = 4$]). Incomplete data for 17 patients in the control group precluded treatment assignment.

HAT-1 Study

The HAT-1 study involved 82 patients with confirmed HIT: 51 patients were assigned to dose regimen A1, 5 to regimen A2, 18 to regimen B, and 8 to regimen C (Tables 1, 4a, and 4b) (Greinacher et al., 1999a). The median duration of treatment was 10 days (range 3–47 days) for regimen A1, 9 days (7–29 days) for A2, 15 days (2–58 days) for B, and 9 days (3–25 days) for C.

HAT-2 Study

The HAT-2 study involved 112 patients with confirmed HIT: 65 patients were assigned to dose regimen A1, 4 to regimen A2, and 43 to regimen B (Tables 4a and 4b) (Greinacher et al., 1999b). The overall median duration of treatment was 11 days (range 0–104 days); for regimen A1, it was 13 days (0–104 days); for A2, 10 days (1–58 days); and for B, 8 days (1–67 days).

HAT-3 Study

The third prospective trial, HAT-3, was the largest and involved 205 patients: 98 patients were assigned to dose regimen A1, 12 to regimen A2, and 84 to regimen B (Lubenow et al., 2005). Ten patients received lepirudin for CPB (regimen C), and one received lepirudin by the sc route. Seventeen patients received more than one treatment cycle. For the efficacy parameters only the first treatment cycle was calculated. For safety analysis, especially allergic reactions, all treatment cycles were included.

All 110 patients in treatment groups A1 and A2 had developed at least one thromboembolic complication (TEC) before the start of lepirudin treatment. The ratio of venous : arterial thrombosis in this patient group was 12 : 1. The median duration of treatment across all treatment arms was 10.0 days: A1, 9 days (1–197); A2, 12 days (5–21); B, 10 days (1–47); and C (followed by regimen B post-CPB surgery), 7 days (1–37). Mean lepirudin doses were 0.11, 0.08, and 0.07 mg/kg/h in groups A1, A2, and B, respectively. During the study, phenprocoumon was given to 121 (59%) patients.

Synopsis of HAT-1, -2, and -3

The patient characteristics for all patients enrolled in the HAT studies are given in Table 3. Categorical data for the 403 patients from diagnosis of HIT and from start of lepirudin treatment are shown in Tables 4a and 4b. In all three studies, patients had a highly elevated risk of new TECs in the time period between diagnosis of HIT and start of lepirudin. The risk for a new TEC was decreased by 92.9%—from 5.1% per patient day during the 1.3 day period between diagnosis of HIT and start of lepirudin treatment to 0.4% during active treatment (Fig. 4). These data support treatment recommendations that alternative anticoagulation should be started as soon as there is strong clinical suspicion of HIT (Warkentin and Greinacher, 2004).

Bleeding was the most important adverse effect of lepirudin treatment. The overall rate of major bleeding among the 403 patients in the three trials was 17.6% (95% confidence interval [CI]: 14.0–21.7%). However, this included the 22 patients requiring CPB (treatment group C), in whom 11 major bleeds occurred during

cardiac surgery. The major bleeding rate was 15.7% if the CPB patients were excluded. In contrast to the efficacy endpoints, all major bleeding episodes occurred during treatment with lepirudin. Five (1.2%) lepirudin-treated patients died from bleeding complications.

There were no major differences in bleeding rates between younger (<65 yr of age) and older (≥65 yr) patients ($p = 0.520$), or between female and male patients ($p = 0.150$). However, renal impairment was associated with an increased rate of bleeding, when comparing patients with serum creatinine values above and below 90 μmol/L ($p < 0.001$).

Antihirudin antibodies were present in 30% of patients at the end of the first treatment cycle and in 70% of reexposed patients. In the three studies, 17 patients (4.2%) experienced allergic reactions. In nine of these, a potential relationship to lepirudin was considered plausible; antihirudin antibodies were detected in five of these nine patients. One of these patients required discontinuation of study drug. No anaphylactic reactions were observed.

B. Comparison with Historical Control Group

A comparison of the composite and individual efficacy endpoints, as well as the major bleeding endpoint, for the lepirudin and control patients groups (categorical data), was performed from time of HIT diagnosis to end-of-observation period (Table 4a), as well as from time of start of treatment (Table 4b). (The latter time frame was also used for the analysis of the argatroban treatment studies (see Chapter 15). No patients were excluded from the categorical analysis.

The composite and single endpoints were also compared with the historical controls from start of treatment using time-to-event analyses (Fig. 5). The composite endpoint occurred less often in the lepirudin-treated patients as compared to controls ($p = 0.04$), primarily due to a reduction in new thrombotic events ($p < 0.001$), while the risk for limb amputation ($p = 0.79$) and death ($p = 0.43$) did not differ significantly (however, the studies were not powered to detect differences in these endpoints). However, the risk for major bleeding was increased in the lepirudin-treated patients ($p = 0.015$), even when the 22 patients who underwent CPB were excluded. Furthermore, 42 patients (11 from HAT-1, 17 from HAT-2, and 14 from HAT-3) were excluded from the time-to-event comparison because the date of HIT confirmation was >21 days before start of treatment ($n = 29$), or because of missing HIT confirmation date or missing date of stopping of lepirudin therapy ($n = 13$).

C. Meta-Analysis of HAT-1, -2, and -3: Patients with HIT and Thrombosis

Table 5 summarizes the results of a meta-analysis of the three HAT studies performed to determine the efficacy and safety of lepirudin in 235 patients with HIT complicated by thrombosis. As in the HAT-1, -2, and -3 studies, the risk for new thrombotic complications (per day) was highest between diagnosis of HIT and start of treatment: 26.2 % of all such events occurred during this pretreatment period.

Efficacy Outcomes

When outcomes were assessed from the start of lepirudin treatment, the combined endpoint for new thrombosis, limb amputation, and death was significantly lower in the lepirudin-treated patients ($n = 235$) than in the controls ($n = 75$) (19.1% vs. 40.0%; $p = 0.002$). This difference was primarily due to a reduction in the number of new thrombosis (6.8% vs. 25.3%; $p = 0.001$). Incidences of limb amputation

TABLE 4a Outcomes from Diagnosis of HIT

	HAT-1, $n=82$					HAT-2, $n=116$					HAT-3, $n=205$					All HAT-studies, $n=403$					Hist. control $n=120$	$p^a=$	Risk reduction%
Treatment	A1	A2	B	C	Total	A1	A2	B	C	Total	A1	A2	B	C	Total	A1	A2	B	C	Total	120	–	–
$n=$	51	5	18	8	82	65	4	43	4[b,c]	116	98	12	84	10	205	214	21	145	22	403			
Death	3	–	3	–	6 (7.3)	8	1	2	–	11 (9.5)	14	2	11	3	30 (14.6)	25	3	16	3	47 (11.2)	21 (17.5)	0.095	36.0
Limb amputation	2	–	2	–	4 (4.9)	4	1	5	–	10 (8.6)	7	–	5	–	12 (5.9)	13	1	12	–	26 (6.5)	8 (6.7)	0.933	3.0
New TEC	3	–	5	–	8 (9.8)	8	1	3	–	12 (10.3)	15	4	9	–	28 (13.7)	26	5	17	–	48 (11.9)	37 (30.8)	<0.0001	61.4
Composite[c]	7	–	8	–	15 (18.3)	17	2	7	–	26 (22.4)	29	6	23	3	61 (29.8)	53	8	38	3	102 (25.3)	3 (44.2)	0.0001	42.8
Major bleeding	7	–	2	2	11 (13.4)	6	1	11	2	20 (17.2)	20	1	12	7	40 (19.5)	33	2	25	11	71 (17.6)	7 (5.8)	0.0015	–67.0

Note: Details of treatment regimens A1, A2, B, and C are given in Table 1.
[a]Comparing all patients in the HAT 1–3 studies with the historical control group by categorical analysis.
[b]The four patients receiving regimen C were not reported in the HAT-2 publication.
[c]Composite of death, limb amputation, and new TEC (maximum, one event per patient).
Abbreviations: HAT, heparin-associated thrombocytopenia; TEC, thromboembolic complication.

TABLE 4b Outcomes from Start of Treatment

	HAT-1, $n=82$					HAT-2, $n=116$					HAT-3, $n=205$					All HAT-studies, $n=403$					Hist. control $n=120$	$p^a=$	Risk reduction %
Treatment	A1	A2	B	C	Total	A	A	B	C	Total	A	A	B	C	Total	A	A	B	C	Total	120	–	–
$n=$	51	5	18	8	82	165	24	43	4[b]	116	198	212	84	10	205	1214	221	145	22	403			
Death	3	–	3	–	6 (7.3)	8	1	2	–	11 (9.5)	14	2	11	3	30 (14.6)	25	3	16	3	47 (11.2)	21 (17.5)	0.095	36.0
Limb amputation	2	–	1	–	3 (3.7)	4	1	4	–	9 (7.8)	6	–	4	–	10 (4.9)	12	1	9	–	22 (5.5)	8 (6.7)	0.618	17.9
New TEC	3	–	5	–	8 (9.8)	7	1	3	–	11 (9.5)	5	–	6	–	11 (5.4)	15	1	14	–	30 (7.4)	30 (25.0)	<0.0001	70.4
Composite[c]	7	–	8	–	15 (18.3)	16	2	6	–	24 (20.7)	18	2	20	3	43 (21.0)	41	4	34	3	82 (20.3)	52 (43.3)	<0.0001	53.1
Major bleeding	7	–	2	2	11 (13.4)	6	1	11	2	20 (17.2)	20	1	12	7	40 (19.5)	33	2	25	11	71 (17.6)	7 (5.8)	0.0015	–67.0

Note: Details of treatment regimens A1, A2, B, and C are given in Table 1.
[a]Comparing all patients in the HAT 1–3 studies with the historical control group by categorical analysis.
[b]The four patients receiving regimen C were not reported in the HAT-2 publication.
[c]Composite of death, limb amputation, and new TEC (maximum, one event per patient).
Abbreviations: HAT, heparin-associated thrombocytopenia; TEC, thromboembolic complication.

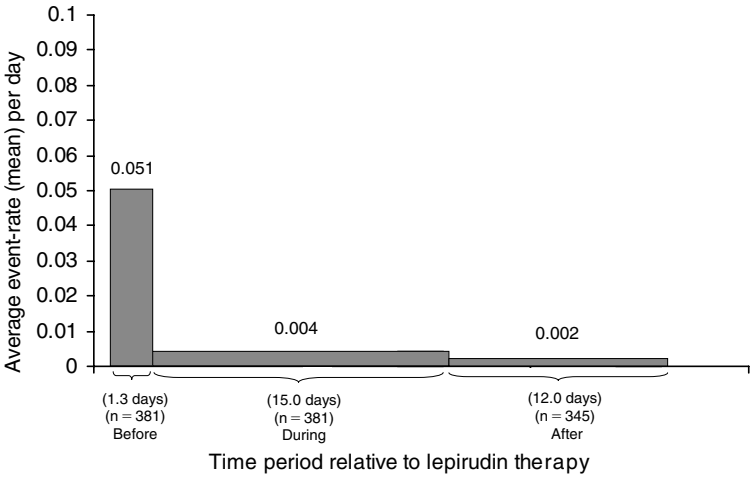

FIGURE 4 Average rate of new thromboembolic complications per day in the HAT-1,-2, -3 studies ($n = 381$ patients). The bar width indicates the mean duration of the observation period (days) and is shown for three time periods: before, during, and after lepirudin therapy. The high average event rate (0.051 [5.1%] event per day during a mean period of 1.3 days) from diagnosis of HIT until start of lepirudin therapy indicates that cessation of heparin alone is insufficient to prevent HIT-associated thrombosis, thus warranting treatment with an alternative anticoagulant if HIT is strongly suspected. *Abbreviations*: HAT, heparin-associated thrombocytopenia; HIT, heparin-induced thrombocytopenia. *Source*: From Lubenow et al., 2005.

(5.5% vs. 8.0%; $p = 0.44$) and death (11.9% vs. 12.0%; $p = 0.98$) did not differ between the lepirudin group and the historical controls (Table 5).

Safety Outcomes

The cumulative incidence of major bleeding was higher in the lepirudin group than in the control group (14.9% vs. 6.7%; $p = 0.064$). The mean treatment duration was 14.2 days corresponding to a risk for major bleeding of 1.05% per patient day.

In a previous meta-analysis of HAT-1 and -2 patients (Greinacher et al., 2000), one of the more important points to emerge was the relationship of aPTT ratios with lepirudin safety and efficacy. For low aPTT ratios (<1.5), the incidence of the combined endpoint was not significantly reduced compared to the control (RR = 0.86; 95% CI, 0.38–1.94; $P = 0.72$). In addition, the risk of bleeding was not significantly greater in the lepirudin group than in the control group (RR = 1.57; 95% CI, 0.52–4.72; $P = 0.42$). At medium aPTT ratios (1.5–2.5), efficacy was significantly greater for the lepirudin-treated patients than for the controls (RR = 0.42; 95% CI, 0.22–0.80; $P = 0.009$), but there was also an increased risk of bleeding (RR = 3.21; 95% CI, 1.72–6.02; $P = 0.0003$). At higher aPTT ratios (>2.5), the efficacy of lepirudin was not enhanced compared with medium aPTT ratios (RR = 0.70; 95% CI, 0.21–2.32; $P = 0.56$). However, there was a marked increase in bleedings with high aPTT ratios (RR = 6.03; 95% CI, 2.34–15.54; $P = 0.0002$).

D. Meta-Analysis of HAT-1, -2, and -3: Patients with Isolated HIT

Each of the HAT trials examined the effects of lepirudin in patients with HIT and isolated thrombocytopenia and in HIT patients with thrombosis. The meta-

TABLE 5 Incidences of Composite and Individual Clinical Endpoints in the HAT Studies and the Drug-Monitoring-Program

Study and endpoint[a]	Observation period	Lepirudin (%)[b]	Historical control (%)[b]	*p*-value (categorical analysis)	*p*-value (time to event analysis)	Risk reduction (%)
HIT with thrombosis HAT-1,2,3	Start of treatment to d 35	$N = 235$	$N = 75$			
Composite	–	19.1	40.0	0.002	0.518	52.3
New thrombosis	–	6.8	25.3	0.001	<0.001	73.1
Limb amputation	–	5.5	8.0	0.438	0.458	31.3
Death	–	11.9	12.0	0.984	0.166	0.8
HIT with thrombosis, DMP (Lubenow et al., 2002)	Start to end of lepirudin treatment	$N = 496$				
Composite	–	21.9	–	–	–	
New thrombosis	–	5.2	–	–	–	–
Limb amputation	–	5.8	–	–	–	–
Death	–	10.9	–	–	–	–
Isolated HIT, HAT-1,2,3 (Lubenow et al., 2004)	Start of treatment to d 35	$N = 91$	$N = 47$			
Composite	–	19.8	29.8	0.187	0.028	33.6
New thrombosis	–	4.4	14.9	0.045	0.020	70.5
Limb amputation	–	3.3	0	0.551	0.242	–
Death	–	14.3	21.3	0.296	0.094	32.9
Isolated HIT, DMP (Lubenow et al., 2002)	Start to end of lepirudin treatment	$N = 612$				
Composite	–	15.7	–	–	–	–
New thrombosis	–	2.1	–	–	–	–
Limb amputation	–	1.3	–	–	–	–
Death	–	12.3	–	–	–	–

[a]Each patient could contribute only once to the composite endpoint (any one of the individual endpoints of new thrombosis, limb amputation, or death).
[b]Number of patients eligible for comparison.
Abbreviations: d, day; DMP, drug-monitoring program; HAT, heparin-associated thrombocytopenia; HIT, heparin-induced thrombocytopenia.

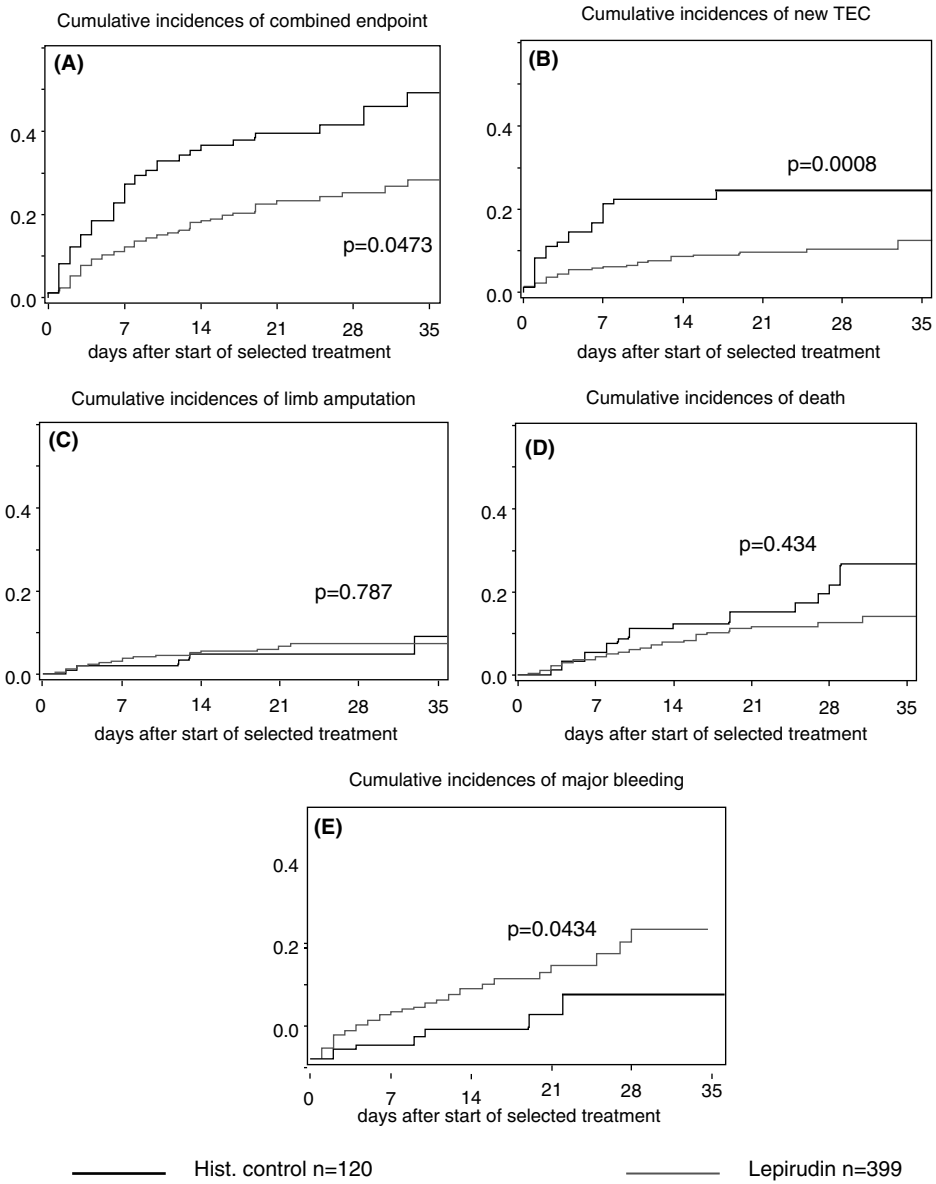

FIGURE 5 Time-to-event analyses of efficacy and safety endpoints in the HAT-1, -2, and -3 studies (combined) in comparison with the historical control group. (**A**) Composite endpoint (new thromboembolic complication, limb amputation, or death); (**B**) new thromboembolic complication; (**C**) limb amputation; (**D**) death; and (**E**) major bleeding. *Abbreviation*: HAT, heparin-associated thrombocytopenia. *Source*: From Lubenow et al., 2005.

analysis of HAT-1 and -2 showed that lepirudin was effective in patients with HIT plus thrombosis (Greinacher et al., 2000). In a meta-analysis of HAT-1, -2, and -3, the safety and efficacy of lepirudin in patients with HIT in the absence of known thrombosis was demonstrated (Lubenow et al., 2004). This meta-analysis included 91 patients treated according to dosing regimen B; of these, 20 had a history of HIT (*not* acute HIT). Patients with recent thrombosis at baseline were excluded from this analysis. Mean duration of treatment was 11.0 days (range 1–68 days). The mean steady state dose was 0.062 ± 0.037 mg/kg/h.

Efficacy Outcomes

Of the 91 patients in this study during treatment with lepirudin, four (4.4%) experienced new thromboses, three (3.3%) underwent limb amputation, and 13 (14.3%) deaths occurred. Most of the deaths were related to underlying disease, not to HIT or treatment with lepirudin. Since patients were counted only once if multiple events occurred, the incidence of the composite endpoint was 18/91 (19.8%) (Table 5). The median platelet count rebounded to 150×10^9/L within 4 days of beginning lepirudin treatment.

Safety Outcomes

Episodes of major bleeding occurred in 13/91 (14.3%) of patients in this meta-analysis (risk for major bleeding per treatment day 1.3%). aPTT ratios above 2.5 were associated with an increased risk of bleeding, and bleeding rates were significantly lower in patients with aPTT <60 s ($p < 0.001$). Nearly all patients with bleeding complications had impaired renal function. Antihirudin antibodies were detected in 26 of the 66 (39.4%) evaluable patients. There were no differences in adverse events or outcomes between patients with and without antihirudin antibodies. No anaphylaxis was observed.

E. Postmarketing Drug Monitoring Program

Preliminary results from 1329 patients treated with lepirudin in a DMP are reported (Lubenow et al., 2002): 496 patients had HIT and thrombosis and 612 patients had isolated HIT. This postmarketing study evaluated the same clinical endpoints as were used in HAT-1, -2, and -3. In this DMP, lepirudin could be started immediately upon clinical diagnosis of HIT, thus avoiding the inherent delay awaiting laboratory confirmation. A total of 382 (77.0%) of the 496 patients with HIT and thrombosis were positive in the HIPA test, while 406 (66.3%) of the 612 patients with isolated HIT were positive in the HIPA test.

Efficacy Outcomes

In the routine clinical settings of the DMP, lepirudin-treated patients with isolated HIT and HIT with thrombosis had the lowest incidence of all clinical endpoints reported with any agent. The incidence of the combined clinical endpoint in the 496 patients with HIT and thrombosis was 21.9%: 26 patients (5.2%) experienced new thrombosis, 29 (5.8%) underwent limb amputation, and 54 patients (10.9%) died. The most common cause of death was multiorgan failure (23/54 patients [42.6%]), emphasizing the serious underlying medical condition of these patients. The incidence of new thrombosis in this study (5.2%) was lower than that observed in the HAT-1 and -2 meta-analysis (10.1%). This may be due to physicians' increased clinical experience with lepirudin, as illustrated by the decision to begin lepirudin treatment immediately upon clinical diagnosis of HIT, thereby improving efficacy and safety outcomes.

The combined endpoint of new thrombosis, limb amputation, and death occurred in 96 (15.7%) of the 612 patients with isolated HIT; 13 patients (2.1%) experienced new thrombosis, 8 (1.3%) underwent limb amputation, and 75 patients (12.3%) died. As seen in the group of patients with HIT plus thrombosis, the largest cause of death in this group was multiorgan failure (39/75 patients, 52.0%).

The overall mortality rate due to new thrombosis in the group of 1108 patients treated with regimen Al or B (thus, excluding patients receiving "miscellaneous" treatments) (Table 5) was low (15 patients, or 1.4%). Efficacy variables in the DMP were even more favorable than those seen in the meta-analyses of the HAT studies. This DMP thus confirms the efficacy of lepirudin in routine clinical practice for both the prophylaxis and the treatment of thromboembolism in patients with HIT.

There were no differences in the mean infusion rates in patients with HIT and thrombosis (0.12 mg/kg/h) and those with isolated HIT (0.11 mg/kg/h) in the DMP. As lepirudin dose is adjusted based on aPTT, the major difference between the two regimens is the initial bolus in HIT patients with acute thrombosis. However, as discussed earlier, in the view of the author the bolus should be avoided in most situations to prevent overdosing.

Safety Outcomes

In the DMP, the incidence of bleeding was greatly decreased when compared to the HAT clinical trials. In the group of 496 patients with HIT plus thrombosis, there were 27 (5.4%) major bleeding episodes, and among the 612 patients with isolated HIT, 36 (5.9%) had major bleeding. Allergic reactions were reported in four (0.8%) patients in the HIT plus thrombosis group and in 1 (0.2%) patient with isolated HIT. No anaphylaxis was reported.

The decreased incidence of bleeding events in the DMP can most likely be attributed to physicians' greater experience with administering lepirudin and monitoring its effects.

F. Experience with Lepirudin in a Large Case Series in France

Tardy and colleagues (2006) reported a retrospective observational analysis involving 181 patients (median age, 67 yr) with HIT, in whom the diagnosis was confirmed in 89.5% by a laboratory assay. The mean treatment dose was only 0.06 $\pm$ 0.04 mg/kg/h, which was much less than the approved dose (0.15 mg/kg/h), as well as the mean doses given in the HAT studies (0.11 mg/kg/h for HIT with thrombosis and 0.07 mg/kg/h for isolated thrombocytopenia). Whereas in the HAT 1–3 studies the rate of new thrombosis was 7.4%, it was somewhat greater (13.8%) in the French cohort. The rate of major bleeding, however, was similar in both studies: 20.4% in the French cohort and 17.6% in HAT 1–3 (although a somewhat broader definition of major bleeding was used in the French study). As in the HAT studies, Tardy et al. also found that moderate to severe impairment of renal function strongly enhanced bleeding risk ($p < 0.001$). They also found that prolonged treatment with lepirudin and a mean dose exceeding 0.07 mg/kg/h were independent risk factors for bleeding. In the context of the HAT 1–3 studies, this is further evidence that the approved dose for lepirudin is too high and that especially in elderly patients, initial lepirudin dosing should be reduced to 0.05–0.10 mg/kg/h iv. In patients with known impairment of renal function, dosing should be reduced even further (Table 2).

G. Comparison with Other Treatments for HIT

Mortality rates in patients with HIT remained at approximately 20–30% for more than a decade (King and Kelton, 1984; AbuRahma et al., 1991; Warkentin and Kelton, 1996; Nand et al., 1997). Notably, these rates are two to three times higher than those observed in the HAT studies. In addition to lepirudin, other drugs with antithrombin activity (e.g., argatroban) or antifactor Xa activity (e.g., danaparoid) may be appropriate for management of HIT (see Chapters 13 and 15–17).

Comparisons of the various clinical trials of agents used to treat HIT need to be interpreted with caution, since there have been no direct comparative trials and the studies employed different designs. Trials of lepirudin and argatroban, however, utilized similar clinical endpoints and historical controls for comparison. The most obvious differences between the lepirudin and the argatroban trials are (1) the need for laboratory confirmation of HIT in the lepirudin trials; (2) treatment duration, which was consistently longer than 10 days in the lepirudin-treated patients but less than 7 days in the argatroban-treated patients (potential to increase apparent efficacy and also bleeding with lepirudin); (3) the observation period, which started at the time of diagnosis in lepirudin-treated patients compared with the time of treatment initiation in the argatroban trials (potential to underestimate the efficacy of lepirudin); and (4) a considerable proportion of patients in the historical control group of the HAT trials had been treated with danaparoid (potential to underestimate the efficacy of lepirudin). To allow a more direct comparison, we reanalyzed the data of the HAT trials as per the argatroban trials, i.e., analyzing those events occurring from start of active treatment only (Tables 4b and 5).

The rates for the combined endpoint were consistently lower in the lepirudin trials than in the two argatroban studies. For patients with isolated HIT, the composite event rate was 19.8% in the HAT-1, -2, and -3 meta-analysis (Lubenow et al., 2004), compared with 25.6% and 28.0% in the two argatroban trials. For patients with HIT and thrombosis, the combined endpoint occurred in 19.1% (meta-analysis) with lepirudin (Lubenow et al., 2005), and in 43.8% and 41.5% of patients treated in the two argatroban trials.

Because of the often critical condition of the patient population under study, the rate of deaths observed for both DTIs is not likely to be solely attributable to treatment failure and may vary considerably with different patient populations. As the argatroban trials included many patients who most likely did not have HIT, the death rate associated with HIT might have been overestimated, as non-HIT patients with a decrease of platelet count are often very sick (e.g., septicemia, DIC). Death rates were 14.3% (meta-analysis) and 12.3% (DMP) for patients with isolated HIT treated with lepirudin, but 18.1% and 23.1% in those treated in the two argatroban trials. In patients with HIT complicated by thrombosis, death rates with lepirudin were 11.9% (meta-analysis) and 10.9% (DMP), compared with 18.0% and 23.1% in the argatroban trials.

Among patients with isolated HIT, limb amputation occurred in 3.3% (meta-analysis) and 1.3% (DMP) when treated with lepirudin, and in 1.9% and 4.2% of those treated with argatroban. The amputation rates were higher in HIT with thrombosis; 5.5% (meta-analysis) and 5.8% (DMP) in those treated with lepirudin, versus 11.1% and 14.8% in those treated with argatroban.

There was also a difference in the incidences of new thrombosis, which is arguably the most important parameter for assessing efficacy of an alternative anticoagulant in HIT. In those with isolated HIT, it was 4.4% (meta-analysis) and

2.1% (DMP) with lepirudin, and 6.9% and 5.8% in those treated with argatroban. In HIT with thrombosis it was 6.8% (meta-analysis) and 5.2% (DMP) for lepirudin, and 14.6% and 13.1% for argatroban-treated patients.

The risk of major bleeding between lepirudin and argatroban appears similar if treatment duration is taken into account. In the lepirudin trials, 17.6% of patients experienced major bleeding (Table 4b) over a mean treatment period of 15 days, i.e., a risk for major bleeding of 1.17% per treatment day. In the argatroban trials, major bleeding occurred in 6.9% (Arg 911) and 5.7% (Arg 915) with a mean treatment period of 6 days in both trials, i.e., a risk for major bleeding of 1.05% per treatment day.

Similar rates of efficacy and safety for the two drugs were also observed in a retrospective chart analysis enrolling 61 lepirudin-treated and 29 argatroban-treated HIT patients in a single center (Smythe et al., 2005). Effective anticoagulation was achieved in 77.8% of argatroban patients and 69.5% of lepirudin patients ($p = 0.61$). Major bleeding occurred in 10.3% and 11.5% of argatroban and lepirudin patients, respectively ($p = 1.0$).

Because there are no prospective data comparing lepirudin and danaparoid for treatment of HIT, we retrospectively compared 126 danaparoid-treated patients with 175 lepirudin-treated patients who fulfilled the same inclusion and exclusion criteria (Farner et al., 2001). In the patients with HIT without TECs at baseline, a time-to-event analysis showed that the cumulative risk of the combined endpoint was higher in danaparoid-treated patients than in the lepirudin-treated patients ($p = 0.02$ by log rank test; hazard ratio [HR] = 2.9 [95% CI 1.1–7.6]; $p = 0.027$). This was due primarily to an increased incidence of new TECs (20% [95% CI 8.4–36.9] for danaparoid vs. 6.3% [95% CI 1.3–17.2] for lepirudin; $p = 0.087$). Of note, patients with isolated HIT usually received only prophylactic-dose danaparoid. In contrast, HIT patients with thrombosis at baseline, and who were therefore treated with a therapeutic-dose regimen of danaparoid, had a similar outcome as patients receiving lepirudin ($p = 0.913$). However, the risk for major bleeding was lower in the danaparoid-treated group ($p = 0.012$) (Farner et al., 2001).

The major conclusions of these comparisons are: (1) HIT patients seem to benefit from a longer treatment period with an alternative anticoagulant, with 10 days better than 5 days; (2) the prophylactic-dose regimen of danaparoid (750 U sc two or three times daily) approved in the European Union for HIT with isolated thrombocytopenia appears to be suboptimal. Thus, patients with acute HIT require anticoagulation generally in therapeutic doses (see also Chapter 12).

H. Antibody Formation

Because hirudin is a protein obtained from a nonhuman species, lepirudin can induce antibody formation in humans. Antibodies are induced by both iv therapeutic-dose and sc prophylactic-dose use (Greinacher et al., 2003a). Antihirudin antibodies have been detected in 44–74% of patients treated with lepirudin (Huhle et al., 1998; Song et al., 1999; Eichler et al., 2000). Of 196 HIT patients treated with lepirudin for 5 or more days, 44% developed antihirudin antibodies of the IgG class (Eichler et al., 2000). Reexposed patients developed antibodies in about 70% of cases (Lubenow et al., 2005). These antibodies were not associated with an increase in thrombin–antithrombin (TAT) complexes (Fig. 6). Antibody formation occurred as early as day 4 and peaked at days 8–9 (Eichler et al., 2000).

Antilepirudin antibodies can extend the half-life of lepirudin (Liebe et al., 2002), most likely by reduced renal filtration of lepirudin–antilepirudin complexes

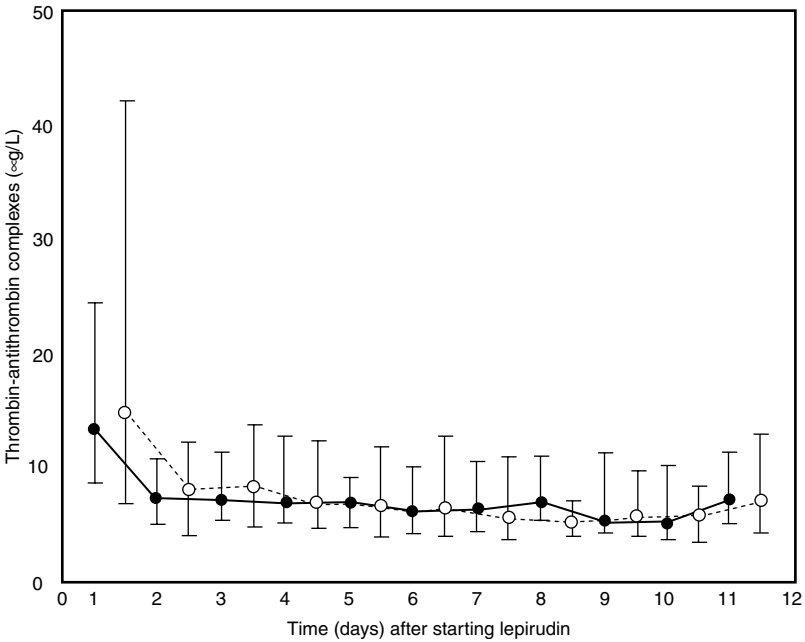

FIGURE 6 TAT complex concentrations in relation to antihirudin antibody formation. TAT complex concentrations did not differ between antihirudin antibody-positive (●, *solid lines*) and antihirudin antibody-negative (O, *dotted lines*) patients (median and 25% and 75% quartiles are given). *Abbreviation*: TAT, thrombin–antithrombin.

(Fig. 7); in about 2–3% of patients with antilepirudin antibodies, an inhibitory effect is seen (Huhle et al., 2001; Fischer et al., 2003). The biological effects of antilepirudin antibodies on anticoagulation can be easily compensated by changes in the lepirudin dose. Thus, ongoing daily aPTT measurements are recommended during lepirudin treatment, even when stable anticoagulation has been observed during the first 5 days.

I. Allergic Reactions

Lepirudin administration during prospective studies in patients with HIT was associated with a low incidence of allergic events, as well as during the much larger clinical trials in patients with ACS. Among the adverse events reported were eczema, rash, pruritus, hot flushes, fever, chills, urticaria, bronchospasm, cough, stridor, dyspnea, angioedema (face, tongue, larynx), and injection-site reactions. Any causal relationship of lepirudin to these adverse events is unclear.

Of 35,000–60,000 patients treated with lepirudin, nine patients were judged to have had severe anaphylaxis in close temporal association with lepirudin use (Greinacher et al., 2003b). All reactions occurred within minutes of iv bolus lepirudin administration, with four fatal outcomes (3 acute cardiorespiratory arrests, one hypotension-induced MI). In these four cases, a previous uneventful treatment course with lepirudin was identified (1–12 wk earlier). In an additional patient with nonfatal anaphylaxis (who did not receive a bolus), we found high-titer IgG antilepirudin antibodies. Since lepirudin had been used in approximately

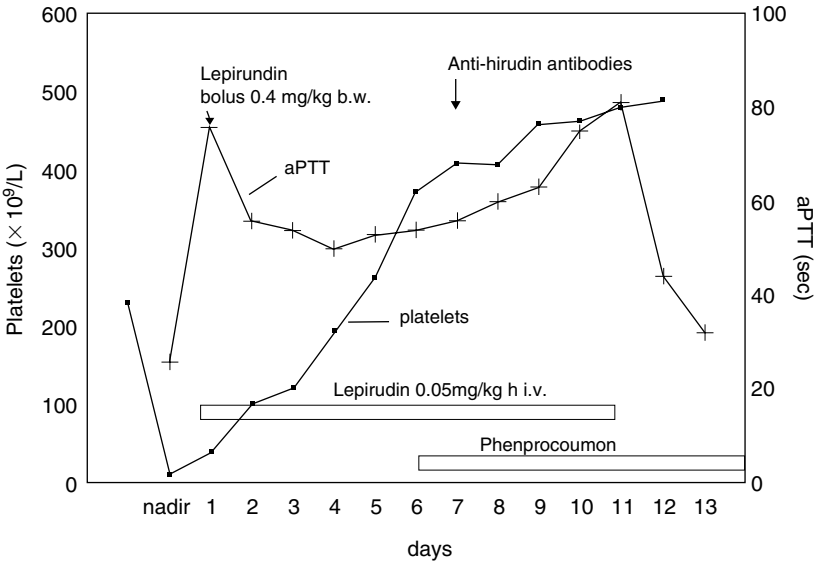

FIGURE 7 A 53-yr-old woman was admitted to the hospital because of an ankle fracture. She received low molecular weight heparin for 10 days, but was switched to unfractionated heparin because of a distal DVT. Ten days later she presented with proximal DVT, pulmonary embolism, and a rapid fall in platelet count from more than 200 to 12×10^9/L. She was switched to iv lepirudin (schedule AI). After normalization of platelet counts, she received overlapping oral anticoagulation (phenprocoumon), with lepirudin stopped when the INR reached 2.0. Antihirudin antibodies were first detected on day 7; at the same time, the aPTT increased despite a stable hirudin dosage of 0.05 mg/kg b.w. per hour. *Abbreviations*: aPTT, activated partial thromboplastin time; DVT, deep vein thrombosis; iv, intravenous; INR, international normalized ratio.

35,000 patients, the risk of anaphylaxis was estimated at 0.015% (5/32,500) in first-exposure and 0.16% (4/2500) in reexposed patients (assuming 7.5% reexposure frequency). One other case has been reported with recurrent anaphylaxis during reexposure (Badger et al., 2004). We and others (Bircher et al., 1996) demonstrated high-titer antihirudin antibodies of the IgG class, but not of the IgE class in patients with hirudin-associated anaphylaxis. IgG-dependent anaphylaxis likely is Fc receptor-mediated and related to infusion dose. Thus, besides reducing bleeding risk, avoiding iv bolus administration of lepirudin should also reduce the risk of severe anaphylactic reactions. There are two patients reported with delayed reactions to hirudin. One patient developed eczematous plaques accompanied by a positive lymphocyte transformation test (Zollner et al., 1996), the other had a granulomatous reaction (Smith et al., 2001). A third patient produced an Arthus-like reaction after intradermal application of lepirudin (Jappe et al., 2002). Approaches to test for these reactions are reviewed in Bircher et al. (2006).

IV. LEPIRUDIN TREATMENT IN OTHER CLINICAL SETTINGS

A. ACSs and Percutaneous Coronary Intervention

Due to their ability to inhibit clot-bound thrombin, DTIs have also been investigated as anticoagulants for ACS and percutaneous coronary intervention (PCI). Lepirudin was examined in large numbers of patients with unstable angina or

suspected acute MI without ST segment elevation in the OASIS-1 (OASIS Investigators, 1997) ($n = 909$) and OASIS-2 ($n = 10{,}141$) trials (OASIS Investigators, 1999). These trials concluded that lepirudin is superior to heparin in preventing ischemic outcomes. A meta-analysis of 11 ACS trials involving over 35,000 patients revealed a 15% reduction in death or MI when bivalent DTIs (lepirudin or bivalirudin) were used to treat ACS patients, compared with heparin (Direct Thrombin Inhibitors Trialists' Collaborative Group, 2002). A retrospective subset analysis of the OASIS-2 trial examined the benefit of lepirudin in 117 ACS patients undergoing PCI within the first 72 hr (Mehta et al., 2002). Lepirudin was superior to heparin in reducing the risk of death or MI at 96 hr ($p = 0.036$) and 35 days ($p = 0.02$). Based on this evidence, lepirudin should be considered a treatment option in ACS patients with HIT.

B. CPB and Vascular Surgery

Lepirudin was initially used to manage CPB patients in the HAT studies (Riess et al., 1995, 1996) and has also been used successfully by other investigators for CPB (Warkentin and Greinacher, 2003). It is now accepted that lepirudin is a suitable alternative for anticoagulation during CPB in patients with acute HIT, provided that ECT monitoring is performed (Koster et al., 1998, 2000a,b; Johnston et al., 1999; Follis and Schmidt, 2000; Latham et al., 2000; Longrois et al., 2000). Neither the activated clotting time (ACT) nor the aPTT is appropriate for monitoring r-hirudin plasma levels in such high-dose situations (see Chapter 19).

Koster and colleagues (2000b) used lepirudin instead of heparin in 57 patients who had clinically diagnosed HIT and required CPB. The primary diagnoses included coronary artery disease ($n = 27$, including eight cases of MI), valvular heart disease ($n = 14$), combined coronary artery and valvular disease ($n = 9$), thoracic aortic aneurysms ($n = 4$), ventricular septal defect resulting from MI ($n = 2$), and atrial tumor ($n = 1$). In that study, anticoagulation was monitored with ECT, and lepirudin was maintained in the range of 3–4 μg/mL. The dose requirement for CPB was 0.016–0.035 mg/kg/min (1.0–2.1 mg/kg/h), with concurrent 24-h blood drainage of 50–2200 mL. Elimination of the drug at the conclusion of CPB was augmented through modified zero-balanced ultrafiltration and forced diuresis. However, drug removal was dependent on the prevailing renal function. Four patients with impaired renal function showed prolonged elimination and bleeding. Of the 57 patients, 54 achieved full recovery and showed no signs of thromboembolism over a 6-mo follow-up. Three patient deaths were unrelated to perioperative management.

For patients undergoing vascular surgery, the dosage of lepirudin should be adjusted for the risk for reocclusion (Hach-Wunderle, 2001). In patients with a low risk of reocclusion (e.g., in the aortic, iliac, and carotid arteries), a bolus of 0.4 mg/kg (reduced in case of renal insufficiency) is given just before the vessel is clamped and is followed postoperatively by either an aPTT-adjusted infusion starting at 0.1 mg/kg/h or 15 mg injected sc b.i.d. (assuming normal renal function). In patients with an increased risk for reocclusion (e.g., undergoing calf-vessel reconstruction or bypass), a preoperative bolus of lepirudin (0.4 mg/kg [less in case of renal impairment]) should be administered, followed by a postoperative infusion of 0.1 mg/kg/h, aPTT-adjusted, for at least 3–4 days. For intraoperative flushing of the vessel during vascular surgery, up to 250 mL (0.1 mg/mL solution) of lepirudin can be used. As patients with acute HIT are at high risk for new TECs, therapeutic levels of anticoagulation should be achieved before surgery and maintained after surgery, at least until platelet counts are normalized.

C. Hemodialysis

Hirudin was the first anticoagulant to be used for hemodialysis, as performed by Haas (1924) in Germany. Because native hirudin preparations were crude and supply of leeches insufficient, hirudin was replaced by heparin to prevent clotting during dialysis.

Management of these patients requires careful dosing and frequent monitoring. HIT patients with transient renal failure are difficult to manage with lepirudin, because substantial dose adjustments are necessary, depending on the extent of renal failure. To reduce bleeding risk, we prefer administering a continuous iv infusion, starting at 0.005 mg/kg/h, with adjustments made according to the aPTT, while others use intermittent iv boluses of 0.005–0.01 mg/kg (Fischer et al., 1999; Kern et al., 1999). Use of lepirudin in renal replacement therapy is reviewed in Chapter 18.

D. Lepirudin in Pregnancy

Data on the treatment of HIT during pregnancy are limited (Lindhoff-Last and Bauersachs, 2002). In general, the use of lepirudin during pregnancy is not recommended, as it crosses the placenta. Zebrafish experiments indicate that thrombin has an important role in early embryogenesis and that inhibition by lepirudin may cause cell regulation defects (Jagadeeswaran et al., 1997). Experiments in rabbits showed a fetal hirudin plasma concentration that was 1/60th that of the maternal concentration (Markwardt et al., 1988), and embryotoxic effects were seen in rabbits at high, but not low, doses (30 mg/kg/day vs. 1–10 mg/kg/day, respectively) (Berlex Laboratories, data on file).

A pregnant woman with systemic lupus erythematosus who was treated with dalteparin developed HIT at week 25. Her platelet count dropped from 230 to 59×10^9/L, after which she was treated with lepirudin (15 mg sc twice daily), with aPTT and ECT used to monitor her dosage. Following delivery by cesarean section, she experienced no postpartum bleeding complications, and treatment with lepirudin was continued for several weeks thereafter (Huhle et al., 2000b). Another pregnant woman with lupus anticoagulant and HIT was successfully treated for 36 wk with lepirudin.

A case report described a breastfeeding woman diagnosed with HIT who was treated with sc lepirudin, 50 mg twice daily (Lindhoff-Last et al., 2000a). No lepirudin was detected in her breast milk, although plasma levels were within therapeutic range. Neither bleeding nor thrombosis occurred in mother or infant.

Lepirudin and danaparoid are each classified by the FDA as pregnancy category B, based on limited animal data. However, danaparoid does not cross the placenta, and it has been used for prophylaxis and therapy of HIT during pregnancy (Greinacher et al., 1993; Dager and White, 2002) (see Chapters 12 and 13).

E. Lepirudin in Children

Although rare in children, HIT is important in the differential diagnosis of thrombocytopenia or unexplained thrombosis in the presence of heparin administration (Ranze et al., 1999; Klenner et al., 2004). Because of the rarity of HIT and its clinical heterogeneity in pediatric patients, it is difficult to design a standardized dosage protocol for lepirudin. Accordingly, current therapeutic recommendations are based on anecdotal experience. Given that children usually have

normal renal function, the short half-life of lepirudin presents an advantage in the event of bleeding complications or the need for invasive procedures. However, the dose required may range between 0.05 and 0.22 mg/kg/h, depending on comorbidity and renal function (Schiffmann et al., 1997; Deitcher et al., 2002; Nguyen et al., 2003) (see Chapter 20).

V. CONCLUSION

The r-hirudin lepirudin is a DTI that provides rapid and effective anticoagulation and significantly reduces the risk of thrombosis in patients with HIT, including those with isolated thrombocytopenia. Less than 10% of all patient groups with HIT developed a new thrombosis after start of active treatment.

Lepirudin is given parenterally by iv infusion or sc injection. Recommended lepirudin dosage schedules have been established (Table 1). Lepirudin has a short half-life, which presents an advantage if invasive surgical procedures are indicated. However, its elimination strongly depends on renal function. The most important lessons learned after approval of the drug is that the approved dosage schedule is too high. The bolus should only be given in life- or limb-threatening thrombosis and also the starting maintenance dose should be greatly reduced (from 0.15 to 0.05–0.10 mg/kg/h), especially in elderly patients. Lepirudin can be used safely and effectively in patients with renal impairment by appropriate dosing according to serum creatinine and regular monitoring. Lepirudin also allows for a safe and uncomplicated transition to warfarin, provided that warfarin is initiated after recovery of the platelet count.

After start of treatment, lepirudin should be monitored every 4 h until a steady state is reached, then daily monitoring of aPTT is recommended with dosage adjustments made as needed to maintain the target aPTT value. Routine monitoring with ECT should be performed in high-dose situations, such as those required during CPB. All functional assays (aPTT, ECT) can give false high levels of anticoagulation in patients with prothrombin or fibrinogen deficiencies. In these patients a chromogenic assay (ECA) is more suitable to avoid underdosing.

The most common adverse event in the prospective clinical trials was bleeding. No antidote exists for the DTIs. Excess lepirudin can be removed by hemofiltration, and rFVIIa may also be used, but clinical data are limited.

Besides the 403 patients with HIT treated in prospective trials, an additional 1329 patients received lepirudin for HIT in a postmarketing surveillance study. Data on these patients, collected under routine clinical conditions, showed the lowest incidence of the clinical endpoints of death, new thrombosis, and amputations, with risk reductions exceeding those reported in the prospective clinical trials. Even more importantly, the incidence of major bleeding was low. These differences support the assumption that outcomes in patients with HIT can be substantially improved by immediately stopping heparin and starting lepirudin when HIT is strongly suspected on clinical grounds, without awaiting results of antibody testing, and that the bleeding risk has been reduced substantially as physicians have learned to handle this agent.

Postapproval observational studies also gave insights in the frequency of rare adverse effects associated with lepirudin treatment, such as anaphylaxis. The results of these trials and the DMP demonstrate that lepirudin is highly effective in reducing the risk of the potentially devastating complications of HIT.

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15 Argatroban Therapy in Heparin-Induced Thrombocytopenia

Bruce E. Lewis
Loyola University Medical Center, Maywood, and Catholic Health Partners, Chicago, Illinois, U.S.A.

Marcie J. Hursting
Clinical Science Consulting, Austin, Texas, U.S.A.

I. INTRODUCTION

The elimination of all heparin sources and the initiation of alternative anticoagulation are recommended for treating patients with heparin-induced thrombocytopenia (HIT), whether or not complicated with thrombosis (Warkentin and Greinacher, 2004). Argatroban is the only direct thrombin inhibitor (DTI) approved in the United States as an anticoagulant for use in patients with HIT in both noninterventional and interventional settings. Two other DTIs are approved in the United States for use in patients with HIT in either the noninterventional (lepirudin; see Chapter 14) or interventional (bivalirudin, see Chapter 16) setting, but not both. Argatroban is indicated in the United States as an anticoagulant for prophylaxis or treatment of thrombosis in patients with HIT and for patients with, or at risk for, HIT undergoing percutaneous coronary intervention (PCI) (Argatroban Prescribing Information, U.S., 2002). It is also available in Austria, Canada, Denmark, Iceland, Germany, The Netherlands, Norway, and Sweden as an anticoagulant for patients with HIT.

In this chapter, the clinical pharmacology of argatroban is reviewed, together with its clinical utility as an anticoagulant in patients with or at risk for HIT. Historically, argatroban was initially known worldwide as "MD-805" and then under the trademark "Novastan." In 2000, the U.S. Food and Drug Administration disallowed the trademark Novastan in the United States because of potential similarities with other named products. Hence, argatroban (generic name) is marketed in the United States without a trademark and under the name "Argatroban" (with a capital A). However, the trademark Novastan continues to be used in some countries, and the trademarks Argatra and Arganova have been introduced in some countries.

II. ARGATROBAN

A. Chemical Description

Argatroban is a synthetic DTI derived from L-arginine (Fig. 1) (Okamoto and Hijikata, 1981; Kikumoto et al., 1984). Argatroban (molecular weight, 526.66) consists of a mixture of 2l-(*R*) and 2l-(*S*) stereoisomers in a ratio of approximately 65:35 (Rawson et al., 1993), with no interconversion between stereoisomers.

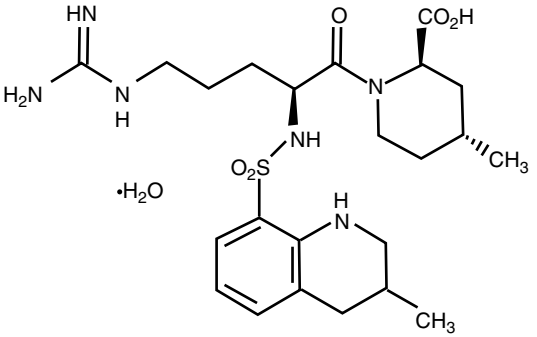

FIGURE 1 Chemical structure of argatroban. Its chemical name is 1-[5-[(ammoimino methyl)amino]-1-oxo-2-[[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-pentyl]-4-methyl-2-piperidinecarboxylic acid, monohydrate, and its molecular weight is 526.66.

B. Clinical Pharmacology

Mechanism of Action

Argatroban is a potent, selective inhibitor of thrombin (Okamoto and Hijikata, 1981; Kikumoto et al., 1984). Argatroban was developed using rational drug design through the mimicry of thrombin substrates. It displays an inhibitory constant (K_i) of 0.04 μmol/L for thrombin and has little or no effect on related serine proteases (K_i values of 5 μmol/L for trypsin, 210 μmol/L for factor Xa, and 800 μmol/L for plasmin) (Kikumoto et al., 1984). Argatroban exerts its anticoagulant effects without need of any cofactor by inhibiting thrombin-catalyzed or induced reactions, such as fibrin formation, activation of factors V, VIII, and XIII, and platelet aggregation (Okamoto and Hijikata-Okunomiya, 1993).

Argatroban effectively inhibits free and clot-bound thrombin (Berry et al., 1994; Hantgan et al., 1998) and is over 500-fold more potent than r-hirudin in its relative ability to inhibit clot-bound versus free thrombin (Berry et al., 1994). Argatroban binds tightly to thrombin (Fig. 2) by inserting the dual hydrophobic moieties on its arginine backbone into deep clefts near the thrombin active site (Banner and Hadvary, 1991). Thus, physiological substrates of thrombin are sterically hindered from access to the catalytic pocket of thrombin. This interaction is reversible, unlike the irreversible interaction between r-hirudin and thrombin (see Chapter 14). The combination of effective inhibition of clot-bound thrombin, reversible binding, and a short elimination half-life (see next subsection) may be particularly beneficial in treating hypercoagulable states, reducing the extension of existing thrombosis, and controlling anticoagulation in the intensive care setting.

Distribution, Metabolism, and Excretion

Argatroban distributes mainly in the extracellular fluid, with a steady state volume of distribution of 174 mL/kg (Swan and Hursting, 2000). It is 54% serum protein-bound (Tatsuno et al., 1986).

Unlike r-hirudin and bivalirudin, argatroban undergoes no significant renal clearance. The main route of metabolism is hydroxylation and aromatization of the 3-methyltetrahydroquinoline ring in the liver (Izawa et al., 1986). In vitro, the human liver microsomal cytochrome P450 3A4/5 (CYP3A4/5) catalyzes the formation of each of the four known metabolites. In plasma, unchanged argatroban is the major component, while the concentration of the primary metabolite (M1),

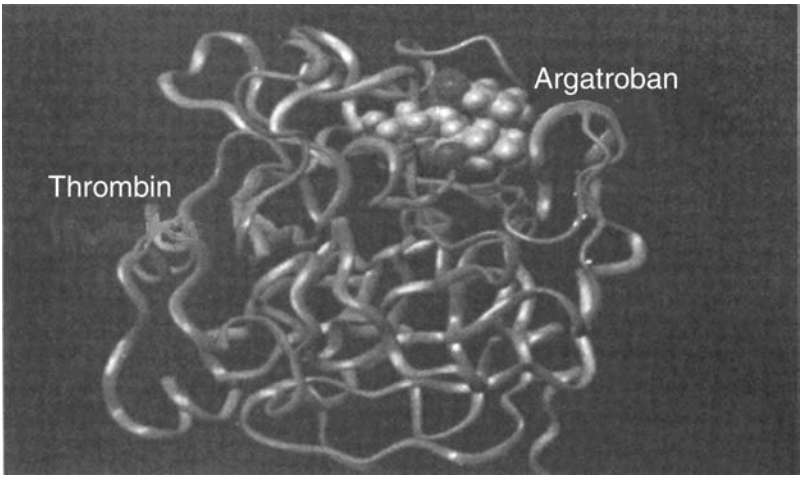

FIGURE 2 (*See color insert.*) Model of the interaction between argatroban and thrombin.

which has three- to fivefold less activity than argatroban, is 0–20% of the parent drug (Ahsan et al., 1997). The other metabolites have not been detected in plasma or feces and are found only in very low quantities in urine. These data, together with the lack of effect of erythromycin, a potent CYP3A4/5 inhibitor, on argatroban pharmacokinetics (Tran et al., 1999) suggest that CYP3A4/5-mediated metabolism is not an important pathway in vivo.

Systemic clearance of argatroban is approximately 5.1 mL/min/kg for infusion doses up to 40 μg/kg/min in healthy volunteers (Swan et al., 2000). Its elimination half-life is 39–51 min (Swan and Hursting, 2000), with excretion primarily via the feces, presumably by biliary secretion.

Pharmacokinetic–Pharmacodynamic Relationship

The pharmacokinetic and pharmacodynamic profiles of intravenous (iv) argatroban are consistent with an anticoagulant agent that is predictable, has a fast onset of action, and is rapidly eliminated (Swan and Hursting, 2000; Swan et al., 2000).

The anticoagulant effects of argatroban are routinely monitored using the activated partial thromboplastin time (aPTT). Higher levels of anticoagulation, such as that required during interventional procedures, are monitored using the activated clotting time (ACT). Argatroban also increases in a dose-dependent fashion the prothrombin time (PT)/International Normalized Ratio (INR), thrombin time, and ecarin clotting time (Nagasawa et al., 1981; Clark et al., 1991; Walenga et al., 1999; Swan et al., 2000; Sheth et al., 2001). High-performance liquid chromatography (Rawson et al., 1993; Walenga et al., 1999) and liquid chromatography/tandem mass spectrometry (Tran et al., 1999) methods for measuring plasma argatroban are described but not practical (or needed) for routine monitoring.

Immediately upon initiation of argatroban infusion, anticoagulant effects are produced as plasma argatroban concentrations begin to rise. Steady-state levels of both drug and anticoagulant effect typically are attained within 1–3 h (faster when

a loading bolus is administered) and maintained with low intra- and intersubject variability until the infusion is discontinued or the dosage adjusted. Plasma drug concentrations increase proportionally with doses up to 40 μg/kg/min and are well correlated with steady-state anticoagulant effects. The relationship at steady state between argatroban dose up to 10 μg/kg/min, plasma argatroban concentration, and aPTT is shown in Figure 3. When infusion is stopped, plasma argatroban concentrations decline rapidly (half-life of 39–51 min), and anticoagulant effects return to pretreatment values with similar effect half-lives (Swan et al., 2000).

Special Populations

Age, gender, and renal function exert no clinically significant effects on the pharmacokinetics or pharmacodynamics of argatroban. Patients with moderate hepatic impairment (Child-Pugh score >6), compared with healthy volunteers, have an approximate fourfold decrease in drug clearance (to 1.5 mL/min/kg) and an approximate threefold increase in elimination half-life (to 152 min) (Swan and Hursting, 2000). Owing to the decreased clearance, a fourfold downward adjustment in argatroban dosage is required for individuals with moderate hepatic impairment. No adjustment in initial argatroban dosage is needed for patients with renal impairment. In patients without hepatic dysfunction undergoing PCI, the pharmacokinetic values of argatroban are similar to those reported in healthy volunteers, and argatroban clearance is unaffected by age, gender, or race (Cox et al., 2004).

Drug–Drug Interactions

No pharmacokinetic or pharmacodynamic drug interactions have been demonstrated between argatroban and aspirin (Clark et al., 1991), erythromycin (Tran et al., 1999), acetaminophen, digoxin, or lidocaine (Inglis et al., 2002). In practice, argatroban coadministered with these frequently used medications should require no dosage adjustments.

No pharmacokinetic interactions have been demonstrated between argatroban and warfarin (Brown and Hursting, 2002). DTIs as a class variably prolong the PT/INR—argatroban to a greater extent than bivalirudin and lepirudin (Warkentin et al., 2005)—and the concomitant use of argatroban and warfarin prolongs the PT/INR beyond that produced by warfarin alone (Hursting et al., 1999; Sheth et al., 2001). Cotherapy compared with warfarin monotherapy exerts no additional effect on vitamin K-dependent factor X levels (Sheth et al., 2001), and INRs >5 commonly occur in patients with HIT during argatroban/warfarin cotherapy (and argatroban monotherapy) without bleeding (Hursting et al., 2005). Hence, the previously established ("traditional") relationship between INR and bleeding risk is altered during combination therapy. Guidelines for monitoring the transition from argatroban to warfarin anticoagulation are presented in Section IV.

Argatroban and a variety of drugs have been evaluated for chemical or physical/visual compatibility at concentrations commonly used in practice. This is important for supporting their simultaneous administration via Y-site injection. Argatroban and eptifibatide or tirofiban are chemically and physically compatible (Patel and Hursting, 2005). Argatroban and abciximab (Patel and Hursting, 2005), fentanyl citrate, midazolam hydrochloride, morphine sulfate, dopamine hydrochloride, dobutamine hydrochloride, phenylephrine hydrochloride, atropine sulfate, hydrocortisone sodium succinate, metoprolol tartrate, diphenhydramine

hydrochloride, verapamil hydrochloride, norepinephrine bitartrate, diltiazem hydrochloride (Hartman et al., 2002), fenoldopam mesylate, lidocaine hydrochloride, milrinone lactate, nitroglycerin, or vasopressin (Honikso et al., 2004) are physically/visually compatible; their chemical stability remains to be established. Argatroban and amiodarone should not be infused through the same iv line because precipitation may occur (Honikso et al., 2004).

C. Other Distinguishing Features

Lack of Cross-Reactivity with HIT Antibody

In contrast with low molecular weight heparin and danaparoid, the DTIs, including argatroban, hirudin, and bivalirudin, bear no structural resemblance to heparin, do not cross-react with HIT antibodies, and have not been associated with potentiation of HIT (Walenga et al., 1996).

Lack of Effect on Clot Strength

Whereas argatroban, lepirudin, bivalirudin, heparin, and fondaparinux each delay clot formation in vitro, the DTIs do not decrease clot rigidity or elasticity (Young et al., 2007; Nielson et al., 2006). The reduced bleeding reported with argatroban and other DTIs versus heparin may relate to the fact that clots form with normal strength (Young et al., 2007).

Lack of Drug-Specific Antibody

Prolonged or repeated exposure to argatroban does not result in the generation of antibodies that alter its anticoagulant activity (Walenga et al., 2002). This has been shown in healthy volunteers, HIT patients, and HIT patients undergoing PCI, and in the postmarketing safety surveillance of over 4800 patients treated in Japan between 1991 and 1998 with argatroban anticoagulation (Walenga et al., 2002). In contrast, approximately 50% of lepirudin-treated patients develop drug-specific antibodies that can occasionally increase plasma lepirudin concentrations, requiring careful monitoring and dose adjustments to avoid bleeding complications (Song et al., 1999; Eichler et al., 2000) (see Chapter 14). An estimated 0.2% of patients re-exposed to lepirudin experience anaphylactoid reactions, possibly death (Greinacher et al., 2003). Bivalirudin cross-reacts in vitro with approximately 51% of anti-lepirudin antibodies (Eichler et al., 2004). Argatroban does not cross-react with anti-lepirudin antibodies and has been used successfully in patients with a history of HIT and anti-lepirudin antibodies (Harenberg et al., 2005).

D. Reversal of Argatroban

Argatroban has a gentle dose-response relationship that offers a wide margin of safety during dose titration (Fig. 3). However, as with any anticoagulant, bleeding is a major safety concern. Excessive anticoagulation, with or without bleeding, may be controlled by discontinuing argatroban or decreasing its infusion dose. Anticoagulant parameters generally return to baseline within 2–4 h after discontinuation of argatroban (Swan et al., 2000; Swan and Hursting, 2000). This reversal takes longer (at least 6 h and up to more than 20 h) in patients with hepatic impairment.

Argatroban has no specific antidote. If life-threatening bleeding occurs and excessive plasma argatroban levels are suspected, argatroban should be discontinued immediately, and the patient should be provided symptomatic and supportive

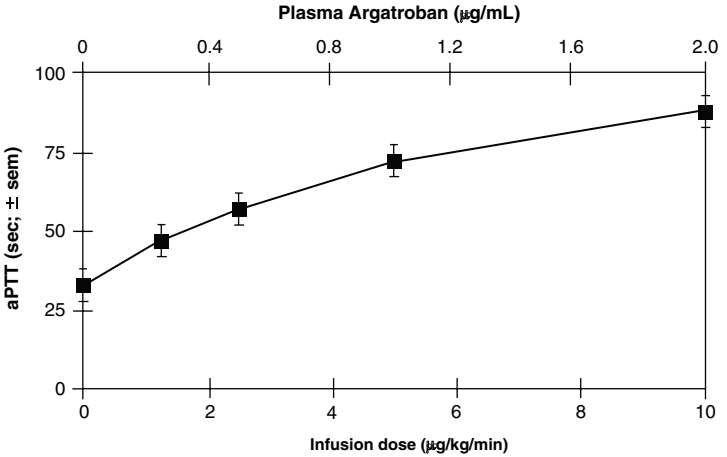

FIGURE 3 Relationship at steady state between argatroban dose, plasma argatroban concentration, and anticoagulant effect (aPTT). Mean (SEM) steady-state plasma argatroban concentrations and aPTT values are for healthy subjects ($n = 9$) administered iv argatroban at doses between 1.25 and 10 μg/kg/min. *Abbreviation*: aPTT, activated partial thromboplastin time. *Source*: Data from Swan et al., 2000.

therapy. Clearance of argatroban by high-flux dialysis membranes is clinically insignificant (de Denus and Spinler 2003; Dager and White, 2003; Murray et al., 2004; Tang et al., 2005). Recombinant factor VIIa has been used to treat argatroban-treated patients with severe bleeding (Malherbe et al., 2004; Alsoufi et al., 2004), although this approach remains to be rigorously evaluated. Fresh frozen plasma has been used successfully following accidental overdose (Yee and Kuter, 2006).

E. Clinical Use of Argatroban

Argatroban therapy has been evaluated, in addition to HIT, in acute myocardial infarction (Theroux, 1997; Jang et al., 1999; Vermeer et al., 2000), unstable angina pectoris (Gold et al., 1993), peripheral arterial obstructive disease (Matsuo et al., 1995), stroke (Kobayashi and Tazaki, 1997; LaMonte et al., 2004a; Sugg et al., 2006), PCI (Herrman et al., 1996; Jang et al., 2004), and hemodialysis (Murray et al., 2004). In each of these settings, argatroban produces predictable anticoagulant effects and is generally safe and well tolerated. In addition to its indications in HIT, argatroban is approved outside the United States for use in nonlacunar stroke (Japan, South Korea), chronic arterial occlusion (Japan, South Korea, China), and hemodialysis of antithrombin-deficient patients (Japan).

III. ARGATROBAN THERAPY OF HIT

A. Overview of Studies

The efficacy and safety of argatroban therapy in patients with clinically diagnosed HIT has been evaluated in the following prospective, multicenter, open-label studies:

- ARG-911, a historical controlled study
- ARG-915, a follow-on study that also used the historical control group from ARG-911 as comparator

- ARG-915X, a Phase III extension of study ARG-915 that allowed physicians continued access to argatroban while it was under regulatory review.

Study ARG-911 has been reported in full (Lewis et al., 2001). Topline data from study ARG-915 (without its extension) as well as safety summaries from ARG-911 plus ARG-915 appear in the product's labeling information (Argatroban Prescribing Information, U.S., 2002). Outcomes of patients with acute HIT from study ARG-915 plus its extension, together simply referred to as "Argatroban-915," have also been reported in full (Lewis et al., 2003). Across these studies, 754 patients received argatroban therapy on 809 separate occasions (Lewis et al., 2000).

When these studies were conducted between 1995 and 1998, no approved alternative agent was available for use as an active comparator, and a randomized, placebo-controlled design was deemed unethical; thus, historical controls were used for comparison. The studies were similar in design with regard to objectives, inclusion and exclusion criteria, the argatroban dosing regimen, and assessments. In each study, patients were assigned at enrollment to one of two prospectively defined study arms: HIT (with isolated thrombocytopenia) or HIT with thrombosis (also referred to as "HIT with thrombosis syndrome" or "HITTS"). The overall study design is presented in Figure 4.

Study Objectives

The objective of study ARG-911 was to evaluate the use of argatroban as an anticoagulant for the prophylaxis of thrombosis in HIT patients and the treatment of HIT patients with thrombosis. Similarly, the objective of studies ARG-915 and ARG-915X was to evaluate the safety and efficacy of argatroban in HIT patients, with or without thrombosis, requiring anticoagulation.

Study Population

Adult patients were eligible if they had a clinical diagnosis of HIT with or without thrombosis. HIT was defined as a platelet count $<100\times10^9$/L, or a 50% decrease in the platelet count after initiation of heparin therapy, with no apparent explanation other than HIT. Patients with a documented history of a positive HIT antibody test who needed anticoagulation were also eligible for the HIT study arm in the

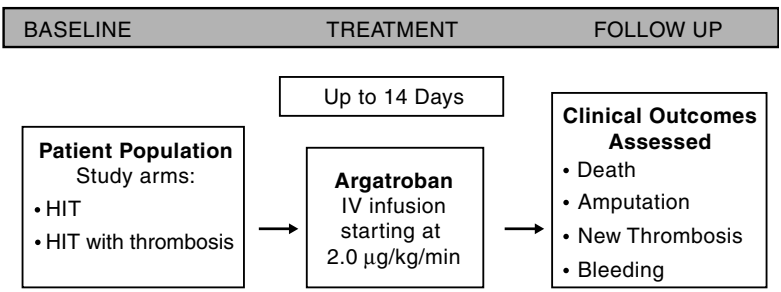

FIGURE 4 Schematic of the study design for ARG-911, ARG-915, and ARG-915X. Patients with a clinical diagnosis of HIT with or without thrombosis were eligible. The starting dose of argatroban, 2.0 μg/kg/min, was titrated to achieve an aPTT 1.5–3.0 times the baseline aPTT (not to exceed 100 s). Outcomes over a 37-day period were compared with those of a historical control group. *Abbreviations*: aPTT, activated partial thromboplastin time; HIT, heparin-induced thrombocytopenia; iv, intravenous.

absence of thrombocytopenia. Patients were excluded if they had an unexplained aPTT greater than two times control at baseline, documented coagulation disorder, or bleeding diathesis unrelated to HIT, a lumbar puncture within the prior 7 days, or a history of previous aneurysm, hemorrhagic stroke, or recent (within 6 mo) thrombotic stroke unrelated to HIT. Reentry of patients into studies ARG-915 and ARG-915X was allowed, although outcomes from initial entries only were included in the primary analyses to avoid potential bias.

The historical control group of ARG-911 consisted of patients at the participating centers who met the same inclusion–exclusion criteria for the study and who were seen prior to the initiation of the study. Controls were treated according to the local standard of practice at the time of HIT diagnosis, with typical treatments being heparin discontinuation and/or oral anticoagulation (Lewis et al., 2001).

Treatment

The treatment group received an initial dose of argatroban 2 μg/kg/min via continuous iv infusion. The aPTT was measured at least 2 h later, and dosage was adjusted (up to 10 μg/kg/min, maximum) until the aPTT was 1.5–3 times the baseline aPTT value (not to exceed 100 s). The aPTT was measured daily and 2 h after each dosage adjustment. Patients remained on argatroban for up to 14 days, until the underlying condition resolved or appropriate anticoagulation was provided with other agents.

Assessments

The primary efficacy assessment was a composite endpoint of all-cause death, all-cause amputation, or new thrombosis within a 37-day study period. Additional analyses included the evaluation of event rates for the components of the composite endpoint and death due to thrombosis. Secondary efficacy endpoints included the achievement of adequate anticoagulation (i.e., an aPTT >1.5 times baseline) and resolution of thrombocytopenia (i.e., platelet count $>100 \times 10^9$/L or >1.5 times baseline by study day 3).

Major bleeding was defined as overt and associated with a hemoglobin decrease >2 g/dL that led to a transfusion of >2 units or that was intracranial, retroperitoneal, or into a major prosthetic joint. Other overt bleeding was considered minor.

B. ARG-911

In study ARG-911, 304 patients having clinically diagnosed HIT ($n = 160$) or HITTS ($n = 144$) received argatroban at a mean dose of 2.0 μg/kg/min for an average of 6 days. This study also enrolled 193 historical controls (HIT, $n = 147$; HITTS, $n = 46$). Although not required for enrollment, laboratory confirmation of HIT antibodies occurred in 57% of the argatroban-treated patients and 77% of controls; the remaining individuals were either never tested or had a negative result (Lewis et al., 2001).

Efficacy

As seen in Table 1, the composite endpoint was reduced significantly in argatroban-treated patients versus controls with HIT (25.6% vs. 38.8%, $p = 0.014$). In HITTS, the composite endpoint occurred in 43.8% of argatroban-treated patients compared with 56.5% of controls ($p = 0.13$). Significant between-group differences by time-to-event analysis of the composite endpoint favored argatroban treatment in HIT ($p = 0.010$, hazard ratio = 0.60; 95% CI, 0.40–0.89) (Fig. 5a) and HITTS ($p = 0.014$, hazard ratio = 0.57; 95% CI, 0.36–0.90) (Fig. 5b).

TABLE 1 Comparisons of Argatroban-Treated Patients with Historical Controls in ARG-911

	HIT, *n* (%)			HIT with thrombosis, *n* (%)		
Parameter	Control ($n = 147$)	Argatroban ($n = 160$)	*p*	Control ($n = 46$)	Argatroban ($n = 144$)	*p*
Composite endpoint[a]	57 (38.8)	41 (25.6)	0.014	26 (56.5)	63 (43.8)	0.13
	Odds ratio = 0.54 (95% CI, 0.33–0.88)			Odds ratio = 0.60 (95% CI, 0.31–1.17)		
Components by severity[b]						
Death (all causes)	32 (21.8)	27 (16.9)	0.31	13 (28.3)	26 (18.1)	0.15
Amputation (all causes)	3 (2.0)	3 (1.9)	1.00	4 (8.7)	16 (11.1)	0.79
New thrombosis	22 (15.0)	11 (6.9)	0.027	9 (19.6)	21 (14.6)	0.49
Death due to thrombosis	7 (4.8)	0 (0.0)	0.005	7 (15.2)	1 (0.7)	< 0.001
Any new thrombosis[c]	33 (22.4)	13 (8.1)	< 0.001	16 (34.8)	28 (19.4)	0.044

[a]All-cause death, all-cause amputation, or new thrombosis within 37-day study period.
[b]Severity ranking: all-cause death > all-cause amputation > new thrombosis; patients with multiple outcomes counted once.
[c]Patient counted only once if multiple events occurred.
Abbreviations: ARG, argatroban; HIT, heparin-induced thrombocytopenia.
Source: Lewis et al., 2001.

Argatroban therapy, compared with controls, significantly reduced death due to thrombosis in each study arm (HIT, $p = 0.005$; HITTS, $p < 0.001$). There were no between-group differences in all-cause mortality. The incidence of amputation (as the most severe outcome) was similar between groups. Argatroban therapy also significantly reduced the percentage of patients experiencing new thrombosis in each study arm (HIT, $p < 0.001$; HITTS, $p = 0.044$).

Argatroban-treated patients achieved therapeutic aPTTs generally at first measure (i.e., within 4–5 h of starting therapy) and maintained these levels throughout infusion. Resolution of thrombocytopenia occurred by day 3 in 53% of argatroban-treated patients with HIT and 58% of patients having HITTS. Compared with controls, argatroban-treated patients had a significantly more rapid rise in platelet counts.

Safety

Major bleeding occurred in 6.9% (21/304) of argatroban-treated patients, compared with 6.7% (13/193) of historical controls. In each group, there were two fatal bleeding events. One patient experienced a fatal intracranial hemorrhage 4 days after discontinuation of argatroban and following urokinase and warfarin therapy; one historical control also experienced a fatal intracranial hemorrhage. Minor bleeding rates were similar between the groups (41%). The most common adverse events among argatroban-treated patients with HIT or HITTS, respectively, were diarrhea (11%) and pain (9%).

C. Argatroban-915

A total of 418 patients with acute HIT ($n = 189$) or HITTS ($n = 229$) were prospectively treated with argatroban in study ARG-915 or its extension (together referred to as "Argatroban-915") (Lewis et al., 2003). The mean argatroban dose was 1.8 μg/kg/min, and the mean duration of therapy was 6 days. Comparisons were

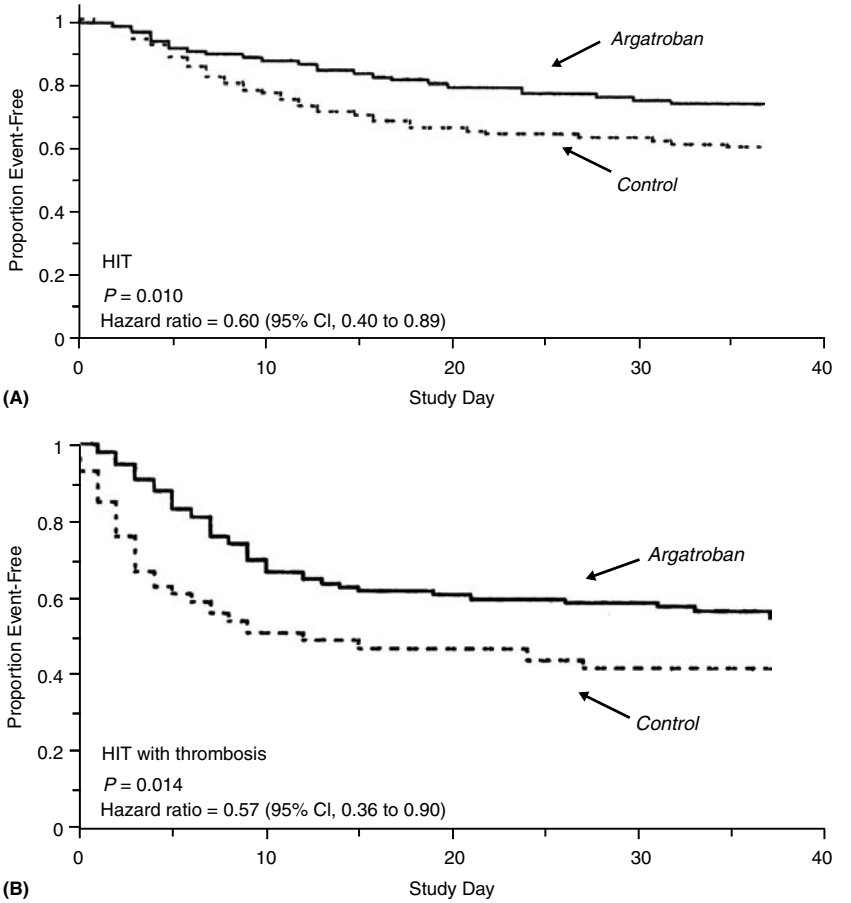

FIGURE 5 Time to first event for the composite endpoint through day 37 in study ARG-911. Significant differences in favor of argatroban therapy were detected in (**A**) the HIT study arm (argatroban group, $n = 160$; historical controls, $n = 147$) and (**B**) the HIT with thrombosis study arm (argatroban group, $n = 144$; historical controls, $n = 46$). *Abbreviations*: ARG-911, argatroban-911; HIT, heparin-induced thrombocytopenia. *Source*: Data from Lewis et al., 2001.

made with 185 historical controls with acute HIT with or without thrombosis (obtained from ARG-911).

Efficacy

Efficacy results (Table 2) were confirmatory and supportive of those from ARG-911. There were significant improvements in the composite endpoint for argatroban-treated patients versus controls among those with HIT (28.0% vs. 38.8%, $p = 0.04$) or HITTS (41.5% vs. 56.5%, $p = 0.07$). Argatroban treatment was significantly favored, compared with control, by time-to-event analysis of the composite endpoint in HIT ($p = 0.02$, hazard ratio = 0.64, 95% CI, 0.43–0.93) or HITTS ($p = 0.008$, hazard ratio = 0.56, 95% CI, 0.36–0.87).

TABLE 2 Comparisons of Argatroban-Treated Patients and Historical Controls with Acute HIT in Argatroban-915

	HIT, *n* (%)			HIT with thrombosis, *n* (%)		
Outcome	Control ($n = 139$)	Argatroban ($n = 189$)	p[a]	Control ($n = 46$)	Argatroban ($n = 229$)	p[a]
Composite endpoint[b]	54 (38.8)	53 (28.0)	0.04	26 (56.5)	95 (41.5)	0.07
Death (all causes)[c]	29 (20.9)	36 (19.0)	0.78	13 (28.3)	53 (23.1)	0.45
Death due to thrombosis	6 (4.3)	1 (0.5)	0.04	7 (15.2)	6 (2.6)	0.002
Amputation (all causes)[c]	4 (2.9)	8 (4.2)	0.57	5 (10.9)	34 (14.8)	0.64
New thrombosis[c]	32 (23.0)	11 (5.8)	<0.001	16 (34.8)	30 (13.1)	<0.001
Major bleeding[d]	12 (8.6)	10 (5.3)	0.27	1 (2.2)	14 (6.1)	0.48
Minor bleeding[d]	57 (41.0)	59 (31.2)	0.08	19 (41.3)	87 (38.0)	0.74

[a]Significance level of $p < 0.05$ for the primary endpoint (composite) and bleeding and $p < 0.0125$ for secondary endpoints (components of composite, and death due to thrombosis).
[b]All-cause death, all-cause amputation, or new thrombosis within 37-day study period.
[c]Outcome categories are not mutually exclusive; within a given category, a patient is counted only once if >1 event.
[d]Patients with > 1 event are counted only once.
Abbreviation: HIT, heparin-induced thrombocytopenia.

Consistent with ARG-911, the positive benefits on the composite endpoint were driven in main part by significant reductions in new thrombosis ($p < 0.001$ in each study arm) (Table 2). There were no significant between-group differences in all-cause mortality or amputation. Argatroban therapy significantly reduced the incidence of death due to thrombosis in patients having HITTS ($p = 0.002$).

Similar, predictable aPTT responses occurred in patients with HIT or HITTS. The target aPTT was typically achieved by first assessment, and mean aPTT values remained generally constant throughout the infusion. Platelet counts recovered more rapidly in argatroban-treated patients than controls ($p < 0.001$ for each study arm).

Safety

Major bleeding rates were not different between argatroban-treated patients and controls in either study arm (Table 2). Twenty-four (5.7%) argatroban-treated patients experienced major bleeding, including a single fatal event in a patient hospitalized for rectal bleeding and who received urokinase. No patient experienced an intracranial hemorrhage. Minor bleeding rates were not different between the groups and were similar to those in ARG-911.

D. Combined Analysis of the Prospective Studies

A secondary, combined analysis of the ARG-911, ARG-915, and ARG-915X studies evaluated the effect of argatroban versus historical control on thrombosis-related (rather than all-cause) outcomes in clinically diagnosed HIT (Lewis et al., 2006). The analysis population included 882 patients (697 argatroban-treated and 185 historical controls), presenting with either HIT or HITTS. The primary endpoint was a 37-day composite of death due to thrombosis, amputation secondary to HIT-associated thrombosis, or new thrombosis.

Argatroban therapy, compared with control, significantly reduced the risk for the thrombotic composite in HIT (hazard ratio, 0.33; 95% CI, 0.20 to 0.54;

TABLE 3 Cox Proportional Hazard Analysis of Argatroban-Treated Patients Versus Historical Control: Combined Data from ARG-911 and ARG-915/X

	HIT		HIT with thrombosis	
Outcome	Hazard ratio (95% CI)	*p*	Hazard ratio (95% CI)	*p*
Thrombotic composite[a]	0.33 (0.20–0.54)	<0.001	0.39 (0.25–0.62)	<0.001
Death due to thrombosis	0.072 (0.009–0.60)	0.015	0.13 (0.045–0.40)	<0.001
Amputation secondary to HIT-associated thrombosis	0.54 (0.15–2.03)	0.36	1.22 (0.44–3.39)	0.71
New thrombosis	0.29 (0.17–0.50)	<0.001	0.32 (0.18–0.55)	<0.001

[a]Death due to thrombosis, amputation secondary to HIT-associated thrombosis, or new thrombosis within a 37-day study period.
Abbreviations: ARG, argatroban; HIT, heparin-induced thrombocytopenia.

$p < 0.001$) and HITTS (hazard ratio, 0.39; 95% CI, 0.25 to 0.62; $p < 0.001$) (Table 3). The antithrombotic benefits remained significant after adjusting for patient age, gender, race, weight, and baseline platelet count. In each HIT presentation, the positive antithrombotic effect was driven by significant risk reductions in new thrombosis and death due to thrombosis (Table 3). The risk of amputation secondary to HIT-associated thrombosis was not different between groups.

E. Patients with a History of HIT Requiring Acute Anticoagulation

A subgroup analysis of the prospective studies of argatroban in HIT identified 36 patients with a history of serologically confirmed HIT who had fully recovered from their initial episode of HIT, had a normal platelet count, and had no exposure to heparin or other parenteral anticoagulants (except argatroban) during their hospitalization (Matthai et al., 2005). Each patient required acute anticoagulation, most often for venous thromboembolism or acute coronary syndrome, and 12 had previously received argatroban. All evaluable patients were successfully anticoagulated. No one had a major bleeding or a new thromboembolic event. There were no adverse events related to reexposure.

F. Argatroban Reexposure

Across the prospective studies of HIT, 55 patients underwent therapy with argatroban on more than one occasion. The argatroban dosing and duration were similar between these patients (repeat group) and patients upon their first exposure (initial group, $n = 754$). Event rates in the repeat group were less than with those in the initial group for the composite endpoint (20% vs. 34%), new thrombosis (3.6% vs. 11.1%), and major bleeding (3.6% vs. 6.6%). The patients reexposed to argatroban had no allergic reactions or apparent differences, relative to the initial group, in adverse experiences (Lewis et al., 2000).

G. Discussion of Prospective Studies of Argatroban in HIT

Consistently in these studies, argatroban therapy, compared with historical controls, produced significant benefits in clinical outcomes in patients having HIT with or without thrombosis. Argatroban, versus control, was effective in reducing the all-cause composite of death, amputation, or new thrombosis as well as the thrombosis-related composite of death due to thrombosis, amputation secondary to HIT-associated thrombosis, or new thrombosis; lowering mortality from thrombosis and preventing new thrombotic events—without increasing bleeding.

Study patients had clinically diagnosed HIT, and laboratory confirmation of HIT was not required for their treatment. This study design simulated the "real world" of managing HIT, wherein guidelines recommend initiating alternative anticoagulation upon strong clinical suspicion, without delay for laboratory confirmation of HIT (Warkentin and Greinacher, 2004). In ARG-911, HIT antibodies were demonstrated in most, but not all, patients. Argatroban therefore is an effective antithrombotic agent in clinically diagnosed, albeit not always laboratory-confirmed, HIT. Argatroban also provided effective anticoagulation in patients with a history of HIT who required acute anticoagulation for a variety of indications.

Across the studies, the overall major bleeding rate was 6% in argatroban-treated patients, similar to that (7%, $p = 0.74$) in the control (Lewis et al., 2006), and no patient experienced intracranial hemorrhage while on argatroban therapy. By indirect comparison, major bleeding associated with lepirudin therapy in HIT is 17.6% (Lubenow et al., 2005). However, these DTIs remain to be compared directly, and conclusions about their relative safety profiles cannot be reached. Argatroban was well tolerated upon reexposure.

These studies supported the approval of argatroban as an anticoagulant for the prophylaxis or treatment of thrombosis in patients with HIT, with the per-protocol dosing regimen adopted as the recommended dosing schedule. As practical experience with argatroban has increased during the past several years, some refinements to its dosing and monitoring regimen have been suggested, as discussed in the following section, which continue to optimize its safety in this setting.

IV. PRACTICAL ASPECTS OF ARGATROBAN DOSING AND MONITORING

A. Duration of Therapy

Because of the persistent high (38% to 76%) risk of thrombosis for at least a month after onset of HIT (Hirsh et al., 2004), nonheparin anticoagulation should be maintained for at least 4 wk (Davoren and Aster, 2006; Arepally and Ortel, 2006). A longer duration, e.g., 3–6 mo, should be considered after an episode of HIT-associated thrombosis. According to treatment guidelines, anticoagulation with an alternative parenteral agent such as argatroban should be continued at least until platelet count recovery (a count of at least 100×10^9/L, preferably 150×10^9/L) and further overlapped with warfarin for a minimum of 5 days and until warfarin effects have been therapeutic for at least 2 days (Warkentin and Greinacher, 2004). These guidelines are important to ensure continuous anticoagulation and to avoid prothrombotic effects of initiating warfarin during acute HIT, e.g., warfarin-induced venous limb gangrene or skin necrosis syndromes (see Chapter 2). In argatroban-treated patients with HIT in study Argatroban-915, mean platelet counts were $>100 \times 10^9$/L after 2 days of therapy and $>150 \times 10^9$/L after 4 days of therapy (Lewis et al., 2003).

B. Dosing and Dosage Adjustments

For prophylaxis or treatment of thrombosis in HIT, the recommended initial dose of argatroban is 2 μg/kg/min (Table 4). Because hepatic impairment decreases argatroban clearance, a reduced initial dose, i.e., 0.5 μg/kg/min, is recommended for patients with at least moderate hepatic impairment, defined as a Child-Pugh score >6 (Swan and Hursting, 2000). This reduced initial dose is also appropriate for patients with hepatic impairment as defined using the routine laboratory measure of

TABLE 4 Dosing Schedules for Argatroban Treatment of Patients with HIT (Approved Indications)

Clinical use	Bolus[a]	IV infusion	Monitoring and adjusting therapy
Prophylaxis or treatment of thrombosis[b,c]	–	2 μg/kg/min (For hepatically impaired patients, reduce initial dose.[d] Patients with renal insufficiency require no initial dosage adjustment.)	Dose adjusted (not to exceed 10 μg/kg/min) to achieve steady state aPTT 1.5–3.0 times the baseline value (not to exceed 100 s)[e,f,g]
PCI[h,i]	350 μg/kg (given over 3–5 min)	25 μg/kg/min	Infusion dose adjusted (15–40 μg/kg/min) to achieve an ACT 300–450 s; additional bolus doses of 150 μg/kg may be given as needed[j,k]

[a]Based on patient's body weight.
[b]Includes patients with active HIT who have isolated thrombocytopenia or associated thrombosis, as well as patients with a documented history of HIT who are no longer thrombocytopenic but require anticoagulation.
[c]Argatroban is approved in the United States as an anticoagulant for prophylaxis or treatment of thrombosis in patients with HIT, and is also available for use in Austria, Canada, Denmark, Iceland, Germany, The Netherlands, Norway, and Sweden as an anticoagulant in HIT.
[d]For patients with moderate hepatic impairment, an initial dose of 0.5 μg/kg/min is recommended.
[e]The aPTT should be checked at least 2 h after the initiation of argatroban or any dosage change.
[f]For patients in studies ARG-911 and ARG-915, the mean ± SEM dose of argatroban was 1.9 ± 0.1 μg/kg/min.
[g]For transferring a patient to warfarin anticoagulant therapy: After substantial resolution of thrombocytopenia, initiate warfarin therapy using the expected daily dose of warfarin (do not use a loading dose) while maintaining argatroban infusion. At least 5 days of warfarin therapy are required to lower functional prothrombin concentrations to a therapeutic, steady state level. For monitoring the conversion to warfarin during coadministration of argatroban at doses up to 2 μg/kg/min, see text and Figure 6.
[h]Argatroban is approved in the United States as an anticoagulant in patients with or at risk for HIT undergoing PCI. Argatroban has not been evaluated in hepatically impaired patients undergoing PCI. These recommendations do not consider the combination use of argatroban with glycoprotein IIb/IIIa antagonists, wherein lower doses of argatroban (e.g., 250–300 μg/kg bolus followed by infusion of 15 μg/kg/min) have been shown to provide effective anticoagulation with an acceptable bleeding risk (Jang et al., 2004).
[i]Includes percutaneous transluminal coronary angioplasty (balloon angioplasty), stent implantation, and atherectomy; oral aspirin 325 mg should be given 2–24 h prior to PCI.
[j]The ACT should be checked 5–10 min following the initial bolus dose and after any additional bolus dose or change in the infusion rate. In studies ARG-216, ARG-310, and ARG-311, the majority of patients required only one bolus dose during the interventional procedure, and the mean ± SEM dose of argatroban was 23.1 ± 0.7 μg/kg/min.
[k]After the procedure, the sheaths should be removed no sooner than 2 h after discontinuing argatroban and when the ACT is <160 s.
Abbreviations: ACT, activated clotting time; aPTT, activated partial thromboplastin time; HIT, heparin-induced thrombocytopenia; IV, intravenous; PCI, percutaneous coronary intervention.

total serum bilirubin (>1.5 mg/dL) (Levine et al., 2006) and for patients with combined hepatic and renal impairment (Williamson et al., 2004; Levine et al., 2006). A conservative, reduced initial dose may also be prudent for patients with heart failure, multiple organ system failure, severe anasarca, or postcardiac surgery, that is, conditions associated with increased hepatic congestion or fluid overload, and possibly decreased argatroban clearance (de Denus and Spinler, 2003; Reichert et al., 2003; Baghdasarian et al., 2004; Levine et al., 2006; Czyz et al., 2006; Koster et al., 2006). The effect of decreased cardiac output or hepatic congestion, particularly in

the absence of abnormal liver function tests, on argatroban pharmacokinetics remains to be prospectively studied.

No initial dosage adjustment is required in patients with renal impairment. Clinical data indicate that argatroban pharmacodynamics and pharmacokinetics are not affected by renal impairment, including severe insufficiency (Swan and Hursting, 2000; Murray et al., 2004; Tang et al., 2005). Creatinine clearance predicts the aPTT-adjusted argatroban dose across a range of renal functioning (Arpino and Hallisey, 2004; Guzzi et al., 2006), with multicenter data indicating that this effect is clinically insignificant, a 0.1 μg/kg/min decrease in dose for each 30 mL/min decrease in creatinine clearance (Guzzi et al., 2006).

The initial dose should be adjusted, as needed, to achieve a target aPTT 1.5–3 times the baseline value. The aPTT should be checked 2 h after initiating therapy or at dose adjustment. Because achievement of steady-state anticoagulation will be delayed in many patients with hepatic impairment, it would be prudent to check their aPTT after at least 4–5 h (Levine et al., 2007). The choice of aPTT reagent does not materially affect assessment of argatroban therapy (Francis and Hursting, 2005). Body mass index does not significantly affect the argatroban dose required to achieve therapeutic aPTTs, and no modification of dosing or monitoring is required for obese patients (Rice et al., 2007). Approximately one in six patients in study ARG-911 maintained their initial argatroban dose for the duration of therapy, indicating that dosage adjustment is often unnecessary (Verme-Gibboney and Hursting, 2003). When dosage adjustment is necessary, the patient's current dose, aPTT, and clinical status (e.g., hepatic function) should be considered. A reasonable increment for most patients is 0.5 μg/kg/min. Smaller increments (e.g., 0.25 μg/kg/min) are appropriate when dosing is already reduced for reasons such as hepatic impairment (Verme-Gibboney and Hursting, 2003). At substantially higher argatroban doses, such as used during PCI, increments of 5 μg/kg/min are recommended (see Sec. V.A). In HIT patients with elevated baseline aPTT due to antiphospholipid antibody syndrome, the successful use of weight-based, fixed-dose argatroban without laboratory monitoring has been reported (Pendleton et al., 2006).

In recent reports of argatroban-treated patients with or at risk of HIT in single centers (Arpino and Hallisey, 2004; Smythe et al., 2005; Kiser et al., 2005; Kodityal et al., 2006) or in a multicenter registry (Bartholomew et al., 2007; Rice et al., 2007), mean or median doses of 0.5–1.2 μg/kg/min yielded target aPTTs. The reasons for the lower dose requirement in these patients, as compared with the ARG-911 and ARG-915 patients, remain unclear. Possibilities include the enrichment of particular patient types, e.g., cardiac surgery patients or patients with hepatic insufficiency, in certain centers; increasing physician preference to target the lower range of therapeutic aPTTs; and/or simply the reflection of more "real world" (outside the bounds of clinical trials) or contemporary experiences. Regardless of the use of a more conservative or less conservative initial dose, the infusion should be adjusted as needed according to the patient's aPTT response. Importantly, argatroban should be initiated upon strong suspicion of HIT, and not delayed pending laboratory diagnostic tests, to reduce thrombotic consequences (Warkentin and Greinacher, 2004) and associated healthcare costs (Arnold et al., 2006).

C. Conversion to Warfarin Anticoagulation

Because argatroban is a DTI, its concomitant use with warfarin prolongs the PT/INR beyond that produced by warfarin alone (Hursting et al., 1999; Sheth et al.,

2001). In the clinical studies of argatroban therapy in HIT, the majority of patients transferred to warfarin therapy for continued anticoagulation, although the method of transition was not specified in the protocols. Even in the absence of guidelines, there was no evidence of systematic underdosing or overdosing of warfarin (Hursting et al., 2005). INRs > 5 commonly occurred during argatroban monotherapy and argatroban/warfarin cotherapy, without major bleeding (Hursting et al., 2005), and among patients with INRs > 4 while on combined argatroban $\leq 2\,\mu g/kg/min$ and warfarin therapy, the thrombotic risk remained greater than the bleeding risk (Bartholomew and Hursting, 2005).

Guidelines for monitoring the transition from argatroban to warfarin using the INR are published (Sheth et al., 2001). The relationship between the INR on cotherapy and the INR on warfarin monotherapy can be used to interpret the INR during the transition period. Specifically, INR on cotherapy increases linearly with the INR on warfarin monotherapy, with the relationship sensitive to the argatroban dose and thromboplastin reagent used, particularly, its International Sensitivity Index (ISI). For argatroban 1–2 μg/kg/min, prediction errors for monotherapy INRs from cotherapy INRs are sufficiently low (±0.4 units) to allow for clinically reliable estimations of a monotherapy INR from a cotherapy INR. For most combinations of argatroban dose 1–2 μg/kg/min and commercial thromboplastins, a cotherapy INR > 4 predicts a monotherapy INR between approximately 2.0 and 3.0, i.e., in the therapeutic range for warfarin monotherapy. In general, after at least 4–5 days of coadministration of warfarin and argatroban at doses up to 2 μg/kg/min, argatroban can be discontinued when the cotherapy INR is >4 (and ideally has been for 2 days). Upon cessation of argatroban, the INR should be checked 4–6 h later, when the effect of argatroban is negligible, to ensure an actual therapeutic value reflective of warfarin monotherapy. For coadministration of warfarin and argatroban at doses >2 μg/kg/min, the argatroban dose should be temporarily (4–6 h) reduced to 2 μg/kg/min. Then the procedure for predicting the warfarin monotherapy INR from the cotherapy INR at doses up to 2 μg/kg/min can be followed. These guidelines are summarized in Figure 6.

Factor assays that are insensitive to argatroban interference, such as the two-stage chromogenic factor X assay, may also be useful for monitoring the transition (Hoppensteadt et al., 1997; Sheth et al., 2001). A chromogenic factor X level of 45% or less is a reliable predictor that the INR will be therapeutic when argatroban therapy is discontinued (Arpino et al., 2005).

D. Conversion to Phenprocoumon or Acenocoumarol Anticoagulation

The pharmacologic interactions between argatroban and the oral anticoagulants phenprocoumon or acenocoumarol are comparable to those described for argatroban and warfarin. Guidelines for the conversion from argatroban to phenprocoumon or acenocoumarol are similar to those for the conversion to warfarin (Harder et al., 2004).

V. ARGATROBAN FOR HIT PATIENTS IN SPECIAL CLINICAL CIRCUMSTANCES

A. Percutaneous Coronary Intervention

Clinical Studies

Argatroban has been evaluated in three multicenter, open-label prospective studies in patients with or at risk of HIT undergoing PCI, including percutaneous

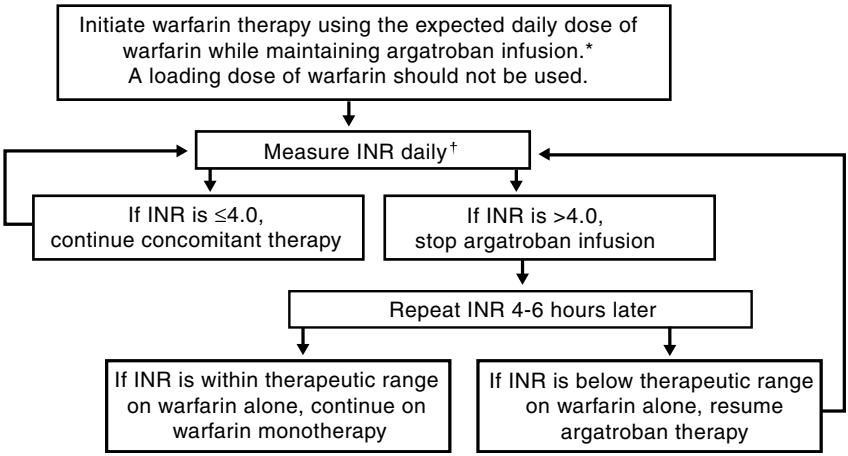

FIGURE 6 Guidelines for conversion from argatroban to oral anticoagulant therapy with warfarin. Warfarin should be initiated only once there has been substantial resolution of the thrombocytopenia, and beginning only with anticipated maintenance doses (≤5 mg/day). Argatroban and warfarin should be overlapped for at least 5 days before discontinuing argatroban. Ideally, the INR should be within the target therapeutic range (i.e., >4.0 during cotherapy for argatroban doses up to 2 μg/kg/ min) for at least the last 2 days of overlap. *For argatroban infusion at ≤2 μg/kg/min, the INR on monotherapy may be estimated from the INR on cotherapy. †If the dose of argatroban is >2 μg/kg/min, temporarily reduce to a dose of 2 μg/kg/min 4–6 h prior to measuring the INR. *Abbreviation*: INR, international normalized ratio.

transluminal coronary angioplasty, stent implantation, or rotational atherectomy. The studies (ARG-216, ARG-310, and ARG-311) were similar in design with respect to eligibility criteria, argatroban dosing regimen, and main outcome assessments, and their pooled analysis has been reported (Lewis et al., 2002). Overall, 91 patients with or at risk of HIT underwent 112 PCIs on argatroban anticoagulation. Patients received 325 mg oral aspirin 2–24 h before PCI. In the catheterization laboratory, patients received iv argatroban at 25 μg/kg/min (initial bolus dose of 350 μg/kg) titrated to achieve an ACT of 300–450 s during PCI (mean infusion dose, 23 μg/kg/min). Additional bolus doses of 150 μg/kg to achieve or maintain the target ACT were allowed, though usually not needed. Target ACT values were achieved typically within 10 min of initiating argatroban and were maintained throughout the infusion. When argatroban was discontinued after the procedure, ACTs rapidly returned to baseline.

Primary efficacy endpoints were subjective assessment of the satisfactory outcome of the procedure and adequate anticoagulation, which occurred in 94.5% and 97.8%, respectively, of patients undergoing their initial PCI with argatroban ($n = 91$) (Table 5). Death (no patients), myocardial infarction (four patients), and revascularization at 24 h after PCI (four patients) occurred in seven (7.7%) patients. Other efficacy endpoints were also consistent with argatroban enabling a satisfactory outcome (Table 5). One patient (1%) experienced major periprocedural bleeding (nonfatal retroperitoneal hemorrhage). No unsatisfactory outcomes occurred during repeat PCIs with argatroban ($n = 21$; mean separation of 150 days from the initial PCI). Overall, the clinical outcomes compared favorably with those reported historically for heparin anticoagulation during PCI.

TABLE 5 Efficacy Assessments in Patients with or at Risk of HIT Undergoing PCI Using Argatroban Anticoagulation

	Number of patients with outcome/total *n* (%)	
Outcome	Initial group	Repeat group
Satisfactory outcome of procedure[a]	86/91 (94.5%)	21/21 (100%)
Adequate anticoagulation[a]	89/91 (97.8%)	21/21 (100%)
Lack of major acute complications[b]	89/91 (97.8%)	21/21 (100%)
Angiographic success[c]	86/88 (97.7%)	20/20 (100%)
Clinical success[d]	86/88 (97.7%)	20/20 (100%)

[a]Primary, subjective outcomes.
[b]No death, emergent coronary artery bypass graft surgery, or Q-wave myocardial infarction during argatroban infusion or 24 h of its cessation (or discharge, whichever came first).
[c]Final stenosis of <50% in at least one lesion attempted, for patients with angiographic data available.
[d]Angiographic success plus the lack of major acute complications.
Abbreviations: HIT, heparin-induced thrombocytopenia; PCI, percutaneous coronary intervention.

In a separate multicenter prospective study of 152 patients (including one patient with previous HIT) undergoing PCI, reduced doses of argatroban were evaluated in combination with the glycoprotein (GP) IIb/IIIa antagonists abciximab ($n = 150$) or eptifibatide ($n = 2$) (Jang et al., 2004). Patients received argatroban as an initial bolus of 250 or 300 μg/kg followed by an infusion of 15 μg/kg/min during PCI. An additional bolus of 150 μg/kg was administered if ACTs 5–15 min after initiating argatroban were <275 s. Median ACTs achieved were approximately 300 s. The primary efficacy composite endpoint of death (no patients), myocardial infarction (four patients), and urgent revascularization (two patients) at 30 days occurred in four (2.6%) patients overall. Two (1.3%) patients had major bleeding (one retroperitoneal, one groin hematoma). Although not specifically conducted in patients with or at risk of HIT, the results suggest that a reduced dose of argatroban may be appropriate if used in combination with GPIIb/IIIa inhibition during PCI.

Argatroban Dosing and Monitoring During PCI

For patients with or at risk for HIT undergoing PCI, argatroban should be started at an infusion dose of 25 μg/kg/min and a bolus of 350 μg/kg given over 3–5 min. The ACT should be checked 5–10 min after the bolus dose is completed. If the ACT is >300 s, the PCI may proceed. If the ACT is <300 s, an additional bolus dose of 150 μg/kg should be given and the infusion dose increased to 30 μg/kg/min. If, however, the ACT is >450 s after the initial bolus, then the infusion dose should be reduced to 15 μg/kg/min. After any additional bolus or dosage adjustment, the ACT should be checked again after 5–10 min to confirm the patient attained a therapeutic ACT. During a prolonged procedure, additional ACTs should be obtained every 20–30 min. For patients requiring anticoagulation after the procedure, argatroban infusion may be continued at a reduced dose such as that recommended for the prophylaxis or treatment of thrombosis in HIT.

These dosing recommendations do not take into consideration the possible combination use of GPIIb/IIIa antagonists. Lower doses of argatroban, e.g., bolus dose of 250–300 μg/kg followed by an infusion dose of 15 μg/kg/min, provide adequate anticoagulation with an acceptable bleeding risk in combination with GPIIb/IIIa antagonists in patients without HIT (Jang et al., 2004).

High doses of argatroban should be avoided in patients with or at risk of HIT who require PCI and have clinically significant hepatic disease, including laboratory evidence such as aspartate aminotransferase or alanine aminotransferase at least three times the upper limit of normal. Argatroban use during PCI has not been studied in such patients (Lewis et al., 2002; Jang et al., 2004).

Because argatroban equally prolongs the Hemochron ACT and HemoTec ACT (Iqbal et al., 2002), investigators in the PCI trials effectively used whichever ACT method was available at their sites to monitor anticoagulation (Lewis et al., 2002; Jang et al., 2004). Sheaths were removed when the ACT was less than 160 s.

B. Peripheral Intervention

Case reports describe the successful use of argatroban anticoagulation in patients with HIT during renal stent implant (Lewis et al., 1997) and carotid stent implant (Lewis et al., 1998). The argatroban dose and target ACT values were the same as those recommended for PCI in the absence of GPIIb/IIIa inhibition.

C. Hemodialysis

Argatroban administration by bolus alone, infusion alone, or bolus plus infusion has been evaluated in a prospective three-way cross-over study of 13 patients with end-stage renal disease who underwent a total of 38 hemodialysis sessions of 3- or 4-h duration (Murray et al., 2004). Although dialysis dose was effectively delivered using each regimen, the most satisfactory intradialysis anticoagulation was achieved using a steady-state infusion of argatroban (2 μg/kg/min begun approximately 4 h before dialysis), or a 250 μg/kg bolus dose at the start of dialysis followed by a continuous 2 μg/kg/min infusion. With those regimens, mean ACTs increased from 131 s at baseline to 200 and 197 s, respectively, after 60 min of dialysis. No dialysis membrane required changing, and one session was shortened by 15 min owing to circuit clotting. There were no thrombotic or bleeding events. Argatroban dialytic clearance was clinically insignificant. Although the study was conducted in patients without HIT, similar dosing regimens may be adequate for inpatients with HIT already at steady-state argatroban levels or outpatients with a history of HIT who require hemodialysis.

The pharmacokinetics, pharmacodynamics, and safety of argatroban during a single renal placement therapy session have been prospectively evaluated in five hospitalized patients with or at risk of HIT (Tang et al., 2005). The patients underwent hemodialysis ($n = 4$) or continuous venovenous hemofiltration ($n = 1$) while receiving continuous iv argatroban 0.5–2 μg/kg/min. ACTs, aPTTs, and plasma argatroban concentrations remained stable during the renal replacement therapy, and hemodialysis was effective as evidenced by the urea reduction ratio. No patient experienced bleeding or thrombosis. Argatroban clearance by the high-flux membranes was clinically insignificant, indicating that in patients with renal failure receiving argatroban therapy for HIT, no dosage adjustment is needed during renal replacement therapy.

A retrospective study evaluated the safety, outcomes, and argatroban dosing patterns in HIT patients requiring renal replacement therapy (Reddy et al., 2005). The patients ($n = 47$) were identified from the prospective ARG-911 and ARG-915 studies of argatroban in HIT. Patients with comorbid hepatic impairment required median lower doses (0.7 μg/kg/min) than patients without hepatic impairment (1.7 μg/kg/min). Two (4%) patients experienced thrombosis while on argatroban.

Major bleeding occurred in three (6%) of 50 treatment courses within a 37-day follow-up period, generally similar to reported bleeding rates in argatroban-treated patients with HIT, irrespective of their renal function. Overall, argatroban provided effective anticoagulation and was well tolerated in this setting, upon initial and repeat administration.

Experience with argatroban in pediatric patients during hemodialysis is limited. A literature analysis identified five pediatric patients with HIT or a history of HIT who underwent hemodialysis using argatroban anticoagulation (Hursting et al., 2006). No patient experienced systemic thrombosis while on argatroban, one patient experienced clotting in the dialysis circuit, and one patient had bleeding that was multifactorial in origin. More recently, a pediatric patient with HIT and thrombosis has been described who underwent continuous renal replacement therapy and extracorporeal membrane oxygenation (ECMO) successfully while on argatroban therapy (Scott et al., 2006).

Argatroban is approved in Japan as an anticoagulant for hemodialysis in patients with congenital or acquired antithrombin deficiency. The recommended dose is generally similar to that used in HIT patients undergoing hemodialysis (Matsuo et al., 1988, 1990; Koide et al., 1995; Tang et al., 2005; Reddy et al., 2005).

D. Stroke

The effect of argatroban anticoagulation on stroke in HIT has been retrospectively evaluated using case records from the prospective studies of argatroban in HIT (LaMonte et al., 2004b). Stroke was present at or within 37 days of study entry in 30 (3.1%) of 960 patients overall. Compared with control therapy, argatroban significantly reduced the risk of new stroke (odds ratio = 0.31, 95% CI, 0.10–0.96, $p = 0.041$) and stroke-associated mortality (odds ratio = 0.18, 95% CI, 0.03–0.92, $p = 0.039$), without increasing intracranial hemorrhage. Of 35 strokes, 33 (94%) were ischemic, with one hemorrhagic stroke in each group. Stroke occurred most often in females, in patients with more severe thrombocytopenia, and within 2 wk of HIT presentation.

In a randomized, double-blind clinical study of patients treated with argatroban versus placebo within 12 h of ischemic stroke onset, there were no significant between-group differences in intracranial hemorrhage or major bleeding rates (LaMonte et al., 2004a). In an ongoing open-label, dose escalation study of argatroban in combination with recombinant tissue plasminogen activator in acute stroke, safety was within acceptable limits in the first treated cohort, and efficacy for producing fast, complete recanalization was promising (Sugg et al., 2006). Although not conducted in patients with HIT, these studies further support the safety of argatroban anticoagulation in patients with stroke. Argatroban is approved for use in nonlacunar stroke in Japan and Korea.

E. Cardiovascular Surgery or ECMO

Case reports describe patients with HIT or a history of HIT in whom argatroban anticoagulation has been used successfully during cardiopulmonary bypass (CPB) (Edwards et al., 2003; Martin et al., 2005), peripheral vascular surgery (Tokuda et al., 2003), carotid endarterectomy (Hallman et al., 2005), and off-pump coronary artery bypass surgery (Arnoletti and Whitman, 1999; Ide et al., 2001; Kieta et al., 2003; Ohno et al., 2003). Prolonged coagulopathy following CPB (Gasparovic et al., 2004), a high bleeding risk in pediatric patients undergoing CPB (Hursting et al., 2006),

and thrombosis during off-pump cardiac surgery (Cannon et al., 2004) have also been reported. Dosing guidelines for argatroban use during adult cardiac surgery that have been proposed based on retrospective analysis of 21 published cases suggest an initial dose of 5 μg/kg/min (and a 100 μg/kg bolus for on-pump surgery), adjusted to target ACTs of 300–500 s for off-pump surgery and 400–600 s for on-pump surgery, with the ACT checked every 15 min (Martin et al., 2007). A safe, effective dose however has not been prospectively established.

For patients with previous HIT who undergo cardiac surgery using brief heparin exposure, treatment guidelines recommend using a nonheparin agent for perioperative anticoagulation (Warkentin and Greinacher, 2004). Reduced doses of argatroban may be needed following cardiac surgery because of possible decreased hepatic perfusion (Reichert et al., 2003; Czyz et al., 2006; Koster et al., 2006).

In vitro studies indicate that argatroban may be more efficacious than heparin in preventing thrombin generation in ECMO circuits (Young et al., 2004). The successful use of argatroban anticoagulation in patients with or at risk of HIT undergoing ECMO has been described, including an adult patient (Johnston et al., 2002) and 12 pediatric patients (Hursting et al., 2006). ECMO was continued in one of the pediatric patients, a neonate, for 78 days (Kawada et al., 2000). In each case, ACTs were typically maintained >180 s, although further study is required to establish dosing recommendations.

F. Other Hypercoagulability States

Argatroban 2 μg/kg/min has been used successfully in a patient with burn-related severe acquired antithrombin deficiency who failed heparin (Gorman et al., 2001). However, no formal studies have been conducted. Argatroban is approved in Japan as anticoagulation for hemodialysis in patients with congenital or acquired antithrombin deficiency.

The effective use of DTIs, including argatroban, has been described in patients with disseminated intravascular coagulation (DIC), including patients with low levels of antithrombin or with suspected HIT (Kumon et al., 1984; Mukundan and Zeigler, 2002). The data, albeit limited, provide evidence that argatroban can improve DIC, and also that DIC in a patient with HIT should not preclude use of argatroban.

G. Pregnant or Nursing Women

Argatroban anticoagulation in pregnant or nursing women has not been studied. Teratology studies in rats reveal no evidence of impaired fertility or fetal harm due to argatroban (Argatroban Prescribing Information, U.S., 2002). Because animal reproductive studies are not always predictive of human response, it is recommended that the drug be used during pregnancy only if clearly needed. The successful use of argatroban therapy for 21 days during the second trimester of pregnancy in a woman with HIT and thrombosis has been described (Francis, 2004).

Argatroban is detected in rat milk (Iida et al., 1986). It is unknown whether argatroban is excreted in human milk, although many drugs are. Hence, it is recommended that a decision be made either to discontinue nursing or discontinue the drug.

H. Geriatric or Pediatric Patients

The pharmacokinetic parameters of argatroban are similar between young adults and elderly volunteers (Swan and Hursting, 2000), and no dosage adjustment is

required for the elderly. The effectiveness of argatroban in HIT was not influenced by patient age (range, 17 to 91 yr) in the ARG-911 and ARG-915 studies (Lewis et al., 2001, 2006). Age was also not a significant factor determining therapeutic argatroban doses or thrombotic risk for elderly patients aged 65 to 93 yr in a multicenter HIT registry (Bartholomew et al., 2007).

A literature review has described 34 pediatric patients aged 1 wk to 16 yr, most with or at risk of HIT, administered argatroban for prophylaxis or treatment of thrombosis or during a variety of procedures, including cardiac catheterization, hemodialysis, ECMO or ventricular assist device support, and CPB (Hursting et al., 2006). Argatroban generally provided therapeutic levels of anticoagulation; by exception, the bleeding risk during CPB was unacceptably high. A prospective study of argatroban in pediatric patients requiring anticoagulant alternatives to heparin has been conducted in the United States, and results are under regulatory review.

VI. CONCLUSION

Argatroban, a synthetic DTI, is an effective anticoagulant with a predictable dose-response effect. This agent offers several theoretical advantages as an anticoagulant for patients with HIT: it inhibits free and bound thrombin, it does not cross-react with HIT antibodies, and its anticoagulant effects are rapidly active and also rapidly reversible. Further, upon prolonged or repeated administration, argatroban is well tolerated, with no alteration in anticoagulant response and no induction of drug-specific antibodies.

In clinical studies, argatroban therapy, compared with historical controls, improves outcomes of HIT, particularly thrombosis and its sequelae, without increasing bleeding risk. Argatroban also provides safe and effective anticoagulation in patients with a history of HIT requiring acute anticoagulation. No intracranial hemorrhage has occurred during argatroban infusion in over 900 patients with HIT, including many with stroke, who have received argatroban during clinical trials. These benefits are achieved when argatroban is administered iv at 2 μg/kg/min, titrated to achieve an aPTT 1.5–3.0 times baseline. Although no initial dosage adjustment is required for patients with renal impairment, an initial dose of 0.5 μg/kg/min is recommended for hepatically impaired patients and may be prudent in patients with conditions associated with hepatic congestion. Also in clinical studies, argatroban at higher doses (25 μg/kg/min, titrated to achieve an ACT of 300–450 s) provides safe and adequate anticoagulation during PCI in patients with or risk of HIT. Lower doses of argatroban in combination with a GPIIb/IIIa antagonist also provide safe and adequate anticoagulation during PCI. The recommended dosing schedules for the approved uses of argatroban in the United States, i.e., prophylaxis or treatment of thrombosis in HIT and during PCI for patients with or at risk for HIT, are summarized in Table 4.

Patients with or at risk of HIT have also successfully undergone hemodialysis using argatroban anticoagulation, and although not studied in prospective clinical trials, peripheral intervention, cardiovascular surgery, and ECMO. Argatroban therefore offers a versatile therapeutic option for the management of patients with or at risk of HIT in diverse clinical settings.

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16 Bivalirudin for the Treatment of Heparin-Induced Thrombocytopenia

John R. Bartholomew
Cleveland Clinic, Cleveland, Ohio, U.S.A.

I. INTRODUCTION

The treatment for heparin-induced thrombocytopenia (HIT) has undergone important changes over the past decade. Until recently, clinicians had very few options for treating this potentially devastating syndrome. Fortunately, with the development of several contemporary anticoagulants, physicians now have a number of novel treatment options. These include danaparoid, an indirect (antithrombin-dependent) factor Xa inhibitor (see Chapter 13) and the direct thrombin inhibitors (DTIs), including recombinant hirudin (r-hirudin) (e.g., lepirudin) (see Chapter 14) and the small molecule DTI, argatroban (Alving, 2003; Chong, 2003; Warkentin, 2003) (see Chapter 15). The synthetic pentasaccharide (fondaparinux), another indirect factor Xa inhibitor, has also been used "off-label" to treat HIT (Spinler, 2006).

More recently, another DTI, bivalirudin (Angiomax), has been approved in the United States for use in HIT, but only for those patients undergoing percutaneous transluminal coronary angioplasty (PTCA). Bivalirudin is also approved in the United States, Canada, New Zealand, Israel, Argentina, and the 25 members of the EU for use as an anticoagulant in patients undergoing PTCA. In the EU it is marketed under the trade name Angiox™ (Warkentin and Koster, 2005). It too has been used "off-label" for HIT, especially in interventional settings (e.g., cardiac surgery) and in patients with combined renal and hepatic dysfunction.

Bivalirudin is a hirulog, i.e., one of a group of drugs designed from the structure of hirudin (analogue of hirudin). It was developed in the early 1990s by the Biogen Corporation (Cambridge, MA, USA), and was originally known as BG8967 or Hirulog. The U.S. Food and Drug Administration (FDA) mandated a name change to avoid confusion with Humalog (recombinant human insulin) when The Medicines Company (Parsippany, NJ, USA) acquired licensure for bivalirudin in 1997. The name was then changed to Angiomax. Currently, the major indications for bivalirudin are for use in patients with unstable angina undergoing PTCA and also with provisional glycoprotein (GP) IIb/IIIa receptor inhibitor treatment to reduce acute ischemic events in select patients undergoing percutaneous coronary intervention (PCI).

Bivalirudin has also been used with favorable results in both "on-pump" and "off-pump" cardiac surgery cases in patients with and without HIT. A clinical trial completed by Merry and colleagues (2004) in New Zealand compared bivalirudin with unfractionated heparin (UFH) (with protamine reversal) in non-HIT patients requiring off-pump coronary artery bypass (OPCAB) surgery. Favorable results, including improved graft patency and comparable hemorrhage and transfusion requirements, led to two subsequent multicenter trials. The *C*ABG

HIT/TS ***O***n- and ***O***ff-Pump ***S***afety and ***E***fficacy (CHOOSE-ON and CHOOSE-OFF studies for patients with HIT, and the ***EV***aluation of Patients during coronary artery bypass graft ***O***perations: ***L***inking ***UT***ilization of bivalirudin to ***I***mproved ***O***utcomes and ***N***ew anticoagulation strategies (EVOLUTION-OFF and EVOLUTION-ON) trials, were conducted to evaluate the safety and efficacy of bivalirudin as an alternative to UFH (and protamine reversal) in the HIT and non-HIT settings, respectively. To date, results of these studies (Dyke et al., 2006, 2007; Smedira et al., 2006; Koster et al., 2007) have revealed comparable safety and efficacy endpoints.

II. BIVALIRUDIN

A. Chemistry

Bivalirudin is a small synthetic 20-amino-acid peptide that is a specific and reversible inhibitor of thrombin (Parry et al., 1994) (Fig. 1). Although it is an analogue of hirudin, its amino acid sequence is considerably shorter. Bivalirudin unites a carboxy-terminal segment of 12 amino acids (dodecapeptide) derived from native hirudin (residues 53–64), plus a sulfated tyrosine at position 63, to an active site-binding tetrapeptide sequence (D-Phe-Pro-Arg-Pro) at its amino terminal (Maraganore et al., 1990; Nawarskas and Anderson, 2001; White and Chew, 2002). Four glycine residues bridge these two segments together. The amino-terminal segment has a high affinity and specificity for binding to the active site of thrombin (Fareed et al., 1999; Sciulli and Mauro, 2002), while the carboxy terminal binds to the fibrinogen recognition site of thrombin at exosite 1 (Thiagarajan and Wu, 1999; Reed and Bell, 2002). One difference between bivalirudin and hirudin is that the binding of bivalirudin to the active site of thrombin is transient, whereas with lepirudin, irreversible thrombin-hirudin complexes are formed (Weitz and Hirsh, 1998; Nawarskas and Anderson, 2001).

Bivalirudin is produced by solid phase peptide synthesis (Maraganore et al., 1990). Its molecular mass is 2180 Da. Bivalirudin has no structural similarity to heparin.

B. Pharmacology

Bivalirudin is a bivalent DTI, i.e., it binds two distinct regions of thrombin: the active (catalytic) site and the fibrinogen-binding site. Moreover, like lepirudin and argatroban, bivalirudin binds to both free (soluble) and clot-bound (fibrin-bound) thrombin. It forms a 1:1 stoichiometric complex that neutralizes thrombin during coagulation and thrombus formation (Maraganore and Adelman, 1996). Thus, bivalirudin inhibits proteolytic cleavage of fibrinogen, thrombin-mediated activation of factors V, VIII, and XIII, and thrombin-induced platelet activation.

Bivalirudin (unlike lepirudin) is a *reversible* inhibitor of thrombin (Fig. 1). It acts initially as a non-competitive inhibitor, rendering thrombin inactive. Circulating proteases (including other thrombin molecules) slowly cleave bivalirudin near the amino-terminal end (between arg_3-pro_4), thus eventually releasing the amino-terminal segment from the active site region of thrombin (Bates and Weitz, 1998; Carswell and Plosker, 2002; Reed and Bell, 2002; Sciulli and Mauro, 2002). This allows thrombin to resume catalytic function.

As mentioned, bivalirudin also inhibits thrombin by the binding of its carboxy-terminal segment to the fibrinogen-binding site on thrombin. This occurs at the same time that the amino-terminal segment attaches to the active site, thus resulting in

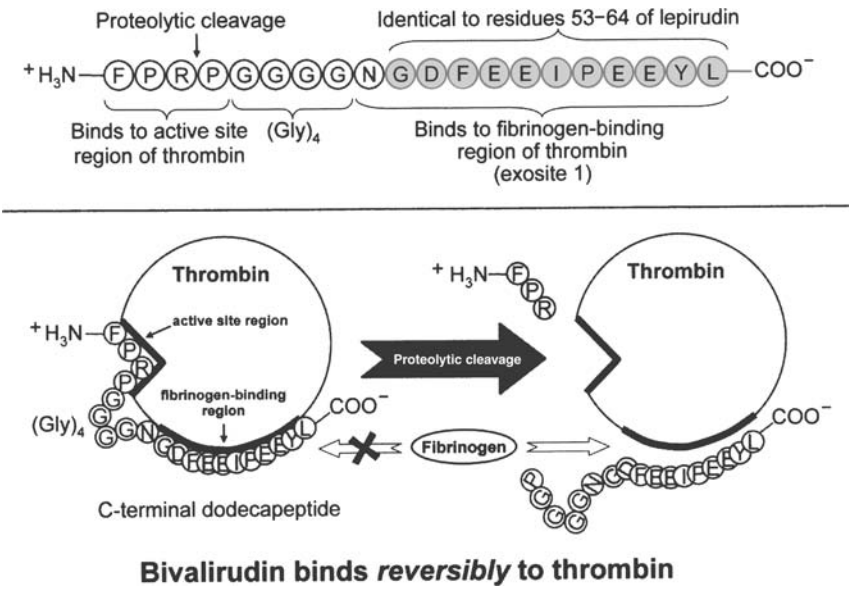

FIGURE 1 The structure of bivalirudin. (*Top*) Bivalirudin is comprised of 20 amino acids, with an N-(amino-)terminal D-Phe-Pro-Arg-Pro (F-P-R-P) region that binds with high affinity to the active site region of thrombin; a $(gly)_4$ (G4) "spacer" region; and a C-(carboxy-)terminal Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu dodecapeptide (N-G-D-F-E-E-I-P-E-E-Y-L) that binds to the fibrinogen-binding region (exosite 1) of thrombin. The 11 C-terminal amino acids (*shaded circles*) correspond exactly to the 53- to 64-amino-acid sequence of lepirudin. Highly-specific, non-competitive binding between bivalirudin and thrombin results. (Not shown is the heparin-binding region [exosite 2] of thrombin.) However, proteases (including other thrombin molecules [not shown]) can cleave the Arg_3-Pro_4 of bivalirudin, leading to loss of antithrombin activity. (*Bottom*) Initially, there is bivalent binding of bivalirudin to thrombin, as shown. Following cleavage at Arg_3-Pro_4, the N-terminal sequence of bivalirudin no longer binds to thrombin, leaving the residual C-terminal dodecapeptide with greatly reduced binding affinity for exosite 1 of thrombin. Thus, the bivalirudin remnant transforms to a competitive inhibitor of thrombin. Other substrates, e.g., fibrinogen, can compete with, and displace, bivalirudin, thus allowing thrombin to resume its prohemostatic functions. *Abbreviations*: Arg (R), arginine; Asn (N), asparagine; Asp (D), aspartic acid; Glu (E), glutamic acid; Gly (G), glycine; Ile (I), isoleucine; Leu (L), leucine; Phe (F), phenylalanine; Pro (P), proline; Tyr (Y), tyrosine.

dual blockage with complete inhibition of thrombin's multiple activities (Sciulli and Mauro, 2002). Once the amino-terminal moiety of bivalirudin is cleaved, however, the carboxy-terminal region acquires low-affinity, weakly competitive binding properties. Fibrinogen can now displace the bivalirudin remnant from thrombin and align itself over the active site to be converted to fibrin (Parry et al., 1994).

Bivalirudin is not inactivated by platelet factor 4 (PF4), nor does it require any cofactor for its activity. It does not bind to red blood cells or proteins other than thrombin.

C. Pharmacokinetics

Bivalirudin has predictable pharmacokinetics and exhibits a linear dose-response relationship when given by the intravenous (iv) route to healthy volunteers with

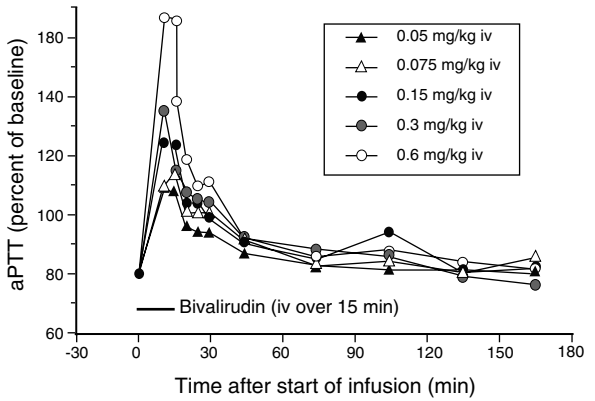

FIGURE 2 Prolongation of the aPTT by increasing doses of bivalirudin. Bivalirudin was given by iv infusion over 15 min to five groups (four subjects each) in doses ranging from 0.05 mg/kg per 15 min up to 0.6 mg/kg per 15 min. Each series of data points represents the mean of four study subjects. *Abbreviation*: aPTT, activated partial thromboplastin time. *Source*: From Fox et al., 1993.

normal renal function. Its half-life is approximately 25 min (Fox et al., 1993; Robson, 2000; Robson et al., 2002; The Medicines Company, 2005). Peak bivalirudin plasma concentrations after a 15 min iv infusion are related to dose and occur within 5 min of completing the infusion (Fig. 2).

Bivalirudin has a volume of distribution of 0.24 L/kg and a clearance rate of approximately 3.4 mL/min/kg (Fox et al., 1993). It is cleared from plasma by both renal mechanisms and cleavage by plasma proteases. Bivalirudin undergoes glomerular filtration, secretion in the proximal convoluted tubule, and reabsorption in the distal convoluted tubule. The peptides are then further degraded within the intracellular lysosomes (Robson, 2000; Robson et al., 2002). In a study by Fox and colleagues (1993), only 20% of bivalirudin was recovered in the urine.

Clearance of bivalirudin is accomplished predominately by proteolytic cleavage within plasma and elsewhere and accounts for approximately 80% of the drug's metabolism (Fox et al., 1993; Scatena, 2000; Robson et al., 2002; Warkentin and Greinacher, 2003). Indeed, proteolysis of bivalirudin appears to result mainly from thrombin, thus providing a mechanism of degradation that is independent of specific organ function (Bates and Weitz, 2000; Koster et al., 2002a,b). This results in degradation to individual amino acids and small, inactive peptide fragments (Carswell and Plosker, 2002).

Patients with renal insufficiency may need dose adjustments for bivalirudin, according to their degree of impairment (Table 1). In a study of 45 patients with normal to severe renal disease, Robson (2000) found that patients with normal kidneys (glomerular filtration rate [GFR] > 90 mL/min) and mildly impaired renal disease (GFR = 60–89 mL/min) had similar renal clearance levels and required no dose adjustments. The clearance rate was reduced by 45% in individuals with moderate renal impairment (GFR = 30–59 mL/min) and by 68% in persons with severe renal impairment (GFR < 30 mL/min). In dialysis-dependent patients, the clearance rate was reduced by 77% (Robson, 2000; Robson et al., 2002). The half-life of bivalirudin in patients with severe renal impairment is prolonged (about 1 h) and in dialysis patients the half-life is approximately 3.5 h (Nawarskas and Anderson, 2001).

Some investigators have suggested that dose reductions might be considered in patients with moderate or severe kidney dysfunction, including those on dialysis (Irvin et al., 1999; Robson, 2000; Robson et al., 2002). However, in the setting of

TABLE 1 Bivalirudin Pharmacokinetic Parameters in Patients with Renal Impairment

Renal function (glomerular filtration rate, mL/min)	Bivalirudin clearance (mL/min/kg)	Half-life (min)
Normal renal function (>90 mL/min)	3.4	25
Mild renal impairment (60–89 mL/min)	3.4	22
Moderate renal impairment (30–59 mL/min)	2.7	34
Severe renal impairment (10–29 mL/min)[a]	2.8	57
Dialysis-dependent (while off dialysis) patients[b]	1.0	210

[a]For PCI, consider reduction in bivalirudin infusion rate to 1.0 mg/kg/h (initial bolus unchanged).
[b]For PCI, reduce bivalirudin infusion rate to 0.25 mg/kg/h (initial bolus unchanged).
Source: Robson, 2000; Robson et al., 2002; and The Medicines Company, 2005.

PTCA, the current package insert states that no dose reduction in the bolus is required, and that only in those individuals with a creatinine clearance <30 mL/min or on hemodialysis should a reduction in the infusion dose be considered (The Medicines Company, 2005) (Table 1).

D. Pharmacodynamics

Bivalirudin produces an immediate effect after iv administration. It causes prolongation of the prothrombin time (PT)/international normalized ratio (INR), activated clotting time (ACT), the activated partial thromboplastin time (aPTT), and the thrombin time (TT) (Fox et al., 1993; Lidon et al., 1993; Sharma et al., 1993; Topol et al., 1993). Although there is some interindividual variability, a dose of bivalirudin given as an infusion of 0.20 mg/kg/h increased the aPTT from 27 to 62 s in one study, while an infusion rate of 1.0 mg/kg/h resulted in an average aPTT of 98 s in another group of patients (Lidon et al., 1993).

The INR is also prolonged somewhat during bivalirudin infusion. In 54 healthy volunteers, a dose of 0.05–0.6 mg/kg of bivalirudin given over 15 min iv increased the INR to between 1.25 and 2.43 (Fox et al., 1993). In a study by Lidon and coworkers (1993), the PT was prolonged to between 12 and 16 s with a dose of 0.20 mg/kg/h, while Francis and colleagues (2004) recently reported the mean INR on monotherapy to be 1.50 (range 1.23–2.18) in 52 patients with suspected HIT treated with bivalirudin. Two abstracts also mention a slight prolongation in the INR (Bufton et al., 2002a,b). Although the increase in the INR seems not to be as great as with the DTI argatroban, physicians need to be aware of DTI-coumarin interactions during overlapping therapy (see Chapter 12).

Two studies have demonstrated differences among the DTIs with respect to their ability to prolong the PT (or INR). Gosselin et al. (2004) performed an in vitro study comparing bivalirudin, lepirudin, and argatroban using pooled normal plasma and 14 PT reagents commercially available in the United States, whereas Warkentin and colleagues (2005) used two reagents (of widely differing international sensitivity index [ISI] values) and compared four DTIs (lepirudin, bivalirudin, argatroban, and melagatran). Both groups reported that argatroban had the

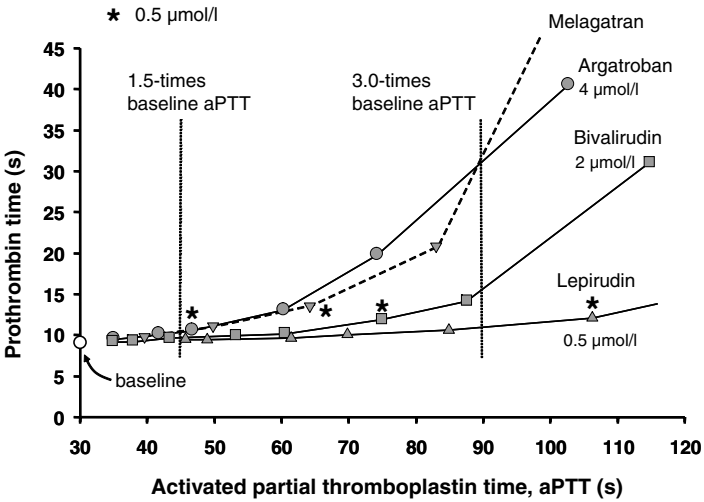

FIGURE 3 Effects of four DTIs on the INR–aPTT relationship. Pooled normal plasma was supplemented with serial twofold increases in the concentrations of each DTI. The asterisk (*) indicates the identical molar concentration (0.5 µmol/L) for each DTI. For a 1.5–3.0-fold increase in the aPTT (the range shown by the vertical dotted lines), the DTIs differ considerably in their ability to prolong the INR, as follows: argatroban > melagatran > bivalirudin > lepirudin. *Abbreviations*: aPTT, activated partial thromboplastin time; DTI, direct thrombin inhibitor; INR, international normalized ratio. *Source*: From Warkentin and Koster, 2005, with modifications.

greatest effect on the INR while lepirudin exhibited the least. Gosselin et al. also found that bivalirudin's effect on the INR was dependent on its concentration and the reagent used, with INR values ranging from 1.10 to 1.53 (Gosselin et al., 2004). Warkentin and Koster found similar results, and noted that PT (INR) prolongation corresponded to the DTI molar concentrations required to prolong the aPTT, i.e., if a higher molar concentration of the DTI is needed to prolong the aPTT, then its effect on PT (INR) prolongation will be relatively greater (Warkentin and Koster, 2005; Warkentin et al., 2005) (Fig. 3).

Bivalirudin decreases fibrinopeptide A levels (a marker of fibrinogen cleavage) in patients with coronary artery disease (Cannon et al., 1993; Ren et al., 1997). It may also increase the bleeding time in some patients (Topol et al., 1993).

Bivalirudin does not inhibit platelet activation or aggregation directly, but it has been shown to inhibit thrombin-mediated platelet aggregation without affecting adenosine 5′-diphosphate (ADP) or collagen-mediated platelet activation (Weitz and Maraganore, 2001; Wiggins et al., 2002; Wittkowsky, 2002) (Fig. 4). Bivalirudin has also been shown to be effective in blocking thrombin activation of both protease-activated receptor (PAR)1 and PAR4-dependent platelet aggregation (Leger et al., 2006).

Recently, Schneider et al. (2006) demonstrated that bivalirudin inhibited thrombin-induced activation of platelets to a greater extent than heparin or heparin plus eptifibatide. Bivalirudin has also been shown to be effective (when used in combination with aspirin and clopidogrel) in suppressing thrombin generation and activity (Keating et al., 2005a). These antiplatelet effects, as well as bivalirudin's

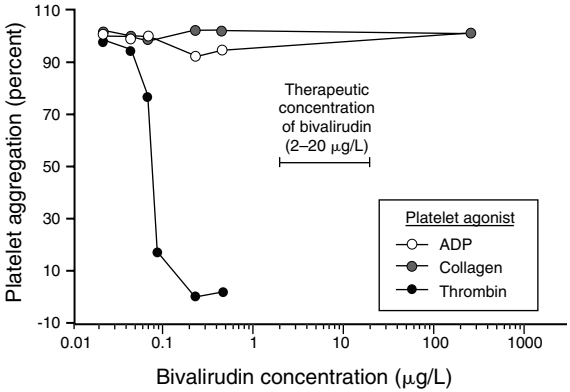

FIGURE 4 The effect of bivalirudin on thrombin-induced platelet aggregation. Bivalirudin completely inhibits thrombin-induced platelet aggregation at concentrations about 1/500 that of therapeutic doses achieved during percutaneous coronary intervention, without significant effect on platelet aggregation by collagen or ADP. *Abbreviation*: ADP, adenosine diphosphate. *Source*: From Wittkowsky, 2002.

recently reported effects on inflammation (decreased concentration of high sensitivity C-reactive protein seen 30 days after PCI), thrombin generation and activity (Keating et al., 2005a), decreased platelet reactivity and platelet-leukocyte aggregates, and leukocyte activation (Keating et al., 2005b) may make it more useful than heparin in the platelet-rich environment of active coronary lesions, where platelet activation and thrombin formation both play significant roles.

The anticoagulant effects of bivalirudin reverse rapidly, with coagulation times returning to baseline within 1–2 h after stopping the infusion (Fox et al., 1993).

E. Dosage

Bivalirudin is approved for iv administration only and dosing regimens for patients undergoing PCI (including patients with HIT) are well established. There are no well established dosing guidelines for other indications (Dager and White, 2002), although dosing regimens for certain indications have been reported. In three patients with venous and arterial thrombosis treated with bivalirudin for HIT, Chamberlin and associates (1994) used doses ranging from 0.05 to 0.20 mg/kg/h. Their goal was to maintain a therapeutic aPTT greater than 50 s. Bufton and coworkers (2002a) used an average dose of 0.27 mg/kg/h in one patient who received bivalirudin for over 2 mo while Berilgen et al. (2003) initiated therapy at a mean dose of 0.16 mg/kg/h in 15 suspected HIT patients maintaining a mean dose of 0.11 mg/kg/h. All patients achieved a therapeutic aPTT within 24 h.

Francis and colleagues (2004) have used bivalirudin in patients with both clinically suspected and confirmed HIT. Initial infusion rates ranged from 0.15 to 0.20 mg/kg/h and their target aPTT was a 1.5- to 2.5-fold prolongation of the baseline aPTT value. Ramirez et al. (2005) reported doses ranging from 0.03 to 0.2 mg/kg/h in 42 patients, many with multiorgan failure who were clinically suspected of, or had a history of, HIT. More recently, Kiser and Fish (2006) reported mean bivalirudin doses for critically ill patients with hepatic and renal dysfunction, including 10 patients receiving continuous venovenous hemofiltration (CVVH) with or without dialysis. They recommended doses of 0.14 mg/kg/h for patients with hepatic dysfunction, 0.03 to 0.05 mg/kg/h in those with renal or combined renal/hepatic dysfunction, and 0.03 to 0.04 mg/kg/h in patients receiving CVVH. Finally, Dang et al. (2006), reviewing their experience with 24 patients who received bivalirudin for confirmed or presumed HIT, found doses ranged from 0.10 to 0.17 mg/kg/h.

Based on these studies, a reasonable regimen might be to start at 0.10 to 0.15 mg/kg/h (no initial bolus) *for non-interventional procedures*, with subsequent adjustments according to aPTT. However in patients with renal or hepatic dysfunction (or both), much lower doses are advised.

For HIT patients undergoing PCI, the dose initially recommended in the "***A***nticoagulant ***T***herapy with ***B***ivalirudin to ***A***ssist in the Performance of Percutaneous Coronary Intervention in Patients with Heparin-induced ***T***hrombocytopenia" (ATBAT) trial was a bolus of 1.0 mg/kg followed by an infusion of 2.5 mg/kg/h for 4 h. This dose was later changed to a bolus of 0.75 mg/kg followed by a 1.75 mg/kg/h infusion over 4 h, based on data from the ***C***omparison of ***A***bciximab ***C***omplications with ***H***irulog Ischemic ***E***vents ***T***rial (CACHET) and ***R***andomized ***E***valuation in ***P***CI ***L***inking ***A***ngiomax to Reduced ***C***linical ***E***vents Trial (REPLACE-1) trials (Mahaffey, 2001; Lincoff et al., 2002a).

Bivalirudin is approved for PTCA in patients with unstable angina The current recommended dose for patients with (near) normal renal function is a bolus of 0.75 mg/kg followed immediately by a continuous infusion at 1.75 mg/kg/h for the duration of the procedure (Sciulli and Mauro, 2002; The Medicines Company, 2005). The bolus is given just prior to angioplasty. Continuation of the infusion for up to 4 h after the procedure is optional, at the discretion of the physician. After completing the 4 h infusion, additional bivalirudin may be given at a rate of 0.20 mg/kg/h for up to 20 h.

Bivalirudin infusion may need to be reduced in patients with moderate to severe renal impairment (GFR < 30 mL/min) (Robson et al., 2002, The Medicines Company, 2005), e.g., to 1.0 mg/kg/h (for GFR < 30 mL/min). In dialysis-dependent patients the dose is reduced to 0.25 mg/kg/h and ideally should be given when the patient is off dialysis (Sciulli and Mauro, 2002; The Medicines Company, 2005) (Table 1).

Allie et al. (2003) used doses similar to the modified ATBAT trial dose for percutaneous transluminal angioplasty (PTA) of the renal and iliac arteries. Following a 0.75 mg/kg iv bolus, bivalirudin was subsequently given by infusion (1.75 mg/kg/h) until completion of the procedure. Similar bivalirudin doses were also used by Shammas and colleagues (2003) in a single-center experience, by Allie et al. (2004) in The ***A***ngiomax ***P***eripheral ***P***rocedure ***R***egistry ***O***f ***V***ascular ***E***vents (APPROVE) Trial involving patients undergoing intervention for renal, iliac, and femoral vessels and by Katzen and colleagues (2005) in patients who underwent peripheral interventions of the lower extremities (iliac, femoropopliteal, or distal), and carotids, vertebrals, renal, aorta, and subclavian vessels. Of note, bivalirudin is not FDA approved for any of these indications.

For cardiac surgery using cardiopulmonary bypass (CPB), i.e., on-pump surgery, about two- to threefold greater levels of anticoagulation are required, compared with OPCAB. (For details regarding these protocols, including important technical considerations for the cardiac surgeon and cardiac anesthesiologist, see Chapter 19; see also Warkentin and Greinacher, 2003; Warkentin and Koster, 2005.)

F. Administration

Bivalirudin is administered iv and produces a rapid anticoagulant effect (Fig. 2). In several small trials, however, it has also been given by subcutaneous (sc) injection. In contrast to its rapid clearance following iv injection, its anticoagulant effects are sustained for several hours following sc administration (Fox et al., 1993) (Fig. 5). The peak anticoagulant effect occurred between 1 and 2 h after sc

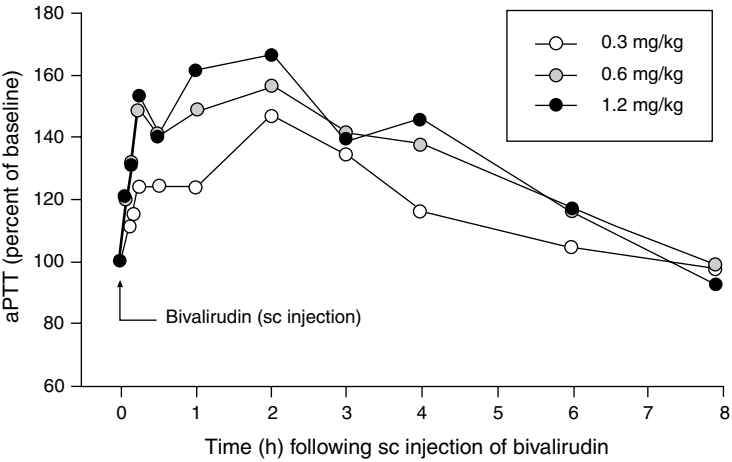

FIGURE 5 Prolongation of the aPTT by administration of sc bivalirudin. Three groups of study subjects containing four subjects each were given an increasing dose of sc bivalirudin. Each line represents the mean value of four study subjects. *Abbreviations*: aPTT, activated partial thromboplastin time; sc, subcutaneous. *Source*: From Fox et al., 1993.

administration in a study of human volunteers, with detectable plasma levels measured up to 6 h post-injection. Following sc injection of 0.3 mg/kg, the aPTT was prolonged to 150 ± 19.4% of the baseline value, and after a 1 mg/kg sc dose, to 176 ± 19.4% of the baseline value; the corresponding INR values increased to 1.18 ± 0.05 and to 1.48 ± 0.17 (Fox et al., 1993). Urinary excretion of the drug was complete by 8–12 h. To date, there are no good efficacy data using the sc route of administration.

A number of drugs commonly used in patients undergoing PCI have been tested for Y-site compatibility with bivalirudin. Testing was for short-term mixing, rather than longer-term interactions (4 h). Drugs found to be compatible with bivalirudin included abciximab, dexamethasone, digoxin, diphenhydramine, dopamine, epinephrine, eptifibatide, esmolol, furosemide, heparin, lidocaine, morphine, nitroglycerin, potassium chloride, sodium bicarbonate, tirofiban, and verapamil (The Medicines Company, 2005; Reed and Bell, 2002). Dobutamine was compatible at concentrations up to 4 mg/mL but incompatible at a concentration of 12.5 mg/mL (Trissel and Saenz, 2002; Hartman et al., 2004).

Trissel and Saenz (2002) looked at the compatibility of bivalirudin with 96 selected drugs including anti-infectives, analgesics, antihistamines, diuretics, steroids, and other supportive care agents by visual observation, turbidity measurement, and electronic particle content assessment. Eighty-seven were compatible with a bivalirudin dilution. Table 2 lists the nine drugs found by Reed and Bell (2002) and Trissel and Saenz to cause haze formation or gross precipitation, and which thus should not be administered in the same line as bivalirudin.

Drug–drug interaction studies have been performed with the thienopyridine derivative ticlopidine, the GPIIb/IIIa inhibitors abciximab, eptifibatide and tirofiban, and low molecular weight heparin (LMWH) and UFH (Reed and Bell, 2002). No pharmacodynamic interactions occurred between bivalirudin and these agents.

TABLE 2 Drugs Incompatible with Bivalirudin

Alteplase
Amiodarone hydrochloride
Amphotericin B
Chlorpromazine hydrochloride
Diazepam
Prochlorperazine edisylate
Reteplase
Streptokinase
Vancomycin hydrochloride

Source: Reed and Bell, 2002; The Medicines Company, 2005.

In patients undergoing PTCA/PCI, coadministration of bivalirudin in conjunction with heparin, warfarin, thrombolytic therapy, or GPIIb/IIIa inhibitors has been associated with increased risk of bleeding compared to patients not receiving these concomitant medications (The Medicines Company, 2005). Aspirin was associated with a mild increase in bleeding times in patients receiving bivalirudin infusions when compared to placebo. These changes were not felt to be clinically significant (Fox et al., 1993).

G. Monitoring

The PT (INR), ACT, aPTT, and TT all rise linearly with increases in the dose of bivalirudin. The ACT can be used to monitor bivalirudin in patients undergoing PTCA, PCI, OPCAB, or PTA, while the aPTT has been used in patients treated for HIT and other non-interventional indications if desired. However, monitoring is optional due to the linear pharmacokinetics of bivalirudin. Dosing in PCI generally results in ACTs above 300 or 350 although dosing is not adjusted based on the ACT, while at lower doses within the range of aPTT monitoring (used for certain non-approved indications), the target range usually is a 1.5- to 2.5-fold increase in the baseline aPTT (Chew et al., 2001).

The ACT and aPTT have limitations, however, and questions regarding their adequacy for monitoring DTI therapy remain, particularly for anticoagulation during CPB (Pötzsch et al., 1997; Koster et al., 2000, 2003a; Despotis et al., 2001; de Denus and Spinier, 2002). As a result, other tests have been developed for monitoring these anticoagulants. One of these, the ecarin clotting time (ECT; see Chapter 19) has been recommended for monitoring during on-pump cardiac surgery.

Koster et al. (2003a) found that an ECT ranging between 400 and 450 s provided acceptable anticoagulation during CPB, noting a close relationship between the ECT (but not the ACT) and bivalirudin concentrations. Nevertheless, a bivalirudin dosing protocol has now been developed that utilizes ACT (rather than ECT) monitoring, which has also provided acceptable monitoring during prospective evaluation for CPB anticoagulation (Dyke et al., 2006) (see Chapter 19). The bivalirudin infusion rate in the EVOLUTION-ON cardiac surgery trial was usually not adjusted according to the ACT; rather, the ACT was used to assure that the protocol-specified dosing achieved an acceptable level of anticoagulation, although physicians could give extra bivalirudin boluses at their discretion (see Chapter 19).

As with other anticoagulants, monitoring may not be reliable if the patient has a lupus anticoagulant, hypofibrinogenemia, elevated fibrinogen-fibrin degradation products, or if the plasma contains heparin (Reid and Alving, 1993). Acquired

coagulopathies are often seen in critically ill patients (a patient group sometimes treated with bivalirudin), and associated low fibrinogen or prothrombin levels may lead to difficulties in judging appropriate drug levels. In these situations, other tests including high-performance liquid chromatography, immunoassays, and chromogenic assays may be superior. Although such assays have been used to measure levels of various DTIs (Griessbach et al., 1985; Bichler et al., 1991; Spannagl et al., 1991; Walenga et al., 1991), these assays are not widely available.

Reid and Alving (1993) developed a quantitative thrombin time (QTT) in which bivalirudin (or hirudin) levels are measured using patient plasma (or whole blood) mixed with human fibrinogen solution, with the clotting time measured after adding human thrombin. The concentration of bivalirudin (or hirudin) is then determined by comparison with a standard curve that is generated by adding known concentrations of bivalirudin to pooled normal plasma.

H. Reversal

There is no specific antagonist to bivalirudin. If renal function is normal, bivalirudin is eliminated rapidly, and its anticoagulant effect clears within a few hours after discontinuing the infusion. Kaplan and Francis (2002) have suggested that recombinant factor VIIa and desmopressin may be of benefit if bleeding occurs. Approximately 25% of bivalirudin can be removed by hemodialysis (Irvin et al., 1999; The Medicines Company, 2005).

Koster and colleagues (2003b) demonstrated that large amounts of bivalirudin can be removed by hemofiltration and plasmapheresis. They utilized five different hemofilters in an in vitro study (conditions mimicking CPB) and observed a correlation between pore size and elimination rate. In their study, 65% of bivalirudin was removed using a hemofilter with a large pore size (65,000 Da) (Mintech Hemocor HPH 700, Minneapolis, MN, USA), an amount comparable to that eliminated with a plasmapheresis filter system (69%). This represents a 50% improvement over the amount of lepirudin that can be removed through filtration (moreover, lepirudin filtration correlates poorly with pore size). These authors suggest that hemofiltration using appropriate filters may be useful for routine management of patients who receive bivalirudin for cardiac surgery.

I. Adverse Effects

Bleeding is the major adverse effect of bivalirudin and occurs more commonly in patients with renal impairment. Injection site pain has been reported in individuals given sc bivalirudin (Fox et al., 1993). Mild headache, diarrhea, nausea, and abdominal cramps have also been reported (Fox et al., 1993). In the ***H***irulog ***A***ngioplasty ***S***tudy (HAS) (now known as the ***B***ivalirudin ***A***ngioplasty ***T***rial [BAT]), the most frequent adverse effects included back pain, nausea, hypotension, pain, and headache. Approximately 5–10% of patients reported insomnia, hypertension, vomiting, anxiety, dyspepsia, bradycardia, abdominal pain, fever, nervousness, pelvic pain, and pain at the injection site (Bittl et al., 1995; Sciulli and Mauro, 2002) (Table 3).

III. CLINICAL USE OF BIVALIRUDIN (NON-HIT PATIENTS)

A. Treatment of Deep Vein Thrombosis

Bivalirudin is not approved for the treatment of venous thromboembolism. However, it has been evaluated in animal models of venous and arterial thrombosis

TABLE 3 Adverse Events Occurring in >5% of Patients Receiving Bivalirudin or Heparin in Randomized Clinical Trials

Event	Bivalirudin ($n = 2161$)	Heparin ($n = 2151$)
Cardiovascular		
Hypotension	262 (12%)	371 (17%)
Hypertension	135 (6%)	115 (5%)
Bradycardia	118 (5%)	164 (8%)
Gastrointestinal		
Nausea	318 (15%)	347 (16%)
Vomiting	138 (6%)	169 (8%)
Dyspepsia	100 (5%)	111 (5%)
Genitourinary		
Urinary retention	89 (4%)	98 (5%)
Miscellaneous		
Back pain	916 (42%)	944 (44%)
Pain	330 (15%)	358 (17%)
Headache	264 (12%)	225 (10%)
Injection site pain	174 (8%)	274 (13%)
Insomnia	142 (7%)	139 (6%)
Pelvic pain	130 (6%)	169 (8%)
Anxiety	127 (6%)	140 (7%)
Abdominal pain	103 (5%)	104 (5%)
Fever	103 (5%)	108 (5%)
Nervousness	102 (5%)	87 (4%)

and in one study involving humans. In a rat model of venous thrombosis using injections of tissue thromboplastin combined with stasis, the administration of bivalirudin demonstrated a dose-dependent interruption of thrombus formation (Maraganore et al., 1991).

Ginsberg et al. (1994a) studied iv and sc injections of bivalirudin in 10 patients with calf-vein thrombosis to determine if single injections could inhibit thrombin generation in a sustained fashion. Prothrombin fragment (F1+2) levels were used as an index of thrombin generation. Significant reductions in F1+2 levels were noted at 6 h postinjection, but by 24 h, levels had increased significantly. These workers speculated that higher doses, more frequent sc injections, or prolonged infusions were required to achieve ongoing inhibition.

B. Prevention of Deep Vein Thrombosis

Bivalirudin (given as sc injection) has been evaluated for prevention of deep vein thrombosis (DVT) in patients undergoing hip or knee surgery. In a phase II, open-label, dose-optimization study of 222 patients, sc bivalirudin was given beginning 12–24 h postoperatively for up to 14 days or until hospital discharge (Ginsberg et al., 1994b). Five dose regimens were used, ranging from 0.3 mg/kg twice a day to 1.0 mg/kg three times a day (Table 4). Patients were evaluated for the occurrence of symptomatic DVT or pulmonary embolism (PE) within 72 h of discontinuing bivalirudin, and assessment of distal or proximal DVT by venography was performed on day 14 or just prior to discharge. Two patients suffered PE while three patients had major bleeding. The rate of DVT ranged from 59% in the lowest-dose regimen to only 17% in the highest-dose regimen (1.0 mg/kg three

TABLE 4 Efficacy and Safety of Bivalirudin in Preventing Deep Vein Thrombosis After Major Hip or Knee Surgery

	Bivalirudin dosing regimen				
Efficacy or safety endpoint	0.3 mg/kg every 12 h	0.6 mg/kg every 12 h	1.0 mg/kg every 12 h for 3 d, then 0.6 mg/kg	1.0 mg/kg every 12 h	1.0 mg/kg every 8 h
n	17	54	40	20	46
Overall DVT rate	10 (59%)	23 (43%)	16 (40%)	7 (35%)	8 (17%)[a]
Proximal DVT rate	7 (41%)	9 (17%)	6 (15%)	4 (20%)	1 (2%)[b]
Pulmonary embolism	0	2 (4%)	0	0	0
Major bleeding	0	1 (2%)	1 (3%)	0	1 (2%)
Minor bleeding	0	2 (4%)	0	1 (5%)	0

Note: Venous thrombosis was documented by bilateral venography or by the occurrence of pulmonary embolism. Of the 222 patients enrolled in the study, 177 patients had technically adequate bilateral venography or clinically documented pulmonary embolism and were considered in the analysis of efficacy. Major bleeding was defined as a fall in hemoglobin level of >2 g/dL or transfusion of >2 units of blood. All other clinically overt bleeding was classified as minor.

[a]Significantly lower overall DVT rate compared with the first four regimens combined: 8/46 (17%) vs. 56/131 (43%); $p < 0.05$.

[b]Significantly lower proximal DVT rate compared with the first regimens combined: 1/46 (2%) vs. 26/131 (20%); $p < 0.01$.

Abbreviation: DVT, deep vein thrombosis.

Source: Ginsberg et al., 1994b.

times a day). Proximal DVT occurred in only 2% of patients in the highest-dose regimen. Bleeding rates were low (<5%) with all regimens.

C. Percutaneous Coronary Intervention

Bivalirudin has been studied for several cardiology indications, including most prominently PCI, but also other non-intervention cardiac situations (Table 5). Bivalirudin has been approved by the FDA for use in patients with unstable angina undergoing PCI. To date, over 1,000,000 patients have been treated with bivalirudin (personal communication with The Medicines Company). Bivalirudin is a safe and effective alternative to heparin in this patient population.

The first clinical study using bivalirudin for coronary angioplasty was reported by Topol and coworkers (1993) in a multicenter, open-label, dose finding trial of 258 patients. The encouraging results led to larger studies of patients requiring urgent angioplasty because of unstable or postinfarction angina, the Hirulog (bivalirudin) Angioplasty Study (for review, see Nawarskas and Anderson, 2001). The primary endpoint was in-hospital death, myocardial infarction (MI), or abrupt vessel closure within 24 h of initiating PCI, or rapid clinical deterioration of cardiac origin. In the original publication, no statistically significant difference in the primary endpoint was noted between bivalirudin and heparin (Bittl et al., 1995), causing the sponsor (Biogen) to abandon further drug development.

Subsequently, The Medicines Company reanalyzed the trial data (including an additional 214 patients analyzed by intention-to-treat principle who were not included in the per-protocol analysis initially reported). In this study, renamed as BAT, the frequency of endpoints (including death, revascularization or MI, and major hemorrhage) were found to be significantly reduced with bivalirudin. Bivalirudin was at least as effective as heparin in preventing ischemic complications

TABLE 5 Major Clinical Studies Using Bivalirudin in Cardiac Patients (PCI and Non-PCI Indications)

Study acronym or description	Trial (Ref.)
PCI indications	
Dose-finding study	Multicenter, open-label study (Topol et al., 1993)
HAS	***H***irulog (Bivalirudin) ***A***ngioplasty study (Bittl, 1995; Bittl et al., 1995)
BAT	***B***ivalirudin ***A***ngioplasty ***T***rial (Bittl et al., 2001)[a]
CACHET	***C***omparison of ***A***bciximab ***C***omplications with ***H***irulog Ischemic ***E***vents ***T***rial (Lincoff et al., 2002b)
REPLACE-1	***R***andomized ***E***valuation in ***P***CI ***L***inking ***A***ngiomax to Reduced ***C***linical ***E***vents (REPLACE)-1 Trial (Lincoff et al., 2002a)
REPLACE-2	***R***andomized ***E***valuation in ***P***CI ***L***inking ***A***ngiomax to Reduced ***C***linical ***E***vents (REPLACE)-2 Trial (Lincoff et al., 2003)
Angiomax in Practice Registry	Cho et al. (2003)
ACS Studies	
ACUITY[b]	***A***cute ***C***atheterization and ***U***rgent ***I***ntervention ***T***riage Strateg***Y*** Trial (Stone et al., 2004, 2006)
TIMI-7	***T***hrombin ***I***nhibition in ***M***yocardial ***I***schemia-7 (Fuchs and Cannon, 1995)
TIMI-8	***T***hrombolysis in ***I***nhibition in ***M***yocardial ***I***schemia-8 (Antman et al., 2002)
HERO-1	***H***irulog ***E***arly ***R***eperfusion/***O***cclusion-1 (White et al., 1997)
HERO-2	***H***irulog ***E***arly ***R***eperfusion/***O***cclusion-2 (White, 2001)

[a]Bittl et al. (1995) reported the first study (combining two randomized, controlled trials) comparing bivalirudin against heparin for PCI; this study, subsequently called the Bivalirudin Angioplasty Trial (BAT), was later reanalyzed (including data from an additional 214 patients) (Bittl et al., 2001).

[b]Approximately 56% of patients in ACUITY underwent PCI, about 11% were triaged to CABG, and the rest were medically managed.

Abbreviations: ACS, acute coronary syndromes; PCI, percutaneous coronary intervention.

in patients who underwent angioplasty for unstable angina and included fewer episodes of major hemorrhage, retroperitoneal bleeding, and need for blood transfusion (Topol et al., 1993; Bittl et al., 1995, 2001; Campbell et al., 2000a; Antman and Braunwald, 2001).

CACHET (phases A, B, and C) evaluated the combination of bivalirudin plus the provisional use of a GPIIb/IIIa inhibitor (abciximab) in comparison to heparin and abciximab in patients undergoing balloon angioplasty and stenting. Bivalirudin was found to be safe and effective with stents and was associated with a lower combined incidence of death, MI, revascularization, or major hemorrhage at 7 days (Nawarskas and Anderson, 2001; Lincoff et al., 2002b; Sciulli and Mauro, 2002).

In the REPLACE-1 trial, heparin was compared to bivalirudin in patients undergoing coronary stenting with any one of the GPIIb/IIIa inhibitors (at the discretion of the physician) in 1056 patients. The combined endpoint of death, MI, or revascularization showed a trend toward a reduction in bivalirudin-treated patients at 48 h (Lincoff et al., 2002a).

The REPLACE-2 trial was a randomized, double-blind, active-controlled trial of 6010 patients who received bivalirudin with provisional use of GPIIb/IIIa blockage or heparin with planned GPIIb/IIIa inhibition. Bivalirudin was found to be superior to heparin alone and as effective as heparin plus GPIIb/IIIa inhibition

for ischemic protection (Lincoff et al., 2003). A significant reduction in the incidence of bleeding and thrombocytopenia were also noted.

Bivalirudin may be a suitable substitute for heparin in patients with chronic renal disease who require PCI because its clearance is primarily determined by proteolysis and not by renal excretion (Robson et al., 2002). ACT monitoring is recommended in patients with chronic renal disease. The dose of bivalirudin may need to be reduced in accordance with the degree of renal impairment, as discussed earlier. If the creatinine clearance is 30 mL/min or less, reduction of the infusion rate to 1.0 mg/kg/h should be considered, while if the patient is on hemodialysis, the infusion should be reduced to 0.25 mg/kg/h (Robson, 2000; Robson et al., 2002; The Medicines Company, 2005) (Table 1).

D. Unstable Angina and Acute MI

Some of the largest experience with bivalirudin is with patients who have had an acute MI or unstable angina. Two open-label, uncontrolled trials were performed to evaluate the efficacy and tolerability of bivalirudin in patients with unstable angina. Sharma et al. (1993) utilized a 5-day infusion of bivalirudin in patients with unstable angina. Their primary endpoints included death, development of an MI, or the need for coronary intervention. Lidon and coworkers (1993) studied 55 patients with unstable angina in a dose-ranging study. As a result of favorable findings in these two trials, the ***T***hrombin ***I***nhibition in ***M***yocardial ***I***nfarction (TIMI) 7 trial comparing four different doses of bivalirudin in combination with aspirin was performed in over 400 patients (Fuchs and Cannon, 1995). The TIMI-8 study compared bivalirudin in a single dose with heparin in patients with unstable angina. This study was prematurely discontinued when Biogen suspended product development. The primary endpoint (all cause mortality and nonfatal MI at 14 days) was lower, and no major bleeding occurred in the bivalirudin group (Antman et al., 2002). These trials suggested that there is a role for bivalirudin in the management of unstable angina.

A number of trials have evaluated the concomitant use of bivalirudin in patients who received streptokinase and aspirin for an acute MI. Lidon et al. (1994) compared bivalirudin to heparin in 45 patients who suffered an acute MI, while Theroux and colleagues (1995) utilized this same strategy in 68 patients. Higher early patency rates and a lower incidence of serious hemorrhage were noted (Nawarskas and Anderson, 2001).

The ***H***irulog ***E***arly ***R***eperfusion/***O***cclusion (HERO) trial randomized 412 patients with acute MI to receive low-dose bivalirudin, high-dose bivalirudin, or heparin (White et al., 1997). Bivalirudin was found to be more effective than heparin in producing early patency rates at a reduced risk for bleeding.

The HERO-2 trial randomized 17,073 patients who received streptokinase to heparin or bivalirudin for 48 h in patients who presented with an acute ST-elevation MI. Bivalirudin did not reduce mortality compared to heparin, but was associated with a 30% reduction in repeat MI, without significant increase in severe or life-threatening bleeding (White, 2001).

A meta-analysis by the Direct Thrombin Inhibitor Trialists' Collaborative Group (2002) based on individual patients' data reported on 11 studies (35,970 patients) receiving either heparin or DTI therapy (relative number of patients treated: hirudin > bivalirudin > argatroban > inogatran > efegatran). Overall, DTI therapy appeared to be superior over heparin for the prevention of MI in patients with acute coronary syndromes (ACS) (although the larger number of

patients treated with hirudin meant that this DTI contributed most to the overall result reported). Bivalirudin was associated with a 56% reduction in major bleeding risk.

The *A*cute *C*atheterization and *U*rgent *I*ntervention *T*riage Strateg*Y* (ACUITY) trial prospectively randomized 13,819 patients with non-ST segment elevation (NSTE) MI/ACS to one of three antithrombotic regiments; UFH or LMWH (enoxaparin) plus IIb/IIIa inhibition; bivalirudin plus GPIIb/IIIa inhibition; or bivalirudin alone. Primary endpoints were a composite ischemia endpoint (death, MI, or unplanned revascularization for ischemia), major bleeding, and the net clinical outcome, defined as the combination of composite ischemia or major bleeding. Coronary angiography was performed in 99% of patients. In this trial, bivalirudin plus GPIIb/IIIa inhibition was associated with rates of ischemia and bleeding that were similar to those of heparin plus GPIIb/IIIa inhibitor. Bivalirudin given without GPIIb/IIIa inhibition was also associated with similar rates of ischemia compared to the heparin plus GPIIb/IIIa inhibitor study arm, but with a significantly lower major bleeding rate (3.0% vs. 5.7%; $p < 0.001$) (Stone et al., 2004, 2006).

E. Percutaneous Transluminal Angioplasty

There is limited experience using bivalirudin in the performance of PTA involving the renal or other peripheral arteries. Allie et al. (2003) performed 180 renal and 75 iliac artery PTAs for patients with severe arterial disease using bivalirudin as the only anticoagulant. Procedural success was achieved in 100% of patients, and no adverse thrombotic events were reported. The authors did note a decrease in sheath removal time, time to ambulation, and length of hospital stay. A decrease in vascular access complications was also seen. Shammas et al. (2003) performed PTA on 48 consecutive patients for lower extremity claudication or ulceration. Although there were two serious in-hospital procedural complications, no patient needed emergent revascularization, nor suffered death or limb loss. Allie and colleagues (2004) also assessed the safety and efficacy of bivalirudin in 505 patients undergoing percutaneous peripheral intervention (PPI) for renal, iliac or femoral disease at 26 centers. Procedural success was achieved in 95% of patients and ischemic events and major hemorrhage rates were low.

Bivalirudin has also been used in carotid artery stenting (Lee et al., 2005; Lin et al., 2005; Finks, 2006). Lee et al. compared bivalirudin to heparin in 46 consecutive patients undergoing carotid artery stenting. Procedural success was 100% in the 24 patients receiving bivalirudin and there were no episodes of major bleeding, vascular complications, strokes, or death. Lin and colleagues performed 200 carotid artery stent procedures on 182 patients, the first 54 receiving heparin anticoagulation. Their protocol was changed, based on results from the REPLACE-2 trial for the next 128 consecutive individuals, resulting in a significant decrease in hemorrhagic complications (Lin et al., 2005). Although none of the series listed above were randomized or double-blind, the authors concluded that bivalirudin was safe, effective, and a reasonable alternative to heparin in peripheral interventions. In addition, the data appear favorable showing lower major bleeding rates and adverse events compared to heparin.

F. OPCAB Surgery

Merry et al. (2004) compared bivalirudin to UFH for OPCAB surgery in a semi-open label (surgeon-blinded), prospective study of 100 patients (half receiving

bivalirudin). The primary endpoint was 12-h blood loss, and secondary endpoints were ischemic complications and coronary artery patency at 12 wk. No deaths were reported. The ACT took longer to return to normal after stopping bivalirudin, when compared to the UFH group (which received protamine reversal). Total blood loss was similar in both groups, however. An intriguing (and potentially important) finding was that graft patency was improved in the patients receiving bivalirudin.

More recently, results from the EVOLUTION-OFF study for OPCAB were published. Smedira et al. (2006) reported on 105 patients randomized to receive bivalirudin and 52 patients who were given UFH. Procedural success rates at 30 days and mortality were identical in both groups, while stroke rates were numerically more frequent in the UFH group (5.5% vs. 0%), and repeat revascularization occurred more often in the bivalirudin group (3% vs. 2%). The authors concluded that while data interpretation should be cautious given the small numbers, this study provides further evidence that bivalirudin is a safe and effective alternative to UFH plus protamine for OPCAB surgery.

G. On-Pump (CPB) Cardiac Surgery

The EVOLUTION-ON study was also recently completed. This trial compared bivalirudin to UFH with protamine reversal in patients undergoing cardiac surgery with CPB (Dyke et al., 2006). Bivalirudin was used in 98 patients vs. UFH and protamine in 52 individuals. There was no significant difference in procedural success (absence of death, non-Q wave MI, stroke, repeat revascularization) in the two groups, although early post-operative blood loss and a numerically higher rate of reoperation for bleeding (5.1% vs. 1.9%) were reported in the bivalirudin group (Warkentin, 2006). Secondary endpoints, including 24-h blood loss and overall incidence of transfusions, were similar between the two study arms (Dyke et al., 2006).

H. Pregnancy and Nursing Mothers

No evidence for impaired fertility or harm to the fetus has been attributed to bivalirudin in teratogenicity studies performed on rats and rabbits using higher doses than recommended for human use (The Medicines Company, 2005). There are no well-controlled studies in pregnant women, however. Given the potential for adverse effects on the neonate and the potential for increased maternal bleeding, bivalirudin and aspirin (normally used together) should be used only if clearly needed (The Medicines Company, 2005). Caution is also advised when giving bivalirudin to nursing women, as it is not known whether bivalirudin crosses the placenta or whether it is excreted in breast milk (Carswell and Plosker, 2002).

I. Other Potential Uses

Bivalirudin has also been studied in animal models for its potential role in both surgical and interventional fields. Its antithrombotic effects were first studied in a baboon carotid endarterectomy model (Kelly et al., 1992). In later studies using endarterectomized rats, significant decreases in platelet deposition with bivalirudin were shown using ^{111}Indium-labeled platelets (Hamelink et al., 1995) and scanning electron microscopy (Jackson et al., 1996).

Bivalirudin has also been studied for prevention of vascular restenosis in a rat carotid artery injury model. Xue and associates (2000, 2001) found that bivalirudin reduced platelet deposition on denuded intima. Platelet-derived growth factor

levels were also decreased following bivalirudin infusion. The authors suggested that balloon catheter injury-induced neointima formation might be suppressed by bivalirudin.

Bivalirudin has been administered to rabbits following balloon injury and reduces vascular restenosis in the femoral artery of angioplasty-injured, diet-induced atherosclerotic rabbits (Sarembock et al., 1996). These studies support the possible role of thrombin in restenosis.

In contrast to the above study, Kranzhofer et al. (1999) administered bivalirudin to rabbits over 3 days immediately after balloon injury to the abdominal aorta and right iliac artery. Markers of inflammation, including intercellular adhesion molecule-1, macrophage colony-stimulating factor, tumor necrosis factor, and interleukin-lβ, were examined by immunohistochemistry. These workers found that bivalirudin did not acutely reduce vascular smooth muscle cell proliferation or inflammation postangioplasty. They did not rule out other mechanisms by which thrombin inhibition could prevent restenosis.

Bivalirudin has also been shown to reduce thrombin-generated increase in levels of plasminogen activator inhibitor-1 (PAI-1) in cultured baboon aortic smooth muscle cells (Ren et al., 1997). Elevated levels of PAI-1 have been found in patients with coronary artery disease (Hamsten et al., 1985; Francis et al., 1988; Sakata et al., 1990), and numerous authors have suggested their role in the development of atherosclerosis and thrombosis (Ren et al., 1997). Bivalirudin may potentially prevent intravascular thrombogenesis through inhibition of thrombin-induced PAI-1 production (Ren et al., 1997; Shen et al., 1998).

Bivalirudin has also been studied in a rat model of endotoxemia and found to increase survival rate in one (but not the other) study (Cicala et al., 1995; Itoh et al., 1996). Bivalirudin reduced endotoxin-induced thrombocytopenia, leukopenia, and fibrinogen consumption, suggesting a possible future therapeutic role in sepsis (Cicala et al., 1995).

IV. BIVALIRUDIN FOR THE TREATMENT OF HIT

A. Miscellaneous Studies

Data on the use of bivalirudin in the treatment of HIT is beginning to accumulate. Chamberlin et al. (1994) reported three patients who received bivalirudin for HIT. One patient was treated for 8 days due to bilateral lower extremity DVTs and recurrent PE with a positive heparin-induced platelet aggregation test, while the other two patients received bivalirudin for arterial ischemia due to HIT. One patient required an above-the-knee amputation and was given bivalirudin (for 12 days) to prevent loss of the other limb, while the other patient had worsening peripheral arterial disease and underwent angioplasty of his right superficial femoral artery using bivalirudin anticoagulation.

In another study, a total of 39 patients with HIT were treated with bivalirudin (Berkowitz, 1999a; Campbell et al., 2000a; Gladwell, 2002). Seventeen patients had acute HIT, while 22 had previous HIT. Patients were treated for a variety of indications (Table 6). There were four deaths (10%), all due to complications from HIT. Revascularization was successful in all but one patient (94%) who had PCI. The one failure was attributed to an unapproachable lesion. Two of the patients required intra-aortic balloon pumps, while another two underwent successful coronary artery bypass surgery. Bleeding complications were usually minor.

More recently, Francis and colleagues (2004) presented their experience using bivalirudin to treat 52 patients with a clinical suspicion of, or at an increased risk

TABLE 6 Indications for Bivalirudin Use in Patients with HIT

Indication	Number treated (%)
PCI	17 (44)
Thrombosis	5 (13)
Coronary artery bypass grafting	4 (10)
PE and DVT	4 (10)
Intra-aortic balloon pump	2 (5)
Cardiac catheterization	2 (5)
Unstable angina	1 (3)
DVT	1 (3)
Pulmonary thromboendarterectomy	1 (3)
Aortic reconstructive surgery	1 (3)
Femoral bypass grafting	1 (3)
Total	39

Abbreviations: DVT, deep venous thrombosis; HIT, heparin-induced thrombocytopenia; PCI, percutaneous coronary intervention; PE, pulmonary embolism.
Source: Campbell et al., 2000a.

for, HIT. Forty-three tested positive for anti-PF4/polyanion antibodies, and 16 had thrombosis preceding bivalirudin treatment. Bivalirudin was given for an average of 8 days, with transition to warfarin (median overlap 4 days) performed in 44 of 52 patients. The authors noted minimal increase in the INR on bivalirudin alone (mean increase 0.33). Minor bleeding was seen in only a few patients and there were no amputations or deaths attributable to HIT.

Berilgen et al. (2003) treated 15 patients with multi-organ failure and suspicion for HIT with bivalirudin. Thirteen of the patients had renal and liver dysfunction, 10 were on dialysis, and eight required mechanical ventilation. Fourteen of the 15 had a positive PF4-dependent enzyme-immunoassay. One new catheter-related superficial thrombophlebitis developed and one patient with previous ischemic extremities required an amputation. Despite six deaths (not related to therapy), the authors concluded that bivalirudin can be safely and effectively administered in HIT patients with both renal and hepatic dysfunction. Dang et al. (2006) used bivalirudin, argatroban or lepirudin in 42 confirmed or presumed HIT patients. A composite of clinical outcomes (DVT, non-fatal MI, non-fatal stroke, limb amputation, and all-cause mortality) were similar in all three groups. The authors concluded that bivalirudin was a viable treatment alternative for the management of HIT.

There are several additional series of critically ill patients with acute HIT (or a history of HIT) with multiorgan failure who received bivalirudin. Ramirez et al. (2005) reported their experience with 42 patients, of whom 78.6% required an intensive care unit (ICU) stay and 14 who had renal or liver dysfunction or both. Nine of their patients with a history of HIT underwent an interventional procedure or surgery requiring bivalirudin administration. Transfusion was required in 28.6% of the patients, five died from multiorgan failure, and three suffered a new thrombotic event during therapy including a cephalic vein thrombosis, left ventricular thrombus, and an ischemic stroke (the latter occurred while the patient was on subtherapeutic doses of bivalirudin). There were no amputations and bivalirudin was felt to be safe and efficacious in both the ICU setting and critically ill individuals.

Similarly, Kiser and Fish (2006) evaluated the safety, effectiveness, and dosing of bivalirudin in 18 critically ill patients with hepatic and/or renal dysfunction (12 had both). The mean duration of bivalirudin therapy was 15 ± 17 days. The authors reported no clinically significant bleeding episodes, there were no amputations or deaths associated with a thrombotic event, and there was only one reported new thrombosis (a lower extremity DVT). Of note, however, three patients had blood clots form on the filters used for CVVH during treatment, and hepatic dysfunction had only a minimal effect on bivalirudin dosing.

There are several single case reports describing bivalirudin use for patients with HIT. Finks (2006) reported the successful use of bivalirudin as a primary anticoagulant during carotid endarterectomy; Robison et al. (2006) used it to maintain anticoagulation during femoral and tibial thromboembolectomy, while Alekshun et al. (2006) used bivalirudin in a patient with idiopathic giant-cell myocarditis requiring emergent biventricular assist device placement who had developed propagating clots in the chamber despite therapeutic heparin anticoagulation; both patients tested positive for anti-PF4/heparin antibodies. There is one report of long-term (2 mo) use of bivalirudin to treat serologically confirmed HIT complicated by recurrent left leg ischemia and arterial thrombosis while on LMWH (Bufton et al., 2002a). This patient received a continuous infusion of bivalirudin (22 mg/h [0.27 mg/kg/h]) using a continuous ambulatory drug delivery (CADD) pump.

Table 7 summarizes theoretical advantages of bivalirudin as a treatment for HIT.

B. Bivalirudin for PCI in HIT

The ATBAT trial was a prospective, open-label study to evaluate the safety and efficacy of bivalirudin in patients with acute HIT or a past history of HIT undergoing PCI (Campbell et al., 2000b; Mahaffey et al., 2003). The primary endpoint was major bleeding within 48 h after completion of the bivalirudin infusion (1.0 mg/kg/h iv bolus followed by 2.5 mg/kg/h by iv infusion for 4 h). This dose was later changed to a 0.75 mg/kg/h iv bolus followed by a 1.75 mg/kg/h infusion for 4 h. Secondary endpoints included event rates for components of the primary endpoint and the ACT, aPTT, and platelet counts (at baseline, pre-PCI/post-PCI,

TABLE 7 Theoretical Advantages of Bivalirudin for Treatment of HIT

Feature of bivalirudin	Comment
Short half-life (25 min)	Avoids need for initial iv bolus; rapid reversal of anticoagulation (useful if patient develops bleeding or if used for intraoperative anticoagulation)[a]
Predominant enzymic metabolism	Minor renal excretion (20%) means that risk of overdosing in renal failure less than with lepirudin; less risk of postoperative bleeding (compared with lepirudin) if used for intraoperative anticoagulation (in case of postoperative renal insufficiency)[b]
Minimal effect on PT/INR	Simplifies transition to oral anticoagulation (compared with argatroban)
Low immunogenicity	Reduced risk of allergy and anaphylaxis (compared with lepirudin)

[a]Possible disadvantages of a short half-life include need for frequent sc administration (e.g., three or four times daily) and rapid loss of anticoagulation (with risk of rebound thrombosis) if prematurely discontinued in patients with acute HIT.

[b]Possible disadvantage of enzymic metabolism includes loss of anticoagulant action in stagnant blood (implications for cardiac anesthesiology) (see Chapter 19).

Abbreviations: HIT, heparin-induced thrombocytopenia; INR, international normalized ratio; iv, intravenous.

and prior to discharge). Clinical success was defined as procedural success without death, emergency bypass surgery, or q-wave MI. Only one of the 52 patients required a blood transfusion (1 U), and procedural and clinical success were achieved in 98% and 96% of the patients, respectively. There were no abrupt closures, nor was thrombus formation reported during or after PCI. One patient died of cardiac arrest about 46 h after successful PCI.

C. Bivalirudin for Cardiac Surgery in HIT

Bivalirudin has been used off-label for cardiac surgery in a number of patients with acute or previous history of HIT, with both "on-pump" and "off-pump" experience reported. Except for several case reports (Spiess et al., 2002; Vasquez et al., 2002; Davis et al., 2003; Koster et al., 2003a; Bott et al., 2003; Gordon et al., 2003; Jabr et al., 2004; Baker et al., 2004; Clayton et al., 2004; Dyke et al., 2005; Veale et al., 2005; Wasowicz et al., 2005)—including two instances in which bivalirudin was used during heart transplantation (Almond et al., 2006; Mann et al., 2005)—experience had been anecdotal in HIT until recent completion of the CHOOSE trials. These employed bivalirudin for anticoagulation in HIT patients undergoing either CPB (CHOOSE-ON) or OPCAB surgery (CHOOSE-OFF). Results of these studies have revealed comparable safety and efficacy endpoints (Koster et al., 2007; Dyke et al., 2007) (see Chapter 19). The rationale for using bivalirudin in these settings included its direct thrombin inhibition without the requirement of a cofactor, its rapid, dose-dependent prolongation of the ACT, its short half-life, lack of structural similarity to heparin (thus, no cross-reactivity with anti-PF4/heparin antibodies), avoidance of protamine use (and its potentially severe adverse reactions), no need for dose reduction in mild renal impairment, and an ability to "reverse" its anticoagulant effect through hemofiltration. Further, there is the potential to avoid HIT antibody formation and, consequently, postoperative HIT.

V. ANTIBIVALIRUDIN ANTIBODIES

Bivalirudin is a relatively small polypeptide and thus is expected to lack significant antigenicity (Fenton et al., 1998). In a study of plasma samples from seven patients, no evidence for antibody formation (IgG, IgM, or IgE) was found (Fox et al., 1993) with plasma samples obtained at 7 and 14 days after iv administration. There was also no evidence for changes in the pharmacokinetics or pharmacodynamics of bivalirudin in their study. One patient exhibited antibody titers of greater than 1:2000 in the assay prior to administration of bivalirudin, although no explanation was given.

In another review of 494 bivalirudin-treated patients from nine different studies, 11 subjects initially tested positive for antibivalirudin antibodies (Berkowitz, 1999b). However, nine of these were found to be false positives on repeat testing. The remaining two (who could not be retested) did not develop any allergic or anaphylactic reactions. In clinical trials of bivalirudin performed from 1993 to 1995, only 1 of 3639 patients (0.03%) experienced an allergic reaction considered by the investigator to be related to study drug. In a study of 222 patients receiving bivalirudin subcutaneously two to three times daily for up to 14 days, no antibody formation occurred up to 6 wk (Ginsburg et al., 1994b; Eichler et al., 2004)

Since bivalirudin shares an 11-amino-acid sequence with hirudin, it is at least theoretically possible that patients with antilepirudin antibodies resulting from treatment with lepirudin could cross-react with bivalirudin. Eichler and colleagues

(2004) found that 22 of 43 (51%) sera containing antilepirudin antibodies showed reactivity in vitro against bivalirudin. This suggests that if bivalirudin is used in patients previously treated with lepirudin, extra caution should be used, e.g., careful anticoagulant monitoring, as antilepirudin antibodies sometimes influence pharmacokinetics.

VI. COST ANALYSIS WITH BIVALIRUDIN

Bivalirudin is the only anticoagulant associated with lower rates of both ischemic and bleeding complications compared to heparin in studies of PCI. These complications are associated with increased morbidity and mortality, as well as higher costs and—as reported by Lauer (2000) and Compton (2002)—have a substantial impact on the cost of PCI, making bivalirudin financially more attractive. Bivalirudin may also be associated with a shorter hospital stay, use of fewer closure devices, lower incidence of hematoma formation, earlier sheath removal, and more selective use of the GPIIb/IIIa inhibitors.

A recent economic evaluation of bivalirudin was reported on the 4651 PCI patients enrolled in the REPLACE-2 trial. In-hospital and 30-day costs were reduced in the bivalirudin group. In addition, regression modeling demonstrated that the hospital savings were not only due to the costs savings of the anticoagulants themselves, but primarily due to the reduction in bleeding and thrombocytopenia that resulted from the use of bivalirudin (Cohen et al., 2004). A more recent prospective economic analysis in the ACUITY trial involving 7851 patients found a shorter overall length of stay (Pinto et al., 2006) and a significant reduction in overall initial hospital costs (personal communications from The Medicine Company), although publication of the hospital- and 30-day costs are pending.

Dang and colleagues (2006) recently reviewed data on 42 hospitalized patients who were treated with bivalirudin, argatroban, or lepirudin for HIT or presumed HIT. Based on average treatment and wholesale price, bivalirudin cost less per day than the other two agents. Potential savings are also possible in patients treated for HIT by reducing its devastating and costly thrombotic complications.

VII. CONCLUSION

Bivalirudin is a unique anticoagulant with a number of already approved and several new potential applications. There is now extensive experience in patients with unstable angina undergoing PTCA, and it has recently received approval by the FDA for PCI in patients with HIT. Data are also accumulating on using bivalirudin for HIT, especially in the critically ill patient with multiorgan failure. It is also being used as an alternative anticoagulant in patients undergoing PTA in the peripheral arteries, including the carotid and renal and in the lower extremities.

Bivalirudin may emerge as the favored alternative anticoagulant to heparin in the setting of cardiac surgery, in patients both with and without HIT (Warkentin and Greinacher, 2003; Dyke et al., 2006; Smedira et al., 2006). Its short half-life, unique metabolism, and means of elimination (enzymic) and low immunogenicity provide it with distinct advantages over heparin and the other DTIs. In addition, its reversible thrombin inhibition may be associated with decreased bleeding risk. Finally, although there are no antidotes available, the potential for reversibility with hemofiltration (which can be used routinely in the postcardiac surgery setting) adds to its attractiveness.

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17 Emerging Anticoagulants and Heparin-Induced Thrombocytopenia: Indirect and Direct Factor Xa Inhibitors and Oral Thrombin Inhibitors

James E. Bradner
Dana-Farber Cancer Institute, Boston, Massachusetts, U.S.A.

John W. Eikelboom
Michael G. DeGroote School of Medicine, McMaster University, Hamilton, Ontario, Canada

I. INTRODUCTION

A. Progress in Drug Development

Historic approaches to anticoagulation have relied on partially purified or chemically derivatized natural products, such as heparin and warfarin. The utility of these agents, however, is limited by interpatient variability in pharmacologic effect, the requirement for laboratory monitoring, and, for warfarin, various food and drug interactions. The association of heparin and warfarin with prothrombotic complications—immune heparin-induced thrombocytopenia (HIT) and warfarin necrosis—are particularly troublesome.

Many novel anticoagulants are under clinical development (Weitz, 2006; Bates and Weitz, 2006). For example, initiation of coagulation by the tissue factor: factor VIIa (TF/VIIa) complex has been targeted by recombinant proteins, such as nematode anticoagulant peptide c2 (rNAPc2; Nuvelo Inc., San Carlos, CA), tissue factor pathway inhibitor (tifacogin; Pfizer Corporation, New York, NY) and inactivated factor VIIa (VIIai; Novo Nordisk A/S, Gentofte, Denmark). In contrast, inhibition of factor IXa has been pursued using a targeted small molecule (TTP889; TransTech Pharma, High Point, NC), a pair of single-stranded DNA aptamers (REG1; Regado Biosciences, Research Triangle Park, NC) and humanized monoclonal antibodies (SB 249417; GlaxoSmithKline Pharmaceuticals, King of Prussia, PA). As these agents are in early development primarily for sepsis and acute coronary syndrome (ACS), they will not be considered further.

B. Rationale for Targeting Factor Xa and Thrombin

Two key pharmacologic targets within the coagulation cascade are factor Xa and factor IIa (thrombin). Table 1 lists several agents, some established, some in development (names italicized), that inhibit factor Xa or IIa (or both), either indirectly through antithrombin (AT; formerly, antithrombin III) or directly (AT-independent). The rationale for targeting these factors follows from their key roles in coagulation. Factor X is synthesized by the liver in a vitamin K-dependent manner. Positioned at the start of the final common pathway, factor X is activated by both the *extrinsic tenase complex* (VIIa/TF) and the *intrinsic tenase complex* (IXa/VIIIa); the resulting factor Xa (a serine protease) then forms the prothrombinase complex with factor Va and ionic calcium on membranes, catalyzing the conversion of prothrombin to

TABLE 1 Parenteral and Oral Anticoagulants that Directly or Indirectly Block Thrombin or Factor Xa

	Indirect (AT-dependent unless indicated otherwise)	Direct (AT-independent)
Thrombin inhibitors	*Odiparcil* (HCII dependent) (oral)	Argatroban *Dabigatran etexilate* (oral) Hirudin derivatives (lepirudin, desirudin) or analogues (bivalirudin) Ximelagatran (oral)[a]
Combined thrombin and factor Xa inhibitors	*Hexadecasaccharide* (SR123781A) Low molecular weight heparins[b] (e.g., enoxaparin, dalteparin) Unfractionated heparin Ultra low molecular weight heparin (*Bemiparin*[c]) Danaparoid[d]	–
Factor Xa inhibitors	Fondaparinux *Idraparinux*[e]	*Apixaban* (oral) *DX 9065a* *Rivaroxaban* (oral)

Note: Agents are parenteral unless indicated otherwise. Agents mentioned in italics are currently being evaluated in randomized control trials.
Several anti-Xa inhibitors in earlier phases of development are included in Table 3.
[a]Ximelagatran has been withdrawn because of hepatotoxicity.
[b]Low molecular weight heparins have an anti-Xa:anti-IIa ratio of between 2:1 and 4:1.
[c]Bemiparin is an ultra low molecular weight inhibitor with an anti-Xa:anti-IIa ratio of about 8:1.
[d]Danaparoid is a mixture of chondroitin sulfate, dermatan sulfate, and heparin sulfate (see Chapter 13). It has an anti-Xa:anti-IIa ratio of >20:1.
[e]Biotinylated idraparinux is currently being evaluated in clinical trials.
Abbreviations: AT, antithrombin; HCII, heparin cofactor II.

thrombin. Thrombin too is a serine protease with even more substrates within the coagulation cascade, notably soluble fibrinogen (converted to insoluble fibrin), and also factors XI, VIII, and V (Fig. 1).

There is debate surrounding the preferential targeting of factor Xa versus IIa, and the role of direct versus indirect (AT-mediated) inhibition. Direct inhibition of thrombin will likely impair both procoagulant (fibrin, XIa, VIIIa, Va generation) and anticoagulant (protein C generation) functions (Fig. 1). However, the pharmacologic utility of commercially available direct thrombin inhibitors (DTIs) suggests that the dominant effect of targeting IIa is anticoagulation. Additionally, thrombin has physiologic roles beyond coagulation that can be abrogated by DTI therapy, such as effects on platelets, wound-healing, and endothelium. In contrast, inhibition of factor Xa is expected to be associated with a purely anticoagulant pharmacodynamic effect, as factor Xa is not known to influence directly natural anticoagulant pathways.

C. Rationale for Exploring Treatment of HIT with New Anticoagulants

HIT is a marked hypercoagulability state with a high risk of venous and arterial thrombosis (see Chapter 2). In most patients with suspected or proven HIT, there is a need for rapidly acting and effective anticoagulation with an alternative non-heparin anticoagulant. In this chapter, the potential role of emerging, novel anticoagulants on the treatment of HIT will be explored. The mechanistic, pharmacologic, and clinical data of agents newly licensed or in a late-stage of clinical development will be summarized. Particular attention will be given to the pentasaccharide anticoagulant, fondaparinux, which has been used for the "off-label" treatment of HIT in a small number of patients.

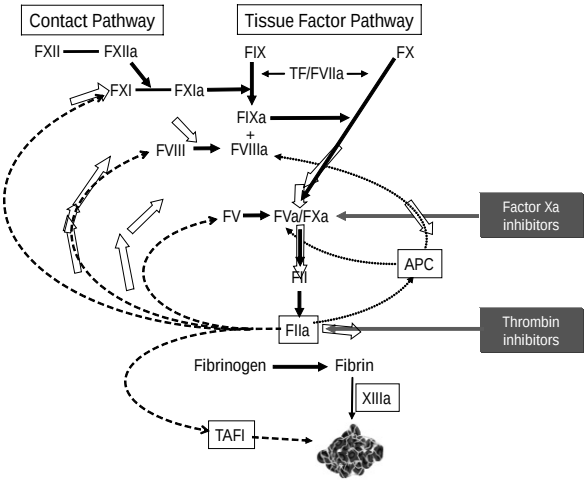

FIGURE 1 Blood coagulation is initiated when factor VII(a) is exposed to membrane-bound TF. The TF/VII(a) complexes activate factor IX (to IXa) and factor X (to Xa), leading ultimately to the formation of thrombin (IIa). Amplification of the process occurs when trace amounts of thrombin formed during the initiation of coagulation activate factor XI (to XIa), and the cofactors, VIII and V, to VIIIa and Va, respectively, in a series of positive feedback loops (denoted by dashed [- - - -] lines), resulting in the formation of the VIIIa/IXa ("tenase") and the Va/Xa ("prothrombinase") complexes, and the generation of large amounts of thrombin, and the formation of a fibrin clot. Thrombin also exerts an inhibitory effect on coagulation by a negative feedback loop (denoted by dotted [· · · ·] lines) that involves activation of the protein C anticoagulant pathway, resulting in degradation of factors Va and VIIIa. Anticoagulant drugs that inhibit factor Xa reduce the amount of thrombin formed. Anticoagulant drugs that inhibit IIa reduce thrombin activity and thus reduce (1) fibrin clot formation, (2) positive feedback amplification of thrombin generation (including contact activation that occurs when blood is exposed to foreign surfaces), and (3) negative feedback via the protein C pathway. *Abbreviations*: APC, activated protein C; TF, tissue factor; TAFI, thrombin activatable fibrinolysis inhibitor.

II. FONDAPARINUX: INDIRECT FACTOR Xa INHIBITOR

A. History

Unfractionated heparin (UFH) is comprised of a highly heterogeneous mixture of linear polysaccharide molecules of variable length (mean, 45–50 saccharide units). Seminal studies by Rosenberg in the 1970s and 1980s defined the structure–function relationship of heparin. The pharmacologic activity of UFH was ascribed to a subpopulation of polysaccharides capable of binding AT (Lam et al., 1976; Rosenberg et al., 1978). Anticoagulation resulted from formation of an inhibitory ternary complex among heparin, AT, and any of five clotting factors (factors IIa, Xa, IXa, XIa, and XIIa). Subsequent studies resolved the minimal chemical motif necessary for anticoagulation as a tetrasaccharide sequence (Rosenberg and Lam, 1979). Based on these findings, synthetic polysaccharides with potent anticoagulant activity were prepared (Choay et al., 1983; Grootenhuis et al., 1995; Petitou et al., 1999). One theoretical benefit of the small size of the synthetic heparin mimetics would be the potential *not* to cause HIT (Petitou et al., 1999).

B. Chemistry

Fondaparinux sodium (Arixtra, formerly Org21540/SR90107A), the first synthetic pentasaccharide anticoagulant, is prepared for injection as a decasodium salt. The

chemical formula is $C_{31}H_{43}N_3Na_{10}O_{49}S_8$, and the molecular mass is 1728 Da (Arixtra Package Insert, 2006). The chemical structure is presented in Figure 5 of Chapter 7.

Fondaparinux is a methyl glycoside analogue designed and optimized based on the AT binding site of UFH. Binding to AT (1:1 stoichiometry) results in the allosteric induction of an irreversible conformational change in AT. This AT conformer exhibits high binding affinity for factor Xa, preventing thrombin generation (Arocas et al., 2001). After covalent binding of Xa to AT, fondaparinux is released without structural alteration, allowing subsequent binding once again to free AT. Unlike UFH and (longer fragments within) low molecular weight heparin (LMWH), binding of fondaparinux to AT does not facilitate inhibition of factor IIa, due to the structural absence of the heparin thrombin-binding domain.

C. Pharmacology

The pharmacodynamic impact of factor Xa inhibition is reduced thrombin generation. Studies performed in vitro with platelet-rich plasma have identified an inhibitory effect on both the initiation and propagation phases of thrombin generation, with reduction in the total amount of thrombin generated (Walenga et al., 1988; Gerotziafas et al., 2004). Animal studies have demonstrated pronounced inhibition of thrombus extension (Amar et al., 1990).

The pharmacokinetics of fondaparinux were determined in young, healthy volunteers and in the elderly (Donat et al., 2002). Following subcutaneous (sc) injection, fondaparinux is absorbed completely, with peak plasma concentrations achieved within 1–3 h. Maximal concentrations and area-under-the-curve correlate linearly with dose, with the half-life being relatively constant in young volunteers (~17 h). In the elderly, the half-life is mildly prolonged (~21 h). The comparatively long elimination half-life permits a once-daily sc dosing schedule, with steady-state achieved following the fourth or fifth daily dose. Fondaparinux is excreted almost completely in the urine without metabolism.

The pharmacology of fondaparinux has not been well defined in special populations, such as pregnant women, children, or persons with severe renal impairment. Data from the orthopedic thromboprophylaxis trials provides insights into the impact of renal impairment on drug clearance (Turpie, 2002). With mild renal impairment (creatinine clearance, 50–80 mL/min), fondaparinux clearance is reduced by 25%; with moderate renal impairment (creatinine clearance, 30–50 mL/min), clearance is 40% reduced (Arixtra Package Insert, 2006). Thus, use of fondaparinux is not advised with severe renal impairment (creatinine clearance, <30 mL/min). The safe, effective use of fondaparinux in pregnant patients has been reported in several case reports (Harenberg, 2007; Mazzolai et al., 2006; Wijesiriwardana et al., 2006). Despite in vitro evidence to the contrary, transplacental passage of fondaparinux has been suggested by the detection of a low anti-factor Xa activity in umbilical cord blood, so caution in this context is warranted (Dempfle, 2004; Lagrange et al., 2002).

D. Dosage and Administration

Fondaparinux is supplied for use as a pre-filled syringe with attached protective needle system. Individual syringes are available for the following doses: 2.5, 5.0, 7.5 and 10.0 mg. The drug is formulated in an isotonic solution of sodium chloride, with a pH varying between 5.0 and 8.0. The dose studied for thromboprophylaxis

was determined by a wide dose-ranging phase II program in orthopedic surgery patients, which led to the selection of a 2.5 mg daily dose (Turpie et al., 2001).

The dose of fondaparinux selected for use in the treatment of venous thromboembolism (VTE) was also selected from a dose-ranging phase II study (Rembrandt Investigators, 2000). Guidance for dose selection was derived from a primary outcome measure of ultrasonographic change in thrombus mass balanced against tolerability data, with the data model supporting a 7.5 mg daily dose. The phase III VTE treatment program validated a simplified weight-based dosing scheme, where patients between 50 and 100 kg received 7.5 mg once-daily. Patients weighing in excess of 100 kg were administered 10.0 mg, whereas patients weighing less than 50 kg received 5.0 mg (Buller et al., 2003, 2004).

E. Monitoring

Monitoring of fondaparinux drug level or pharmacodynamic effect was not required during the clinical studies establishing its efficacy. Consequently, monitoring has not been required by regulatory agencies. The pharmacodynamic effect of fondaparinux can be monitored using commercial assays for anti-factor Xa activity, although calibration to a standard curve derived from fondaparinux is required. However, the clinical utility of such monitoring is presently unclear (Klaeffling et al., 2006).

Fondaparinux does not prolong the bleeding time (Boneu et al., 1995). Subtle prolongation of the prothrombin time (PT; 1 s) and activated partial thromboplastin time (aPTT; 4 s), as well as a mild reduction in factor VIII have been reported (Smogorzewska et al., 2006).

F. Reversal

No antidote exists for fondaparinux. Studies performed in vitro have demonstrated the capacity of recombinant heparinase to depolymerize and inactivate fondaparinux (Daud et al., 2001). However, pharmaceutical preparations of this enzyme are not available. Notably, protamine sulfate does not neutralize the anti-factor Xa effect of pentasaccharides (Bernat and Herbert, 1996). A clinical study of healthy volunteers treated with fondaparinux suggests that recombinant factor VIIa may counteract its anticoagulant effects (Bijsterveld et al., 2002). A case report describes use of recombinant factor VIIa and tranexamic acid to help manage an orthopedic patient with hemorrhagic shock complicating fondaparinux use (Huvers et al., 2005).

G. Adverse Effects

The most common reported adverse effect of fondaparinux is bleeding. In the large, prospective efficacy studies in VTE, the incidence of major bleeding among patients treated with (therapeutic-dose) fondaparinux was approximately 1% (Buller et al., 2003, 2004). In a registration study of patients undergoing elective total knee replacement, a statistically significant increase in bleeding, as defined by the bleeding index, was noted with fondaparinux, compared to enoxaparin, with no increase in fatal or clinically relevant bleeding (Bauer et al., 2001). A meta-analysis of the phase III registration program studies identified a modest increase in major bleeding (compared with enoxaparin) but without an increase in bleeding leading to death, requiring surgical intervention or bleeding in a critical site (Turpie et al., 2002a). Caution should be used in patients with cutaneous hypersensitivity to UFH, as the literature presents conflicting information regarding the tolerability of fondaparinux in this setting (Hirsch et al., 2004; Jappe et al., 2004; Utikal et al., 2005).

III. CLINICAL USE OF FONDAPARINUX

A. Prevention of VTE After Orthopedic Surgery

The clinical utility of fondaparinux as a method of thromboprophylaxis following orthopedic surgery was established by four, large phase III studies in which patients were randomized to receive either fondaparinux or enoxaparin. Two studies (EPHESUS, PENTATHLON 2000) were performed in patients undergoing elective hip replacement (Lassen et al., 2002; Turpie et al., 2002b). With a primary outcome measure of venographically evident deep-vein thrombosis (DVT) and symptomatic VTE, fondaparinux demonstrated superior efficacy compared to once-daily dosing of enoxaparin (40 mg; EPHESUS) and comparable efficacy to twice-daily dosing (30 mg b.i.d.; PENTATHLON 2000). Major bleeding was not statistically different between the two groups. In a study of patients undergoing elective knee replacement surgery (PENTAMAKS), fondaparinux demonstrated superior efficacy (defined above) compared to enoxaparin (Bauer et al., 2001).

A fourth registration study (PENTHIFRA) enrolled patients undergoing hip fracture surgery (Eriksson et al., 2001). Here, fondaparinux demonstrated a marked reduction in postoperative DVT and VTE compared to enoxaparin (8.3% vs. 19.1%; $p < 0.001$). Bleeding was not significantly different between the two treatment groups. To investigate the utility of extended prophylaxis following hip fracture surgery, a fifth orthopedic trial was undertaken (PENTHIFRA-Plus) (Eriksson and Lassen, 2003). Patients completing a standard course of therapy with 2.5 mg of fondaparinux given for 6–8 days were randomized to additional therapy versus placebo for 19–23 days. The primary efficacy outcome, symptomatic or venographically evident VTE, was markedly reduced by extended therapy (1.4% vs. 35%; $p < 0.001$). Fondaparinux received approval from the U.S. Food and Drug Administration (FDA) for the prevention of VTE following major hip or knee surgery (Table 2).

B. Prevention of Venous Thrombosis in Other Clinical Settings

Additional clinical studies have examined the utility of fondaparinux in the prevention of VTE following general surgery and in the medical patient. In general surgery patients, the PEGASUS study compared the efficacy of 2.5 mg fondaparinux versus dalteparin administered preoperatively and then once daily at a dose of 5000 IU (Agnelli et al., 2005). The primary endpoint was venographically evident DVT and symptomatic VTE to day 10. The objective of this non-inferiority study was met, without a significant increase in major hemorrhage, prompting approval of fondaparinux by the FDA for the prevention of VTE following abdominal surgery.

In a population of medically ill patients, fondaparinux was compared to placebo in the prevention of venographically detected DVT and symptomatic VTE to day 15 of blinded therapy (Cohen et al., 2006). This international study of 849 patients illustrates the activity of fondaparinux in medical thromboprophylaxis, with a reduction in symptomatic and venographically evident VTE. The approval for fondaparinux in this clinical setting is under review.

C. Treatment of VTE

The treatment of VTE was assessed in two large, international studies (Buller et al., 2003, 2004). The primary outcome measure for both non-inferiority studies was the incidence of symptomatic VTE during a total treatment period of 3 mo.

TABLE 2 Indications for the Use of Fondaparinux

Indication	Dose and administration	U.S. approval
Venous thromboembolism prevention		
Hip fracture	2.5 mg/d sc o.d. for up to 32 days	Yes
Elective hip or knee replacement	2.5 mg/d sc o.d. for up to 11 days	Yes
Abdominal surgery	2.5 mg/d sc o.d. for up to 10 days	Yes
Medical	2.5 mg/d sc o.d.	Under review
Venous thromboembolism treatment		
Acute treatment of deep vein thrombosis or pulmonary embolism	5 mg (<50 kg), 7.5 mg (50–100 kg), or 10 mg (>100 kg) sc o.d. in fixed weight adjusted dose for minimum of 5 days and until INR $\geqslant$ 2.0 on two occasions 24 h apart	Yes
Acute coronary syndrome		
Unstable angina/non-ST elevation MI	2.5 mg/d sc o.d. for 8 days	Under review[a]
ST elevation MI	2.5 mg/d iv bolus followed by 2.5 sc o.d. for 8 days	Under review[a]

[a]Undergoing expedited review by the Food and Drug Administration as of March 2007.
Abbreviations: INR, international normalized ratio; MI, myocardial infarction; o.d., once-daily; sc, subcutaneous; iv, intravenous.

In MATISSE-DVT, patients with acute DVT were randomized to receive either sc fondaparinux, given once daily, or enoxaparin, given twice daily at a dose of 1 mg/kg. Patients in both arms of the study were transitioned to vitamin K antagonist therapy for the remainder of the protocol. There was no significant difference in efficacy (fondaparinux 3.9%; enoxaparin 4.1%) or tolerability.

In MATISSE-PE, patients with symptomatic acute pulmonary embolism were randomized to once-daily sc fondaparinux versus standard therapy with UFH. Again, all patients were transitioned to oral therapy with a vitamin K antagonist. The incidence of recurrent VTE was not significantly reduced in patients treated with fondaparinux (3.8%) compared to UFH (5.0%). Notably, patients with a serum creatinine over 2.0 mg/dL were excluded from this study, which leaves some uncertainty regarding the tolerability of fondaparinux in patients with severe renal dysfunction. Based on the favorable results of the MATISSE studies, fondaparinux was approved for the treatment of VTE (Table 2).

D. Treatment of ACS

Recent clinical studies have explored the use of fondaparinux in the treatment of ACS, based on the rationale that antithrombotic therapy with UFH and LMWH is beneficial in this clinical context, as well as percutaneous coronary intervention (PCI). A pilot study was performed in patients undergoing elective or urgent PCI (Mehta et al., 2005). Fondaparinux was administered at either 2.5 or 5.0 mg, by intravenous (iv) infusion, and compared to iv UFH. Comparable efficacy was observed using a composite endpoint (all-cause mortality, infarction, revascularization, or need for glycoprotein IIb/IIIa antagonist therapy). However, a reduced incidence of hemorrhage in patients receiving 2.5 mg (3.4%) versus 5.0 mg (9.6%) prompted further evaluation of the 2.5 mg dose of fondaparinux in registration studies.

The OASIS-5 study compared the efficacy of fondaparinux (2.5 mg o.d.) versus enoxaparin (1 mg/kg twice-daily) in patients with ACS (Yusuf et al., 2006a). In this large (n = 20,078) international study, patients were randomized and treated

for 6–8 days. The primary endpoint of death, infarction or ischemia was examined at day 9, without a significant difference noted between the groups. However, significant differences in mortality were noted at 30 days and at 180 days, favoring treatment with fondaparinux. Major bleeding was also markedly reduced by the use of fondaparinux (2.2% vs. 4.1%; $p < 0.001$) and most of the excess deaths in the enoxaparin group occurred in patients who experienced bleeding.

With an interest in establishing the efficacy of fondaparinux in the treatment of ST-segment myocardial infarction (MI), fondaparinux was studied in another large (n = 12,092) randomized control trial (RCT) (Yusuf et al., 2006b). Patients were randomized to fondaparinux (2.5 mg o.d.) administered for an average of 8 days or usual care, either UFH or no anticoagulation (placebo). The primary endpoint of this study, the composite of death or recurrent infarction at 30 days, was reduced by treatment with fondaparinux (9.7% vs. 11.2%; p = 0.008). The benefit of therapy was durable through the study to final follow-up at 3–6 mo, including a significant reduction in mortality. This large study allows for speculative examination of clinically meaningful subpopulations. Of note, there was no efficacy benefit among those patients undergoing primary therapy with PCI, whereas patients receiving thrombolytic therapy as well as those who received no reperfusion therapy derived a significant benefit from fondaparinux.

Together, these studies have defined a striking activity and tolerability of fondaparinux in the cardiac patient. Fondaparinux is currently undergoing expedited FDA review for the treatment of ACS (as of March 2007).

IV. CLINICAL USE OF FONDAPARINUX IN THE TREATMENT OF HIT

A. Rationale

Fundamental to the development of HIT is the binding of (anionic) sulfated polysaccharides (UFH or LMWH) to (cationic) PF4, resulting in an allosteric modulation of PF4 structure that presents neoantigens to the immune system. One motivation for the development of pentasaccharide anticoagulants was the inference that these comparatively small (~1700 Da) compounds might not associate significantly with PF4, potentially avoiding or minimizing risk of HIT (Petitou et al., 1999; see also Chapter 7). There is growing evidence supporting this thesis, including numerous in vitro studies, and (more importantly) early favorable experience with fondaparinux in the treatment of HIT (Table 3). The rationale for the use of fondaparinux in treating HIT derives from its efficacy in numerous (non-HIT) clinical settings and the evidence that pentasaccharide anticoagulants do not cross-react significantly with anti-PF4/heparin antibodies.

B. Laboratory Studies of Fondaparinux in HIT

Shortly after the identification of PF4/heparin as the major antigen in HIT (see Chapters 5–7), various structural determinants for antigen formation were clarified by Greinacher and colleagues (1994, 1995). Among these, increasing polysaccharide molecular weight and degree of sulfation increased the ability to form antigens with PF4 that were recognized by HIT antibodies.

In addition, several studies described a lack of in vitro cross-reactivity when HIT antibodies were tested in the presence of fondaparinux. Elalamy et al. (1995) examined the ability of plasma (or purified IgG) from 25 patients with documented HIT to induce aggregation of platelets from healthy volunteers in the presence of

TABLE 3 Clinical Studies of Fondaparinux in the Treatment of HIT-Associated Thrombosis

Publications

Author	Journal	Year	#	History[a]	HIT	HIT-T	Confirmation	Dose	Duration	Recovery[b]	Plts[c]	VTE	Amp	Death	Hem
D'Amico et al.	J Thromb Haemost	2003	1	–	1	–	PAT(Dir)	2.5 mg	N/R	N/R	0	0	0	0	0
Parody et al.	Haematologica	2003	1	–	1	–	EIA	0.5 mg[d]	N/R	2 days	0	0	0	0	0
Harenberg et al.	Haematologica	2004	8	6	2	–	HIPA	2.5 mg	7–14 days	N/R	0	0	0	0	0
Haase et al.	Nephrol Dial Transplant	2005	1	–	–	1	Negative	2.5 mg	10 wk	<1 wk	0	0	0	0	0
Kuo and Kovacs	Thromb Haemost	2005	5	–	–	5	EIA	7.5 mg	N/R	2–9 days	0	0	0	1[e]	0
D'Angelo et al.	Thromb Haemost	2006	1	–	–	1	EIA	7.5 mg	25 days	N/A	0	0	0	0	0
Bradner et al.	Personal communication[f]	2007	38	–	36	2	EIA	2.5–7.5 mg	5–71 + days	4.8 days	0	1	0	8[e]	0

Abstracts

Author	Conference	Year	#	HIT	HITT	Confirmation	Dose	Duration	Recovery[b]	Plts[c]	VTE	Amp	Death	Hem
Boshkov et al.	ASH	2004	1[g]		1	N/R	0.15 mg/kg	3 mo	N/A	0	0	0	0	0
Grabowski and Bussel	ASH	2006	2[g]		2	N/R	N/R	4.5–8 mo	N/R	0	0	0	0	0
Piovella et al.	ASH	2006	20	3	17	N/R	2.5–7.5 mg	4–25 days	N/R	1	0	0	0	1

[a]Number of patients with a history of HIT.
[b]Time to platelet count recovery, determined only from those patients treated with fondaparinux while thrombocytopenic.
[c]Number of patients with persistent or recurrent thrombocytopenia while on fondaparinux.
[d]Dose of fondaparinux adjusted due to renal failure.
[e]Deaths were not perceived by investigators to result from fondaparinux.
[f]Bradner JE, Yeh RW, Hallisey RK, Kuter DJ. Fondaparinux in the treatment of heparin-induced thrombocytopenia (unpublished observations).
[g]Pediatric patient.
Abbreviations: Amp, limb amputation; EIA, (PF4-dependent) enzyme-immunoassay; Hem, major hemorrhage; HIPA, heparin-induced platelet activation test (washed platelet assay); HIT, isolated HIT (i.e., HIT without thrombosis); HIT-T, HIT-associated thrombosis; PAT(Dir), platelet aggregation test (direct, i.e., using the patient's own platelets); N/R, not reported by investigators; VTE, venous thromboembolism.

UFH (100% of donor platelet samples aggregated), LMWH (76%), danaparoid (8%) or pentasaccharide (0%). Thus, platelet aggregation was induced in all samples in the presence of UFH but was not induced in the presence of fondaparinux.

These observations were confirmed shortly thereafter by another group in France using enzyme-linked immunoassay (EIA) (Amiral et al., 1997). Plasmas from 49 patients with HIT (confirmed by platelet aggregometry) were compared with respect to their ability to induce the binding of antibodies to immobilized PF4 in the presence of increasing concentrations of fondaparinux or UFH. Although antibodies were fixed by a broad range of concentrations of UFH, no antibody binding was detected in the presence of fondaparinux.

A subsequent prospective study tested the cross-reactivity of fondaparinux with sera obtained from 39 patients with laboratory-confirmed HIT and 15 unaffected controls (Savi et al., 2005). Three functional (platelet activation) tests for cross-reactivity were performed by independent laboratories: the serotonin-release assay, the heparin-induced platelet activation assay (both the aforementioned are washed platelet assays) and conventional platelet aggregometry (using platelet-rich plasma). Although cross-reactivity between HIT plasma and UFH was observed in 75 of 94 assays (79.8%), only three of 91 assays (3.3%) conducted in the presence of fondaparinux were reported as positive. The authors concluded that fondaparinux was essentially non-reactive with HIT sera.

Two recent studies have investigated the potential for formation of PF4/fondaparinux complexes. Rauova et al. (2006) investigated the biophysical structure and polysaccharide determinants of ultra-large PF4 complexes (ULC). Using high performance liquid chromatography (HPLC) size exclusion chromatography and electron microscopy, the authors observed that ULC formation was formed preferentially by UFH and less well by LMWH. In contrast, fondaparinux was incapable of forming ULC. However, using atomic force microscopy (AFM) and photon correlation spectroscopy, Greinacher et al. (2006) provided evidence that fondaparinux can induce some formation of PF4 clusters, although this effect was much less marked than for UFH and LMWH.

These studies support the concept that fondaparinux exhibits no (or negligible) in vitro cross-reactivity with HIT antibodies, although the AFM studies suggest that it might provoke an immune response against PF4-dependent antigens. Indeed, in the PENTAMAKS and PENTATHLON orthopedic thromboprophylaxis RCTs, anti-PF4/heparin antibodies were generated at similar frequencies in both the fondaparinux and enoxaparin study arms, although none of the 2700 patients studied developed clinical HIT (Warkentin et al., 2005). Interestingly, the antibodies that were generated recognized PF4 in the presence of UFH and LMWH, but not in the presence of fondaparinux, even when the blood samples were obtained from patients who had formed antibodies while receiving fondaparinux (Fig. 2). In a smaller study, Pouplard et al. (2006) also observed a low frequency of anti-PF4/heparin antibody formation among post-orthopedic surgery patients undergoing fondaparinux thromboprophylaxis. One interpretation of these data is that anti-PF4/heparin antibodies formed in patients treated with fondaparinux likely would not cause clinical HIT even in the presence of fondaparinux, given the negligible degree of in vitro cross-reactivity. It should be further noted that neither of the above studies *proves* immunogenicity of fondaparinux, as a background frequency of "spontaneous" antibody formation after orthopedic surgery has not been ruled out (Warkentin et al., 2005, 2006). To date, there is only a single published case of possible fondaparinux-induced HIT (Warkentin et al., 2007).

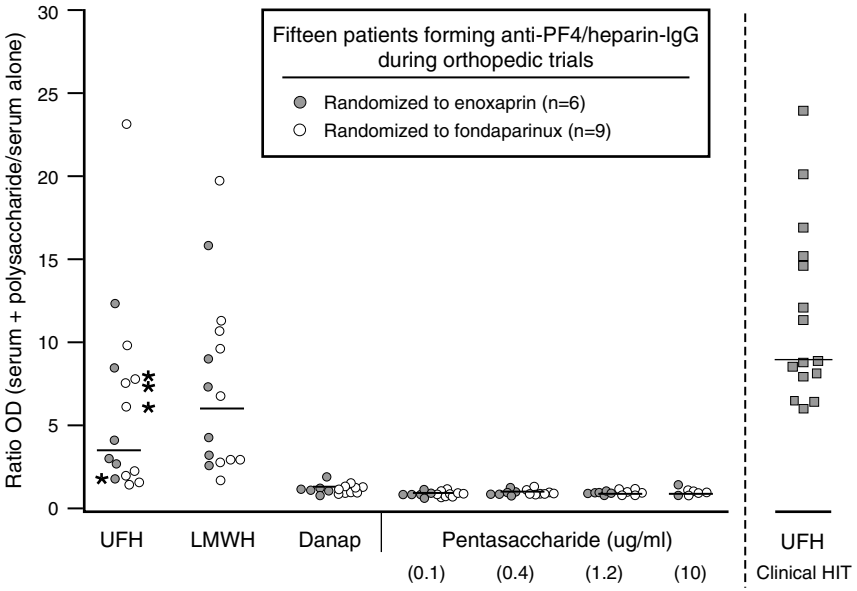

FIGURE 2 Ratio of antibody binding to PF4/polysaccharide complexes compared to PF4 alone by fluid-phase EIA. Results of fluid-phase EIA testing for sera from 15 patients who formed anti-PF4/heparin IgG antibodies (detected using solid-phase EIA) while receiving enoxaparin ($n = 6$, closed circles) or fondaparinux ($n = 9$, open circles). The data are expressed as ratios of binding to PF4 in the presence of polysaccharide (UFH, 0.6 IU/mL, LMWH 0.5 anti-Xa U/mL, danaparoid 0.1 anti-Xa U/mL, and fondaparinux, 0.1, 0.4, 1.2, and 10.0 μg/mL) over the baseline (buffer). Horizontal bars indicate medians. Asterisks indicate the four samples that tested positive (in the presence of UFH) in the platelet activation assay. For comparison, results are also shown for 15 patients with clinical HIT. Statistically significant increases in reactivity (null hypothesis, mean ratio of *OD* [presence of drug]/*OD* [presence of buffer] = 1) for the 15 sera obtained from patients in the orthopedic trials were observed for UFH ($p = 0.0032$), LMWH ($p = 0.0004$), danaparoid ($p = 0.0016$), but not with fondaparinux at any concentration ($p > 0.05$). Whereas 14 of 15 sera from patients in the orthopedic trials exhibited more than twofold greater reactivity than baseline against PF4/LMWH, none reacted similarly against PF4/fondaparinux ($p = 0.0002$ by McNemar's test, two-tailed). *Abbreviations*: EIA, enzyme-linked immunoassay; HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; UFH, unfractionated heparin. *Source*: From Warkentin et al., 2005.

C. Observational Studies

The first published case of fondaparinux use in a patient with HIT was by Dr. Elbio D'Amico et al. (2003). A patient with paroxysmal nocturnal hemoglobinuria and Budd-Chiari syndrome developed HIT following administration of LMWH (dalteparin 5000 IU sc q12h). The patient experienced spontaneous platelet count recovery, and received thrombolytic therapy. While receiving prophylactic doses of fondaparinux, the platelet count remained unchanged and the patient was transitioned uneventfully to oral anticoagulant therapy.

There are now at least six published reports (D'Amico et al., 2003; D'Angelo et al., 2006; Haase et al., 2005; Harenberg et al., 2004; Kovacs, 2005; Parody et al., 2003), three abstracts (Boshkov et al., 2004; Grabowski and Bussel, 2006; Piovella et al., 2006) and one unpublished case series describing the use of fondaparinux to

treat HIT (Table 3). The case series involved 38 patients with HIT who were treated with fondaparinux at the Massachusetts General Hospital (MGH) between January 1, 2002, and August 1, 2004 (Table 3). This series represents two cohorts of patients treated either with fondaparinux after initial treatment with a DTI ($n = 17$) or receiving fondaparinux for the initial treatment of HIT ($n = 21$). Platelet count recovery data from the MGH series are presented in Figure 3, representing normalized, daily platelet counts of patients who received fondaparinux during the acute thrombocytopenic phase. The platelet recovery and the low incidence of thromboses in the treated cohorts (2/38) are consistent with the conclusion that there is no clinical cross-reactivity between fondaparinux and HIT antibodies. None of the studies reported prolonged or recurrent thrombocytopenia during fondaparinux treatment. An important limitation of these studies is that relatively few patients underwent functional testing using platelet activation assays that have relatively high diagnostic specificity for HIT (see Chapter 10).

D. Prospective Evaluation

The results of retrospective studies must be interpreted with caution because the reports are subject to publication bias (studies with "positive" outcomes are more likely to be submitted and accepted for publication than studies with "negative" outcomes). There also was marked heterogeneity among the studies regarding patient eligibility, the methods used to diagnose HIT, the treatment regimens employed, and the monitoring of the response to treatment. For these reasons, a prospective evaluation of fondaparinux efficacy and safety is required. Fondaparinux may have advantages over more established therapies for HIT, including the potential for a lower incidence of major bleeding, easier transition to oral warfarin (if desired),

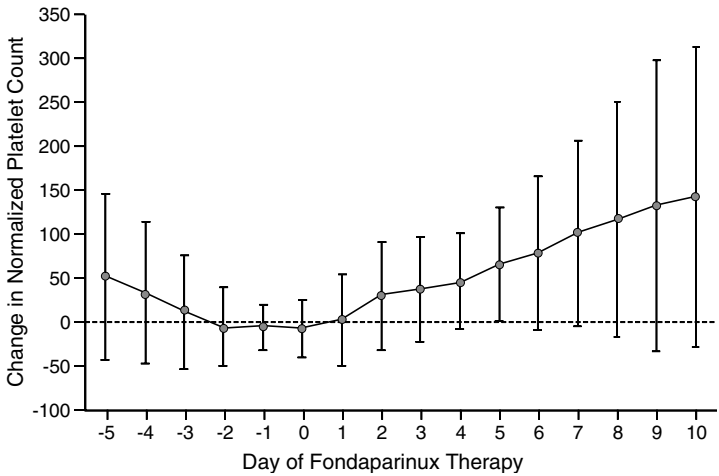

FIGURE 3 Twenty-two patients with HIT were treated with fondaparinux at (or near) the platelet count nadir following exposure to UFH or LMWH. Platelet count recovery is illustrated by this graph, which compares mean platelet counts for the population. Platelet data have been normalized by subtracting the individual value of the patient's platelet count at first administration of fondaparinux from each preceding and subsequent measurement. Error bars represent one standard deviation from the mean. *Abbreviations*: UFH, unfractionated heparin; LMWH, low molecular weight heparin; HIT, heparin-induced thrombocytopenia.

TABLE 4 Emerging and Established Parenteral and Oral Factor Xa Inhibitors[a]

Agent	Company	Phase	Therapeutic applications evaluated to date
Oral			
Apixaban	BMS	Phase II	ACS, AF, VTE prevention in orthopedic surgery
Du176b	Daiichi Sankyo	Phase II	VTE prevention after orthopedic surgery
Rivaroxaban	Bayer	Phase II/III	ACS, AF, DVT treatment, PE treatment, VTE prevention after orthopedic surgery
LY 517717	Lilly	Phase II	VTE prevention after orthopedic surgery
PD 0348292	Pfizer	Phase II	VTE prevention after orthopedic surgery
YM 150	Astellas	Phase II	AF, VTE prevention after orthopedic surgery
	GSK	Phase II	VTE prevention after orthopedic surgery
Parenteral			
DX-9065a	Daiichi Sankyo	Phase II	ACS
Fondaparinux	GSK	Phase IV	ACS, DVT treatment, PE treatment, VTE prevention after orthopedic surgery, general surgery, and in medical patients
Idraparinux[b]	Sanofi-aventis	Phase III	AF, DVT treatment, PE treatment
Otamixaban	Sanofi-aventis	Phase II	ACS

Note: The only factor Xa inhibitor currently approved for clinical use is fondaparinux.
[a]Does not include agents in phase 1.
[b]Biotinylated idraparinux is currently being evaluated in clinical trials.
Abbreviations: ACS, acute coronary syndrome; AF, atrial fibrillation; DVT, deep vein thrombosis; PE, pulmonary embolism; VTE, venous thromboembolism.

sc administration allowing for outpatient therapy, and lower cost. There are however, important unresolved issues including the appropriate dosing regimen of fondaparinux for the severe hypercoagulability state of HIT, as well as whether the 4–5-day delay to reach peak steady-state levels of fondaparinux is adequate for a condition in which maximal thrombin generation occurs at diagnosis. A single-arm phase II evaluation of fondaparinux in the treatment of isolated HIT is currently being planned.

V. DIRECT INHIBITORS OF FACTOR Xa

A. History

Historical antecedents for the development of direct factor Xa inhibitors and the rationale for targeting factor Xa are discussed above. The success of fondaparinux has fueled the development of a large number of *direct* factor Xa inhibitors that are currently in various stages of clinical development (Table 4). Fondaparinux must be administered parenterally, which is inconvenient for long-term administration, and because the greatest clinical need for new anticoagulants is for a replacement for warfarin, the present focus of drug development is on *oral* direct factor Xa inhibitors.

To date no oral direct factor Xa inhibitors have been approved for clinical use. This section will focus on those agents that are most advanced in clinical development.

B. Pharmacology

Direct factor Xa inhibitors bind to the active site of factor Xa and block the interaction of factor Xa with its substrates (Hirsh et al., 2005). Unlike indirect factor Xa inhibitors which are catalytic and result in AT-mediated irreversible inhibition of free factor Xa, the direct factor Xa inhibitors are reversible, and not only inhibit free factor Xa, but also inactivate factor Xa bound to platelets within the prothrombinase complex (Table 5). This represents an important theoretical

TABLE 5 Comparison of Indirect and Direct Factor Xa Inhibitors

Characteristic	Indirect	Direct
Inhibitory mechanism	AT-dependent Catalytic	AT-independent Stoichiometric
Binding	Irreversible (i.e., covalent AT-Xa complexes are formed)	Reversible
Drug target	Free Xa	Free and tissue-bound Xa

Abbreviation: AT, antithrombin.

advantage of direct factor Xa inhibitors over the indirect inhibitors, because prothrombinase-bound factor Xa that is not inhibited can continue to generate thrombin and thereby promote thrombus formation. Because of the pharmacological differences between direct and indirect factor Xa inhibitors, it cannot be assumed that direct factor Xa inhibitors will achieve the same success as indirect factor Xa inhibitors such as fondaparinux.

C. Direct Factor Xa Inhibitors in Clinical Development

Factor Xa inhibitors currently being evaluated in clinical trials are listed in Table 4. Among the most advanced in clinical development are rivaroxaban (Johnson & Johnson Pharmaceutical Research & Development, Raritan, NJ) and apixaban (Bristol-Myers Squibb, New York, NY).

Rivaroxaban is a selective competitive direct factor Xa inhibitor with high bioavailability (60–80%) (Kubitza and Haas, 2006). Maximum plasma drug levels occur 2–3 h after oral administration, and the terminal half-life is 6–9 h. It is excreted by both renal (66%) and fecal/biliary (28%) routes, and is administered once or twice daily. Rivaroxaban causes dose-dependent prolongation of the PT and aPTT (Kubitza and Haas, 2006). Rivaroxaban has shown promising results in phase II trials for the prevention and treatment of VTE in patients undergoing orthopedic surgery and is currently being tested in phase II trials for the management of ACS, in phase III trials for the prevention and treatment of VTE, and in phase III trials for the prevention of stroke or systemic embolism in patients with atrial fibrillation (AF).

Razaxaban is an orally active direct factor Xa inhibitor that was being developed by Bristol-Myers Squibb, but was discontinued after the three higher doses in a dose ranging study to prevent VTE were stopped because of increased bleeding (Kubitza and Haas, 2006). Apixaban is a follow-up compound to razaxaban and is currently entering clinical trials for the prevention of VTE, for the management of ACS, and for the prevention of stroke or systemic embolism in patients with AF.

D. Role of Direct Factor Xa Inhibitors in the Treatment of HIT

Oral direct factor Xa inhibitors have not been evaluated for the treatment of HIT but are attractive for this indication because the small-molecule factor Xa inhibitor compounds are not expected to associate with PF4 or to cross-react with anti-PF4/heparin antibodies. Oral direct factor Xa inhibitors will not cause coumarin-induced microthrombosis (a particular risk of HIT) and are therefore potentially suitable for use during the acute or subacute phase of HIT.

VI. ORAL DIRECT INHIBITORS OF FACTOR IIa

A. History

DTIs were developed to overcome the limitations of the heparin/AT complex to inactivate tissue-bound thrombin (Weitz and Buller, 2002). The prototype DTI is hirudin, a naturally occurring 65-amino acid polypeptide first isolated from the salivary gland of medicinal leeches but now manufactured using recombinant DNA technology. Hirudin and argatroban are approved for the treatment of HIT (see Chapters 14 and 15), argatroban is also approved for use in patients with or at risk of HIT undergoing PCI (see Chapter 15), and bivalirudin is licensed as an alternative to heparin in patients with or without HIT undergoing PCI (see Chapter 16). Hirudin, argatroban, and bivalirudin are all parenteral agents and do not address the unmet need for an oral anticoagulant to replace warfarin. Several oral DTIs have been developed; ximelagatran was the first orally available DTI and dabigatran etexilate is currently being evaluated in phase III clinical trials.

B. Pharmacology

Thrombin bound to fibrin or fibrin degradation products is protected from being inhibited by AT-heparin but is susceptible to inactivation by DTIs (Weitz and Buller, 2002). Thus, DTIs block both free and fibrin-bound thrombin, which is an important theoretical advantage over heparin because fibrin-bound thrombin can continue to promote thrombus formation. Because DTIs do not bind plasma proteins, they also produce a more predictable anticoagulant response than heparin (Weitz and Buller, 2002).

C. Oral DTIs in Clinical Development

Ximelagatran is a prodrug of melagatran, a dipeptide mimetic of the portion of fibrinopeptide A that interacts with the active site of thrombin and blocks the enzyme's interaction with its substrate (Linkins and Weitz, 2005). After oral ingestion, ximelagatran undergoes rapid biotransformation to melagatran. Melagatran is eliminated via the kidneys and has a half-life in the plasma of 4–5 h. It has no known interactions with food, drugs or alcohol and is administered twice-daily.

Ximelagatran has been extensively evaluated in large phase III RCTs for the prevention and treatment of VTE, for the prevention of stroke or systemic embolism in AF, and for the management of MI. Despite favorable efficacy and bleeding results, ximelagatran was not approved for use in North America because 5–10% of patients developed abnormal liver function tests, typically between 6 wk and 6 mo of treatment, and several deaths possibly related to hepatotoxicity occurred. The drug has been withdrawn from European markets (Bauer, 2006).

Dabigatran etexilate is a prodrug of dabigatran, a specific, competitive, and reversible inhibitor of thrombin. Dabigatran etexilate is rapidly absorbed after oral administration and is converted to dabigatran. The plasma half life is approximately 8 h after a single dose and 14–17 h after multiple doses. Dabigatran is renally cleared. Promising results of phase II trials of dabigatran etexilate for the prevention of VTE in major orthopedic surgery, for the initial and long-term management of VTE, and for the prevention of stroke and systemic embolism in AF have prompted several phase III trials currently in progress (Bauer, 2006). There are no reports to date of liver toxicity with dabigatran etexilate.

D. Role of DTIs in the Treatment of HIT

Oral DTIs have not been evaluated for the treatment of HIT but share the potential of the parenteral DTIs for this indication. Parenteral DTIs are widely used for the

management of HIT, particularly in the United States where danaparoid is not available. The availability of an oral DTI alternative to warfarin may reduce the potential for HIT-associated microthrombosis, as has been reported during warfarin administration during the acute or subacute phase of HIT (see Chapters 2 and 12).

VII. SUMMARY

It is likely that new parenteral and oral anticoagulants that selectively target factor Xa or thrombin will increasingly replace heparin and warfarin in the future. As a consequence of this evolution in anticoagulant practice, the use of UFH is likely to decline, resulting in a reduction in the incidence of HIT, and there is likely to be an expansion of the treatment options for HIT. Fondaparinux has an extensive track record as a highly effective and safe parenteral anticoagulant, which is replacing UFH and LMWH across a broad spectrum of clinical indications. Fondaparinux does not cross-react with anti-PF4/heparin antibodies and there are promising albeit limited data supporting its effectiveness and safety for the treatment of HIT. New oral factor Xa inhibitors and oral DTIs are theoretically attractive for the treatment of HIT, but further evaluation is needed for all of these agents.

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18 Hemodialysis in Heparin-Induced Thrombocytopenia

Karl-Georg Fischer
University Hospital Freiburg, Freiburg, Germany

I. HEPARIN-INDUCED THROMBOCYTOPENIA IN HEMODIALYSIS PATIENTS

Given the major role of unfractionated heparin (UFH) for anticoagulation in hemodialysis (HD), it is important to define the potential impact of immune heparin-induced thrombocytopenia (HIT) in contributing to morbidity and mortality in patients with dialysis-dependent renal failure.

To date, there is only one study reporting the incidence of HIT in patients being newly treated with HD. Six of 154 patients (3.9%) were clinically suspected of having developed HIT because of a fall in the platelet count accompanied by clotting of the dialyzer and extracorporeal circuit (Yamamoto et al., 1996). The clinical diagnosis was confirmed by the detection of HIT antibodies in all but one patient. Only one patient developed organ damage from thrombosis (myocardial infarction and stroke). All six patients were switched to an alternative anticoagulant and did not suffer from thromboembolic events in the follow-up period. Compared with the incidence of HIT of 2.7% found in 332 hip surgery patients undergoing thromboprophylaxis with UFH (Warkentin et al., 1995), the incidence of HIT in acute HD patients thus appears to be similar, regardless of the underlying cause of renal dysfunction (Finazzi and Remuzzi, 1996).

Several cross-sectional studies of HIT and anti-platelet factor 4 (PF4)/heparin antibody formation in HD patients report detection of antibodies in up to 18% of HD patients receiving UFH (Table 1). For patients undergoing HD using low molecular weight heparin (LMWH), the frequency in one study was 0.3% (1/133) (Boon et al., 1996). Only in a few patients did HIT antibodies cause thrombocytopenia or thromboembolic events.

Tentative conclusions suggested by these studies are that only a few patients who form anti-PF4/heparin antibodies in association with HD develop clinical events and that these are more likely to be clotting of the dialyzer and extracorporeal circuit than symptomatic thrombosis affecting the patient. It is also possible that the risk of clinical HIT is higher in patients starting HD (the population studied by Yamamoto et al.) than in patients in the long-term phase of HD (as per the remaining studies). Anecdotal case reports of HIT complicating HD also seem frequently to include patients undergoing short-term HD (Matsuo et al., 1989; Hall et al., 1992; Nowak et al., 1997; Gupta et al., 1998), or HD given in a postoperative setting (Hartman et al., 2006). Given the low rate of thromboembolic events in HD patients having antibodies in the absence of thrombocytopenia, switch to alternative anticoagulants has been considered justified only if clinical symptoms or signs of HIT occur (Greinacher et al., 1996).

Another complicating feature is that UFH can exert a platelet proaggregatory effect even in the absence of anti-PF4/heparin antibodies, particularly in critically

TABLE 1 Frequency of Anti-PF4/Heparin Antibodies and Clinical HIT in Hemodialysis Patients

		Frequency of HIT antibodies		Frequency of HIT	
Reference	N[a]	Antigen test	Functional test	Thrombocytopenia	Thrombosis
De Sancho et al., 1996	45	0% (0/45)	NA	NA	NA
Yamamoto et al., 1996	154	6.8% (5/73)	NA	3.8% (6/154)[b]	3.8% (6/154)[c]
Greinacher et al., 1996	165	NA	4.2 (7/165)	0% (0/165)	0% (0/165)
Boon et al., 1996	261	1.9% (5/261)	NA	0% (2/261)	0% (0/261)
Sitter et al., 1998	70	2.8% (2/70)	NA	0% (0/70)	0% (0/70)
Luzzatto et al., 1998	50	12% (6/50)	NA	0% (0/50)	0% (0/50)
O'Shea et al., 2002	88	1.1% (1/88)	NA	0% (0/88)	0% (0/88)
Peña de la Vega et al., 2005	57	3.5% (2/57)	NA	1.7% (1/57)	0% (0/57)
Palomo et al., 2005	207	17.9% (37/207)	5.8% (12/207)	18.5% (29/156)	12.4% (21/207)
Carrier et al., 2006	419	12.9% (54/419)	0% (0/419)	NA	–[d]

[a]All patients are chronic hemodialysis patients except Yamamoto et al. (acute HD patients, $n = 50$; chronic HD patients, $n = 104$).
[b]Defined as a greater than 20% reduction in platelet counts.
[c]Dialyzer clotting defined as a consequence of HIT (other forms of thrombosis observed only in one patient).
[d]Of 419 patients, 107 patients had access thrombosis. However, no significant correlation was observed between presence of HIT antibodies and access thrombosis.
Abbreviations: HD, hemodialysis; HIT, heparin-induced thrombocytopenia; NA, not available.

ill patients undergoing continuous venovenous HD (Burgess and Chong, 1997); this heparin-induced platelet proaggregatory effect is less marked with LMWH, and absent or negligible with danaparoid sodium.

II. CLINICAL PRESENTATION OF HIT IN HD PATIENTS

The diagnosis of HIT and respective management decisions should be primarily based on clinical criteria (Lewis et al., 1997). A further consideration in HD patients is that the procedure of HD itself is associated with a relative decrease in platelet count, even when so-called biocompatible dialyzer membranes are used (Beijering et al., 1997; Schmitt et al., 1987). Furthermore, the fall in platelet count in HD patients developing HIT may be only moderate (Matsuo et al., 1997).

The occurrence of fibrin formation, or even frank clotting of the extracorporeal circuit despite apparent sufficient anticoagulation, should lead to suspicion of possible HIT (Koide et al., 1995). One of the most serious complications, occlusion of vascular access (the "Achilles' heel" of HD), may also indicate HIT, and it has been described for both native fistulae as well as prosthetic grafts (Hall et al., 1992; Laster et al., 1989). However, whereas vascular access thrombosis frequently occurs in HD patients, it appears questionable whether HIT increases the risk of this complication (Carrier et al., 2006; Chang and Parikh, 2006; Nakamoto et al., 2005; O'Shea et al., 2002; Palomo et al., 2005).

The occurrence of an anaphylactoid reaction, sometimes termed an "acute systemic reaction," upon initiating HD with UFH or LMWH is a sign of possible acute HIT (Hartman et al., 2006), and represents systemic manifestations of rapid platelet activation (see Chapter 2). Symptoms and signs include fever, chills,

tachycardia, and dyspnea, sometimes mimicking pulmonary embolism ("pseudo-pulmonary embolism").

Severe skin necrosis, even in the presence of normal platelet counts, has been reported in association with the presence of HIT antibodies in patients after both short- and long-term HD (Bredlich et al., 1997; Leblanc et al., 1994).

Recently, data have been reported concerning the impact of elevated absorbance in the anti-PF4/heparin enzyme(-linked) immuno(sorbent)assay (EIA, or ELISA) test below the threshold permitting serologic diagnosis of HIT on cardiovascular outcome in HD patients (Peña de la Vega et al., 2005). Absorbance values in the EIA of 57 patients were ranked in tertiles. Patients then were followed for a median of 798 days. After risk adjustment, patients in the highest tertile (still below the threshold of HIT positivity) had a 2.47-fold greater risk ($p = 0.03$) of all-cause mortality and a 4.14-fold greater risk of cardiovascular mortality ($p = 0.02$) compared to the lower tertiles. Further studies are necessary to confirm this finding and to clarify if it is a mere epiphenomenon, e.g., of endothelial damage or, in contrast, represents a causal role of heparin-dependent antibodies for the poor cardiovascular outcome of HD patients. In the latter case, antibody levels might be worthwhile to be measured on a regular basis in HD patients (Chang and Parikh, 2006), although the crucial issue of whether benefit would result from substituting heparin with alternative anticoagulants for HD would remain unresolved.

Rarely, patients can develop HIT after years of regular long-term maintenance HD. Tholl et al. (1997) reported a patient who developed HIT following surgery after 9 yr of long-term intermittent HD performed with UFH. In this patient, an anaphylactic reaction to heparin, accompanied by a platelet count fall, led to the diagnosis of HIT. It is possible that the surgery itself contributed to HIT antibody formation, as the highest reported rates of HIT are in postoperative patients receiving UFH (see Chapter 3).

Unfortunately, HD complications associated with HIT are not very specific. Thus, the clinician must consider other factors that could compromise patency of the extracorporeal circuit (e.g., low blood flow, high ultrafiltration rate, excess turbulence within the circuit, or foam formation with blood–air interfaces in the drip chambers). The quality of the vascular access plays a crucial role in this. Other patient-related factors include low arterial blood pressure, high hematocrit, and the need for intradialytic blood transfusion or lipid infusion (Hertel et al., 2001). In addition to insufficient anticoagulation, these factors should be ruled out first as the underlying causes of clotting within the extracorporeal circuit. Given the long-term implications of labeling HD patients as having HIT, laboratory testing for HIT antibodies should only be performed when HIT is clinically suspected (O'Shea et al., 2003).

III. MANAGEMENT OF HD IN HIT PATIENTS

A. Discontinuation of Heparin Treatment

As HIT is frequently associated with potentially life-threatening thrombotic events (Warkentin et al., 1995; Warkentin and Kelton, 1996), discontinuation of heparin treatment and initiation of adequate alternative anticoagulation is generally considered mandatory (Warkentin et al., 1998). Thus, heparin must not be added to any flushing solution, and no heparin-coated systems can be used. Indeed, heparin flushes and heparin-coated devices can both initiate and sustain HIT (Moberg et al., 1990; Kadidal et al., 1999).

B. Unsuitable Approaches

Low Molecular Weight Heparin

LMWH is not recommended as an alternative anticoagulant for managing acute HIT. In vitro tests for HIT antibodies show a high degree of cross-reactivity between UFH and LMWH (Greinacher et al., 1992b; Vun et al., 1996). Furthermore, in vivo cross-reactivity manifesting as persistent or recurrent thrombocytopenia or thrombosis during LMWH treatment of HIT appears to be common (Greinacher et al., 1992a; Horellou et al., 1984; Roussi et al., 1984). Because non-heparin anticoagulants are available, LMWH probably should not be used even if in vitro cross-reactivity is reported to be negative.

Regional Heparinization

Regional heparinization is defined as application of heparin at the inlet of the extracorporeal circuit and its neutralization by protamine at the outlet of the circuit. However, its use in HIT is problematic because of the potential for heparin "contamination" of the patient, as well as for heparin "rebound anticoagulation" (recurrence of heparin anticoagulation owing to shorter half-life of protamine compared with heparin) (Blaufox et al., 1966). Moreover, direct injurious effects of protamine on the clotting cascade can occur. Consequently, this regimen is not recommended for HD of patients with HIT.

Aspirin

Acetylsalicylic acid has been used as an antiplatelet agent together with continued anticoagulation with UFH for HD of patients with HIT (Hall et al., 1992; Janson et al., 1983; Matsuo et al., 1989). This approach is not recommended for at least two reasons: (1) protection against heparin-induced platelet activation may be incomplete or absent, as aspirin's effects on blocking the thromboxane-dependent pathway of platelet activation does not reliably inhibit platelet activation by HIT antibodies (Kappa et al., 1987; Polgár et al., 1998; Selleng et al., 2005); and (2) the bleeding risk of uremic patients is increased.

HD Without Anticoagulant

HD without an anticoagulant (Romao et al., 1997) is not ideal for maintenance HD. Without anticoagulation, the artificial surfaces become coated, first by plasma proteins, followed by adhesion and activation of platelets, with accompanying activation of the coagulation cascade (Basmadjian et al., 1997). This will markedly reduce dialysis quality in removal of fluid and solutes long before clotting of the circuit is visible. Moreover, this approach may aggravate HIT-associated thrombosis. However, in patients at high risk of bleeding (e.g., owing to hepatic disorders or multiorgan failure, or those requiring surgery), temporary HD without anticoagulant may be appropriate.

C. Adequate Anticoagulants for HD in HIT Patients

Patients with renal failure show plasma hypercoagulability as well as uremic platelet defects, both of which can be worsened by HD (Ambühl et al., 1997; Sreedhara et al., 1995; Vecino et al., 1998). Therefore, selection of an appropriate anticoagulant in HD patients who also suffer from HIT is difficult.

Reports on specific anticoagulant strategies in HIT are anecdotal. Large studies, especially those comparing different anticoagulant regimens, are lacking. Therefore, no treatment recommendations based on level A or B evidence (i.e.,

randomized trials) can be provided. Furthermore, because UFH is the routine anticoagulant in use for HD, considerable additional time, effort, and costs are usually required to manage a new anticoagulant for HD, especially during initial use. Ideally, therefore, a center should try to gain experience with a single appropriate alternative anticoagulant for management of these difficult patients. Fear of inducing bleeding should not be used to justify under-anticoagulation, with the potential risk for thrombotic complications.

Danaparoid Sodium

Danaparoid sodium (Orgaran, formerly known as Org 10172) is the alternative anticoagulant that has been most widely used for management of HD in patients with HIT (Chong and Magnani, 1992; Greinacher et al., 1992a, 1993; Henny et al., 1983; Magnani, 1993; Magnani and Gallus, 2006; Neuhaus et al., 2000; Ortel et al., 1992; Roe et al., 1998; Tholl et al., 1997; Wilde and Markham, 1997). However, danaparoid is currently not available in the United States (see Chapter 13). Some of its characteristics require specific attention:

1. The anticoagulant activity of danaparoid can be monitored only by measurement of antifactor Xa levels based on a danaparoid calibration curve; however, many laboratories do not routinely perform these assays. Except for an emergency situation, such as when HIT is strongly suspected and danaparoid is the only available alternative, HD should not be performed without monitoring the antifactor Xa activity to evaluate the dose required for adequate anticoagulation. Once the optimal dose is identified, it can often be used without alteration for several subsequent HD sessions, provided no bleeding or inappropriate clotting occurs and no surgical intervention is scheduled. Periodic measurement of antifactor Xa activity thereafter to validate the dosing of danaparoid is recommended. For maintenance HD without complications, single determination of pre-HD antifactor Xa activity probably suffices. If there are concerns about adequate or excess anticoagulation, then monitoring of levels at three time points is appropriate (e.g., 30–60 min pre-HD, 30 min after beginning HD, and just before completion).
2. Regarding the pharmacokinetics of danaparoid, renal excretion accounts for approximately 40–50% of total plasma clearance; accordingly, diminished clearance of antifactor Xa activity occurs in HD patients (Danhof et al., 1992). The elimination half-life of the antifactor Xa activity (about 24 h in healthy individuals) (Danhof et al., 1992) may reach as high as 4 days (unpublished observations of the author). Thus, significant antifactor Xa levels can be detected in patients undergoing HD with danaparoid even during the interdialytic interval. Whether this yields clinical benefit, such as decreased risk of thrombosis or greater maintenance of vascular access, is unknown. An increase in interdialytic bleeding episodes has not been reported.
3. Given its pharmacokinetics, danaparoid is given by initial bolus in intermittent HD, which normally is sufficient to prevent clotting within the extracorporeal circuit during the procedure. Danaparoid anticoagulation may also be useful in critically ill patients on continuous renal replacement therapy. In 13 consecutive intensive care unit (ICU) patients clinically suspected to have HIT, danaparoid was administered by initial bolus followed by continuous infusion (Lindhoff-Last et al., 2001). This regimen was sufficient to prevent clotting within the extracorporeal circuit both in continuous venovenous hemofiltration (eight

patients) and in continuous venovenous HD (five patients), respectively (Lindhoff-Last et al., 2001). Thromboembolic complications did not occur. Despite a mean danaparoid infusion rate of approximately 140 U/h, which is markedly reduced compared to the recommendation of the manufacturer, major bleeding was observed in six of 13 patients (which could be explained by disseminated intravascular coagulation in five patients). However, HIT was confirmed by antibody detection in only two patients. Thrombocytopenic patients not having the prothrombotic state of acute HIT likely are at increased bleeding risk. Therefore, dosing of danaparoid in ICU should be based on the individual patient's risk of bleeding versus thrombosis. With regard to invasive procedures, the long half-life of danaparoid should be considered.

4. No antidote to danaparoid exists. Accordingly, we evaluated hemofiltration as a potential means to rapidly reduce danaparoid plasma concentration. Whereas five different high-flux hemodialyzer membranes did not allow for danaparoid filtration, a plasmapheresis membrane was capable of removing danaparoid from the blood compartment (Schneider et al., 2004). Hence, plasmapheresis may be a way to reduce danaparoid levels in situations of overdosing or bleeding. Again, careful dosing of danaparoid is important to avoid bleeding.
5. HIT antibodies potentially cross-react with danaparoid. Although the respective clinical risk has been claimed to be less than 5% (Warkentin et al., 1998; Magnani and Gallus, 2006), individual patients, nevertheless, may be threatened if this condition occurs. As positive in vitro cross-reactivity is of uncertain clinical significance (Warkentin, 1996; Wilde and Markham, 1997; Newman et al., 1998), attention should focus on platelet count monitoring. A further fall in platelet count, or new fibrin deposits and clot formation within the extracorporeal circuit after application of danaparoid, may indicate clinically relevant cross-reactivity. To differentiate in vivo cross-reactivity from "under-anticoagulation" owing to insufficient dosage, determination of anti-factor Xa levels and HIT antibody cross-reactivity studies are needed.

Table 2 lists dose recommendations for use of danaparoid for HD as provided by the manufacturer. The recommendations should be considered as guidelines and not followed uncritically in any individual patient. If applied with appropriate care, danaparoid provides adequate anticoagulation for HD of HIT patients with a favorable benefit/risk ratio, even during long-term use.

Recombinant Hirudin

Native hirudin was the first anticoagulant used for HD over 75 yr ago (Haas, 1925). In recent years, interest in its use for HD has redeveloped because of the availability of recombinant preparations, as well as the clinical need for managing patients with HIT. A preparation of recombinant hirudin (r-hirudin), lepirudin (Refludan® or HBW023), has been used successfully in humans for anticoagulation of both intermittent (Bucha et al., 1999a; Nowak et al., 1992, 1997; Steuer et al., 1999; Vanholder et al., 1994; Van Wyk et al., 1995) and continuous HD (Fischer et al., 1999; Schneider et al., 2000; Saner et al., 2001; Vargas Hein et al., 2001).

For use of r-hirudin anticoagulation in HD, some aspects should be specifically addressed. For further information on r-hirudin in renal insufficiency, the reader is referred to a recent review (Fischer, 2002):

1. As there is repetitive exposure to r-hirudin when used for regular, intermittent HD, immunogenicity of r-hirudin is of particular interest. Initially, r-hirudin

appeared to be a weak immunogen (Bichler et al., 1991). However, recent studies revealed frequent development of antihirudin antibodies (AHAb) in patients receiving lepirudin for more than 5 days (Huhle et al., 1999, 2001; Song et al., 1999; Eichler et al., 2000). In addition, allergic reactions (including fatal anaphylaxis) to r-hirudin have been reported (Huhle et al., 1998; Eichler et al., 2000; Greinacher et al., 2003).

r-Hirudin is increasingly used for alternative anticoagulation in HD. Here, repetitive application of r-hirudin in patients on an intermittent maintenance HD regimen is likely to favor both induction and boostering of an immune response against the drug. As prospective studies evaluating sufficient numbers of HD patients on r-hirudin anticoagulation for the generation of AHAb are lacking, the incidence of AHAb and related adverse clinical events in this patient population remain to be elucidated.

Studies of HIT patients treated with lepirudin suggest that AHAb sometimes reduce renal lepirudin clearance (Huhle et al., 1999; Eichler et al., 2000). Indeed, marked reduction of renal lepirudin clearance due to monoclonal AHAb has been demonstrated in rats with normal renal function (Fischer et al., 2003). This was accompanied by a significant increase of both maximal plasma concentration and area under the curve of the alternative anticoagulant when compared to non-AHAb-treated animals. In chronic renal failure patients undergoing HD this may not be an issue. However, even small reductions in residual renal function have been shown to account for relevant prolongation of r-hirudin decay in plasma (Bucha et al., 1999a; Vanholder et al., 1997). Further reduction of renal r-hirudin clearance due to AHAb thus may influence r-hirudin dosing in these patients.

In acute renal failure requiring HD treatment for a prolonged period, reduction of renal r-hirudin clearance attributable to AHAb may be more relevant. Here, in patients suffering from multiorgan failure, the r-hirudin dosage required for sufficient anticoagulation was reduced significantly compared with the dosage needed in patients with normal renal function. In addition, r-hirudin dosage varied markedly depending on the residual renal function (Fischer et al., 1999). AHAb are likely to reduce further the amount of r-hirudin required, and thus may complicate anticoagulation in this challenging patient population.

The animal study also showed a significant decrease in the volume of distribution of lepirudin at steady state in the presence of AHAb (Fischer et al., 2003). Hence, even if further reduction of renal r-hirudin clearance owing to AHAb was negligible, major alterations in r-hirudin plasma concentration could still occur.

2. There remains debate as to which laboratory parameter is best suited for monitoring r-hirudin treatment. Initial studies addressing this in HD patients yielded conflicting results (Vanholder et al., 1994, 1997; Van Wyk et al., 1995). However, it now appears that the ecarin clotting time (ECT) (Nowak and Bucha, 1996) and chromogenic substrate assays (Griessbach et al., 1985) measure the r-hirudin plasma concentration with adequate precision over a wide concentration range and correlate well with each other (Hafner et al., 2000, 2002). However, as these tests are often not available, monitoring of r-hirudin anticoagulation is usually performed with the activated partial thromboplastin time (aPTT). A meta-analysis of two lepirudin treatment trials for HIT revealed a suitable aPTT ratio for reducing clinical thromboembolic complications to be

TABLE 2 Anticoagulation in Hemodialysis of HIT Patients—Dosage Examples of Suitable Alternative Anticoagulants

Agent	Dialysis procedure			Bolus	Continuous infusion	Monitoring parameter[a]	Target range
Danaparoid sodium (Org10172, Orgaran®)	Intermittent HD (every second day)	Before first 2 HDs		3750 (2500)[b,c]	–	Anti-Xa activity	0.5–0.8[d,e]
		Subsequent HD	Predialytic anti-Xa activity[d,f]				
			<0.3	3000 (2000)	–		
			0.3–0.35	2500 (1500)	–		
			0.35–0.4	2000 (1500)	–		
			>0.4	0[g]	–		
	Intermittent HD (daily)	First HD		3750 (2500)[b,c]	–	Anti-Xa activity	0.5–0.8[d,e]
		Second HD		2500 (2000)	–		
		Subsequent HD		See above	–		
	Continuous HD/HF		Initial bolus	2500 (2000)[b,c]			
			First 4 h	–	600 (600)[c,h]	Anti-Xa activity	0.5–1.0[d,i]
			Next 4 h	–	400 (400)[c,h]		
			Subsequently	–	200–600[g,h,j] (150–400)[c,g,h,j]		
Lepirudin (HBW023, Refludan®)	Intermittent HD (every second day)			0.08–0.15[k,l,m]	–	aPTT ratio[n,o,p]	2–3[e,q]
						Hirudin conc.[r]	0.5–1.2[e,s]
	Continuous HD[t,u]		Initial bolus	0.01[k,l,m,v]	–	aPTT ratio[n,o,p]	1.5–2.0[w,x]
			Subsequent boluses	0.005–0.01[k,l,m,v]	–		
			Alternatively	–	0.005–0.01[k,l,m,v]		

Argatroban (MD-805, Novastan®, Argatra®)	Intermittent HD[t] (every second day)	250 μg/kg	1.7–3.3 μg/kg/min	aPTT ratio[o]	1.5–3.0[q]	

Note: Many of the approaches discussed in this chapter have not been formally studied, none has been approved yet. Treatment examples are given based on a limited number of cases successfully treated with the respective regimen. The different anticoagulants thus cannot be uncritically applied in the dosage given here. The choice of anticoagulant should depend on the experience of the center and the anticoagulant monitoring available. Doses for danaparoid as given by the manufacturer.

[a]Monitoring the condition of the dialyzer after a HD session as well as the time required for termination of bleeding of the fistula should be included as well.

[b]Dosage given in anti-Xa units (bolus).

[c]Dosage in brackets for patients with body weight <55 kg.

[d]Data given in U/mL.

[e]Peak activity determined after approximately 30 min of HD; this level is not required throughout the whole HD session.

[f]Determination 30–60 min before start of the respective HD session.

[g]If fibrin deposition in the dialyzer or clots in the extracorporeal circuit occur, addition of 1500 anti-Xa units as a single bolus.

[h]Dosage given in anti-Xa units/h (infusion).

[i]To achieve the same anti-Xa activity, smaller doses may be required in hemo*dialysis* as compared to hemo*filtration*.

[j]Maintenance dosage dependent on actual anti-Xa activity; determination every 12 h (provided that no bleeding or clotting occurs).

[k]Dosage given in mg/kg body weight for hemodialysis performed with polysulfone high-flux hemodialyzers.

[l]The dosage required to reach the target range may vary, for example due to residual renal function or the type of dialyzer used (cf. text).

[m]If larger doses are needed to achieve the target range or to avoid clotting of the extracorporeal circuit, changing to another type of dialyzer may be helpful.

[n]In our center aPTT is determined with the BCS coagulometer and Pathromtin SL as aPTT reagent (both Dade-Behring, Liederbach, Germany).

[o]According to the literature alternative tests such as Ecarin Clotting Time (ECT) or Activated Clotting Time (ACT) also appear suitable for monitoring.

[p]It is unclear which test is best-suited to monitor anticoagulation with r-hirudin, as no test has been prospectively evaluated in HD patients so far.

[q]A peak aPTT of 100 s should not be exceeded.

[r]Determination in plasma by chromogenic assays.

[s]Data given in μg/mL.

[t]The agent has not yet been formally studied in continuous hemodialysis procedures.

[u]This approach has been successfully performed in a number of patients in our center without adverse events.

[v]Dosage given for anuric patients; in case of polyuria a higher dosage may be required; the required daily dosage may vary significantly between patients.

[w]As patients requiring continuous procedures often are at an increased risk of bleeding, a lower aPTT is to be preferred (50–70 s).

[x]To be initially controlled every 4–6 h to avoid overdosage especially in patients at bleeding risk.

Abbreviations: aPTT, activated partial thromboplastin time; conc., concentration; HD, hemodialysis; HF, hemofiltration; Xa, clotting factor Xa.

between 1.5 and 2.5, which was associated with only a moderately increased bleeding risk (Greinacher et al., 2000). Control of r-hirudin treatment by the aPTT is problematic: there is considerable assay variability among patients and different aPTT reagents (Nurmohamed et al., 1994; Hafner et al., 2000; Lubenow and Greinacher, 2000). In contrast to the foregoing tests, correlation between aPTT and plasma r-hirudin concentration is not linear over a broad concentration range. Instead, linear correlation is observed only with r-hirudin concentrations up to 0.5 μg/mL (Nowak and Bucha, 1996), a concentration often insufficient for HD. Above this concentration, the correlation between aPTT and r-hirudin concentration is poor (Nowak and Bucha, 1996; Hafner et al., 2000), especially for aPTT values of more than 70 s (Lubenow and Greinacher, 2000). Nevertheless, because of its wide availability, aPTT monitoring of r-hirudin treatment is likely to remain common. If available, ECT or chromogenic assays are preferred. A frequent problem in ICU patients with HIT and renal failure are low prothrombin levels, which can cause falsely high values in the aPTT and ECT during therapy with lepirudin or argatroban (risk of underdosing).

3. The elimination of r-hirudin is markedly prolonged in renal impairment. Nowak et al. (1992) reported elimination half-lives of up to 316 h in HD patients. Vanholder et al. (1997) found a prolongation of r-hirudin half-life by a factor of 31 in HD patients compared with healthy controls. Both studies showed a correlation between the residual creatinine clearance and the r-hirudin clearance, in that a minor improvement in creatinine clearance resulted in a shorter elimination half-life of r-hirudin. This was confirmed in a study of HD patients repetitively anticoagulated with r-hirudin (Bucha et al., 1999a). As with HD patients treated with danaparoid, r-hirudin-treated patients remain anticoagulated during the interdialytic interval (Nowak et al., 1997). Because various organs may metabolize hirudin (Grötsch and Hropot, 1991), other factors affecting metabolic clearance of hirudin may be present in patients with end-stage renal failure.

   In patients suffering from acute renal failure, further deterioration or partial recovery of renal function frequently occurs (Fischer et al., 1999). Hence, r-hirudin anticoagulation should be closely monitored in these patients for timely dose adjustments. Preferably, r-hirudin should be given in repeated small boluses, rather than administered continuously, to minimize bleeding risk (Fischer et al., 1999; Kern et al., 1999). For the same reason, use of r-hirudin permeable high-flux hemodialyzers for patients with HD-dependent acute renal failure is recommended, especially as the patients often need vessel punctures, biopsies, or surgical interventions.

   Given the prolonged half-life of r-hirudin in renal impairment, use of polyethylene glycol-hirudin (molecular mass 17 kDa), which has an even greater elimination half-life compared with uncoupled r-hirudin (Pöschel et al., 2000), does not seem appropriate for HD, as bleeding risk likely would be increased.

4. Pharmacokinetics of r-hirudin are also influenced by the type of dialyzer used. The pharmacology of r-hirudin (molecular mass ~7 kDa; volume of distribution 0.20–0.25 L/kg b.w.; low protein binding) should favor its elimination by high flux hemodialyzers with a nominal cutoff point of approximately 60 kDa. Indeed, most high-flux hemodialyzers are permeable to r-hirudin, whereas most of the low-flux hemodialyzers tested appear to be r-hirudin-impermeable (Bucha et al., 1999b; Frank et al., 1999, 2002; Benz et al., 2007; Koster et al.,

2000). However, high-flux hemodialyzers vary considerably in their capacity to filter r-hirudin (Fischer, 2002; Willey et al., 2002). Further, a specific type of hemophan low-flux dialyzer has been reported to show high permeability for r-hirudin (Nowak et al., 1997), whereas a specific type of polysulfone high-flux dialyzer, with a cutoff point of approximately 50 kDa, did not filter r-hirudin from the circulation (Vanholder et al., 1997). Thus, knowledge of the actual filtration characteristics for r-hirudin of a given type of hemodialyzer improves safety of treatment with r-hirudin in HD.

5. r-Hirudin overdosing or unexpected drug accumulation can lead to severe bleeding (Fischer et al., 2000; Kern et al., 1999; Müller et al., 1999). In this situation, r-hirudin can be removed from the circulation using hemofiltration (Bauersachs et al., 1999; Fischer et al., 2000; Mon et al., 2006). However, several hours may be needed to lower r-hirudin plasma levels by 50%, even at high ultrafiltration rates. Thus, careful r-hirudin dosing is of utmost importance. Recent case reports have shown application of recombinant factor VIIa to be of additional value in patients suffering from renal insufficiency and post-operative bleeding upon lepirudin anticoagulation (Hein et al., 2005; Oh et al., 2006). In the presence of AHAb, hemofiltration may no longer suffice to eliminate r-hirudin (Fischer et al., 2003). Here, plasmapheresis may be the only means to clear r-hirudin from the circulation. Preliminary studies in animals suggest that a possible future treatment might be use of certain AHAb with r-hirudin-neutralizing capacity (Liebe et al., 2001).

Table 2 lists dosing recommendations for use of lepirudin for HD. The recommendations should be considered as guidelines and not followed uncritically in any individual patient. In summary, r-hirudin is a valid alternative anticoagulant for HD procedures in HIT patients, but it should be used with caution and careful monitoring.

Argatroban

Argatroban (Novastan®; Argatra®; MD-805) is a potent arginine-derived, synthetic, catalytic site-directed thrombin inhibitor lacking antiplatelet and antifibrinolytic activities (Koide et al., 1995; Matsuo et al., 1992). This agent is approved as alternative anticoagulant for HIT in the United States, Canada and a number of European countries (see Chapter 15). It does not cross-react with HIT antibodies. Apart from an even better relative ability to inhibit fibrin-bound versus soluble thrombin (Berry et al., 1996; Lunven et al., 1996), the principal advantages of argatroban over heparin are similar to r-hirudin (Markwardt, 1991; Matsuo et al., 1992). However, argatroban is metabolized primarily by the liver, and its half-life is only moderately extended in patients with renal insufficiency, i.e., a half-life of 64 ± 35 min in patients with creatinine clearance of 0–29 mL/min versus 47 ± 22 min in patients with creatinine clearance >80 mL/min ($p = 0.58$) (Swan and Hursting, 2000). Also, argatroban dialytic clearance by high-flux membranes is regarded as being clinically insignificant (Murray et al., 2004; Tang et al., 2005).

After argatroban proved to be a valuable anticoagulant in HD (Matsuo et al., 1986), it was applied successfully to HIT patients undergoing this procedure (Koide et al., 1995; Matsuo et al., 1992). In a retrospective analysis of 47 patients with HIT and renal failure requiring renal replacement therapy (with at least 11 patients receiving continuous venovenous or arteriovenous HD), argatroban provided effective anticoagulation with an acceptable safety profile (Reddy et al., 2005). Initially,

argatroban was given according to current dosing recommendations used for the prophylaxis or treatment of thrombosis in HIT, i.e., 2 μg/kg/min (or 0.5 μg/kg/min if hepatically impaired), adjusted to an aPTT 1.5–3-times baseline (see Chapter 15). For adjustment to reach the target aPTT range, argatroban dosing had to be more frequently adjusted downwards than upwards. A recent prospective crossover study of 12 maintenance HD patients showed three different argatroban dosing regimens (bolus alone, infusion alone, or bolus plus infusion) to be safe and well tolerated (Murray et al., 2004). Recent studies show pharmacokinetics of argatroban not to be significantly influenced by different degrees of renal insufficiency. Argatroban dose adjustments were not found to be necessary in these patients (Tang et al., 2005; Guzzi et al., 2006). In contrast, others show argatroban dosing to clearly depend on renal function (Arpino and Hallisey, 2004).

In ICU patients suffering from renal, but not measurable liver insufficiency, however, dose reductions may be frequently necessary (unpublished observations of the author). Based on this experience, in ICU patients, we start argatroban at a reduced dose, provided there is no acute thrombosis. Careful monitoring and dosing is required. Similar experiences have also been reported by others (de Denus and Spinler, 2003; Guzzi et al., 2006; Reichert et al., 2003). Here, decreased cardiac output or hepatic congestion have been posited to cause reduced argatroban requirements (Guzzi et al., 2006).

Argatroban has also proved effective and safe in HD patients with antithrombin deficiency (Ota et al., 2003). Whether anticoagulation with argatroban alone is always sufficient to prevent clotting in the extracorporeal circuit is unclear: in one HD patient treated with argatroban, marked spontaneous platelet aggregation occurred, perhaps due to HIT together with additional platelet activation known to occur in HD (Koide et al., 1995). Because platelet aggregation could not be suppressed by argatroban alone in this patient, aspirin was added to achieve patency of the extracorporeal circuit.

Periodic monitoring of the anticoagulant activity of argatroban is recommended (Matsuo et al., 1992) using for example the aPTT (Koide et al., 1995; Matsuo et al., 1992), the ECT (Berry et al., 1998), or the activated clotting time (ACT) (Murray et al., 2004; Tang et al., 2005).

Argatroban appears to be at least as well suited as r-hirudin for anticoagulation of HIT patients requiring HD. Its predominant hepatic elimination favors argatroban for alternative anticoagulation in chronic renal failure. Its role and dosing in ICU patients suffering from acute renal failure remain to be defined.

Table 2 lists dosing recommendations for use of argatroban for HD. The recommendations should be considered as guidelines and not followed uncritically in any individual patient. In particular, ICU patients often do not require full dose argatroban.

Vitamin K Antagonists

For HIT patients requiring long-term anticoagulation, vitamin K antagonists (coumarins) are usually given. Although coumarins decrease hemostasis and thrombosis, fibrin formation within the extracorporeal circuit is not always sufficiently blocked. In these cases, additional low-dose intravenous anticoagulation with UFH is usually given for regular maintenance HD. However, in HIT patients requiring HD, alternative low-dose anticoagulation has not been formally studied. The need for additional intravenous anticoagulation depends on the increase of the international normalized ratio (INR), which should be checked regularly

before HD. Priming of the extracorporeal circuit by addition of a compatible anticoagulant to the filling solution with subsequent washout before start of the respective HD session may be of value in diminishing the risk of "overanticoagulation."

D. Other Approaches

Dermatan Sulfate

Dermatan sulfate is a natural glycosaminoglycan that selectively inhibits both soluble and fibrin-bound thrombin through potentiation of endogenous heparin cofactor II. It does not interfere with platelet function. Dermatan sulfate has been used successfully to anticoagulate patients with HIT (Agnelli et al., 1994), and has also been applied successfully as an anticoagulant for HD (Boccardo et al., 1997).

Nafamostat Mesilate

Nafamostat mesilate (FUT-175), a synthetic nonspecific serine protease inhibitor with a short half-life, has been evaluated for regional HD in patients at risk of bleeding (Akizawa et al., 1993). It has also been applied occasionally to HIT patients on HD (Koide et al., 1995). However, owing to significant clot formation at the dialyzer outlet, despite a 2-fold prolongation of aPTT, reported both in HIT and non-HIT patients (Koide et al., 1995; Matsuo et al., 1993; Takahashi et al., 2003), this anticoagulant cannot currently be recommended for HD of HIT patients.

Prostacyclin

Prostacyclin (PGI_2, epoprostenol), a potent antiplatelet agent with a short half-life, has been evaluated both as a substitute for, and as an adjunct to, standard heparin for HD of patients with acute or chronic renal insufficiency (Turney et al., 1980; Smith et al., 1982; Samuelsson et al., 1995). Adverse effects, such as nausea, vomiting, and hypotension, can be avoided by dose reduction, use of bicarbonate- instead of acetate-containing dialysate, or infusion of the drug at the inlet of the extracorporeal circuit. Because of its mode of action, prostacyclin cannot inhibit activation of coagulation during HD (Rylance et al., 1985; Novacek et al., 1997). Moreover, in a HIT patient receiving continuous venovenous HD, prostacyclin was unable to suppress platelet consumption effectively after heparin had been reinstituted, owing to a false-negative platelet aggregation assay (Samuelsson et al., 1995). Prostacyclin thus does not seem to be a suitable antithrombotic agent for HD in HIT. Whether it may be a useful adjunct in selected cases remains to be clarified.

Regional Citrate Anticoagulation

Anticoagulation by regional citrate is based on the concept of inhibition of clotting by chelation of ionized calcium, and it was first developed as an alternative anticoagulant regimen in HD patients at risk of bleeding (Pinnick et al., 1983). Metabolic alkalosis, hypernatremia, alterations in calcium homeostasis, and hyperalbuminemia are reported side effects that are generally manageable (Ward and Mehta, 1993; Flanigan et al., 1996; Janssen et al., 1996). Regional citrate anticoagulation is a valuable approach in experienced centers. Efficient and safe long-term citrate anticoagulation in a HIT patient over a period of 9 mo was reported (Unver et al., 2002). Regional citrate anticoagulation is a treatment option only in patients with a history of HIT as it does not suppress the prothrombotic state in acute HIT.

Fondaparinux

Despite not being formally approved, fondaparinux has occasionally been used for alternative anticoagulation in HIT patients (see Chapter 17). As fondaparinux is

predominantly excreted by the kidneys, its dose is to be reduced for anticoagulation in patients with renal insufficiency and for HD procedures. Recently, successful anticoagulation with fondaparinux has been described in a maintenance HD patient with symptomatic HIT (Haase et al., 2005). The role of fondaparinux for anticoagulation in HD procedures of HIT patients requires further evaluation.

IV. RESUMPTION OF HEPARIN ANTICOAGULATION FOR HD IN A PATIENT WITH PREVIOUS HIT

HIT antibodies are surprisingly transient, and usually become undetectable within a few weeks or months following an episode of HIT (Warkentin and Kelton, 2001). Moreover, after their disappearance, the antibodies do not usually recur (or are regenerated in low levels) if a deliberate rechallenge with heparin is administered, such as for cardiac or vascular surgery (see Chapters 12 and 19) (Pötzsch et al., 2000; Warkentin and Kelton, 2001). This experience at least suggests the possibility that resumption of heparin for HD may be feasible too following an episode of HIT. Recently, Hartman et al. (2006) reported such a strategy in three patients who developed HD-associated HIT. Once the HIT antibodies became undetectable, the investigators resumed anticoagulation of HD using the LMWH, nadroparin (the standard anticoagulant used at their center in Antwerp, Belgium). More recently, Matsuo and colleagues (2007) successfully reintroduced HD with UFH in a patient who had developed HIT in the setting of acute HD about 6 mo earlier. These rechallenges were successful, with none of the patients developing recurrent HIT. However, before rechallenge with LMWH or UFH for HD anticoagulation can be recommended more generally, carefully designed systematic studies are necessary, balancing the costs and risks of indefinite anticoagulation with non-heparin agents against the potential risk of recurrent HIT.

V. SUMMARY

An alternative anticoagulant is required for HD in patients with HIT. Appropriate agents include danaparoid sodium, r-hirudin derivatives such as lepirudin, or argatroban, as these appear to suppress clot formation in HD at doses that do not substantially increase bleeding risk. As these results are based on experience with a limited number of patients, larger prospective trials are needed to define the best treatment options in this setting. Even today, though, HIT should no longer be a life-threatening problem for patients requiring dialysis.

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19 Management of Intraoperative Anticoagulation in Patients with Heparin-Induced Thrombocytopenia Undergoing Cardiovascular Surgery

Andreas Koster
Deutsches Herzzentrum Berlin, Berlin, Germany

Bernd Pötzsch
Rheinische Friedrich-Wilhelms-University Bonn, Bonn, Germany

Katharina Madlener
Kerckhoff-Klinik, Bad Nauheim, Germany

I. INTRODUCTION

Immediate cessation of, and avoidance of reexposure to, heparin are important principles underlying the management of patients with immune-mediated heparin-induced thrombocytopenia (HIT) (Chong and Berndt, 1989). Because further antithrombotic therapy is often necessary for these patients, several alternative anticoagulant strategies have been developed (see Chapters 12–17). However, patients with HIT who require cardiovascular surgery present exceptional problems. Apart from the inherent disturbances of the hemostatic system in this patient population, considerable activation of the hemostatic system results from the surgical trauma itself. Further, during cardiopulmonary bypass (CPB), there is exposure of blood to the large non-endothelial surfaces of the CPB circuit and reinfusion of tissue factor-activated blood aspirated from the operative field into the CPB system. This profound hemostatic activation requires potent high-dose anticoagulation in order to prevent thrombosis within both the CPB system and the patient (Edmunds, 1993; Slaughter et al., 1994). Anticoagulation with unfractionated heparin (UFH), point-of-care monitoring by activated clotting time (ACT) systems, and reversal via the antidote protamine comprise a longstanding and well-established strategy permitting cardiovascular surgery. This approach is so universally entrenched that there is very minor experience with all other forms of anticoagulation in this patient setting.

Any alternative anticoagulant considered for HIT patients should ideally meet certain requirements. First, the agent should be effective in minimizing activation of coagulation during CPB and in preventing thrombus formation. Second, a rapid and simple method of monitoring its anticoagulant effects should be available, so as to avoid inappropriate under- or over-anticoagulation. Finally, rapid and complete reversibility of the anticoagulant effects is important to minimize postoperative bleeding complications. Unfortunately, no existing agent meets all of these requirements.

II. ALTERNATIVE STRATEGIES FOR ANTICOAGULATION IN CARDIAC SURGERY WITH AND WITHOUT CPB

A variety of approaches to perform anticoagulation in HIT patients who require cardiac surgery with and without CPB has been reported, including the use of danaparoid, the thrombin inhibitors lepirudin and bivalirudin, and the combination of UFH with short-acting antiplatelet agents, such as platelet glycoprotein (GP) IIb/IIIa antagonists or prostaglandins. Experience with a planned reexposure to UFH to permit CPB in patients with a previous history of HIT, but who no longer have detectable HIT antibodies at the time of subsequent UFH reexposure, will also be discussed.

A. Danaparoid Sodium

Danaparoid is a low molecular weight heparinoid, which achieves its anticoagulant effect predominately by inhibition of coagulation factor Xa. The plasma anti-factor Xa activity half-life is approximately 20 h and somewhat dependent on renal function. No antidote is available. Monitoring of its anticoagulant effect is performed by measuring plasma anti-factor Xa activity. In the United States, danaparoid is not currently available. However, in several other jurisdictions, danaparoid is available and indeed approved for prophylaxis and treatment of HIT (see Chapter 13).

Danaparoid has been used in various protocols for CPB anticoagulation in HIT patients (Magnani, 1993; Magnani et al., 1997; Magnani and Gallus, 2006). However, due to its long plasma elimination half-life, lack of a reversal agent, and the fact that danaparoid cannot be monitored with current point-of-care tests such as the ACT, its use during cardiovascular surgery with CPB is problematic, including a high major bleeding rate (42%), as well as potential for CPB thrombosis due to inadequate dosing (Magnani and Gallus, 2006).

In contrast, in a controlled prospective trial comparing danaparoid (bolus of 40 U/kg) and UFH in non-HIT patients undergoing coronary artery bypass grafting (CABG) without use of CPB (so called off-pump coronary artery bypass [OPCAB] grafting surgery), danaparoid was effective and associated with bleeding rates comparable to that seen with UFH, with only a minor increase in transfusion requirements (Carrier et al., 2003). Anecdotal experience with danaparoid in the setting of OPCAB for a patient with acute HIT has also been reported (Warkentin et al., 2001).

In peripheral vascular surgery, danaparoid has also been used with a single intravenous (iv) bolus of 2250 U. The reported incidence of major bleeding complications was about 6% (Magnani and Gallus, 2006).

Conclusion

Based on these results, we believe that danaparoid should not be used for anticoagulation during CPB. However, danaparoid is an option in HIT patients undergoing OPCAB surgery and in vascular surgery.

B. Recombinant Hirudin

Recombinant hirudin (r-hirudin), a direct thrombin inhibitor (DTI) naturally produced by the salivary gland of the leech (*Hirudo medicinalis*), is now approved in most countries for clinical use by the iv route. Hirudin is a single-chain polypeptide of 65 amino acids (7000 Da) that forms a tight 1:1 stoichiometric

complex with thrombin, thereby occupying both the fibrinogen-binding site and also blocking access to its catalytic site. As a result, all of the thrombin-catalyzed procoagulant reactions, such as conversion of fibrinogen to fibrin, activation of coagulation factors V, VIII, and XIII, and thrombin-induced platelet activation, are inhibited. Although two hirudins are approved (lepirudin, desirudin), data on use in cardiac surgery are only available for lepirudin.

Because of its potent anticoagulant effect, r-hirudin has been studied as an anticoagulant for use in open heart surgery in both dogs (Walenga et al., 1991) and pigs (Riess et al., 1997). In both animal models, effective CPB anticoagulation could be achieved by administration of r-hirudin as a bolus injection (1 mg/kg b.w.) followed by a continuous infusion of 1 mg/kg b.w., started after initiation of CPB, and continuing until end of CPB. In humans, however, recovery of hirudin in the plasma following b.w.-adjusted dosing shows a high interindividual variability (Koza et al., 1993). Therefore, a fixed-dose protocol for r-hirudin in the CPB setting bears the risk of both inadequate anticoagulation and overdosing. Although the latter is complicated by excessive and potentially fatal postoperative bleeds, the former may result in the occurrence of thromboembolic complications while on pump, including catastrophic total pump occlusion.

To establish a treatment schedule that is adjusted to the individual's response to hirudin, we investigated different monitoring systems for hirudin plasma levels. Several in vitro and in vivo experiments demonstrated that the ACT and activated partial thromboplastin time (aPTT) were not sufficiently sensitive to monitor hirudin plasma levels (Pötzsch et al., 1997). However, reliable results were obtained by using the whole blood ecarin clotting time (ECT) (Pötzsch et al., 1997; Koster et al., 2000a).

Ecarin is a prothrombin-activating enzyme, derived from the venom of the snake, *Echis carinatus,* that activates prothrombin to an intermediate product, meizothrombin (Nishida et al., 1995). Meizothrombin expresses only moderate clotting activity, but is fully reactive toward, and thus inhibited by, hirudin. As a result, in r-hirudin-containing plasma, meizothrombin forms stable 1:1 complexes with r-hirudin. Only when hirudin is neutralized does clotting become initiated, either by meizothrombin or by subsequently generated thrombin. Ecarin is available from commercial sources.

Table 1 outlines the whole blood ECT method, which we perform using the KC10a coagulometer (Pötzsch et al., 1997).

The method is easily adaptable to any other coagulometer. A calibration curve is constructed by using citrate-anticoagulated whole blood spiked with r-hirudin to achieve final concentrations of 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 μg/mL. A reliable ECT requires adequate prothrombin levels, which can be reduced in severely ill patients and/or by hemodilution after beginning CPB. This problem can be overcome by mixing patient blood with normal human plasma (1:1).

Critical levels of r-hirudin during CPB were established in an in vitro CPB setting and in a first series of HIT patients undergoing cardiac surgery

TABLE 1 Whole Blood Ecarin Clotting Time

50 μL citrate-anticoagulated whole blood to be analyzed
+ 50 μL standard normal human plasma
Incubate for 1 min at 37°C
+ 50 μL ecarin solution (20 U/mL) containing 0.025 M calcium chloride
Determination of the clotting time

TABLE 2 Treatment Protocol for r-Hirudin (Lepirudin) Anticoagulation During CPB

Initial lepirudin dosing (pre-CPB)	
Initial iv lepirudin bolus:	0.25 mg/kg body weight
Initiate continuous iv infusion:[a]	30 mL/h (0.5 mg/min.)
Lepirudin added to priming solution:	0.2 mg/kg body weight
Target lepirudin plasma levels:[b]	>2.5 μg/mL before start of CPB
	If <2.5 μg/mL, give additional bolus (10 mg)
Lepirudin dosing and monitoring while on CPB	
Frequency of lepirudin level monitoring:	Every 15 min using ECT
Intraoperative dose adjustments, based on ECT:	
Lepirudin plasma level	Dosing modification
>4.5 μg/mL	Reduce infusion rate by 10 mL/h
3.5–4.5 μg/mL	No change in infusion rate
<3.5 μg/mL	Increase infusion rate by 10 mL/h
Special steps toward end of CPB	
Stop lepirudin infusion 15 min before anticipated end of CPB	
After disconnection of CPB, administer 5 mg hirudin to the heart-lung machine to avoid clot formation	

[a]50 mg of lepirudin are dissolved in 50 mL 0.9% sodium chloride.
[b]The target lepirudin level pre-CPB (>2.5 μg/mL) is lower than the ones sought during CPB (3.5–4.5 μg/mL) because of the addition of lepirudin to the pump circuit volume (0.2 mg/kg body weight).
Abbreviations: CPB, cardiopulmonary bypass; ECT, ecarin clotting time; iv, intravenous.

(Pötzsch et al., 1993; Riess et al., 1995, 1996). Clot formation in the CPB apparatus was seen at levels of r-hirudin below 1.8 μg/mL, and increasing levels of fibrinopeptide A (an indicator of thrombin-mediated fibrinogen cleavage) occurred at r-hirudin plasma levels less than 2.0 μg/mL. Based on these results, the therapeutic level of r-hirudin during CPB was set between 3.5 and 4.5 μg/mL. Higher intraoperative levels of r-hirudin could be complicated by a higher postoperative bleeding risk, especially because no antidote is available.

A treatment protocol based upon the ECT-monitoring of hirudin levels is given in Table 2. The data obtained from 10 patients with HIT, treated with r-hirudin for heart surgery, demonstrated that stable r-hirudin plasma levels in the range from 3.5 to 5.0 μg/mL could be obtained using the ECT-adjusted treatment schedule. Because of the relatively short half-life of r-hirudin of approximately 1 h, plasma levels of r-hirudin declined rapidly after stopping its infusion. However, in renally impaired patients, r-hirudin can accumulate, leading to postoperative bleeding (Koster et al., 2000b). In this situation, elimination can be augmented by the use of hemofilters, e.g., as modified ultrafiltration after termination of CPB (Koster et al., 2000c).

To date, the clinical data demonstrate that r-hirudin is a suitable alternative for anticoagulation of CPB in selected HIT patients. The ECT provides adequate monitoring and allows an adjusted treatment schedule with apparently minimal risk for thrombotic problems on pump. Because of the relatively short half-life, plasma levels of r-hirudin decline rapidly after stopping its infusion. However, there are two key aspects, which must be considered for safe management of CPB with r-hirudin. As no commercial point-of-care test for measurement of the ECT is available, care must be taken that reliable measurement of the ECT in the operating room can be provided. Moreover, due to the dramatic prolongation

of the r-hirudin plasma half-life to >100 h in case of renal failure and associated risk of severe hemorrhage, only patients at low risk for postoperative renal impairment should be selected for this strategy.

The experience with the use of lepirudin for OPCAB surgery is limited to a small number of case reports. The dosages used varied from 0.2 to 0.4 mg/kg bolus followed by a continuous infusion of 0.15 mg/kg/h (Iqbal et al., 2005). Therefore, as no adequate dose finding study has been performed to date, this strategy should only be used if no other option is available. With regard to the use of lepirudin in vascular surgery, only one report describes the use for prosthetic replacement of an abdominal aneurysm. In this case a single bolus of 0.25 mg/kg was used which was not followed by a continuous infusion; this provided adequate anticoagulation during an aortic cross-clamp time of 65 min (Koster et al., 2000d). However, vascular surgery patients often have concomitant diabetes, with the potential for lepirudin accumulation due to renal impairment.

Conclusion

Based on current data, we conclude that lepirudin can be safely used for anticoagulation during CPB in patients with unimpaired renal function, provided there is reliable ECT monitoring. For OPCAB surgery and vascular surgery, the limited available data suggest that lepirudin should only be used if no other option is available.

C. Bivalirudin

Bivalirudin (Angiomax, The Medicines Company, Parsippany, NJ) is a short-acting, bivalent, reversible DTI (see Chapter 16). Its pharmacokinetics are characterized by rapid onset of effect and a short half-life of approximately 25 min. The drug's elimination is predominaty achieved by proteolytic cleavage and to a minor extent by renal excretion. These pharmacologic features, particularly its rapid elimination essentially independent of specific organ involvement, renders bivalirudin a potentially valuable alternative to UFH/protamine for high-dose anticoagulation during cardiac surgery with and without CPB (Warkentin and Koster, 2005).

Bivalirudin has been assessed in large trials (>40,000 patients) in patients undergoing percutaneous coronary intervention (PCI), including formal evaluation in patients with or at risk for HIT. Bivalirudin is approved by the U.S. Food and Drug Administration for angioplasty in patients with or without HIT (see Chapter 16).

Based on the favorable results in PCI, a large program was started for assessment of safety and efficacy of bivalirudin use in cardiac surgery. The first of these investigations was performed in OPCAB surgery in non-HIT patients, and involved a comparison between bivalirudin (given in the PCI dose) and UFH/protamine. This investigation revealed comparable results with respect to safety parameters such as perioperative hemorrhage and transfusion requirements. Interestingly, bivalirudin demonstrated improved efficacy, as shown by enhanced graft patency (Merry et al., 2004).

A second pilot investigation assessing the use of bivalirudin in cardiac surgery with CPB also demonstrated an acceptable safety and efficacy profile (Koster et al., 2003a). Based on these data, dosing schemes, perfusion strategies and monitoring recommendations were formulated (Koster et al., 2004a, 2005; Veale et al., 2005; Zucker et al., 2005) (Table 3). Due to the fact that in areas of

stagnant blood bivalirudin is cleaved by thrombin, surgical and perfusion practice must be adjusted to the unique pharmacology of the drug. In particular, any area of stasis within the CPB circuit must be avoided and cardiotomy suction from the operating field minimized whenever possible (Table 3).

After defining dosing protocols, and monitoring guidelines and surgical/perfusion strategies, two multicenter investigations were begun with the goal of obtaining regulatory approval for cardiac surgery in HIT patients, either with CPB (CHOOSE-ON study) or OPCAB surgery (CHOOSE-OFF study). These investigations (The acronym CHOOSE, indicates *C*ABG *H*IT/TS *O*n- and *O*ff-Pump *S*afety and *E*fficacy) were accompanied by "back up safety studies" in non-HIT patients that compared bivalirudin with UFH/protamine in OPCAB surgery (EVOLUTION-OFF study) and in CPB (EVOLUTION-ON study) surgery. (The acronym, EVOLUTION, indicates *EV*aluation of Patients during coronary artery bypass graft *O*perations: *L*inking *UT*ilization of bivalirudin to *I*mproved *O*utcomes and *N*ew anticoagulant strategies.) Results of the two EVOLUTION studies are published and reveal comparable safety and efficacy endpoints (Smedira et al., 2006; Dyke et al., 2006). Results of the CHOOSE-ON study also demonstrated an acceptable safety and efficacy profile even in patients with impaired renal function (Koster et al., 2007). The results of the CHOOSE-OFF study also showed good results in safety and efficacy data (Dyke et al., 2007).

In contrast to this relatively large pool of data in cardiac surgery, in vascular surgery no data are available. Theoretically, however, use of bivalirudin for this indication is problematic, since clamping of the vessel results in ischemia (stimulating generation of thrombin) and stagnation (preventing influx of fresh non-degraded bivalirudin molecules), with the potential for bivalirudin concentrations to decrease to critically low levels.

Conclusion

To date bivalirudin is the only alternative anticoagulant being assessed in prospective trials for intraoperative anticoagulation during cardiac surgery. Based on the currently available data, bivalirudin can be recommended as a first-line option in HIT patients undergoing OPCAB surgery. It also is the drug-of-choice in a large variety of procedures in cardiac surgery requiring CPB, at least where experience exists in adjusting surgical and perfusion techniques to reflect the unique pharmacology of the agent (Table 3).

As there are no data regarding use of bivalirudin for vascular surgery, no recommendations can be given, and some general skepticism regarding feasibility of bivalirudin in this indication is warranted.

D. Argatroban

Argatroban is a synthetic, small-molecule (532 Da) DTI derived from *L*-arginine that binds reversibly to thrombin. Its half-life is about 40–50 min. The potential of argatroban to be an effective anticoagulant in patients with HIT has been documented by the studies of Lewis et al. (1997a,b, 2001, 2003) (see Chapter 15). Its feasibility for anticoagulation of HIT patients is *after* cardiac surgery, with dosing reduced to 0.5–1 μg/kg/min (Koster et al., 2006). However, only limited information (<20 patients) is available for use during cardiac surgery (Kawada et al., 2000; Furukawa et al., 2001; Edwards et al., 2003; Kieta et al., 2003; Ohno et al., 2003; Cannon et al., 2004; Gasparovic et al., 2004) and no standardized

TABLE 3 Treatment Protocol for Bivalirudin Anticoagulation During CPB and OPCAB Surgery

I. CPB

Dosing of bivalirudin

Initial iv bivalirudin bolus: 1.0 mg/kg body weight and initiate continuous iv infusion: 2.5 mg/kg/h

Bivalirudin added to pump circuit volume: 50 mg

ACT: A prolongation of ≥2.5-fold baseline ACT level (varies between different commercially available assays) indicates adequate anticoagulation with bivalirudin

Bivalirudin dosing and monitoring while on CPB

Continue i.v. infusion: ≥2.5 mg/kg/h

Frequency of bivalirudin level monitoring: Every 30 min by ACT

A prolongation of ≥2.5-fold baseline ACT level (varies between different commercially available assays) indicates adequate anticoagulation with bivalirudin. Keep bivalirudin infusion rate constant, and only increase bivalirudin infusion rate if the ACT levels decrease below target (alternatively, maintain the same infusion rate but give repeat fractionated boluses of 0.25 mg/kg to maintain ACT in therapeutic range); do not reduce infusion rate if ACT exceeds target

Performance of CPB

Due to the unique pharmacology of bivalirudin, stasis in the CPB circuit should be avoided/minimized. This can be achieved by the following strategies: the use of closed systems whenever possible; the creation of shunting lines from the arterial filter to the cardiotomy reservoir; intermittent compression of the collapse venous reservoir with flushing back in the hard shell cardiotomy reservoir to provide flow of systemic blood in the cardiotomy reservoir and to maintain bivalirudin levels; storage of excessive blood in citrated bags instead of the hard shell cardiotomy reservoir; or processing excessive blood with the use of cell savers. The use of cardiotomy suction should be minimized whenever possible and replaced by the use of a cell saver to avoid aspiration and systemic infusion of ″activated″ blood from the operative field. If ″blood cardioplegia″ is used, constant flow should be provided in the lines to avoid clot formation and danger of thromboembolism of coronary arteries

Temperature

Due to the enzymic metabolism of bivalirudin, hypothermia may lead to drug accumulation. Therefore, in the absence of a specific test to monitor bivalirudin levels, periods of hypothermia should be brief, and if possible only mild hypothermia (30–34°C.) should be instituted

Extracorporeal elimination

Bivalirudin elimination can be enhanced *after* CPB by the use of modified ultrafiltration using standard commercially available hemofilters. However, due to the possibility of eliminating bivalirudin via hemofiltration, this procedure is discouraged *during* CPB (Koster et al., 2003b, 2004a)

Management of circuit after CPB

After cessation of CPB, the venous line should be infused into the patient, the system refilled with saline, the arterial and venous line reconnected, and circulation of the closed system started to avoid stasis and thrombosis of the system. With the beginning of recirculation, a bivalirudin bolus of 50 mg, followed by a continuous infusion of 50 mg/h, should be added to provide adequate bivalirudin concentrations. When it is definitively determined that CPB will not need to be reestablished, this volume has to be processed with a cell saver before reinfusion to avoid overdosage with bivalirudin

Cell saver

A cell saver should be used with sodium citrate as anticoagulant for flushing line

II. OPCAB surgery

Dosing of bivalirudin

Bolus: 0.75 mg/kg followed by continuous infusion of: 1.75 mg/kg/h (stop infusion approx. 20 min before end of grafting)

ACT: > 300 s when measured with the ACT+ device (Hemochrone Jr, NJ, USA)

Considerations of graft handling

Assessments of grafts for patency and leakage should be performed with saline or, if bivalirudin-containing blood is used, grafts should thereafter be flushed with saline and ″bulldogged″ while applying pressure on the saline syringe. If a left or right internal thoracic artery is used for grafting, the vessel should be transected shortly before grafting in order to avoid stasis and potential risk of thrombus formation in the graft

Abbreviations: ACT, activated clotting time; CPB, cardiopulmonary bypass; OPCAB, off-pump coronary artery bypass.

dosing/monitoring protocol exists. Therefore, argatroban cannot be recommended for these indications. However, because of its short half-life and hepatobiliary excretion, argatroban can be expected to gain a pivotal role in peripheral vascular surgery where a large percentage of patients have renal impairment, thus precluding use of lepirudin. Studies to establish argatroban dosing protocols and to provide safety and efficacy data for this indication are desirable.

E. Platelet Inhibition as a Strategy to Permit Heparinization for CPB

Another approach for managing CPB in a patient with acute or previous HIT is to combine full heparinization with one or more antiplatelet agents. Following surgery, an alternative non-heparin anticoagulant (e.g., a DTI or danaparoid) is initiated as soon as deemed safe.

Several groups of investigators have used iloprost for this situation (Kappa et al., 1985; Long, 1985; Palmer Smith et al., 1985; Addonizio et al., 1987; Kraenzler and Starr, 1988), following the original observation by Olinger et al. (1984) that iloprost inhibited heparin-dependent platelet activation in the presence of HIT serum. Iloprost is a stable analogue of prostacyclin; thus, it stimulates adenylate cyclase, resulting in increased platelet cAMP levels, which prevents platelet activation by various platelet agonists, including HIT antibodies. In one larger case series Antoniou et al. (2002) preoperatively determined the individual concentrations of iloprost in vitro to inhibit the HIT induced platelet activation and thereafter used these individual concentrations to attenuate the HIT reaction during CPB.

Recently, this approach has experienced a resurgence with epoprostenol sodium (Flolan), a freeze-dried preparation of prostacyclin itself (Mertzlufft et al., 2000; Aouifi et al., 2001). Epoprostenol is approved for use in patients with primary pulmonary hypertension. Its very short half-life (6 min) means that continuous iv infusion is necessary. Complete inhibition of heparin-dependent platelet aggregation by HIT antibodies is generally achieved by doses ranging from 15 to 30 ng/kg/min. One protocol that does not require intraoperative monitoring of platelet aggregation gradually increases epoprostenol infusion (in 5 ng/kg/min increments made at 5-min intervals) until the target rate (30 ng/kg/min) is reached, whereupon standard-dose UFH anticoagulation is commenced (Aouifi et al., 2001). The epoprostenol infusion is continued until 15 min following reversal of UFH with protamine. The major adverse effect is vasodilatation, leading to severe hypotension that requires intraoperative vasopressors.

Another strategy is the combination of the short-acting GPIIb/IIIa inhibitor, tirofiban, with UFH for anticoagulation during CPB (Koster et al., 2000e, 2001a,b). Tirofiban is predominantly eliminated by the kidneys, and has a plasma half-life of about 2 h. However, in contrast to prostaglandins, tirofiban exhibits no effect on vascular tone. Tirofiban is given 10 min before standard-dose UFH as a 10 μg/kg bolus followed by 0.15 μg/kg/min continuous infusion. The tirofiban infusion is stopped 1 h before end of surgery. UFH is neutralized with protamine as per usual. Using this treatment protocol, no thromboses occurred. However, in patients with severe renal impairment, tirofiban persists in the circulation and can cause major bleeding refractory to platelet transfusions: three such cases led the manufacturer to discourage use of this off-label protocol (Warkentin and Greinacher, 2003). In such patients, extracorporeal elimination of tirofiban (e.g., ultrafiltration at the end of CPB or modified zero-balanced ultrafiltration after CPB) appears to be an appropriate strategy to augment tirofiban elimination and prevent excessive hemorrhage (Koster et al., 2004b).

However, although there are no reports about thromboembolic complications following these strategies, theoretically there is a danger that HIT-associated platelet activation or thromboembolism might occur when platelet function recovers and platelet factor 4 (PF4)-heparin-antibody complexes still circulate. Therefore, immediately postoperatively iv thrombosis prophylaxis should be started with argatroban, lepirudin or danaparoid.

With regard to OPCAB surgery and vascular surgery no data are available. In theory, UFH plus antiplatelet therapy could be used during these procedures.

Conclusion

Based on the current available data, we believe that the strategy of UFH plus antiplatelet therapy, particularly during CPB is easy to perform and is associated with a minimal risk of bleeding complications, even in extended, complex surgeries. Tirofiban might be preferred in hemodynamically unstable or hypotensive patients, whereas prostaglandins might be advisable for procedures that also require profound reduction of the pulmonary artery pressure, such as in heart transplantation or implantation of a left ventricular assist device. However, as HIT might be only attenuated, with the theoretical potential for a prothrombotic state during recovery of platelet function, these strategies are not be the first choice in a patient with acute HIT.

Concerning OPCAB surgery and vascular surgery, it should be noted that it is difficult to establish hemofiltration for augmented elimination of tirofiban intraoperatively.

III. USE OF HEPARIN FOR CPB IN PATIENTS WITH A PREVIOUS HISTORY OF HIT

An intriguing option for patients with a history of HIT, but in whom HIT antibodies can no longer be detected, is to consider reexposure to UFH for CPB, and to avoid heparin completely both before surgery (e.g., at heart catheterization) and in the postoperative period. This approach has been used successfully (Makhoul et al., 1987; Pötzsch et al., 2000; Selleng et al., 2001; Warkentin and Kelton, 2001), and is based on the following rationale. First, HIT antibodies are transient, and are usually not detectable after several weeks or a few months following an episode of HIT (see Chapter 2). Thus, no immediate problems would be expected in a patient without residual HIT antibodies. Second, it appears that a minimum of 5 days are required before clinically significant levels of HIT antibodies are generated following any episode of heparin treatment (Warkentin and Kelton, 2001). In the event that a recurrent immune response to PF4-heparin is induced by reexposure to heparin during CPB, it is unlikely that the newly generated antibodies will contact exogenously administered heparin. As a consequence, platelet activation by HIT antibodies should not occur, and thus the thrombotic risk should not be increased. Pötzsch et al. (2000) reported 10 patients with a documented history of HIT, but no detectable HIT antibodies at the time of the proposed surgery, who thus underwent CPB anticoagulation with heparin. In none of the 10 patients was a thromboembolic complication or prolonged thrombocytopenia observed. Further, no increase in anti-PF4/heparin antibody concentrations occurred during 10-day follow-up. These data are consistent with the findings of Warkentin and Kelton (2001), who also observed no evidence for a rapid "anamnestic" type of immune response when heparin reexposure was used

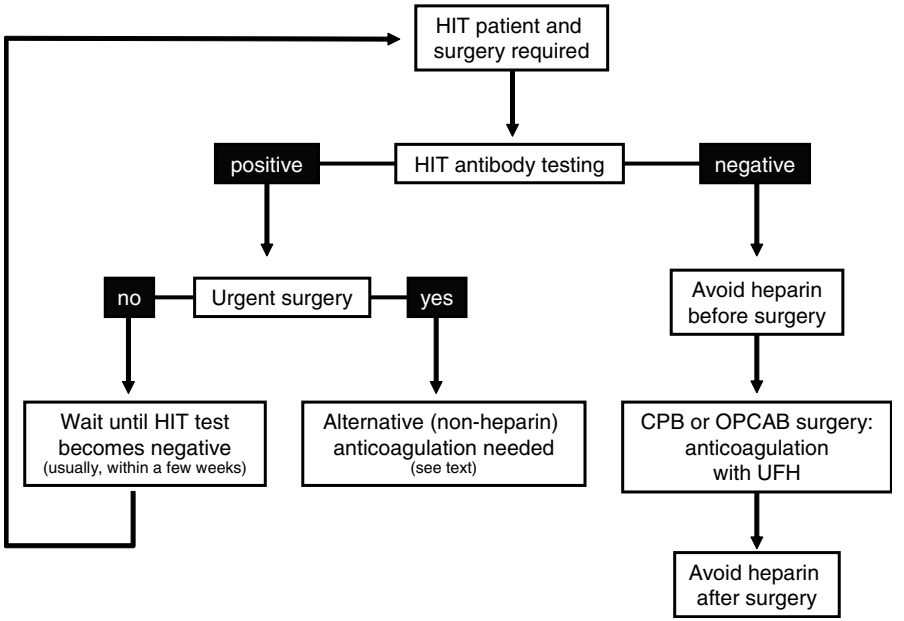

FIGURE 1 Algorithm for decision making for alternative anticoagulation in HIT patients. ″Negative″ testing for HIT antibodies includes a weak (gray zone) enzyme immunoassay result plus a negative functional test using washed platelets, such as the serotonin release assay or heparin-induced platelet activation assay (see Chapter 10). *Abbreviations*: CPB, cardiopulmonary bypass; OPCAB, off-pump coronary artery bypass; UFH, unfractionated heparin.

as a strategy for intraoperative anticoagulation for cardiac and vascular surgery in patients with previous HIT.

We recommend that HIT antibody-negative patients with a history of HIT who require CPB for heart surgery should be treated according to established heparin protocols (Fig. 1). The use of heparin should be restricted to the operative period itself; if necessary, postoperative anticoagulation should be achieved with an alternative anticoagulant (see Chapters 12–17).

Testing for HIT antibodies in patients with a history of HIT before anticipated heparin reexposure at heart surgery should be performed using one or more sensitive tests (see Chapter 10). Particularly in cardiac surgical centers where there is limited experience with non-heparin anticoagulation for CPB, risk–benefit considerations strongly favor a brief use of heparin for these patients. For example, a patient who developed near-fatal CPB circuit thrombosis during danaparoid anticoagulation had had HIT 11 yr earlier and had no detectable HIT antibodies at the time danaparoid was used (Grocott et al., 1997).

Studies using enzyme immunoassays show that the frequency of anti-PF4/heparin antibody formation following heart surgery (Visentin et al., 1996; Bauer et al., 1997; Warkentin and Sheppard, 2006) is as high as 70% (see Chapter 3). Even with the washed platelet serotonin-release assay, platelet-activating antibodies are detected in 13–20% of patients (Bauer et al., 1997; Warkentin et al., 2000). However, despite this high seroconversion rate, only about 1–3% of patients who

receive further postoperative anticoagulation with UFH develop HIT. Currently, there is no convincing evidence that patients who form anti-PF4/heparin antibodies in the absence of thrombocytopenia are at increased risk for thrombosis (Bauer et al., 1997; Trossaert et al., 1998; Warkentin et al., 2000). However, postoperative cardiac surgical patients who develop clinical HIT appear to be at increased risk for both venous and arterial thrombotic events (Walls et al., 1990; Van Dyck et al., 1996; Pouplard et al., 1999). Therefore, if HIT is clinically highly suspected or confirmed, an alternative non-heparin anticoagulant should be initiated (see Chapter 12).

A. Final Recommendations

In patients with a history of HIT and a negative antibody test result at time of surgery, anticoagulation during surgery should be performed with UFH (Fig. 1). Postoperatively, if further anticoagulation is needed, an alternative non-heparin anticoagulant should be given. In patients with detectable antibodies, the operation should be postponed whenever possible to allow for disappearance of the antibodies (40–100 days). Surgery is likely also safe if the antibodies are of the (non-platelet-activating) IgA and/or IgM classes, or if the IgG antibodies are "weak" (i.e., negative testing in the platelet serotonin release assay) and have gray zone results in the enzyme immunoassay. It is reasonable to repeat testing for HIT antibodies at short intervals (e.g., every 2 or 3 wk), as antibodies can disappear quickly in some patients. Thereafter, surgery is performed with UFH, as above.

In patients with a positive antibody test before surgery requiring urgent operation, an alternative approach should be used. Based on current data, bivalirudin appears to be the first-line strategy for OPCAB surgery, and for a large variety of standard CPB procedures (CABG, isolated valve surgery, combined CABG and valve procedures). However, consideration should be given to the modifications of CPB and surgical practice that are necessary due to the pharmacology of bivalirudin (Table 3). Lepirudin should only be used in patients with normal renal function and a low probability of developing perioperative renal impairment. Moreover, point-of-care monitoring with the ECT is a further obligation for use of lepirudin in this indication. The heparinoid, danaparoid, cannot be recommended for CPB procedures but might be an option in OPCAB and peripheral vascular surgery.

In complex procedures or institutions with minor experience in alternative anticoagulation strategies, risk reduction might be achieved best by combination of UFH with a short-acting potent antiplatelet agent in order to attenuate the HIT reaction. The safest class of agents appears to be prostaglandins as the elimination half-life is very short, and major bleeding complications appear to be uncommon. However, their potent hypotensive effect should be considered. The short-acting platelet GPIIb/IIIa antagonist tirofiban may also be used for this purpose, if there is a low probability that the patient will develop perioperative renal failure.

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20 Heparin-Induced Thrombocytopenia in Children

Anne F. Klenner and Andreas Greinacher
Ernst-Moritz-Arndt-University-Greifswald, Greifswald, Germany

I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) can occur in children, with the potential for severe venous and arterial thrombotic complications (Table 1). Unlike in adults, few data exist regarding pediatric HIT. Only 104 children have been reported with HIT between 1990 and 2006 (Martchenke and Boshkov, 2005; Oriot et al., 1990; Potter et al., 1992; Murdoch et al., 1993; Boon et al., 1994; Klement et al., 1996; Wilhelm et al., 1996; Butler et al., 1997; Schiffmann et al., 1997; Barth, 1998; Sauer et al., 1998; Scurr et al., 1998; Bocquet et al., 1999; Saxon et al., 1999; Weigel et al., 1999; Neuhaus et al., 2000; Ranze et al., 1999, 2001; Girisch et al., 2001, 2002; Severin and Sutor, 2001; Zöhrer et al., 2001; Deitcher et al., 2002; Schmugge et al., 2002; Severin et al., 2002a; Boshkov et al., 2002, 2003a,b, 2004; Gatti et al., 2003; Schlegel and Hurtaud-Roux, 2003; Klenner et al., 2003a, 2004; Newall et al., 2003; Nguyen et al., 2003; Porcelli et al., 2003; Alsoufi et al., 2004; Dager and White, 2004; Lischetzki et al., 2004; Malherbe et al., 2004; Mejak et al., 2004; Rischewski et al., 2004; Tcheng and Wong, 2004; Verso et al., 2004; Frost et al., 2005; Grabowski et al., 2005; Iannoli et al., 2005; John and Hallisey, 2005; Bidlingmaier et al., 2006; Knoderer et al., 2006).

II. PATHOPHYSIOLOGY

Studies of the pathophysiology of HIT have been performed using adult blood. In our laboratory, pediatric and adult HIT sera react similarly in various in vitro assays. Therefore, it seems reasonable to infer that the pathophysiology of HIT in children resembles that in adults (see Chapters 4–9).

III. FREQUENCY

Eleven studies have addressed the frequency of HIT in children.

A. Pediatric Intensive Care Unit Patients

Spadone and coworkers (1992) collected cases of suspected HIT in a neonatal intensive care unit (ICU) between 1988 and 1990. Of 1329 newborns enrolled, about 70% received unfractionated heparin (UFH), either 0.5–1.0 IU/mL added to central venous or peripheral/umbilical artery catheters or via flushing of peripheral venous catheters (10 IU/mL UFH-saline every 4 h). In 34 (3.7%) newborns, HIT was suspected because the platelet count fell to less than 70×10^9/L or because of new thromboembolic events. In 14 of these 34 infants, HIT antibodies

TABLE 1 Clinical Complications of HIT in Children ($n = 104$)

Complication	Absolute	Percentage
Venous thrombosis		
Iliac vein	11	10.5
Femoral vein	9	8.6
Inferior vena cava	9	8.6
Pulmonary embolism	8	7.6
Progression of venous thrombosis	8	7.6
Superior vena cava	5	4.8
Calf vein	5	4.8
Subclavian vein	4	3.8
Jugular vein	3	2.8
Rare: innominate vein, pulmonary vein, arm veins, renal vein, dural sinus veins	8	7.6
Arterial thrombosis		
Femoral artery	3	2.8
Iliac artery	2	<2
Foot arteries	2	<2
Rare: renal artery, arterial embolism	2	<2
Others		
Clotted lines (ECMO, hemodialyzer, catheters)	9	8.6
Bleeding	7	6.7
Intracardiac thrombi	5	4.8
Neurological deficits	4	3.8
Clotted shunt	4	3.8
Decreased ventricular function	4	3.8
Reoperation	2	<2
Skin necrosis	2	<2

Note: Patients may have had more than one complication.
Abbreviations: ECMO, extracorporeal membrane oxygenation; HIT, heparin-induced thrombocytopenia.

were detected by platelet aggregation assay (incidence $14/930 = 1.5\%$). However, this study has several limitations. It is an observational study without a defined protocol. As differentiation of HIT from other causes of thrombocytopenia or thrombosis is difficult and the specificity of the applied platelet aggregation test for HIT antibodies may be low in ICU patients (see Chapter 10), the incidence of HIT might have been overestimated.

In a retrospective cohort study in a pediatric ICU, 57 patients developed arterial and/or venous thrombosis among 612 children treated with UFH for more than 5 days (Schmugge et al., 2002). In 14 children (2.3%), HIT was suspected based on thrombosis and a platelet count below 150×10^9/L (or platelet fall exceeding 50%) occurring after 5 or more days of UFH use. In six patients (1.0%), HIT antibodies were demonstrated by platelet factor 4 (PF4)-dependent enzyme immunoassay (EIA), using adult cutoff values in determining a positive assay result. The eight other patients with clinically suspected HIT had antibody levels below adult cutoff. Eleven of the 14 patients had received UFH following cardiac surgery. Four were newborns and five others were also under 1 yr of age (mean age, 6.5 mo).

Newall et al. (2003) retrospectively collected cases of HIT in a tertiary pediatric hospital. During the 2-yr study, 116 patients received UFH over a 7-day period (25 reexposures). HIT was suspected in four patients who received therapeutic-dose UFH and developed a platelet count fall of more than 85% of the pre-heparin value. Three of the patients were tested for HIT antibodies, with one positive result (incidence 1/116 = 0.9%).

Etches et al. (2003) conducted a prospective pilot study to determine the incidence of HIT in a pediatric intensive care population. Patients received UFH during cardiopulmonary bypass (CPB) and continuous intravenous (i.v.), intraarterial infusion, and/or subcutaneous injection. During a 41-mo study period, 233 patients with a median age of 2.3 yr were enrolled. Three of 233 study patients had a positive HIT assay (by platelet lumi-aggregometry), giving a seroconversion incidence of 1.3%. All three patients were post-cardiovascular surgery. None of the HIT assay-positive patients showed a 50% or greater decrease in platelet count, and none had clinically evident thrombosis.

In a randomized, double-blind, placebo-controlled trial in neonatal ICU patients receiving either i.v. UFH or saline to prolong patency of peripheral venous catheters (Klenner et al., 2003b), of 108 neonates receiving UFH (0.5 IU/mL) and 105 receiving saline for at least 5 days, none developed HIT or anti-PF4/heparin antibodies (assessed by EIA). This suggests that the incidence of HIT is lower in neonatal ICU patients than previously reported. However, no neonates following cardiac surgery were enrolled. The results of this study were confirmed by a smaller trial (Kumar et al., 2004). None of 42 newborns receiving UFH for prolonging patency of a central venous access line developed heparin-dependent antibodies, either in the anti-PF4/heparin EIA or in a functional (platelet activation) assay. Although 57% of the newborns developed thrombocytopenia, none had clinical suspicion of thrombosis.

B. Pediatric Cardiac Surgery Patients

Boshkov et al. (2003a) reported a retrospective case series in pediatric cardiac surgery patients. HIT antibodies were demonstrated by positive functional assay in five of 433 children following open heart surgery (incidence, 1.2%). Martchenke and colleagues (2004) found an incidence of HIT with thrombotic complications of 2.5% in pediatric patients after congenital heart surgery. Boning et al. (2005) performed a retrospective analysis to identify the incidences of HIT and of anti-PF4/heparin antibodies in pediatric patients undergoing cardiac surgery. There were 559 cardiac procedures with extracorporeal circulation using heparin in 415 patients with congenital heart defects performed over a 2-yr period. The 144 patients undergoing a scheduled second procedure on extracorporeal circulation were screened preoperatively. Of these 144 patients, 41 underwent a third procedure and were screened before each procedure for the presence of anti-PF4-heparin antibodies and clinical signs of HIT. The incidence of anti-PF4/heparin antibodies was 1.4% (2/144). In none of the patients did clinical HIT occur.

Punzalan and colleagues (2005) performed a prospective study in children receiving heparin during CPB. Of 30 children between 2 days and 50 mo of age, one patient had a borderline positive result and another a positive anti-PF4/heparin EIA; both tested negative by a functional test (serotonin release assay). Neither patient developed thrombosis.

C. Pediatric Dialysis Patients

Skouri et al. (2006) performed a prospective study in the pediatric hemodialysis unit, evaluating 38 children between 1 and 16 yr of age (mean, 10.45 yr) undergoing chronic hemodialysis thrice weekly. Patients received i.v. UFH as a single bolus (70 IU/kg body weight). Plasma samples were tested for antibodies by PF4/heparin-EIA and by a functional assay utilizing washed platelets, the heparin-induced platelet activation assay (HIPA). Of 38 patients, nine patients (21%) tested positive by EIA and/or HIPA, but none had thrombocytopenia or clinical thrombosis. Sequential EIAs performed every 3 mo in seven of the eight patients with antibodies detected by EIA showed gradual reductions in antibody levels in six children, with a persistently positive EIA seen in only one patient at 1-yr follow-up.

IV. CLINICAL PRESENTATION

Between 1990 and 2006, 104 children have been reported with HIT. Fourteen (13.4%) were newborns, 43 (41.3%) were children aged 1 mo to 3 yr, 20 (19.2%) were between 4 and 11 yr of age, and 27 (25.9%) ranged in age from 12 to 18 yr (Fig. 1). In most newborns and young children (under 4 yr of age) HIT occurred after cardiac surgery (40/57 = 70.1%). In contrast, among 27 children aged 12 yr or older, HIT complicated the use of UFH given because of preceding thrombosis

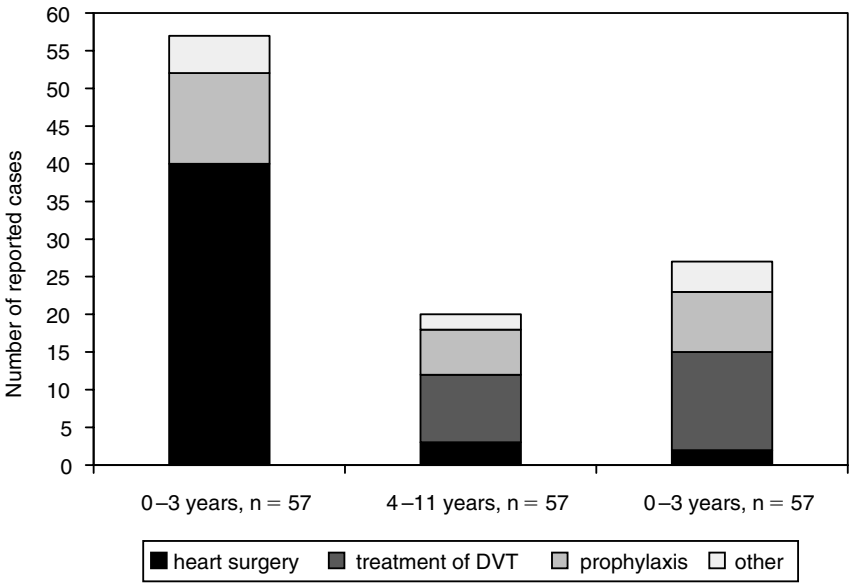

FIGURE 1 Reasons for preceding heparin therapy in children with HIT. Among the various age groups, the reasons for heparin therapy that led to HIT varied considerably: whereas newborns and infants usually developed HIT after cardiac surgery, among teenagers, HIT more often complicated the use of heparin during treatment of thrombosis.

in 13 (48.1%) patients, and following use of antithrombotic prophylaxis in eight (29.6%); only two of the older children had undergone cardiac surgery.

Five patients developed HIT during low-dose UFH given for catheter patency (4.8%). Hemodialysis or hemofiltration accounted for UFH use in eight (7.6%) patients. In 23 (22.1%) of the 104 patients, the laboratory test for HIT was negative or not performed.

The most frequent manifestation of HIT in children was a decrease in platelet count (83/104, 79.8%). HIT was associated with thromboembolic complications in about two-thirds of the patients, most commonly involving iliac and femoral veins, the inferior vena cava, and pulmonary embolism (Table 1). Less commonly, intracardiac thrombi or neurological events occurred, or clotting of the dialyzer. Only about 9% (9/104) of patients developed arterial thrombosis. Thus, there is a strong preponderance of venous thrombosis in pediatric HIT.

Thirteen (12.5%) of the 104 children died (Butler et al., 1997; Weigel et al., 1999; Deitcher et al., 2002; Boshkov et al., 2003a; Klenner et al., 2003a; Newall et al., 2003; Porcelli et al., 2003; Alsoufi et al., 2004; Mejak et al., 2004; Martchenke and Boshkov, 2005; Bidlingmaier et al., 2006), and three required amputations. In four children, only partial recanalization of thrombosed veins occurred.

This summary does not include the 14 newborns reported by Spadone and colleagues (1992). These workers primarily observed arterial thrombosis, with at least 11 (78.6%) developing aortic thrombosis (one infant died without imaging studies). Two newborns with thrombosis had normal platelet counts. Eleven (78.6%) survived, the remaining three developing mesenteric ischemia. Arterial thrombosis likely was related to umbilical artery catheters (used in all but one of the 14 neonates). In adults, intravascular catheters are a risk factor for HIT-associated thrombosis (Hong et al., 2003), but whether the arterial thrombi observed by Spadone et al. (1992) indeed were HIT-related is unclear.

V. LABORATORY TESTING

As in adults, no data exist to justify routine screening for HIT antibodies during heparin use in children. Testing for HIT antibodies should be used to exclude or confirm clinically suspected HIT. During UFH therapy, platelet counts should be monitored regularly (see Chapter 3), particularly between days 5 and 14 of heparin use [when >90% of HIT begins (see Chapter 2)].

For laboratory testing, functional and antigen tests are available (see Chapter 10). Commercial antigen assays (ElAs) are often used and are especially appropriate for neonates and infants because small blood volumes are needed (<100 μL vs. >1 mL for most platelet activation assays). However, the appropriate cutoff level that defines a positive EIA result suitable for children is debated. In a retrospective study, Schmugge and coworkers (2002) investigated cutoff levels for children using a commercial EIA (Asserachrom, Stago). Among 612 children, HIT was suspected in 14 because of thrombocytopenia and thrombosis. Positive test results (using the adult cutoff) were seen in six of the 14 patients. In the remaining eight children with suspected HIT, test results ranged from 26% to 80% of the adult cutoff level, i.e., levels that were higher than among controls (with wide overlap).

A retrospective analysis performed by Risch and colleagues (2003) of the same 612 pediatric ICU patients initially reported by Schmugge et al. (2002) addressed whether there was an association between anti-PF4/heparin antibody levels and thrombosis. Ten patients who developed thrombosis without thrombocytopenia

constituted the study group and were compared with 19 matched controls with neither thrombosis nor thrombocytopenia. All 29 subjects had lower antibody levels than the adult cutoff level. However, median assay results were significantly higher in the thrombosis patients than in controls (51% vs. 23% of the manufacturer's cutoff; $p = 0.004$). The authors concluded that there might be an association between anti-PF4/heparin antibody levels and thrombosis, even in the absence of thrombocytopenia or a positive test result (by conventional criteria).

However, in our randomized, double-blind trial (Klenner et al., 2003b), none of the infants developed anti-PF4/heparin antibodies using the adult cutoff [UFH group: mean optical density (OD), 0.020; maximum, 0.328; saline group: mean OD, 0.019, maximum, 0.239; PF4/polyvinyl sulfonate EIA (GTI, Inc., Wausheka, WI)]. Minor increase in OD (>0.100) occurred in six patients (three in each group) (Fig. 2). Therefore, these minor increases in OD are unlikely to be related to UFH use and could represent a nonspecific increase in antibody levels in ill patients (acute phase reaction). Among the subjects receiving placebo, all OD values were below 0.400 (the accepted adult cutoff value), suggesting that this level is also appropriate for neonates.

The limitations of antigen assays observed in adults likely also apply to children. In some cases, the antigen assay could be false-negative if HIT antibodies recognize a non-PF4-dependent antigen (Greinacher et al., 1994) (see Chapters 5 and 6). Thus, a functional test for HIT antibodies should be performed when HIT

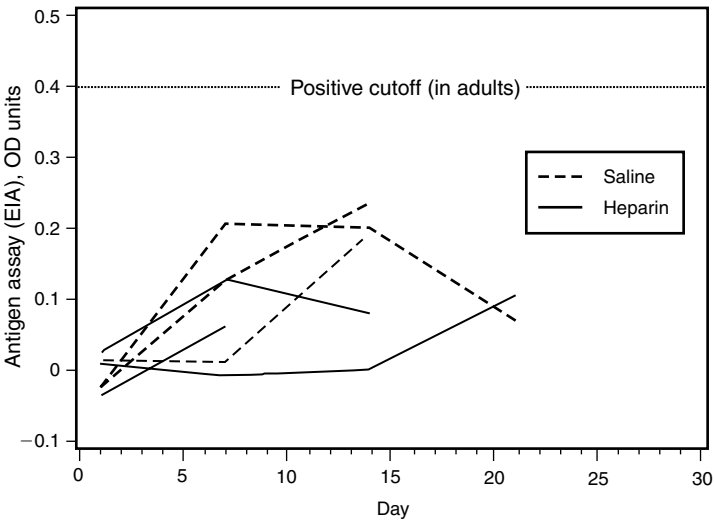

FIGURE 2 Six neonates with rising absorbance levels in PF4-dependent EIA. Six of 213 neonates participating in a randomized, double-blind trial comparing heparin with normal saline for maintenance of peripheral venous catheter patency developed a rise in absorbance of more than 0.100 OD units by PF4/polyvinyl sulfonate EIA (GTI, Inc., Waukesha, WI). All OD values were less than the positive adult cutoff (0.400 OD units). No differences were observed between patients receiving heparin (*solid lines*) compared to patients receiving saline (*dotted lines*). As the maximum OD in saline controls was 0.239, the 0.400 cutoff seems appropriate also for pediatric patients. *Abbreviations*: EIA, enzyme-immunoassay; PF4, platelet factor 4; OD, optical density. *Source*: Klenner et al., 2003b.

remains strongly suspected despite a negative EIA. However, when the pretest probability of HIT is low, a negative antigen test usually excludes HIT.

VI. THERAPY OF PEDIATRIC HIT

Numerous case reports describe the occurrence of new or recurrent thromboembolic events during continued or repeated use of heparin in adult patients with acute HIT. Further, thrombocytopenia usually persists if heparin is not stopped. Thus, all heparin should be discontinued in patients strongly suspected of having HIT, including heparin "flushes," heparin-coated catheters, and heparin-containing blood products (Severin et al., 2002b) (see Chapter 12). As in adults, low molecular weight heparin (LMWH) should not be used to treat acute HIT in children (see Chapter 12).

Because HIT is a prothrombotic ("hypercoagulability") state with high risk of thromboembolic complications, alternative anticoagulation is usually required after stopping heparin. In adults, there are prospective studies of anticoagulation for HIT patients using danaparoid, lepirudin, or argatroban. However, in children, experience with these agents is anecdotal and heterogeneous. Expert opinions are available in reviews (Young, 2004; Balasa, 2005; Bidlingmaier et al., 2006; Hursting et al., 2006; Risch et al., 2006).

Danaparoid use has been reported in 39 patients (with additional aspirin, thrombolysis, or lepirudin given in some cases). In two children, danaparoid was stopped because of apparent cross-reactivity, with further anticoagulation with lepirudin. Fourteen patients received lepirudin (one combined with aspirin). Eight children were treated with LMWH. Three infants received aspirin, three were given argatroban plus aspirin, one argatroban and fondaparinux, and 15 were treated with argatroban alone. Nineteen children received oral anticoagulants and six received thrombolytics. [Note, however, that oral anticoagulants should not be given during acute HIT (see Chapters 2 and 12).] In five children, no anticoagulant was given. In 15 cases, alternative anticoagulation is not mentioned.

A. Danaparoid

Danaparoid (see Chapter 13) is a mixture of low molecular weight glycosaminoglycans that catalyze the inactivation of factor Xa (FXa) by antithrombin (formerly, antithrombin III). It has relatively minor anti-factor IIa activity. Dosing schedules for adults (appropriately weight-adjusted for the child) can be used for guidance. For antithrombotic prophylaxis, 10 IU/kg body weight given twice daily by subcutaneous injection is recommended. For therapeutic anticoagulation in pediatric HIT patients, an initial i.v. bolus of 30 IU/kg is followed by continuous infusion of 1.2–2.0 IU/kg/h (Monagle et al., 2004). The anti-FXa level should be measured during treatment for optimal dosing. Target levels of anti-FXa activity are 0.4–0.6 IU/mL for standard and 0.5–0.8 IU/mL for higher danaparoid doses (Severin et al., 2002b).

B. Lepirudin

Lepirudin (see Chapter 14) is a direct inhibitor of free and clot-bound thrombin through noncovalent, irreversible binding. In adults with HIT complicated by thrombosis, the approved dose is an initial bolus of 0.4 mg/kg followed by continuous i.v. infusion (0.15 mg/kg/h) adjusted by activated partial thromboplastin

time (aPTT). The usual target aPTT ratio should be 1.5–2.5 times the normal laboratory mean aPTT. Dosing in children is based on anecdotal experience. Schiffmann et al. (1997) gave a bolus of lepirudin (0.2 mg/kg) and a continuous infusion (ranging between 0.1 and 0.7 mg/kg/h) adjusted by aPTT. Severin et al. (2002b) achieved therapeutic anticoagulation with a continuous infusion of 0.1 mg/kg/h in a 15-yr-old boy, and with an infusion rate of about 0.15 mg/kg/h in an 8-yr-old girl. In an 11-yr-old girl, 0.15–0.22 mg/kg/h was given. In a premature infant, Nguyen and coworkers (2003) gave a 0.2 mg/kg bolus followed initially by 0.1 mg/kg/h infusion rate; the dose was adjusted daily based on the aPTT, and 0.03–0.05 mg/kg/hr provided adequate anticoagulation. Since pharmacokinetics depend largely on renal function, we recommend starting lepirudin with an i.v. infusion of 0.10 mg/kg/h (if renal function is normal) and to adjust the dose according to aPTT 4 h later, without initial bolus. This minimizes both risk of overdosing and anaphylaxis (see Chapter 14).

C. Argatroban

Argatroban (see Chapter 15) is a synthetic direct thrombin inhibitor that binds reversibly to the active site of thrombin. In adults, the recommended initial dose of argatroban is 2 μg/kg/min given by continuous i.v. infusion and adjusted by aPTT (target range, 1.5–3 times the baseline aPTT). Argatroban also prolongs the international normalized ratio (INR), which makes subsequent transition to vitamin K antagonist therapy, if required, more difficult.

Safety and efficacy of argatroban in pediatric patients have not been established. However, there are several case reports and a chart review (Liedel et al., 2003). Hursting et al. (2006) conducted a comprehensive search and critical analysis of the literature on argatroban use in 34 children. Overall, the patients were between 1 wk and 16 yr of age. All patients received a continuous i.v. infusion of argatroban, titrated to achieve a target aPTT. The aPTT-adjusted doses ranged from 0.1 to 12 μg/kg/min. Four patients also received an initial argatroban bolus of 75–200 μg/kg. Bleeding occurred in three patients while on argatroban. In neonates (Kawada et al., 2000; Okada et al., 2000; Boshkov et al., 2003a,b) an argatroban bolus of 200–250 μg/kg is reported followed by a continuous infusion rate of 7.5–10 μg/kg/min. Of note, this dose is much higher than the 1–2 μg/kg/min recommended in adults. In a newborn, argatroban was used for anticoagulation during extracorporeal membrane oxygenation (ECMO). After an initial bolus of 200 μg/kg, a continuous infusion at a rate of 3.0–7.5 μg/kg/min was started. During use of a ventricular assist device (VAD), safe anticoagulation with argatroban could be achieved in this infant with an infusion rate of 0.05–1.8 μg/kg/min (Mejak et al., 2004).

The literature reviewed by Hursting et al. (2006) provides information regarding argatroban dosing during CPB, ECMO, VAD use, hemodialysis, and cardiac catheterization. In general, although generally safe anticoagulation with argatroban was reported, further evaluation of the efficacy and safety of argatroban in pediatric patients is needed to make further recommendations.

D. Coumarin

Oral anticoagulants of the coumarin class (warfarin, phenprocoumon) are not appropriate for therapy of acute HIT (see Chapter 12). HIT patients are at relatively high risk of developing coumarin-induced microthrombosis (venous limb gangrene and skin necrosis syndromes (see Chapter 2). Therefore, coumarin

should be delayed until the patient is adequately anticoagulated with danaparoid, lepirudin, or argatroban, and the platelet counts have substantially recovered (usually $>150 \times 10^9$/L).

E. Fondaparinux

Fondaparinux is a selective but indirect inhibitor of FXa by catalyzing antithrombin. It is approved for thromboprophylaxis in adults undergoing orthopedic surgery and abdominal surgery, and for treatment of adults with deep-vein thrombosis and pulmonary embolism (see Chapter 17). The agent does not cross-react with HIT antibodies. There are only two reports of fondaparinux use in children (Boshkov et al., 2004; Young and Nugent, 2004). In both cases, therapeutic levels could be achieved with a dose of 0.15 mg/kg given once daily. No adverse effects were noted during a period of several months. Clinical trials with fondaparinux are warranted before any further recommendations of its use in children can be made.

F. Cardiac Interventions in Children with HIT

Recommendations concerning anticoagulation in children with HIT undergoing cardiac surgery have been published in recent reviews (Alsoufi et al., 2004; Greinacher and Klenner, 2005; Boshkov et al., 2006). In patients with a history of HIT who need repeat cardiac surgery, the intraoperative use of UFH is recommended for anticoagulation during CPB, if a sensitive assay excludes the presence of HIT antibodies (Warkentin and Greinacher, 2004). This is because rapid recurrence of HIT antibodies (before postoperative day 5) will not occur. Further, whereas UFH is the standard anticoagulant for CPB, and its effects can be readily antagonized (protamine), there is minimal experience with newer anticoagulants for CPB (particularly in children) and no antidotes exist. However, for pre- and postoperative anticoagulation, a non-heparin anticoagulant should be given.

For patients with acute HIT or patients with persistently circulating platelet-activating antibodies, this approach cannot be used. Therefore these patients require alternative anticoagulation during cardiac surgery. The most practical approach (when feasible) is to postpone surgery until the antibodies disappear or reach very low levels (usually, within 4–10 wk). After their disappearance, heparin can be used (discussed above). If surgery cannot be delayed, an alternative non-heparin regimen can be used (see Chapter 19). In children, Boshkov et al. (2002) started argatroban infusion with a 250 μg/kg bolus followed by continuous infusion of 10 μg/kg/min in a 6-mo old child with HIT for anticoagulation during CPB.

For patients with subacute or previous HIT who require cardiac catheterization, the use of an alternative anticoagulant such as bivalirudin, argatroban, lepirudin, or danaparoid is recommended over the use of heparin (as heparin use might boost antibody levels, complicating use of heparin for subsequent surgery). Porcelli and coworkers (2003) gave 150 μg/kg of argatroban i.v. over 10 min at the start of cardiac catheterization in a 6-yr-old boy with HIT and congenital heart disease. No continuous infusion of argatroban was given due to relatively brief procedure. In a 14-mo-old boy with tetralogy of Fallot and HIT after cardiac surgery, danaparoid was used for cardiac catheterization (Girisch et al., 2001), with a loading dose (30 U/kg) followed by an i.v. infusion (2 U/kg/h).

Boning et al. (2005) reported four children with anti-PF4/heparin antibodies without clinically manifest HIT requiring cardiac surgery with CPB. In these four patients, surgery was performed using lepirudin. Three of the four children had

an uneventful procedure and postoperative course. In one patient, after total cavopulmonary connection, reoperation was necessary on postoperative day 7 because of partial thrombosis of the lateral tunnel.

VII. PREVENTION OF HIT IN CHILDREN

Since the pivotal trial in adult orthopedic patients (Warkentin et al., 1995), it is known that LMWH induces HIT less frequently than does UFH. In children, HIT appears to occur most often among the very young following cardiac surgery, and among adolescents given UFH to treat spontaneous thrombosis. Data from Pouplard and colleagues (1999) suggest that HIT might also occur less with LMWH than with UFH thromboprophylaxis after cardiac surgery. This approach should be investigated in children.

Similarly, in the second group of at-risk pediatric patients (adolescents with thrombosis), it is possible that the frequency of HIT would be reduced if LMWH is given instead of UFH. Pharmacokinetic studies of LMWH in infants and children have been conducted for several LMWH preparations. The safety and efficacy of prophylactic and therapeutic doses of LMWH in children have been evaluated in clinical trials for a variety of conditions. LMWH is safe and effective for anticoagulation of infants and children of varying age (Albisetti and Andrew, 2002; Monagle et al., 2004; Sutor et al., 2004; Merkel et al., 2006; Massicotte et al., 2003a,b,c).

VIII. SUMMARY

HIT appears to be rare in children. The incidence depends somewhat on patient age and indication for heparin. Two major pediatric at-risk groups are apparent: newborns/infants after cardiac surgery (incidence ~1%), and adolescents treated with UFH for spontaneous thrombosis. HIT can be life-threatening in children (~12% mortality). Venous thrombosis is the most frequent HIT-associated complication. For laboratory confirmation of HIT, antigen assays are most appropriate (small blood volumes required). Although there are conflicting data on the optimal laboratory cutoff for antigen assays, a randomized, double-blind clinical trial suggests that the cutoff level established in adults is also appropriate for children. There are no prospective studies of alternative anticoagulants in children with HIT. Most available data are for lepirudin, danaparoid, and argatroban. Greater use of LMWH in children may lead to a reduced risk of HIT, as is seen in adults.

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21 A Clinician's Perspective on Heparin-Induced Thrombocytopenia: Paradoxes, Myths, and Realities

Lawrence Rice
The Methodist Hospital, Weill Cornell Medical College, Houston, Texas, U.S.A.

I. INTRODUCTION

I had no choice but to develop an interest in heparin-induced thrombocytopenia (HIT). As a medical resident in 1976, I had just read papers in the *New England Journal of Medicine* and *Annals of Internal Medicine* (Babcock et al., 1976; Bell et al., 1976) championing different mechanisms of this phenomenon, when a 50-yr-old man came under my care at the Veterans Administration hospital with deep-vein thrombosis (DVT) following back surgery. After intravenous (iv) heparin was infused for 1 wk, he experienced worsening leg signs, new pulmonary emboli (PE), and a fall in platelet count from 441 to 83 $\times$ 10^9/L. Proud of my astuteness, I immediately stopped the heparin, gave a "loading dose" of warfarin (10 mg), protected his lungs with an inferior vena cava (IVC) filter, and confirmed the diagnosis by observing that heparin produced in vitro aggregation of normal platelets in the presence of the patient's platelet-poor plasma. Within days, a necrotic thigh lesion emerged, sepsis and death ensued, leaving me to marvel at the incredibly bad luck that could deal a person both HIT and warfarin-induced skin necrosis. Today we understand that these two drug reactions are not coincidental and that the warfarin therapy likely contributed to the catastrophic outcome. We published this case 27 yr later, including it within a case series illustrating the dangers of warfarin use during acute HIT (Srinivasan et al., 2004).

Sealing a personal abiding clinical interest in HIT were five more cases I encountered during my first 3 mo of Hematology Fellowship. As impressionable as my first case was, each of these proved more dramatic and more challenging than the last, such that I can still recount many details. For example, one middle-aged man was admitted for leg pain and swelling, and received heparin while awaiting a contrast venogram. The test was postponed through the weekend when he was mistakenly served breakfast. All signs and symptoms resolved in the interim, and the venogram proved negative, but on discharge, walking to the door, he collapsed. Brought back to life by electric countershock, he was found to have suffered a massive anterior myocardial infarction (MI); also discovered was a precipitous fall in the platelet count on a blood sample drawn that morning but not checked until post-arrest (Rice and Jackson, 1981). Another of the patients received heparin for an MI only to develop thrombocytopenia and fatal bowel necrosis 1 wk later. By that time, case series from the University of Missouri had delineated clinical features of the HIT syndrome and highlighted the importance of thromboembolic complications (Rhodes et al., 1977; see Chapter 1).

I first presented Grand Rounds on HIT more than 25 yr ago, also seeking speaking opportunities at state, national, and international society meetings. A small cadre of "HITophiles" naïvely believed that just getting the word out would greatly diminish the amputations, strokes, PE and deaths afflicting these patients. At lectures, questioners frequently protested that this syndrome had to be incredibly rare or downright imaginary. One venerated practitioner at my hospital assured the audience that he had never seen a case despite using heparin for decades; I restrained myself from pointing out that his resident had just shared with me hospital records indicating that one of his patients had developed low platelets and recurrent multiple arterial and venous clots while receiving more and more heparin. Perhaps we should have known that as awareness increased, case numbers would escalate—my colleagues and I collected 50 cases with thrombotic complications at our institution from the mid-1970s to the mid-1980s (which we felt represented an enormous problem), but by the mid-1990s we were seeing that number of patients each and every year.

My interest in HIT has opened vistas that cannot be gleaned in medical school, concerning issues of medical education, medical orthodoxies and their evolution, medical economics, medicolegal challenges, and relationships between academic medicine, clinical medicine, and industry. It is troubling that even today, some otherwise competent physicians cannot recognize overt HIT in their patients, and that many textbooks (in critical care medicine, vascular medicine, etc.) continue to provide scant guidance. While some new anticoagulant strategies in cardiology quickly become standard practice, sometimes despite marginal benefit, why are contemporaneous advances in the understanding of HIT relatively ignored? Did HIT find a crack, some manner of "perfect storm," as Hematology/Oncology training shifted emphasis to solid tumors and thrombosis and thought-leaders left the intensive care unit for the laboratory? Whatever the other factors, I believe that a major impediment to the widespread recognition of, and proper treatment for, HIT patients continues to be numerous paradoxes and myths that surround this disorder. Awareness of these paradoxes and myths may allow physicians to advance past these obstacles (Rice, 2004).

II. HIT PARADOXES

1. Heparin, the most powerful anticoagulant of the twentieth century, which has saved countless lives and limbs, *also can cause the most extreme hypercoagulable state, costing thousands yearly their lives and limbs*.
2. Despite the fall in platelet count during use of heparin, patients rarely develop bleeding; rather, attempts to correct the platelet count with platelet transfusions could worsen the prothrombotic problem.
3. This is a humoral immune reaction, yet it usually does not recur with future heparin exposure, and classic anamnestic responses do not appear to occur.
4. This drug reaction continues for a time even after the drug has been stopped, and even asymptomatic patients who have already recovered from their only initial manifestation of the reaction (thrombocytopenia) may develop a thrombotic event following platelet count recovery.
5. One would think that medical professionals (doctors, nurses, pharmacists) would be especially attuned to this problem, given that it is common, serious, treatable, preventable, a major source of malpractice litigation, and iatrogenic,

but many are not, and scant attention has been paid in textbooks and in medical school curricula.

III. HIT MYTHS

A. HIT Is Rare

This has been one of the most detrimental myths, leading health professionals not to learn about the problem and not to be vigilant for it. In fact, HIT occurs in up to 3–5% of patients receiving 1 wk or more of prophylactic-dose unfractionated heparin (UFH) for postoperative thromboprophylaxis, as well as in about 1% of patients receiving iv UFH for treatment of DVT, PE, unstable angina, or other indications (see Chapter 3). Any heparin exposure can cause HIT, with documented cases following single or brief exposures to heparin (Warkentin and Bernstein, 2003). In my two large hospitals, we have recognized more than 100 cases of bona fide HIT yearly for several consecutive years.

B. Heparin Flushes Are Needed to Maintain Catheter Patency and Are Benign

We reported decades ago that the small amounts of heparin used in catheter flush solutions sometimes caused the full-blown HIT syndrome and more often promulgated the syndrome (Rice and Jackson, 1981; Rice et al., 1986, 1988), observations that have been verified by others (Doty et al., 1986). Even the small amounts of heparin that leaches from heparin-bonded central venous or pulmonary artery catheters has been reported to cause full-blown HIT (Laster and Silver, 1988). It is clear from a meta-analysis of randomized trials that there is no advantage to heparin flush over saline in maintaining patency of traditional "hep lock" peripheral venous catheters (Randolph et al., 1998). The data are scant with regard to other types of catheters (e.g., intra-arterial catheters, percutaneously inserted central catheters), but further exploration of risks/benefits are warranted. Clearly, it is important to eliminate all exposures to heparin that are unnecessary and also to eliminate heparin completely when HIT is suspected.

C. We Can Just Use Low Molecular Weight Heparin and Forget About HIT

Low molecular weight heparin (LMWH) preparations cause HIT about one-tenth as often as does UFH (Martel et al., 2005). Nevertheless, given that there are millions of patients exposed to LMWH each year, it should not be surprising that I regularly encounter some patients with HIT who have been exposed exclusively to LMWH. Importantly, once a patient has HIT, LMWH is contraindicated, as the antibodies often cross-react with LMWH, leading to more complications (see Chapter 12).

D. This Cannot Be HIT Because It Is Too Early, Too Late, or the Platelets Are Not Low Enough, or They Are Too Low

"Classic" HIT ensues 5–10 days (occasionally, a few days later) after the beginning of a course of heparin, but rapid-onset HIT occurs sooner, sometimes within minutes of heparin administration, if the patient has had prior sensitization to heparin within the preceding 3 mo (Warkentin and Kelton, 2001a; Mims et al., 2004). While the degree of thrombocytopenia with HIT is often moderate (median platelet count, 60×10^9/L), 10% of patients may experience severe thrombocytopenia (platelet count less

than 20×10^9/L) (Warkentin, 2003, 2007); remarkably such patients rarely bleed, even though many are fully anticoagulated, and in fact those patients with the lowest platelet counts have the highest risk for thromboembolic complications and thus the most dire need for alternative anticoagulation. Another 10% of HIT patients may have nadir platelet counts that fall within the normal range, but usually these are patients that had substantially elevated platelet counts a few days earlier (Warkentin et al., 2003). Thus, one should not completely dismiss the possibility of HIT when temporal features or the magnitudes of the platelet count decline are atypical.

E. This Cannot Be HIT Because the Patient Is Not on Heparin Now

The syndrome of "delayed-onset HIT" was elucidated a few years ago, and is now increasingly recognized around the world (Warkentin and Kelton, 2001b; Rice et al., 2002). The patients have been off heparin for a few days or more, often recuperating at home from a benign hospital course that included heparin exposure, then they return to hospital with an arterial or venous thrombotic event. Upon return, the platelet count is often (although not necessarily) low. These people are often given heparin for their presenting thrombosis, which invariably leads to an abrupt fall in platelet count, frequently clinical deterioration, and substantial mortality. Invariably, there are high-titer antibodies against PF4-heparin complexes. The message to emergency room doctors, intensivists, and hospitalists is to consider the possibility of delayed-onset HIT—and not to initiate heparin reflexively—when a recently hospitalized patient returns with thrombosis. Of note, the U.S. Food and Drug Administration recently notified healthcare professionals about the revision to the warning section of the prescribing information for heparin indicating this possibility of delayed-onset HIT (December 8, 2006).

F. We Can Wait for the Test to Come Back

The time just after heparin is stopped may be the most dangerous for the emergence of thromboemboli, because heparin may be exerting some protective anticoagulant effect at the same time it is feeding a prothrombotic maelstrom. The protocol for the lepirudin registration trials in Europe called for heparin to be stopped when HIT is suspected, but lepirudin was initiated only after obtaining positive serologic results; a 6% per day thrombosis event rate was observed while physicians awaited the test results (Greinacher et al., 2000). The initial suspicion for HIT depends on clinical features, such as the 4 T's (*T*hrombocytopenia, *T*iming, *T*hrombosis, o*T*her causes of thrombocytopenia unlikely) (Lo et al., 2006); when HIT is reasonably suspected, an alternative anticoagulant should be initiated, and the results of serologic tests considered later.

G. If the Test Is Positive, the Patient Has HIT

Serologic tests are important in confirming the diagnosis and should be ordered whenever the diagnosis is reasonably suspected. Nevertheless, the practical limitations of currently available tests must be appreciated by clinicians. In terms of sensitivity-specificity tradeoff, reproducibility, and availability of results in "real time," no assay is highly satisfactory. The commercially available enzyme(-linked) immunosorbent assays (EIAs or ELISAs) are the most widely used, and have the advantages of standardization, ease of performance, wide availability, and high sensitivity, but a major problem is the very high rate of "false positives" for

diagnosing clinical HIT. A question has emerged of where appropriate cut-off values should be between positive and negative defined in differing clinical situations. For example, 1–2 wk after heart surgery, 25–70% of patients will have a "positive" EIA test, but only 3% to 5% of these will actually have clinical HIT. Interpretation of the EIA is greatly aided if the EIA optical density (a proxy for antibody titer) is taken into account, as most true positives are high titer, or if a functional (platelet activation) assay, such as the platelet serotonin release assay (SRA), is positive. It is an unfortunate fact of life that interpreting HIT serologic tests is much like interpreting many other tests obtained in clinical medicine: one has to understand the disease process, its likelihood, and the limitations of testing (see Chapter 10). As always, the interpretation of the test begins with clinical judgment and assessment of pre-test probability. Accordingly, information on the temporal course of platelet counts in relation to heparin exposure, or a scoring system such as the 4 T's, can be invaluable (Lo et al., 2006; see Chapter 3). There is little question that the overly sensitive EIAs can lead to HIT overdiagnosis by the unwary, sometimes producing its own untoward consequences.

H. We Can Just Stop the Heparin

Now that awareness and recognition of HIT are increasing, this myth may be generating the most harm. When Warkentin and Kelton (1996) followed up 62 patients with serologically confirmed "isolated HIT" (HIT with no thrombosis) whose UFH had been stopped, and in whom either no anticoagulant or warfarin was initiated or continued, 53% developed new clots, usually in the first 2 wk; in 3 (5%), the new clot was manifest as sudden death. Other case series have confirmed the high risk for new thromboemboli after heparin is stopped (Wallis et al., 1999; Lewis et al., 2001). Indeed, it seems wise to investigate systematically the lower limbs for DVT when isolated HIT is diagnosed. In getting physicians thinking beyond paradox 4 that stopping the drug will end the danger, one will also confront the "minor paradox" that prophylactic doses of anticoagulation will not suffice in isolated HIT (Farner et al., 2001; Warkentin, 2001; Kodityal et al., 2003) and the myth that HIT with thrombosis is somehow a different disorder than HIT without thrombosis—the only real difference is that clots have not yet appeared in the latter. Continuing to promulgate type I and type II HIT terminology (see Chapter 1) further confuses physicians into believing that some cases of true HIT are benign and do not require intervention (Rice, 2004). My colleagues and I see case after case of new devastating thromboses appear after doctors have recognized HIT and stopped the heparin, but failed to institute an alternative anticoagulant; only then do they consult a hematologist. We have seen many clots emerge after the platelet count has recovered to normal.

I. We Can Just Give Warfarin

Unlike most anticoagulants, warfarin does not inhibit any activated coagulation factors, and thus will not inhibit the hypercoagulable state that characterizes acute HIT. Worse, warfarin will produce an early and rapid decrease of the short-lived vitamin K-dependent natural anticoagulant factor, protein C. Thus, in the extreme prothrombotic milieu of HIT, warfarin's earliest effects will be to precipitate or exacerbate thromboembolic phenomena, including microvascular thrombosis. The syndrome of venous limb gangrene first came to light as a complication of warfarin use in the setting of HIT, and may be a more common cause of limb loss in HIT than arterial thrombosis (Warkentin et al., 1997). Examples of "classic"

warfarin-induced central skin necrosis are also recognized as complications of HIT (Srinivasan et al., 2004). Observations made in HIT patients have taught us about warfarin's significant risks when used in any active procoagulant process, especially when it is used unopposed, early and/or in excessive doses. (Ironically, supratherapeutic levels of anticoagulation—as judged by the international normalized ratio [INR]—are a surrogate marker for very low protein C levels, and correlate with increased risk of microvascular thrombosis.) The magnitude of warfarin danger during acute HIT is such that current treatment guidelines recommend reversal with vitamin K if a patient with HIT has already begun warfarin therapy: this not only prevents the exacerbation of thrombotic complications per se, but also prevents underdosing of alternative anticoagulants due to warfarin's contribution to the prolongation of global coagulation tests used for monitoring (Warkentin and Greinacher, 2004) (see Chapter 12).

J. We Can Protect the Patient with an Inferior Vena Cava Filter

The scant available evidence for the efficacy and safety of IVC filters best support use when anticoagulation is contraindicated in a patient at high risk for PE or when adequate anticoagulation therapy has failed. With HIT, anticoagulation with an alternative agent is strongly indicated, not contraindicated. My colleagues and I have seen half a dozen patients in whom an IVC filter was placed in the extreme hypercoagulable situation of acute HIT, who promptly clotted the filter and developed venous limb gangrene (Rice L, Baker KR, McCarthy JJ, unpublished observations).

K. Alternative Anticoagulants Are Expensive and Do Not Improve Outcomes with HIT

The only randomized controlled trial of HIT therapies (danaparoid versus dextran-70) experienced very slow patient recruitment, probably because of the widely perceived (and ultimately demonstrable) superiority of danaparoid (Chong et al., 2001) (see Chapter 13). In the pivotal studies of the direct thrombin inhibitors, lepirudin and argatroban, it was deemed unethical to have placebo controls, and so historical controls were used (see Chapters 14 and 15). Nevertheless, clear benefit has been demonstrated consistently, not only for the designated primary composite endpoints (all-cause mortality, limb amputation, new thromboemboli) but particularly for the endpoint in which an effective antithrombotic agent would be expected to show the most impact, namely new thromboemboli (Lewis et al., 2006). (This is because most deaths in HIT patient series are due to non-thrombotic events, such as multi-organ failure, cancer, and other non-HIT co-morbidities, and amputations are often performed on limbs already doomed by the time of initiation of alternative anticoagulation, and perhaps too because these prospective cohort studies were done before warfarin's adverse effect profile was appreciated.) There is no question of efficacy of alternative anticoagulants among those with experience managing HIT, who have often witnessed dramatic reversals of the thrombotic "storm" with therapy.

L. HIT and Its Complications Are Inevitable, Unpredictable, and Cannot Be Prevented

LMWHs have a number of advantages over UFH, one being the lower risk of inducing HIT by one order of magnitude (Martel et al., 2005). Fondaparinux (like

LMWH) also has a low risk of causing significant antibody formation, and in addition (unlike LMWH) does not cross-react with pathogenic HIT antibodies: thus, fondaparinux should have an even lower—perhaps negligible—risk of causing HIT (Warkentin et al., 2005). In my opinion, either LMWH or fondaparinux should be preferred to UFH in the great majority of situations where anticoagulation is indicated, outside of the cardiovascular operating room, cardiac catheterization lab, hemodialysis unit, and, perhaps, renally impaired or high bleeding risk critical care patients. Such a change in practice has the potential to diminish greatly both HIT incidence and sequelae. Short-sighted administrators cannot be allowed to "save money" by divorcing pharmacy acquisition costs for UFH from the institution's costs of monitoring for, treating, and defending lawsuits arising from HIT. The last bastion of UFH use is likely to be cardiac surgery employing extracorporeal circulation, because of the established experience with heparin, including reliable intraoperative monitoring and its rapid reversibility with protamine, although even here potentially safer alternative anticoagulants are being studied (see Chapter 19). Furthermore, appropriate monitoring of platelet counts in patients at risk for HIT, followed by appropriate action when thrombocytopenia occurs, is likely to reduce the thromboembolic catastrophes that might otherwise occur; this is likely to be advanced by "systems-based" approaches (see Chapter 3).

IV. HIT REALITIES

Medical professionals have to be highly knowledgeable about HIT, a relatively common and serious clinical problem. It can be prevented by avoiding unnecessary heparin exposures (e.g., heparin flushes), by increasing, where appropriate, the use of LMWH or fondaparinux rather than UFH, and through appropriate platelet count monitoring. In addition to the lack of attention traditionally devoted to HIT in medical curricula and textbooks, obstacles to addressing the problem include greater awareness of the paradoxes and myths surrounding it. HIT produces the most extreme prothrombotic diathesis, so upon reasonable clinical suspicion, an alternative anticoagulant must be initiated. Key to preventing catastrophes is knowledge, vigilance, and maintenance of a high degree of suspicion: HIT must be a prime consideration whenever a patient in the hospital (or recently hospitalized) suffers a fall in platelet count or a new venous or arterial thrombotic event. The temporal relationship of such events to heparin exposure has to be analyzed. Physician thinking must get past the notion that this drug reaction can be reversed simply by stopping the drug. By appreciating the paradoxes and exposing the myths, we can move forward, particularly now that effective agents and strategies are available for prevention and treatment.

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Appendices

APPENDIX 1. TEN CLINICAL "RULES" FOR DIAGNOSING HIT

Rule 1

A thrombocytopenic patient whose platelet count fall began between days 5 and 10 of heparin treatment (inclusive) should be considered to have HIT unless proved otherwise (first day of heparin use is considered "day 0").

Rule 2

A rapid fall in the platelet count that began soon after starting heparin therapy is unlikely to represent HIT unless the patient has received heparin in the recent past, usually within the past 30, and latest, 100 days.

Rule 3

A platelet count fall of more than 50% from the postoperative peak between days 5 and 14 after surgery associated with heparin treatment can indicate HIT even if the platelet count remains higher than 150×10^9/L.

Rule 4

Petechiae and other signs of spontaneous bleeding are not clinical features of HIT, even in patients with very severe thrombocytopenia.

Rule 5

HIT is associated with a high frequency of thrombosis despite discontinuation of heparin therapy with or without substitution by coumarin: the initial rate of thrombosis is about 5–10% per day over the first 1–2 days; the 30-day cumulative risk is about 50%.

Rule 6

Localization of thrombosis in patients with HIT is strongly influenced by independent acute and chronic clinical factors, such as the postoperative state, arteriosclerosis, or the location of intravascular catheters in central veins or arteries.

Rule 7

In patients receiving heparin, the more unusual or severe a subsequent thrombotic event, the more likely the thrombosis is caused by HIT.

Rule 8

Venous limb gangrene is characterized by (1) in vivo thrombin generation associated with acute HIT; (2) active DVT in the limb(s) affected by venous gangrene; and (3) a supratherapeutic INR during coumarin anticoagulation. This syndrome can be prevented by (1) delaying initiation of coumarin anticoagulation during acute HIT until there has been substantial recovery of the platelet count (to

at least 150×10^9/L) while receiving an alternative parenteral anticoagulant (e.g., lepirudin, argatroban, danaparoid), and only if the thrombosis has clinically improved; (2) initiating coumarin in low, maintenance doses (e.g., 2–5 mg warfarin); (3) ensuring that both parenteral and oral anticoagulant overlap for at least 5 days, with at least the last 2 days in the target therapeutic range; and (4) if applicable, physicians should reverse coumarin anticoagulation with intravenous vitamin K in a patient recognized with acute HIT after coumarin therapy has been commenced.

Rule 9

Erythematous or necrotizing skin lesions at heparin injection sites should be considered dermal manifestations of the HIT syndrome, irrespective of the platelet count, unless proved otherwise. Patients who develop thrombocytopenia in association with heparin-induced skin lesions are at increased risk for venous and, especially, arterial thrombosis.

Rule 10

Any inflammatory, cardiopulmonary, or other unexpected acute event that begins 5–30 min after an intravenous heparin bolus should be considered acute HIT unless proved otherwise. The postbolus platelet count should be measured promptly and compared with prebolus levels, because the platelet count fall is abrupt and often transient.

APPENDIX 2. ESTIMATING THE PRETEST PROBABILITY OF HIT: THE FOUR T's

	Points (0, 1,or 2 for each of 4 categories: maximum possible score=8)[a]		
	2	1	0
Thrombocytopenia (acute)	>50% platelet fall (nadir $\geq 20 \times 10^9$/L)	30–50% platelet fall (or >50% fall due to surgery); or nadir $10–19 \times 10^9$/L	<30% platelet fall; or nadir $\leq 10 \times 10^9$/L
Timing[b] of platelet count fall, thrombosis, or other sequelae (first day of heparin course = day 0)	Clear onset between days 5–10 or $\leq$1 day (if heparin exposure within past 30 days)	Consistent with day 5–10 fall, but not clear (e.g., missing platelet counts) or $\leq$1 day (heparin exposure within past 31–100 days) or platelet fall after day 10	Platelet count fall $\leq$4 days without recent heparin exposure
Thrombosis or other sequelae (e.g., skin lesions, ASR)	New thrombosis; skin necrosis; ASR after iv heparin bolus	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis (not yet proven); asymptomatic upper-limb DVT	None
o**T**her cause of thrombocytopenia not evident	No explanation (besides HIT) for platelet count fall is evident	Possible other cause is evident	Definite other cause is present

[a]Pretest probability score: 6–8 = high; 4–5 = intermediate; 0–3 = low.

[b]First day of immunizing heparin exposure considered day zero; the day the platelet count begins to fall is considered the day of onset of thrombocytopenia (it generally takes 1–3 more days until an arbitrary threshold that defines thrombocytopenia is passed). In general, giving heparin during or soon after surgery is most likely to induce immunization. The scoring system shown here has undergone minor modifications from previously published scoring systems (see Chapter 2).

Abbreviations: ASR, acute systemic reaction; DVT, deep venous thrombosis.

APPENDIX 3. RECOMMENDATIONS FOR PLATELET COUNT MONITORING FOR HIT

1. Monitoring for typical-onset HIT: stratifying the intensity of platelet count monitoring for HIT based upon its risk
   A. Patients at highest risk for HIT (1–5%) (e.g., postoperative patients receiving prophylactic-dose UFH after major surgery, patients receiving therapeutic-dose UFH): monitoring during heparin therapy, at least every second day from day 4 to day 14[a,b]
   B. Patients at intermediate risk for HIT (0.1–1%) (e.g., medical/obstetrical patients receiving prophylactic-dose UFH, or postoperative patients receiving prophylactic-dose LMWH, or postoperative patients receiving intravascular catheter ″flushes″ with UFH): monitoring during heparin therapy, at least every 2 or 3 days from day 4 to day 14[a], when practical[c]
   C. Patients at low risk for HIT (<0.1%) (e.g., medical/obstetrical patients receiving prophylactic- or therapeutic-dose LMWH, or medical patients receiving only intravascular catheter ″flushes″ with UFH): routine platelet count monitoring is not recommended[d]
2. Monitoring for rapid-onset HIT: for a patient recently exposed to heparin (within the past 100 days), a repeat platelet count within 24 h following reinitiation of heparin
3. When to suspect HIT

   A relative (proportional) platelet count fall of 50% or greater that is otherwise clinically unexplained should be considered suspicious for HIT, even if the platelet count nadir remains above 150×10^9/L.

   For any patient who develops thrombosis during (day 5 to 14) or within several days after stopping heparin therapy, or who develops an unusual clinical event in association with heparin therapy (e.g., inflammatory or necrotic skin lesions at heparin injection sites, acute systemic reaction post-intravenous heparin therapy), a repeat platelet count should be measured promptly and compared with recent values.

Note: These recommendations parallel those of the Seventh American College of Chest Physicians (ACCP) Concersus Conference on Antithrombotic and Thrombolytic Therapy (Warkentin and Greinacher, 2004).

[a]The crucial time period for monitoring ″typical-onset″ HIT is between days 4 to 14 (first day of heparin = day 0), where the highest platelet count from day 4 (inclusive) onwards represents the ″baseline.″ Platelet count monitoring can cease before day 14 when heparin is stopped.

[b]Once-daily platelet count monitoring is reasonable in patients receiving therapeutic-dose UFH given that daily blood draws required for aPTT monitoring are usually required.

[c]Frequent platelet count monitoring may not be practical when UFH or LMWH is given to outpatients.

[d]Monitoring as per ″intermediate″ risk is appropriate if UFH was given before initiating LMWH.

Abbreviations: HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; UFH, unfractionated heparin.

Source: Adapted from Warkentin and Greinacher, 2004.

APPENDIX 4. TREATMENT RECOMMENDATIONS

RECOMMENDATION GRADES

Grade 1B and Grade 1C:
Strong recommendations, which apply to most patients in most circumstances; and
Grade 2C:
weak recommendations, other alternatives may be equally reasonable.

THERAPY OF (IMMUNE) HIT

Discontinuation of Heparin for Clinically Suspected HIT

Recommendation. All heparin administration should be discontinued in patients clinically suspected of having (immune) HIT (grade 1C).
Recommendation. A clearly visible note should be placed above the patient's bed stating "NO HEPARIN: HIT" (grade 2C).
Recommendation. Heparin can be restarted in patients proved not to have HIT antibodies by a sensitive platelet activation assay or a PF4-dependent antigen assay (grade 1C).

Anticoagulation of the HIT Patient with Thrombosis

Anticoagulants Evaluated for Treatment of HIT

Recommendation. Therapeutic-dose anticoagulation with a rapidly acting anticoagulant, e.g., danaparoid (grade 1B), lepirudin (grade 1C), or argatroban (grade 1C), should be given to a patient with thrombosis complicating acute HIT. Treatment should not be delayed pending laboratory confirmation in a patient strongly suspected (or confirmed) to have HIT.

Danaparoid Cross-Reactivity

Recommendation. In vitro cross-reactivity testing for danaparoid using HIT patient serum or plasma is not recommended prior to danaparoid administration (grade 1C).

Anticoagulation of the HIT Patient Without Thrombosis

Recommendation. Patients suspected to have acute HIT should undergo imaging studies for lower limb DVT, especially those at highest risk for venous thromboembolism, such as postoperative patients (grade 1C).
Recommendation. Alternative therapeutic-dose anticoagulation with an appropriate anticoagulant, such as danaparoid, lepirudin, or argatroban, should be considered in patients strongly suspected (or confirmed) to have HIT even in the absence of symptomatic thrombosis. Anticoagulation should be continued at least until recovery of the platelet counts to a stable plateau (grade 1C).

Longer-Term Anticoagulant Management of the HIT Patient with Thrombosis

Transition to Vitamin K Antagonist (Coumarin) Therapy

Recommendation. To minimize the risk of coumarin necrosis in a patient with acute HIT, vitamin K antagonist (coumarin) therapy should be delayed until the patient is adequately anticoagulated with a rapidly acting parenteral anticoagulant, and not until there has been substantial platelet count recovery (at least $> 150 \times 10^9/\mathrm{L}$). The vitamin K antagonist should be started in low maintenance doses (e.g., $\leq 5\,\mathrm{mg}$

warfarin), with at least 5 days of overlap with the parenteral anticoagulant (including at least 2 days in the target-therapeutic range), and the parenteral anticoagulant should not be stopped until the platelet count has reached a stable plateau (Grade 1C).

Recommendation. Oral or iv vitamin K should be given to reverse coumarin anticoagulation in a patient recognized as having acute HIT after coumarin has been commenced (grade 1C).

Recommendation. Prothrombin complex concentrates should not be used to reverse coumarin anticoagulation in a patient with acute or recent HIT unless bleeding is otherwise unmanageable (grade 2C).

Management of the Patient with a Low or Intermediate Probability of HIT (Pending Results of HIT Antibody Testing)

Recommendation. In a patient with a low probability for HIT (e.g., 4T's score ≤ 3) pending the results of laboratory testing for HIT antibodies, we suggest either continuing the use of heparin or using alternative, non-heparin anticoagulation in prophylactic, rather than in therapeutic, doses (assuming there is no other reason for therapeutic-dose anticoagulation) (Grade 1C).

Recommendation. In a patient with an intermediate probability for HIT (e.g., 4T's score of 4 or 5), who has an alternative explanation for thrombocytopenia and who does not require therapeutic-dose anticoagulation for other reasons, we suggest alternative anticoagulation in prophylactic, rather than in therapeutic, doses (Grade 2C).

Reexposure of the HIT Patient to Heparin

Heparin Reexposure of the Patient with Acute or Recent HIT

Recommendation. Deliberate reexposure to heparin of a patient with acute or recent HIT for diagnostic purposes is not recommended. Rather, the diagnosis should first be excluded or confirmed in most situations by testing acute patient serum or plasma for HIT antibodies using a sensitive activation or antigen assay (Grade 1C).

Heparin Reexposure of the Patient with a History of Remote HIT

Recommendation. Heparin should not be used for antithrombotic prophylaxis or therapy in a patient with a previous history of HIT, except under special circumstances (e.g., cardiac or vascular surgery) (Grade 2C).

HIT IN SPECIAL CLINICAL SITUATIONS

Cardiac or Vascular Surgery

Management of the Patient with Acute or Recent HIT

Recommendation. Alternative anticoagulation should be used for heart or vascular surgery in a patient with acute or recent HIT with detectable heparin-dependent, platelet-activating antibodies. Either bivalirudin or lepirudin are appropriate alternatives for intraoperative anticoagulation, provided that appropriate, rapid-turnaround laboratory monitoring and blood product support to manage potentially severe bleeding complications are available. Another approach is to give heparin together with a potent antiplatelet agent (Grade 2C).

Management of the Patient Following Disappearance of HIT Antibodies

Recommendation. In a patient with a previous history of HIT, heart or vascular surgery can be performed using heparin, provided that HIT antibodies are absent

(by sensitive assay) and heparin use is restricted to the surgical procedure itself (Grade 1C).

HIT During Pregnancy

Recommendation. If available, danaparoid (and possibly fondaparinux) is preferred for parenteral anticoagulation of pregnant patients with HIT, or in those who have a previous history of HIT (Grade 2C).

ADJUNCTIVE THERAPIES

Medical Thrombolysis

Recommendation. Regional or systemic pharmacological thrombolysis should be considered as a treatment adjunct in selected patients with limb-threatening thrombosis or pulmonary embolism with severe cardiovascular compromise (Grade 2C).

Surgical Thromboembolectomy and Fasciotomies

Recommendation. Surgical thromboembolectomy is an appropriate adjunctive treatment for selected patients with limb-threatening large-vessel arterial thromboembolism. Thrombocytopenia is not a contraindication to surgery. An alternative anticoagulant to heparin should be used for intraoperative anticoagulation (Grade 1C).

Intravenous Gammaglobulin

Recommendation. ivIgG is a possible adjunctive treatment in selected patients requiring rapid blockade of the Fc receptor-dependent platelet-activating effects of HIT antibodies (e.g., management of patients with cerebral venous thrombosis, severe limb ischemia, or very severe thrombocytopenia) (Grade 2C).

Plasmapheresis

Recommendation. Plasmapheresis, using plasma as replacement fluid, may be a useful adjunctive therapy in selected patients with acute HIT and life- or limb-threatening thrombosis who are suspected or proved to have acquired deficiency of one or more natural anticoagulant proteins (Grade 2C).

Antiplatelet Agents

Dextran

Recommendation. Dextran should not be used as primary therapy for acute HIT complicated by thrombosis (Grade 1B).

Acetylsalicylic Acid, Dipyridamole, and Clopidogrel

Recommendation. Antiplatelet agents, such as aspirin or clopidogrel, may be used as adjuncts to anticoagulant therapy of HIT, particularly in selected (arteriopathic) patients at high risk for arterial thromboembolism. The possible benefit in preventing arterial thrombosis should be weighed against the potential for increased bleeding (Grade 2C).

Platelet Glycoprotein IIb/IIIa Inhibitors

Recommendation. GPIIb/IIIa inhibitors should be considered as experimental treatment in HIT and used with caution if combined with anticoagulant drugs (Grade 2C).

CAVEATS FOR THE TREATMENT OF HIT

Low Molecular Weight Heparin

Recommendation. LMWH should not be used to treat patients with acute HIT (Grade 1C).

Vitamin K Antagonists

Recommendation. Vitamin K antagonist (coumarin) therapy is *contraindicated* during the acute (thrombocytopenic) phase of HIT. In patients who have already received coumarin when HIT is diagnosed, reversal with vitamin K is recommended.

Platelet Transfusions

Recommendation. Prophylactic platelet transfusions are relatively contraindicated in patients with acute HIT (Grade 2C).

APPENDIX 5. DANAPAROID DOSING SCHEDULES IN HIT PATIENTS

Clinical indication	Danaparoid dosing schedule
Prophylaxis of VTE	750 U sc b.i.d. or t.i.d. for patients with history of HIT or who have low suspicion for HIT. For patients with (confirmed or strongly suspected) acute HIT with or without thrombosis, use treatment doses (see below)
Treatment of VTE or arterial thromboembolism	2250 U iv bolus[a] followed by 400 U/h for 4 h, 300 U/h for 4 h, then 150–200 U/h for $\geq$ 5 days, aiming for a plasma anti-Xa level of 0.5–0.8 U/mL; subcutaneous administration[b]: 1500–2250 U sc b.i.d.
Embolectomy or other peripheral vascular surgery	Preoperative: 2250 U iv bolus[a]; intraoperative flushes: 750 U in 250 mL saline, using up to 50 mL; postoperative: 750 U sc t.i.d. (low-risk patients) or 150–200 U/h (high-risk patients) beginning at least 6 h after surgery
Intermittent hemodialysis (on alternate days)	3750 U iv before first and second dialyses; 3000 U for third dialysis; then 2250 U for subsequent dialyses, aiming for plasma anti-Xa level of $<$ 0.3 U/mL predialysis, and 0.5–0.8 U/mL during dialysis.
CRRT	2250 U iv bolus, followed by 400 U/h for 4 h, then 300 U/h for 4 h, then 150–400[c] U/h aiming for a plasma anti-Xa level of 0.5–0.8 U/mL
CPB	125 U/kg iv bolus after thoracotomy; 3 U/mL in priming fluid of apparatus; 7 U/kg/h iv infusion commencing after CPB hookup, and continued until 45 min before expectation of stopping CPB
Cardiac catheterization	Preprocedure: 2250 U iv bolus (3000 U if 75–90 kg and 3750 U if $>$ 90 kg)
PCI or intra-aortic balloon pump	Preprocedure: bolus as per foregoing Postprocedure: 150–200 U/h for 1–2 days post-PCI (or until removal of balloon pump)
Catheter patency	750 U in 50 mL saline, then 5–10 mL per port, or as required
Pediatric dosage considerations	Refer to Bidlingmaier et al., 2006 (see Chapter 13 for full reference); see also Chapter 20

Note: Compatibility with intravenous solutions: Danaparoid is compatible for dilution with the following solutions: saline, dextrose, dextrose—saline, Ringer's, lactated Ringer's, 10% mannitol. Preparation of solution for infusion: One option is to add four ampules containing 3000 U (i.e., 750 anti-Xa U/0.6 mL ampule) of danaparoid to 300 mL of intravenous solution, i.e., a solution that comprises 10 U danaparoid per milliliter of intravenous solution: thus, an infusion rate of 40 mL/h corresponds to a dose of 400 U/h: 20 mL/h to a dose of 200 U/h, and so on.

[a]Adjust iv danaparoid bolus for body weight: $<$ 60 kg, 1500 U; 60–75 kg, 2250 U; 75–90 kg, 3000 U; $>$90 kg, 3750 U.

[b]Danaparoid should be given iv during the acute (thrombocytopenic) phase of HIT (see above).

[c]Initially up to 600 U/h may be only required if the filter has recently shown excessive clotting. Once filter life is restored to normal, the rate can be lowered.

Abbreviations: b.i.d., twice daily; b.w., body weight; CPB, cardiopulmonary bypass surgery; CRRT, continuous renal replacement therapy; HIT, heparin-induced thrombocytopenia; iv, intravenous; PCI, Percutaneous coronary intervention; t.i.d., three times daily; VTE, venous thromboembolism.

APPENDIX 6. DOSING SCHEDULES FOR LEPIRUDIN TREATMENT OF PATIENTS WITH HIT

	Bolus[a,b]	IV infusion[a,b]	Target aPTT ratio[c]
Dose recommended in all HIT patients without renal impairment	None[d]	0.05–0.10 mg/kg b.w./h[d]	1.5–2.5 (0.6–1.0 μg/mL)
HIT with isolated thrombocytopenia (dose regimen B in HAT trials)	None[e]	0.10 mg/kg b.w./h[e]	1.5–2.5 (0.6–1.0 μg/mL)
HIT and thrombosis (dose regimen A1 in HAT trials)	(0.40 mg/kg b.w. iv[e])	0.15 mg/kg b.w./h[e]	1.5–2.5 (0.6–1.0 μg/mL)
Thrombosis prophylaxis in patients with a history of HIT	15 mg sc b.i.d.[f]	–	–
HIT with thrombosis and concomitant thrombolysis (dose regimen A2 in HAT trials)	(0.20 mg/kg b.w. iv)[e]	0.10 mg/kg b.w./h[e]	1.5–2.5
Renal dialysis every alternate day	0.10 mg/kg b.w. iv predialysis	–	2.0–2.5
CVVH	–	0.005 mg/kg b.w./h (initial rate)	1.5–2.5
PCI (Mehta et al., 2002); UA or acute MI without ST elevation (OASIS-2, 1999)	0.40 mg/kg b.w. iv	0.15 mg/kg b.w./h	1.5–2.5
Vascular surgery (Hach-Wunderle, 2001)	0.40 mg/kg b.w. iv	0.10 mg/kg/h	1.5–2.5
Vascular surgery (intraoperative vessel flushes)	Use up to 250 mL (0.1 mg/mL solution)	–	–
Postoperative anticoagulation	–	0.10 mg/kg b.w./h	1.5–2.5
Cardiac surgery using CPB (dose regimen C in HAT trials) (see also Chapter 19)	0.25 mg/kg b.w. iv[e] 0.20 mg/kg b.w in the priming fluid	0.50 mg/min[a,g]	Monitored by ECT: >2.5 μg/mL before start of CPB; 3.5–4.5 μg/mL during CPB[h]

Note: Repeat aPTT determinations should be made 4–6 h after any dose adjustment.
[a]A maximum body weight of 100 kg should be used for dose calculations.
[b]Adjust for renal insufficiency.
[c]The ratio is based on comparison with the normal laboratory mean aPTT. If Actin FS or Neothromtin reagents are used, the aPTT target range is usually 1.5–3.0.
[d]This is the author's recommended starting dose in all HIT patients, unless life-or limb-threatening thrombosis is present.
[e]Used in the HAT-1, -2, and -3 trials.
[f]Tested in a prospective, randomized trial after orthopedic surgery with desirudin (Eriksson et al., 1996, 1997).
[g]Stop 15 min before and of CPB; put 5 mg into CPB after disconnection to avoid clotting of pump.
[h]The target lepirudin level pre-CPB (>2.5 μg/mL) is lower than the level sought during CPB (3.4–4.5 μg/mL) because of the addition of lepirudin to the pump priming fluid (0.2 mg/kg b.w.).
Abbreviations: aPTT, activated partial thromboplastin time; b.w., body weight; CPB, cardiopulmonary bypass; CVVH, continuous venovenous hemofiltration; ECT, ecarin clotting time; iv, intravenous; MI, myocardial infarction; PCI, percutaneous coronary intervention; UA, unstable angina.
Source: See Chapter 14 for references cited.

APPENDIX 7. INITIAL LEPIRUDIN DOSING IN RENAL DYSFUNCTION

Serum creatinine mg/dL (μmol/L)	Initial iv infusion rate (subsequently adjusted to aPTT) (mg/kg/h)
1–1.58 (90–140)	0.05
1.58–4.52 (140–400)	0.01
>4.52 (>400)	0.005

Abbreviations: aPTT, activated partial thromboplastin time; iv, intravenous.

APPENDIX 8. DOSING SCHEDULES FOR ARGATROBAN TREATMENT OF PATIENTS WITH HIT (APPROVED INDICATIONS)

Clinical use	Bolus[a]	IV infusion[a]	Monitoring and adjusting therapy
Prophylaxis or treatment of thrombosis[b,c]	—	2 μg/kg/min (For hepatically impaired patients, reduce initial dose.[d] Patients with renal insufficiency require no initial dosage adjustment.)	Dose adjusted (not to exceed 10 μg/kg/min) to achieve steady state aPTT 1.5-3.0 times the baseline value (not to exceed 100 s)[e,f,g]
Percutaneous coronary intervention (PCI)[b,h,i]	350 μg/kg (given over 3-5 min)	25 μg/kg/min	Infusion dose adjusted (15–40 μg/kg/min) to achieve an ACT 300–450 s; additional bolus doses of 150 μg/kg may be given as needed[j,k]

Note: See chapter 15 for references cited below.

[a]Based on patient's body weight.

[b]Includes patients with active HIT who have isolated thrombocytopenia or associated thrombosis, as well as patients with a documented history of HIT who are no longer thrombocytopenic but require anticoagulation.

[c] Argatroban is approved in the United States as an anticoagulant for prophylaxis or treatment of thrombosis in patients with HIT, and is also available for use in Austria, Canada, Denmark, Iceland, Germany, Netherlands, Norway, and Sweden as an anticoagulant in HIT.

[d]For patients with moderate hepatic impairment, an initial dose of 0.5 μg/kg/min is recommended. A conservative, reduced initial dose may also be prudent for patients with heart failure, multiple organ system failure, severe anasarca, or postcardiac surgery, that is, conditions associated with increased hepatic congestion or fluid overload, and possibly decreased argatroban clearance (de Denus and Spinler, 2003; Reichert et al., 2003; Baghdasarian et al., 2004; Levine et al., 2006; Czyz et al., 2006; Koster et al., 2006).

[e]The aPTT should be checked at least 2 h after the initiation of argatroban or any dosage change.

[f]For patients in studies ARG-911 and ARG-915, the mean ± SEM dose of argatroban was 1.9 ± 0.1 μg/kg/min.

[g]For transferring a patient to warfarin anticoagulant therapy: After substantial resolution of thrombocytopenia, initiate warfarin therapy using the expected daily dose of warfarin (do not use a loading dose) while maintaining argatroban infusion. At least 5 days of warfarin therapy are required to lower functional prothrombin concentrations to a therapeutic, steady state level. For monitoring the conversion to warfarin during coadministration of argatroban at doses up to 2 μg/kg/min, see text and Fig. 15.6.

[h]Argatroban is approved in the U.S. as an anticoagulant in patients with or at risk for HIT undergoing PCI. Argatroban has not been evaluated in hepatically impaired patients undergoing PCI. These recommendations do not consider the combination use of argatroban with glycoprotein IIb/IIIa antagonists, wherein lower doses of argatroban (e.g., 250–300 μg/kg bolus followed by infusion of 15 μg/kg/min) have been shown to provide effective anticoagulation with an acceptable bleeding risk (Jang et al., 2004).

[i]Includes percutaneous transluminal coronary angioplasty (balloon angioplasty), stent implantation, and atherectomy; oral aspirin 325 mg should be given 2–24 h prior to PCI.

[j]The ACT should be checked 5–10 min following the initial bolus dose and after any additional bolus dose or change in the infusion rate. In studies ARG-216, ARG-310, and ARG-311, the majority of patients required only one bolus dose during the interventional procedure, and the mean ± SEM dose of argatroban was 23.1 ± 0.7 μg/kg/min.

[k]After the procedure, the sheaths should be removed no sooner than 2 h after discontinuing argatroban and when the ACT is <160 s.

Abbreviations: ACT, activated clotting time; aPTT, activated partial thromboplastin time; HIT, heparin-induced thrombocytopenia; iv, intravenous; PCI, percutaneous coronary intervention.

APPENDIX 9. DOSING SCHEDULE FOR BIVALIRUDIN TREATMENT OF PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION

Clinical use	Bolus	IV infusion	Monitoring and adjusting therapy
PTCA[a]	0.75 mg/kg (given just prior to angioplasty)	1.75 mg/kg/h[b]	Nil
PTCA[a] (CrCl < 30 mL/min)	Same as above	1.0 mg/kg/h[b,c]	Nil
PTCA[a] (dialysis-dependent)	Same as above	0.25 mg/kg/h[d]	Nil

[a]Angiomax is indicated for patients with or at risk of HIT/HITTS undergoing PCI (non-HIT indications for bivalirudin are not listed here).

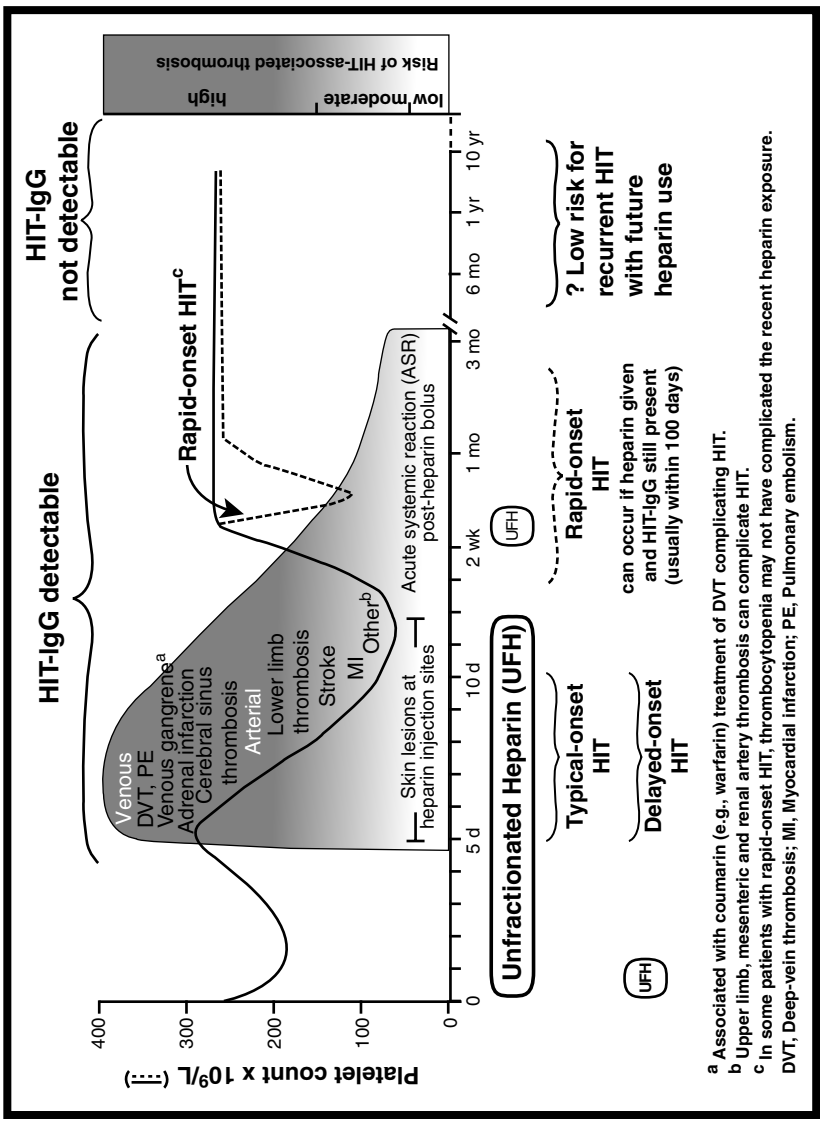
[b]The post-bolus IV infusion is usually continued for the duration of the procedure; continuation of the infusion at this dose for up to 4 hours after the procedure is optional, at the discretion of the physician; after completing the 4-h infusion, additional bivalirudin may be given at a rate of 0.20 mg/kg/h for up to 20 h.

[c]According to the U.S. package insert, a reduction in the infusion dose should be considered if the CrCl is less than 30 mL/min.

[d]According to the U.S. package insert, if a patient is on hemodialysis, the infusion dose should be reduced to 0.25 mg/kg/h.

Abbreviations: CrCl, creatinine clearance; HIT, heparin-induced thrombocytopenia; HITTS, heparin-induced thrombocytopenia/thrombosis syndrome; PTCA, percutaneous transluminal coronary angioplasty.

APPENDIX 10. TIMELINES OF AN EPISODE OF HIT



APPENDIX 11. INTEGRATED DIAGNOSTIC AND TREATMENT APPROACH FOR SUSPECTED HIT

Suspicion of HIT based upon the 4 **T**'s	Score	Pre-test Probability Score Criteria 2	1	0
Thrombocytopenia	☐	>50% platelet fall (nadir ≥20 x 10^9/L)	30-50% platelet fall (or >50% fall due to surgery) or nadir 10-19 x 10^9/L	<30% platelet fall or nadir <10 x 10^9/L
Timing of onset of platelet fall	☐	day 5-10, or ≤day 1 with recent heparin[a]	>day 10 or timing unclear (but fits with HIT)	≤day 4 (no recent heparin)
Thrombosis or other sequelae	☐	new thrombosis, (proven); skin necrosis, or ASR[b]	progressive, recurrent, or suspected thrombosis; erythematous skin lesions	none
O**T**her cause of platelet fall	☐	none evident	possible	definite
Total Pre-test Probability Score	☐	periodic reassessment as new information can change pre-test probability (e.g., positive blood cultures)		

Total Pre-test Probability Score

High			Moderate		Low				
8	7	6	5	4	3	2	1	0	

Stop heparin[c], give alternative non-heparin anticoagulant danaparoid[d] or lepirudin[e] or argatroban[f] (or bivalirudin[g] or fondaparinux[h])

Physician judgment

Continue (LMW) heparin; or consider non-heparin anticoagulant

therapeutic dose; prophylactic dose (if otherwise appropriate)

HIT Test

Positive test for HIT antibodies
Continue non-heparin anticoagulant until platelet count recovery

Negative test for HIT antibodies
Consider continuing or switching back to (LMW) heparin[i]

Imaging studies for lower-limb DVT (high frequency of subclinical DVT)

Thrombosis[j]
If HIT, continue non-heparin anticoagulant until platelet count recovery, then **cautious coumarin overlap**[k]

No Thrombosis
If HIT, consider anticoagulating until platelet count recovery, even if no thrombosis apparent (± coumarin[k])

[a] Recent heparin indicates exposure within the past 30 days (2 points) or past 30-100 days (1 point)

[b] ASR, acute systemic (anaphylactoid) reaction following i.v. heparin bolus

[c] Stop all heparin, including low molecular weight heparin, catheter "flushes" and, possibly, heparin-coated catheters

[d] Danaparoid: usual i.v. bolus, 2250 U (body weight 60-75 kg) followed by infusion (400 U/hr for 4 hr, then 300 U/hr for 4 hr, then 200 U/hr, adjusted by antifactor Xa levels); this therapeutic-dose regimen is appropriate both for isolated HIT and for HIT complicated by thrombosis; prophylactic-dose danaparoid (750 U b.i.d. or t.i.d.) appropriate when probability of HIT is not high

[e] Lepirudin: approved for treatment of thrombosis complicating HIT. Note that recommended dosing is lower than package insert: 0.05-0.10 mg/kg/h adjusted to 1.5-2.5X baseline aPTT or mean of the laboratory normal range (see Chapter 14); treat isolated HIT (0.05-0.10 mg/kg/h, aPTT-adjusted); to avoid overdosing, it may be preferable to omit the initial bolus, and begin with i.v. infusion, except in patients with life- or limb-threatening thrombosis; reduce dose for renal insufficiency

[f] Argatroban: approved for isolated HIT and HIT complicated by thrombosis (2 μg/kg/min i.v., adjusted to 1.5-3.0 × patient's baseline aPTT or the mean of the laboratory normal range); reduce dose in critically ill or in heart failure (e.g., 1 μg/kg/min) and in patients with hepatobiliary compromise (0.25 μg/kg/min); increases INR more than other direct thrombin inhibitors, thus care required in managing coumarin overlap (see below)

[g] Bivalirudin: no bolus, infusion 0.10 mg/kg/hr adjusted by aPTT; limited experience (off-label)

[h] Fondaparinux: dosing for HIT not established; limited experience (off-label)

[i] Depending on physician confidence in the laboratory's ability to rule out HIT antibodies (usually, negative PF4-dependent enzyme-immunoassay and/or washed platelet activation assay performed by an experienced laboratory)

[j] Some thrombin may require special treatment, e.g., thrombectomy for large limb artery thrombosis

[k] Do not begin coumarin until there is substantial recovery of platelet count (usually, >150); begin coumarin in low, maintenance doses only, with minimum 4-5 day overlap with alternative anticoagulant; stop coumarin when INR therapeutic at least 2 days

APPENDIX 12. SIX TREATMENT PRINCIPLES OF HIT[a]

TWO DO's

Do stop all heparin (including heparin flushes, low-molecular-weight heparin, etc.)[b]
Do start an alternative, non-heparin anticoagulant[c] (usually in therapeutic doses[d,e])

TWO DON'Ts

Don't administer coumarin (warfarin) during the acute thrombocytopenic phase of HIT[f] (give vitamin K if coumarin has already been given when HIT is diagnosed)
Don't give prophylactic platelet transfusions[g]

TWO DIAGNOSTICS

Test for HIT antibodies[h]
Investigate for lower-limb deep-vein thrombosis (e.g., duplex ultrasound)[i]

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[a]These principles apply when HIT is strongly-suspected or confirmed.
[b]Sometimes HIT begins after all heparin has been stopped (″delayed-onset HIT″).
[c]Danaparoid (Chapter 13), lepirudin (Chapter 14), and argatroban (Chapter 15) are three alternative, non-heparin anticoagulants that are approved for treatment of HIT, although approval status and drug availability varies in different jurisdictions.
[d]Therapeutic-dose regimens include aPTT-adjusted iv dosing schedules for lepirudin and argatroban; for danaparoid, there is evidence that a therapeutic-dose regimen (e.g., initial iv bolus, then 400 U/h iv x 4 h, followed by 300 U/h iv x 4 h, followed by 200 U/h iv (with subsequent dose adjustments made using anti-factor Xa levels, if available) is more effective than low-dose danaparoid (e.g., 750 U b.i.d. or t.i.d. by subcutaneous injection).
[e]There is evidence that therapeutic-dose anticoagulation of ″isolated HIT,″ i.e., HIT recognized because of thrombocytopenia and in the absence of clinically-apparent thrombosis, reduces risk of subsequent thrombosis.
[f]Coumarin is a risk factor for microvascular thrombosis, e.g., venous limb gangrene; further, aPTT prolongation by coumarin can lead to underdosing of lepirudin or argatroban therapy.
[g]Petechiae and other signs of thrombocytopenic bleeding are not characteristic of HIT.
[h]PF4-dependent enzyme-immunoassays have high sensitivity (>98%) for clinical HIT; however, their diagnostic specificity is lower than washed platelet activation assays. In experienced laboratories, the latter ″functional″ (platelet activation) assays also have high sensitivity for diagnosis of clinical HIT (see Chapter 10).
[i]Up to 50% of patients with isolated HIT have deep-vein thrombosis.
Abbreviations: aPTT, activated partial thromboplastin time; b.i.d., twice daily, b.w., body weight; HIT, heparin-induced thrombocytopenia; iv, intravenous; PF4, platelet factor 4; t.i.d., three times daily.

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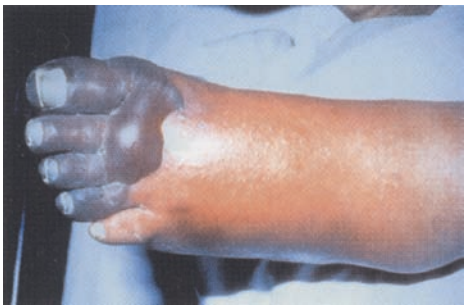


FIGURE 2.10 Warfarin-associated venous limb gangrene. Progression of deep vein thrombosis to acral necrosis (leading to below-the-knee amputation) occurred despite the presence of palpable arterial foot pulses in this 49-yr-old woman with HIT treated with warfarin (international normalized ratio = 7.2 at the onset of limb gangrene).

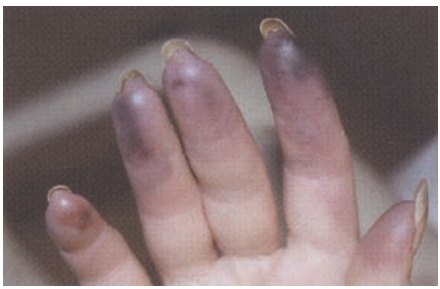
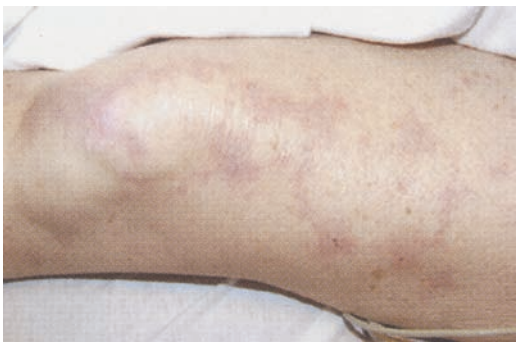
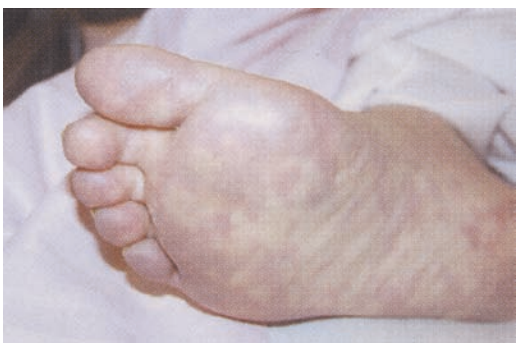


FIGURE 2.12 Warfarin-associated multiple digital necrosis of the right hand in a 61-yr-old woman with paraneoplastic Raynaud's phenomenon and adenocarcinoma-associated thrombotic endocarditis who developed HIT following aortic valve replacement surgery (see text for additional clinical details).



(A)



(B)

FIGURE 2.13 Clinical manifestations of DIC. (**A**) Livedo reticularis. (**B**) Patchy ischemic necrosis of right foot. This 70-yr-old woman developed HIT-associated DIC with hypofibrinogenemia, elevated INR, and reduced antithrombin and protein C activity levels 9 days after emergency cardiac surgery for cardiac catheterization-associated dissection of the left main coronary artery (see text for additional clinical information).

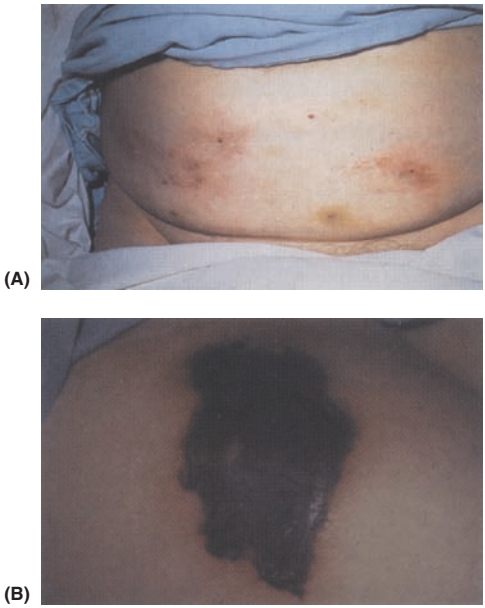


FIGURE 2.14 Heparin-induced skin lesions. (**A**) Heparin-induced erythematous plaques: UFH injections into the lower abdomen resulted in painful erythematous plaques beginning on day 7 of sc UFH treatment; at this time, the platelet count fell only by 9% from 340 to 311 $\times$ 10^9/L. HIT antibody seroconversion from a negative baseline was shown using the serotonin release assay (from 0% to 84% serotonin release). (**B**) Heparin-induced skin necrosis: UFH injections into the right anterior thigh led to skin necrosis: a large black eschar with irregular borders is surrounded by a narrow band of erythema. The platelet count fell to 32 $\times$ 10^9/L; despite stopping heparin, the patient developed symptomatic proximal deep vein thrombosis 10 days later. *Abbreviations*: HIT, heparin-induced thrombocytopenia; UFH, unfractionated heparin.

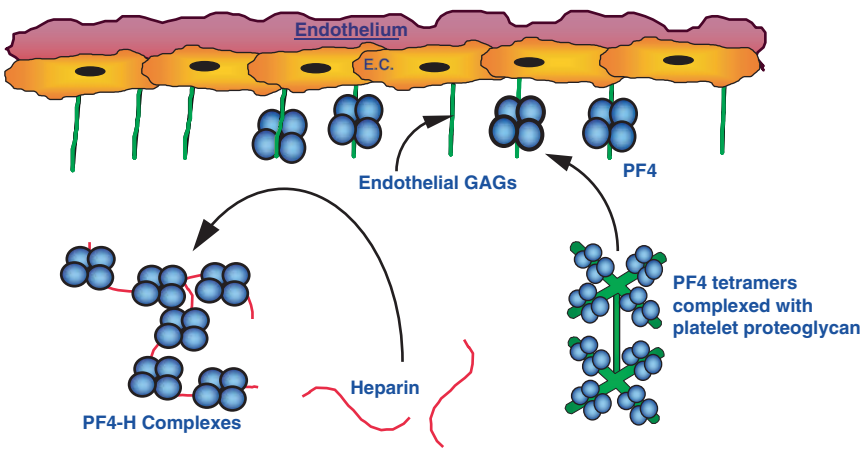


FIGURE 5.1 Release of PF4 from platelets as a high molecular weight complex of eight tetramers with a proteoglycan dimer; PF4 binds to endothelial cell GAGs, for which it has a greater affinity, but it is displaced by heparin, which exhibits a higher affinity for PF4. *Abbreviations*: GAGs, glycosaminoglycans; PF4, platelet factor 4; PF4-H, PF4 complexed to heparin.

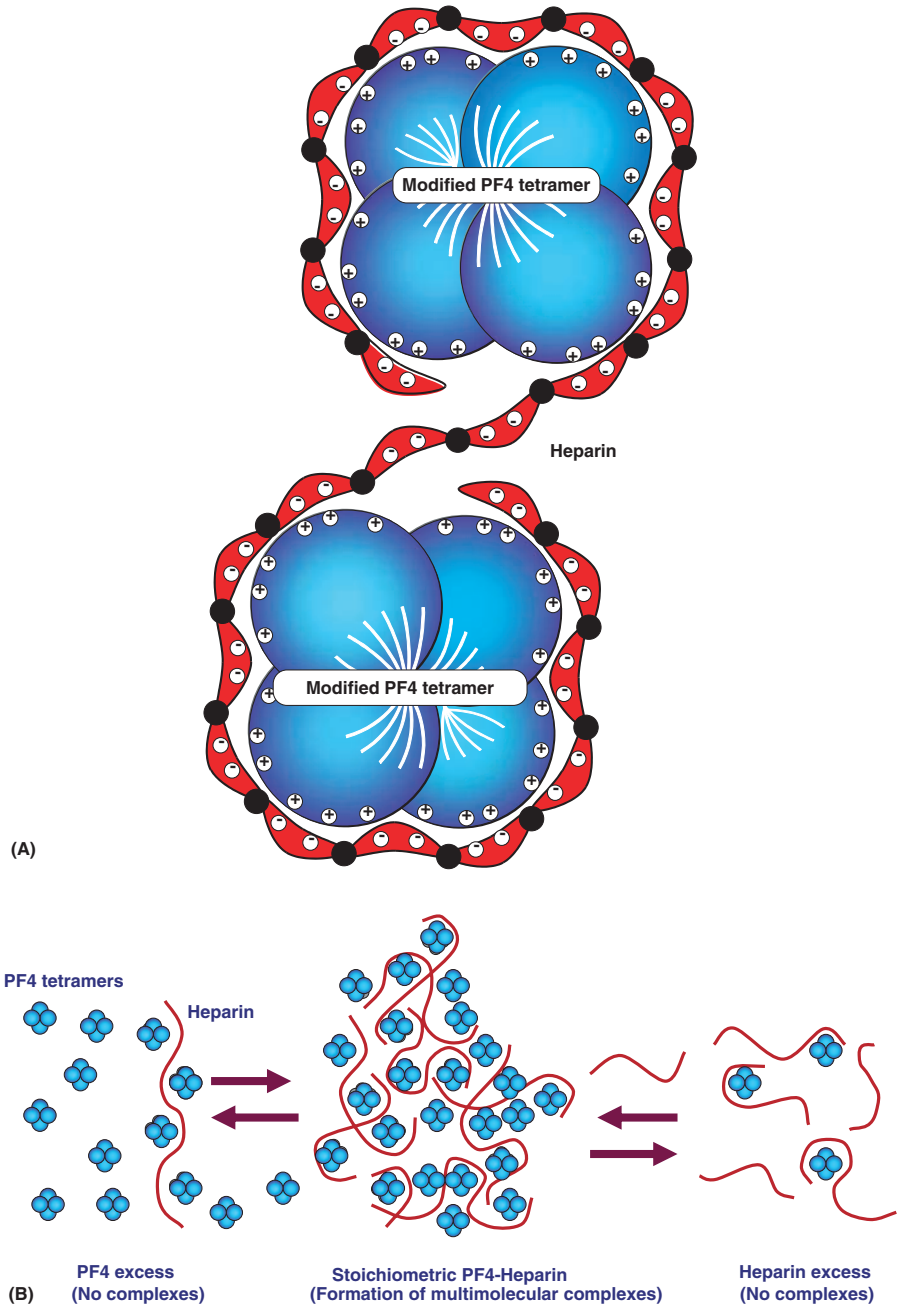


FIGURE 5.2 Schema showing the ″modifications″ of the PF4 tetramer following its tight binding with heparin at stoichiometry and exposure of neoepitopes (**A**) and depicting the formation of heparin and PF4 complexes at different concentrations of heparin and PF4 (**B**). In the presence of stoichiometric concentrations of both substances, multimolecular complexes are formed. Heparin then wraps around the PF4 tetramer, altering its structure and rendering it antigenic. *Abbreviation*: PF4, platelet factor 4.

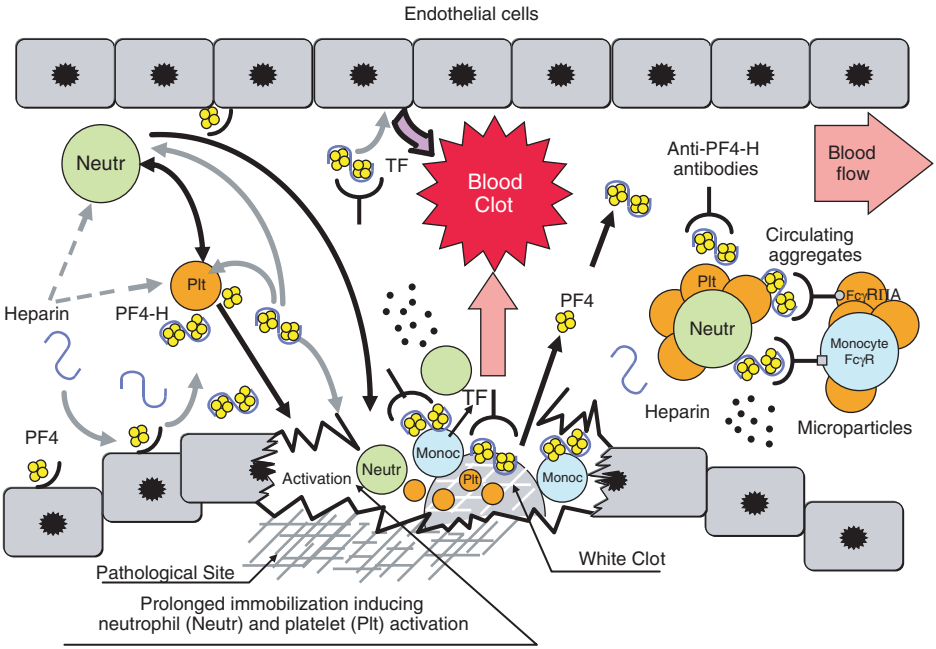


FIGURE 5.3 Cell–cell interactions in the neighborhood of blood activation or inflammation sites: Presence of heparin-dependent antibodies increases the amount of cells available at these sites, amplifies cell–cell interactions and cellular activation, and can lead to blood clotting or release of circulating cell aggregates. The procoagulant effect is enhanced by release of tissue factor (from endothelial cells and monocytes) and generation of microparticles. *Abbreviations*: IL-8, interleukin-8; PF4, platelet factor 4; PF4-H, heparin-platelet factor 4; TF, tissue factor.

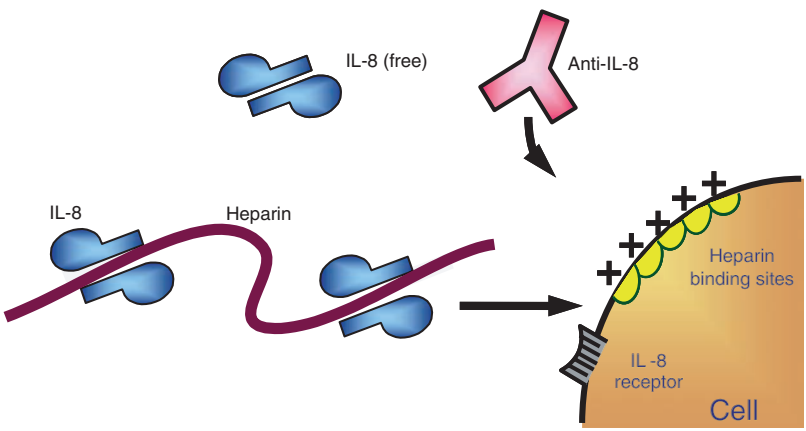


FIGURE 5.4 Possible effect of heparin for carrying preexisting antibodies to IL-8 onto platelets (and other blood cells), through the heparin binding sites or through the IL-8 receptors, targeting the deleterious consequences of these antibodies onto these cells. *Abbreviation*: IL-8, interleukin-8.

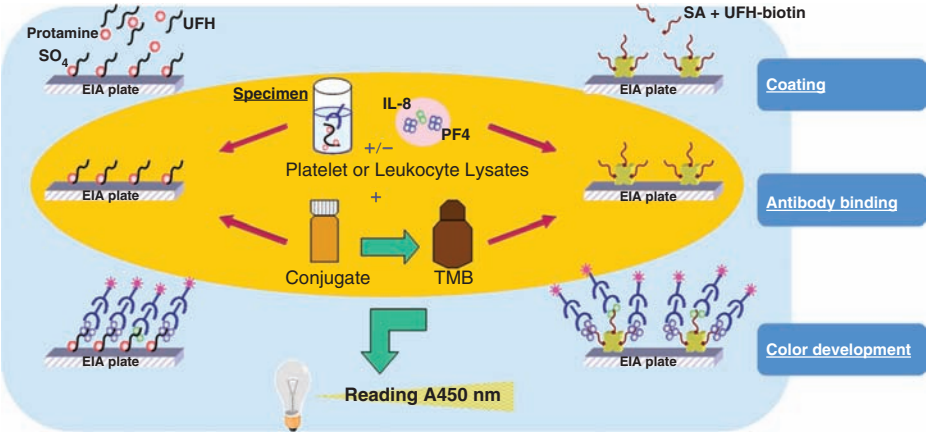


FIGURE 5.5 Assay for testing heparin-dependent antibodies, associated with HIT, by their binding to functionally available heparin through the heparin cofactor antigen (usually PF4); heparin is coated in a large excess as a complex with aprotinin or biotinylated and reacted with coated streptavidin. *Abbreviations*: EIA, enzyme-immunoassay; IL-8, interleukin-8; PF4, platelet factor 4; SA, streptavidin; TMB, tetramethyl benzidine; UFH, unfractionated heparin.

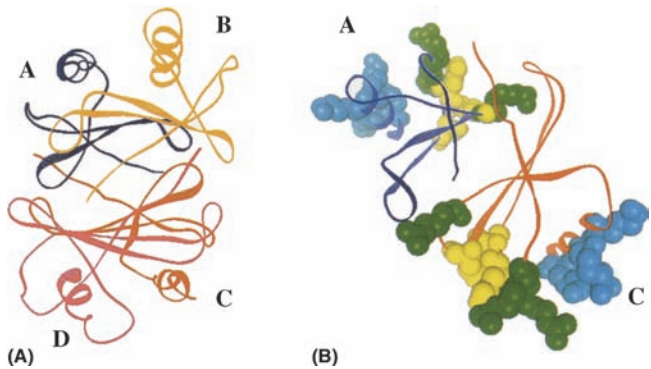


FIGURE 6.2 (**A**) Computer-generated model (WebLab ViewerPro; Molecular Simulation Inc., San Diego, CA) of the human PF4 tetramer, based on the crystallographic coordinates. (**B**) AC dimer view of human PF4: the amino acid residues crucial for heparin binding are displayed. *Abbreviation*: PF4, platelet factor 4.

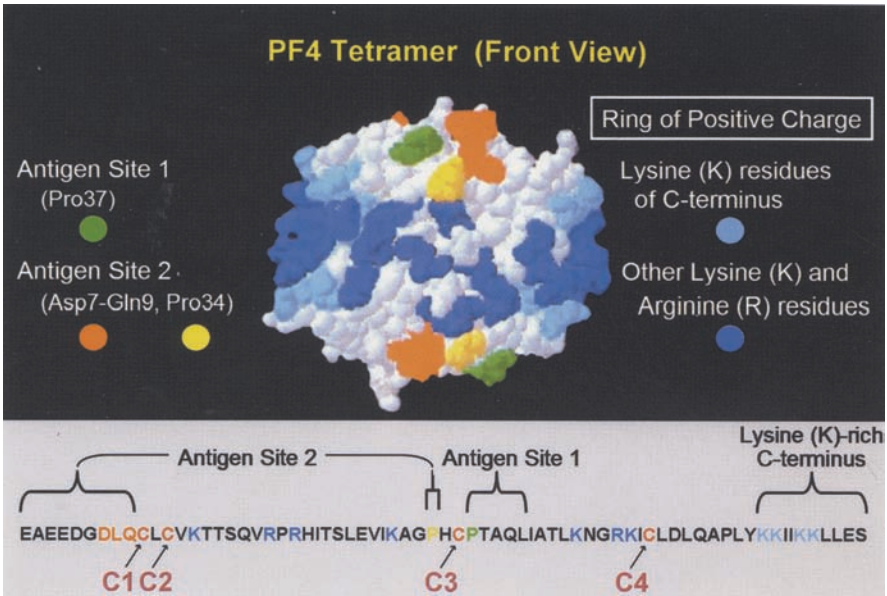


FIGURE 6.4 Primary and secondary structure of PF4 in relation to HIT neoepitopes. (*Top*) 3D representation of the PF4 tetramer, indicating two neoepitope sites (per monomer). The ″ring of positive charge″ is formed by lysine residues in the C-terminus (*light blue*) and other lysine and arginine residues (*dark blue*). (*Bottom*) The linear sequence of the 70-amino acid polypeptide of a single PF4 molecule is shown. *Abbreviation*: PF4, platelet factor 4.

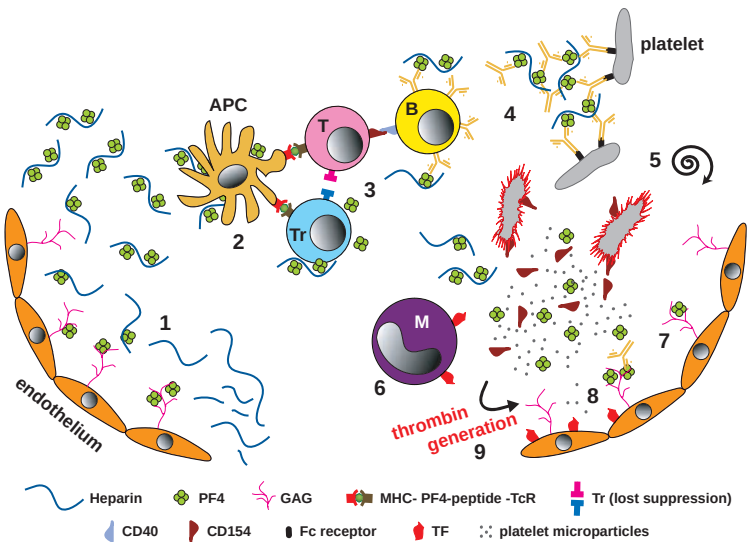


FIGURE 6.6 Proposed model of pathogenesis in HIT and thrombosis. PF4 is postulated to be both the target for the antibody (when complexed with heparin) and a modulator of T-cell responsiveness (see text for additional details). *Abbreviations*: APC, antigen-presenting cell; GAG, glycosaminoglycan; HIT, heparin-induced thrombocytopenia; MHC, major histocompatibility complex; PF4, platelet factor 4; TCR, T-cell receptor; Tr, T regulatory; TF, tissue factor.

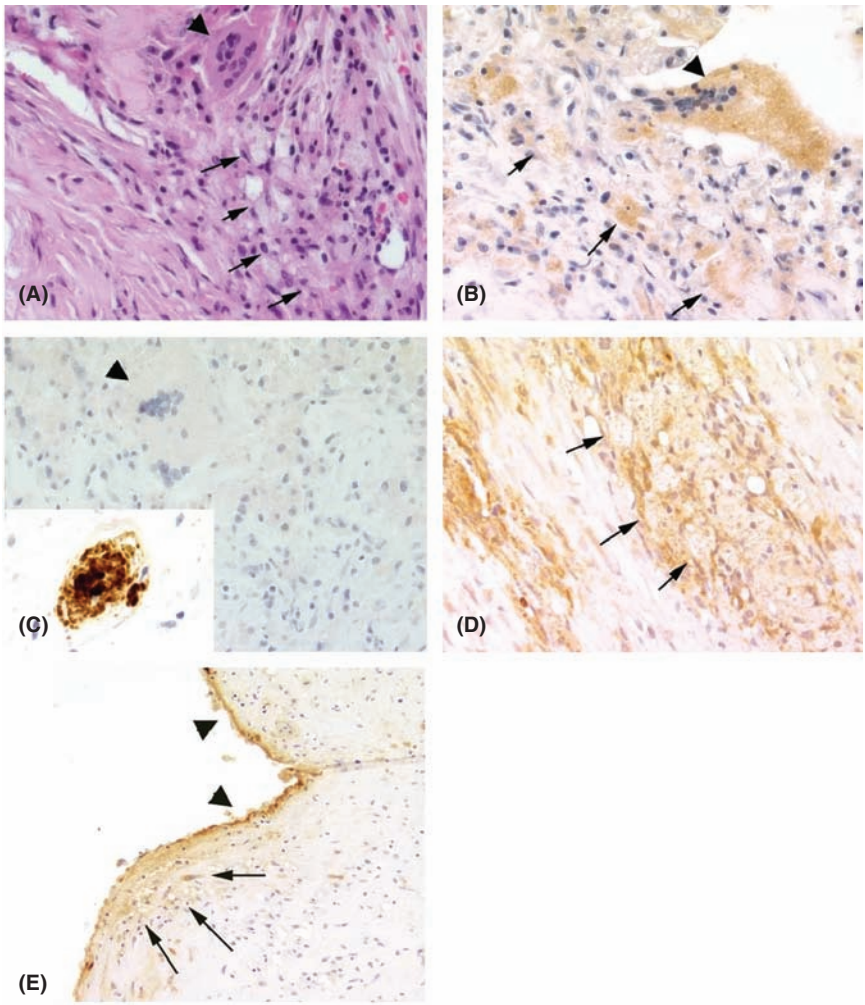


FIGURE 9.2 Atherosclerotic tissue is invested with PF4. (**A**) Photomicrograph (H&E stain) demonstrating the appearance of a group of foamy macrophages, which contain bubbly, vacuolated cytoplasm and indistinct nuclei (*arrows*). A giant cell is indicated by the arrow-head. (**B**) Photomicrograph of immunohistochemical staining for CD68, which is specific for cells of the macrophage lineage. Foamy macrophages (*arrows*) and a giant cell (*arrowhead*) is indicated. (**C**) Photomicrograph of immunohistochemical staining for CD41, specific for platelets. A small vessel filled with red blood cells and platelets serves as an internal positive control (*inset*). (**D**) This photomicrograph demonstrates the anti-PF4 staining the foamy macrophages. Note that the morphology of these cells is identical to those in panels (**A** –**C**), with vacuolated cytoplasm that stains for PF4 (*arrows*). (**E**) Photomicrograph demonstrating PF4 staining of an early atherosclerotic lesion from a carotid artery obtained at autopsy. The endothelium (*arrowheads*) and subendothelial macrophages (*arrows*) of an early lesion stain intensely positive with anti-PF4. Images are 40X (**A**–**D**) or 20X (**E**) original magnification. *Abbreviation*: PF4, platelet factor 4.

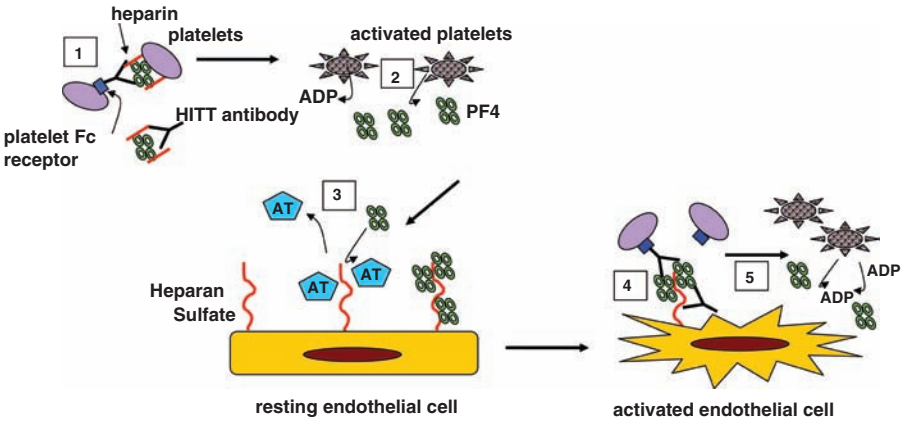


FIGURE 9.3 Model of HIT antibody interactions with endothelial cells. (1) HIT antibodies bind to antigen (multimolecular PF4/heparin complexes) localized to platelets. (2) Platelet activation occurs after Fc receptor binding, leading to platelet granule release. (3) Released PF4 binds to platelets and endothelial cell HS displacing AT from endothelial cells. (4) Antigenic complexes on endothelial cells bind HIT antibodies. (5) HIT antibody binding to endothelial cells leads to endothelial cell activation and further platelet activation. *Abbreviations*: ADP, adenosine diphosphate; AT, antithrombin; HIT, heparin-induced thrombocytopenia; HS, heparan sulfate; PF4, platelet factor 4.

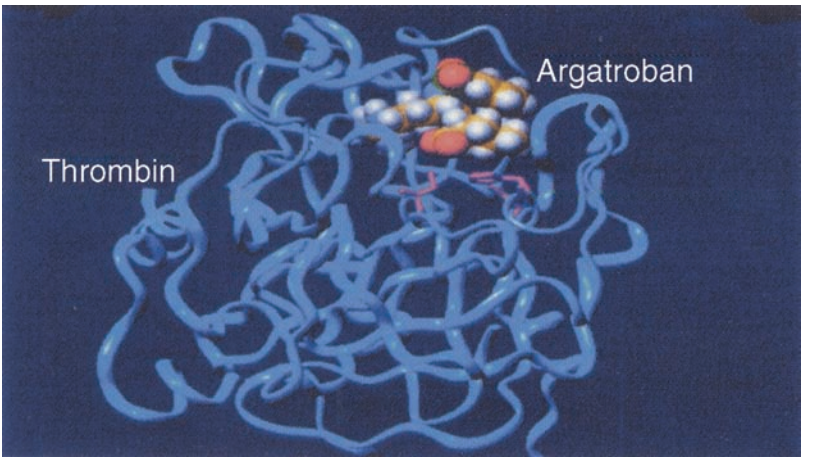


FIGURE 15.2 Model of the interaction between argatroban and thrombin.

Cardiology

about the fourth edition...

Although first reported in 1973, immune heparin-induced thrombocytopenia (HIT) remains one of the most potentially devastating and frequent adverse drug reactions encountered by physicians. This *Fourth Edition* reinforces its standing as the leading guide to the accurate diagnosis and management of HIT by identifying key signs and symptoms of this disorder and providing clear intervention strategies, including detailed information on the use of alternative anticoagulants to manage these critical circumstances.

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about the editors...

THEODORE E. WARKENTIN is Professor, Department of Pathology and Molecular Medicine, and Department of Medicine, McMaster University; and Associate Head, Transfusion Medicine, Hamilton Regional Laboratory Medicine Program; and Hematologist, Service of Clinical Hematology, Hamilton Health Sciences, all in Hamilton, Ontario, Canada. The author, coauthor, or editor of more than 200 professional publications, journal articles, and book chapters, Dr. Warkentin is a Fellow of the Royal College of Physicians and Surgeons of Canada and the American College of Physicians. Dr. Warkentin received the M.D. degree from the University of Manitoba, Winnipeg, Canada, and completed postgraduate studies in internal medicine and clinical hematology, respectively, at the University of Toronto, Ontario, Canada, and McMaster University, Hamilton, Ontario, Canada.

ANDREAS GREINACHER is Director of the Department of Transfusion Medicine, Institute for Immunology and Transfusion Medicine, Ernst-Moritz-Arndt-University-Greifswald, Greifswald, Germany. Dr. Greinacher received the M.D. degree from the Julius Maximilian University, Würzburg, Germany, and finished training in clinical immunology and transfusion medicine at the Justus-Liebig University, Giessen, Germany. He is the author, coauthor, or editor of more than 200 professional publications, journal articles, and book chapters.

Front cover: Iceberg model of HIT. The two top levels depict HIT (with and without associated thrombosis); the lower three levels infer sensitivity/specificity profiles of three different assays for HIT antibodies (see Chap. 3). Courtesy of Jo-Ann I. Sheppard, B.Sc.

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Telephone House
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