TRANSLATIONAL RESEARCH IN CORONARY ARTERY DISEASE

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Pathophysiology to Treatment

Edited by

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125, London Wall, EC2Y 5AS
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

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ISBN: 978-0-12-802385-3

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress.

Publisher: Mica Haley
Acquisition Editor: Stacy Masucci
Editorial Project Manager: Shannon Stanton
Production Project Manager: Julia Haynes
Designer: Matt Limbert

Typeset by MPS Limited, Chennai, India
www.adi-mps.com

Printed and bound in the USA

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Preface

These first years of the new millennium have witnessed an explosion in translational research in the field of cardiovascular disease in general and coronary artery disease in particular. During this time, our understanding of the pathophysiology, the available diagnostic modalities, and the appropriate therapeutic interventions has changed considerably. This is a time, too, during which the promise of stem cell therapy has remained unfulfilled, largely as a result of an insufficient knowledge of the many signaling pathways that underpin the programming, homing, and differentiation of pluripotent cell lines. Despite this, remarkable advances have been made in vascular biology and the genetics of coronary disease, our understanding of lipid chemistry and inflammation, the electrophysiological manifestations of ischemic disease, and the development of novel means of revascularization and treatments for ischemic shock. These new developments promise to completely reinvent our approach to the prevention, diagnosis, and treatment of coronary disease over the course of the decade to come. They have also laid the groundwork for a more carefully designed series of experiments and clinical trials that will finally realize the benefits of cellular plasticity as a therapeutic modality.

This is as it needs to be, for despite the significant improvements made in the diagnosis and treatment of coronary disease in developed nations, the emergence of coronary disease in the developing world is now becoming a major public health problem.

Given the complexity of human biology, it is a truly remarkable thing that the human organism responds as well as it does to the pharmacologic and interventional strategies that have been developed for the treatment of coronary disease over the course of the last half century. For those of us privileged to have had our careers unfold during this time of unparalleled growth in knowledge and therapeutic potential, it has been an extraordinary journey. What we have witnessed to date, however, serves only as a prelude to even more remarkable discoveries that will build exponentially on what we now know.

This book has attempted to encapsulate in one volume the major advances of the recent past in order to provide the reader with a concise, but comprehensive, view of where we are and where we are going. To this end, we have organized it in such a fashion as to begin with the most promising work in basic science, subsequent to which we transition into diagnostic modalities and ultimately into new therapies. We have concluded with a chapter on biostatistics which presents the reader with a precise review of the techniques currently used for the development and analysis of clinical data.

We have crafted this volume to be of particular use to cardiovascular scientists and practitioners alike as well as to biomedical faculties and students of all stripes who have an interest in learning about and furthering the progress of coronary artery research. We hope that you will find it useful in your own education as well as the education of others who care for our patients and who continue to develop and improve the therapies for them.

Wilbert S. Aronow and John Arthur McClung

Biographies

Wilbert S. Aronow, MD, is Professor of Medicine at New York Medical College/Westchester Medical Center, Valhalla, NY, USA. Dr Aronow received his MD from Harvard Medical School. He has edited 13 books and is author or coauthor of 1453 papers, 301 commentaries or Letters to the Editor, and 1004 abstracts and is presenter or copresenter of 1374 talks at meetings. Dr Aronow is a Fellow of the ACC, the AHA, the ACP, the ACCP, the AGS (Founding Fellow of Western Section), and the GSA. He has been a member of 112 editorial boards of medical journals, coeditor of two journals, deputy editor of one journal, executive editor of three journals, associate editor for nine journals, and guest editor for seven other medical journals. He has received each year from 2001 to 2015 an outstanding teacher and researcher award from the medical residents and from 2001 to 2015 from the cardiology fellows at Westchester Medical Center/New York Medical College. He has received awards from the Society of Geriatric Cardiology, the Gerontological Society of America, New York Medical College including the 2014 Chancellor's Research Award, the F1000 Faculty Member of the Year Award for the Faculty of Cardiovascular Disorders in 2011, 2013, and 2014, the Walter Bleifeld Memorial Award for distinguished contributions to clinical research from the International Academy of Cardiology in July, 2010, and a Distinguished Fellowship Award from the International Academy of Cardiology in July, 2012. He has been a member of four national guidelines committees including being a coauthor of the 2010 AMDA guidelines for heart failure, cochair and first author of the 2011 ACC/AHA expert consensus document on hypertension in the elderly, coauthor of the 2015 AHA/ACC/ASH scientific statement on treatment of hypertension in patients with coronary artery disease, and is currently a member of the writing group of the ACC/AHA guideline for the management of patients with hypertension. He was a coauthor of a 2015 position paper from the International Lipid Expert Forum. He was a consultant to the ACP Information and Educational Resource (PIER) on the module of aortic stenosis. He is currently a member of the Board of Directors of the ASPC, and a member of the ACCP Cardiovascular Medicine and Surgery Network Steering Committee.

John Arthur McClung, MD, is Professor of Clinical Medicine in the School of Medicine and Professor of Clinical Public Health in the School of Health Sciences and Practice at New York Medical College in Valhalla, New York, where he also serves on the clinical faculty of the Westchester Medical Center. Dr McClung received his AB from the Johns Hopkins University in 1971 and his MD from New York Medical College in 1975, where he received the Sprague Carlton Award and the Cor et Manus Award of Distinction. He has been on the faculty of New York Medical College since 1979 and served as its Chief of the Critical Care Section of the Department of Medicine from 1982 until 1990. In 1988, he completed the Intensive Bioethics Course at Georgetown University and went on to the Advanced Bioethics Course in 1990. He is board certified in Internal Medicine, Cardiovascular Disease, and Echocardiography and currently serves as the Director of the Noninvasive Cardiology Laboratory at the Westchester Medical Center, a position that he has held since 2006. He is a past Director of the Cardiovascular Fellowship Training Program at New York Medical College from 2001 until 2014, and was a member of the New York Medical College Committee for the Protection of Human Subjects from 1987 until 2008, serving as its chair for the last 2 years. He is currently a member of the New York Academy of Sciences and a Fellow of the American College of Physicians, the American College of Cardiology, the American Heart Association and its Council on Clinical Cardiology, and the American Society of Echocardiography. He is a past Fellow of the Society for Cardiac Angiography and Interventions and serves on the Board of Directors of the Physicians' Home since 2009. He is a member of the Iota Chapter of AΩA and is a past Councilor for the New York State Chapter of the American College of Cardiology, where he served as the chair of its nominating committee. He has served as a manuscript reviewer for the *European Journal of Endocrinology*, *Drugs & Aging*, *Catheterization and Cardiovascular Diagnosis*, the *Journal of Clinical Ethics*, and *Cardiology in Review*. In 1990, Dr McClung founded the Division of Clinical Ethics of the Department of Medicine at New York Medical College and served as its chief until 1995. He has published articles and book chapters on endothelial function in diabetes mellitus, heme oxygenase, cardiomyopathy, multiple topics in echocardiography, and multiple topics in clinical cardiology. He has also published articles and book chapters on ethical issues in the areas of cardiopulmonary resuscitation, bioethics consultation, and end of life care.

CHAPTER

1

Endothelial Biology: The Role of Circulating Endothelial Cells and Endothelial Progenitor Cells

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In Lewis Carroll's Alice in Wonderland, the king responds to the query of the White Rabbit as to where to begin by saying, "Begin at the beginning… and go on till you come to the end: then stop." When dealing with cell turnover, the definition of the beginning is an open question, as a result of which, simply for purposes of discussion, this review will begin with the endothelial progenitor cell and go on from there.

WHAT ARE ENDOTHELIAL PROGENITOR CELLS?

Since Asahara et al. first isolated and described a population of what were termed "endothelial progenitor cells" (EPCs) in the peripheral blood at the end of the last century [1,2], a wealth of research has been generated that has further characterized these cells and in so doing raised more questions about both their identity and their behavior. Asahara's original work identified a population of cells that were CD34 positive as well as vascular endothelial growth factor receptor-2 (VEGFR-2) positive that were capable of differentiating into endothelial cells *in vitro*, migrating *in vivo* to sites of vascular injury, and that enhanced the formation of new endothelium when infused into an organism. Given that both CD34 and VEGFR-2 are also expressed on mature endothelial cells, Peichev et al. demonstrated a population of circulating cells that also expressed CD133 in contradistinction to presumably mature human umbilical vein cells (HUVECs) which were CD133 negative [3].

Concurrently, Gehling et al. isolated CD133+ cells from peripheral blood that, when plated on fibronectin for 14 days, were able to generate colony-forming units (CFUs) of apparently both hematopoietic and endothelial lineage cells [4]. Shortly thereafter, Hill et al. described a similar, but not identical, assay in which circulating mononuclear cells were cultured for 2 days with the nonadherent cells and were subsequently plated on fibronectin. Colonies were counted 7 days later and demonstrated an endothelial phenotype by histochemical staining for von Willebrand factor, VEGFR-2, and CD31 [5]. The number of colonies generated correlated negatively with the Framingham risk score and positively with the flow-mediated brachial index. Other investigators, using a similar technology, demonstrated that these cells could be incorporated into the damaged endothelium of a ligated left anterior descending coronary artery in a rat model [6]. A commercial assay using this technology was subsequently devised that used a 5-day protocol and has subsequently become known as the CFU-Hill Colony Assay.

In contradistinction to the CFU assay, Lin et al. plated human monocytes from which the nonadherent cells were removed at 24 h [7]. The remaining adherent cells were cultured and observed to have expanded significantly in bone marrow (BM) transplant recipients over the course of a month. Similarly, Vasa et al. evaluated

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00001-2

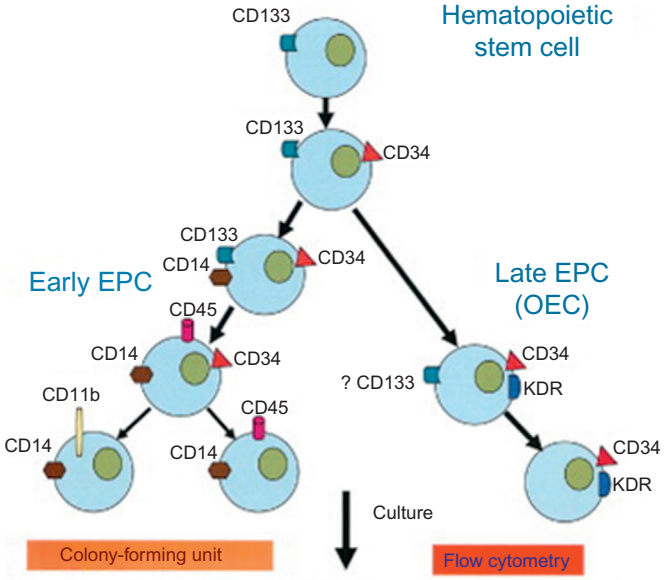
the migratory capability of monocytes cultured for 2 days on fibronectin in which the adherent cells were isolated rather than the nonadherent cells [8]. These cells demonstrated significant migratory potential that appeared to be inversely proportional to the number of risk factors in a population of patients with coronary artery disease (CAD).

Hur et al. plated monocytes on endothelial basal medium and noted the appearance of spindle shaped cells similar to the original Asahara reports that increased in number for 14 days, after which replication ceased and the cells gradually disappeared by 28 days [9]. Another population of cells appeared after 2–4 weeks of incubation that rapidly replicated and demonstrated no evidence of senescence. These "late" EPCs, in contradistinction to "early" EPCs, were observed to successfully form capillaries when plated on Matrigel and were more completely incorporated into HUVEC monolayers. Notwithstanding, both early and late EPCs were equally effective at improving perfusion to an ischemic limb in a mouse model. Combining these two populations of cells was even more effective at enhancing ischemic limb perfusion [10].

Late EPCs have also been described as "outgrowth endothelial cells" (OECs) or "endothelial colony-forming cells" (ECFCs) by other investigators [7,11]. Using the approach of Lin and Vasa in which nonadherent cells were discarded and adherent cells were retained, investigators were able to culture a subpopulation of cells that appeared to be identical to Hur's late EPCs, both morphologically and in their migratory behavior. Late EPCs appear to be distinctly superior to other EPC subpopulations in promoting angiogenesis, both *in vitro* and *in vivo* [12]. In addition to having a much higher rate of proliferation and resistance to apoptosis, this subpopulation has also been noted to have increased telomerase activity [11].

Sieveking et al. generated both early and late EPCs out of a single population of mononuclear cells that were plated on fibronectin with the nonadherent cells removed after 24 h [13]. Both early and late EPCs were observed to be CD34, CD31, CD146, and VEGFR-2 positive, however, only early EPCs expressed CD14 and CD45. Late EPCs formed branched interconnecting vascular networks, while early EPCs were observed to exhibit a marked augmentation of angiogenesis by a paracrine mechanism. These results are summarized in Figure 1.1 [14].

Thus, there appear to be at least four different methodologies for isolating putative EPCs from monocytes plated on fibronectin. The CFU assay cultures cells that are not adherent to the medium which form colonies at 4–9 days that are consistent with an early EPC phenotype. Hur et al. were able to grow both early and late EPCs from monocytes that were not separated out by their ability to either adhere or not to adhere to the medium. Sieveking et al. were able to grow both early and late EPCs from only adherent monocytes. Hence, it appears that nonadherent cells can generate only early EPC colonies, while adherent cells have the capability of generating both early EPC and OEC (Figure 1.2).



Early EPC	Characteristics	Late EPC
3–5 days	Duration	7–14 days
Heterogenous	Morphology	Homogenous
$CD14/CD45/KDR_{Low}$ CD31/vWf	Antigen markers	CD34/KDR CD31/vWf
+++	Secretion of angiogenic cytokines	+
Low	*In vivo* angiogenic effects	High

FIGURE 1.1 Antigenic cell surface markers of "Early" EPC and "Late" EPC (OEC). "Early" EPCs form CFUs and most of these go on to have hematopoetic rather than endothelial phenotypes. OEC, outgrowth endothelial cells; KDR, VEGFR-2. *Source: From Ref. [14]. Used by permission.*

In addition to BM-derived cells, a recent study isolated a rare vascular endothelial stem cell in the blood vessel wall of the adult mouse that is CD117+ and c-kit+, and has the capacity to produce tens of millions of daughter cells that can generate functional blood vessels *in vivo* that connect to the host circulation [16]. The cellular regeneration of both the vascular and other components of a mouse digit tip in the context of CFU transplantation has been found to be composed of tissue-derived cells only [17].

All of these various cells have reparative capability when acting together, but precisely how this occurs is a matter of intense current research.

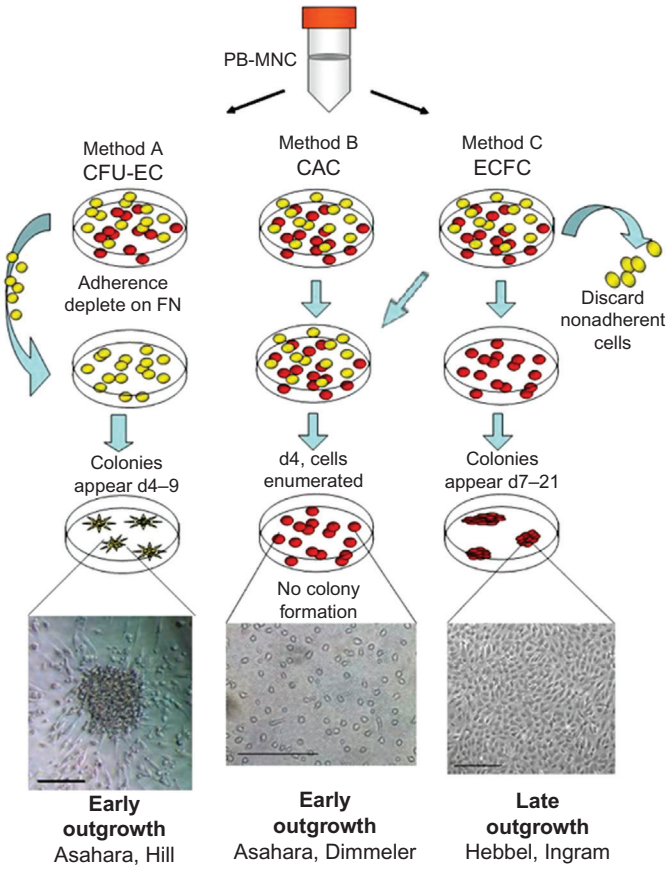


FIGURE 1.2 Methodologies for the generation of various cell types from tissue culture of BM-derived circulating mononuclear cells. PB-MNC, peripheral blood mononuclear cells; CFU-EC, CFU endothelial cells; CAC, circulating angiogenic cells; ECFC, OEC; FN, fibronectin. *Source: Adapted by permission from Macmillan Publishers Ltd; Ref. [15].*

PARACRINE EFFECTS OF BM-DERIVED CELLS

CFU derived early EPCs secrete a number of agents, among them matrix metalloproteinase (MMP)-9, interleukin (IL)-8, macrophage migration inhibitory factor (MIF), angiopoeitin-1 (Ang-1), and thymidine phosphorylase (TP) in higher amounts than in early EPCs from adherent monocytes [18]. Early EPCs cultured from nonadherent monocytes secrete VEGF, stromal cell-derived factor-1 (SDF-1), insulin-like growth factor-1 (IGF-1), and hepatocyte growth factor (HGF) [19]. Early EPCs cultured from adherent monocytes secrete VEGF, HGF, granulocyte colony stimulating factor (G-CSF), and granulocyte macrophage colony stimulating factor (GM-CSF) [20].

Both VEGF and SDF-1 promote migration and tissue invasion of progenitor cells to a site of injury as well as enhance migration of mature endothelial cells [21–23]. IGF-1 promotes angiogenesis and inhibits apoptosis [24]. HGF markedly enhances angiogenesis [25]. G-CSF and GM-CSF enhance the migration of endothelial cells, and both have anti-inflammatory activity on vascular endothelium as well [26–28]. MMP-9 appears to be required for EPC mobilization, migration, and vasculogenesis, and IL-8 enhances both endothelial cell proliferation as well as survival [29,30]. Among other things, MIF appears to induce EPC mobilization [31]. Ang-1 is expressed from hematopoetic stem cells [32]. Along with VEGF, Ang-1 has been implicated in the recruitment of vasculogenic stem cells, and when BM mononuclear cells are enhanced by Ang-1 gene transfer, angiogenesis is improved both qualitatively and quantitatively [33,34]. TP has been demonstrated to both enhance endothelial cell migration and protect EPCs from apoptosis [18,35].

Early EPCs also have been shown to be repositories of both eNOS and iNOS which play a role in ischemic preconditioning and chronic myocardial ischemia, respectively [36,37]. More recently, prostacyclin (PGI_2) has been identified as being secreted in very high levels by late (OEC) EPC [38].

MECHANISMS, KNOWN AND UNKNOWN

Mobilization of BM-Derived Cells

Under stable physiologic conditions, circulating EPC precursors exist in a niche in the BM that is defined by a combination of low oxygen tension, low levels of reactive oxygen species (ROS), and high levels of SDF-1 [39–41]. In the face of myocardial ischemia, VEGF and SDF-1 are expressed in human and rat models, respectively [42,43]. This, as well as vascular trauma, initiates a complex mechanism that involves the release of multiple chemokines [44–46]. Among other effects, this release activates the phosphoinositide 3-kinase (PI3K)/Akt pathway to increase the production of nitric oxide (NO) which in turn activates MMPs [47,48]. MMPs, and in particular MMP-9 via release of soluble kit ligand, disrupt the integrins that form the scaffold that retains the stem cells in the marrow, allowing them to respond to the enhanced SDF-1 gradient and move out into the circulation (Figure 1.3) [50,51]. Once released from the marrow, development of these cells is enhanced, in part, by the release of Ang-1 by pericytes and by EPC themselves which can also enhance their survival by means of the downstream activation of the PI3K/Akt pathway (Figure 1.4) [53].

Vasculogenesis and Angiogenesis

Originally thought by Asahara et al. to be a process mediated solely by BM-derived cells, vasculogenesis refers to *de novo* vessel formation by *in situ* incorporation,

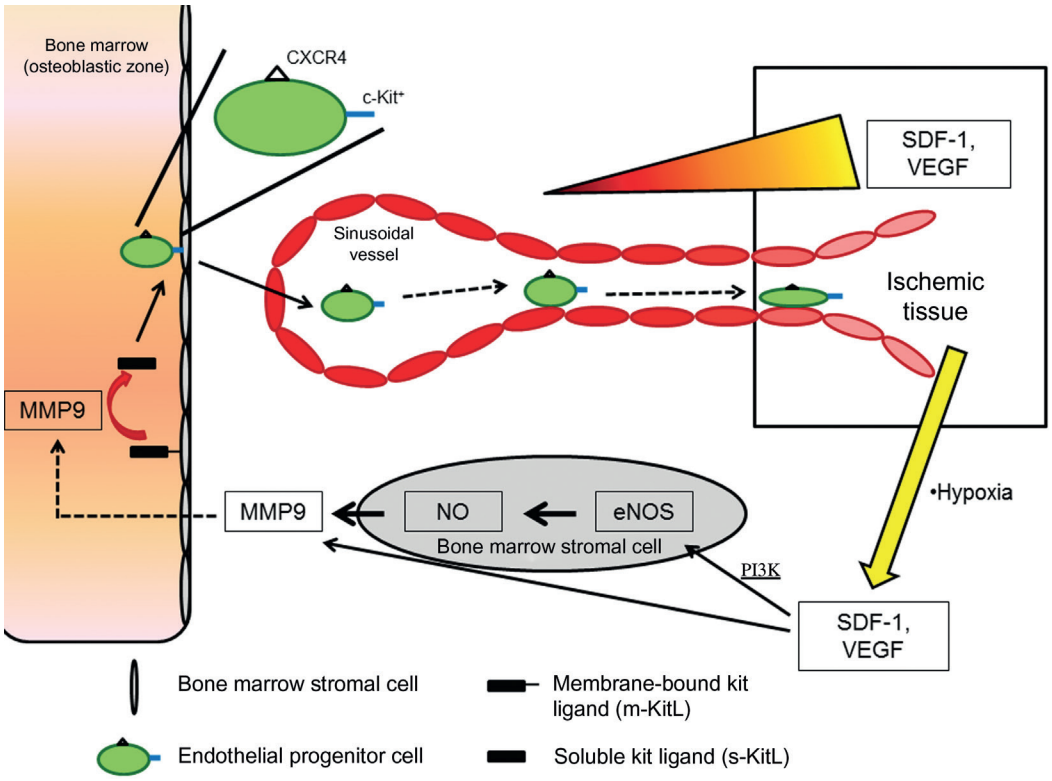


FIGURE 1.3 Schematic representation of the mobilization of BM-derived EPC by ischemic stimuli. Ischemia activates the PI3K/Akt pathway to increase the production of NO which in turn activates MMP-9 disrupting the integrin scaffold in the BM and allowing EPC to respond to the enhanced SDF-1 gradient and move out into the circulation. *Source: Adapted from Ref. [49]. Used by permission.*

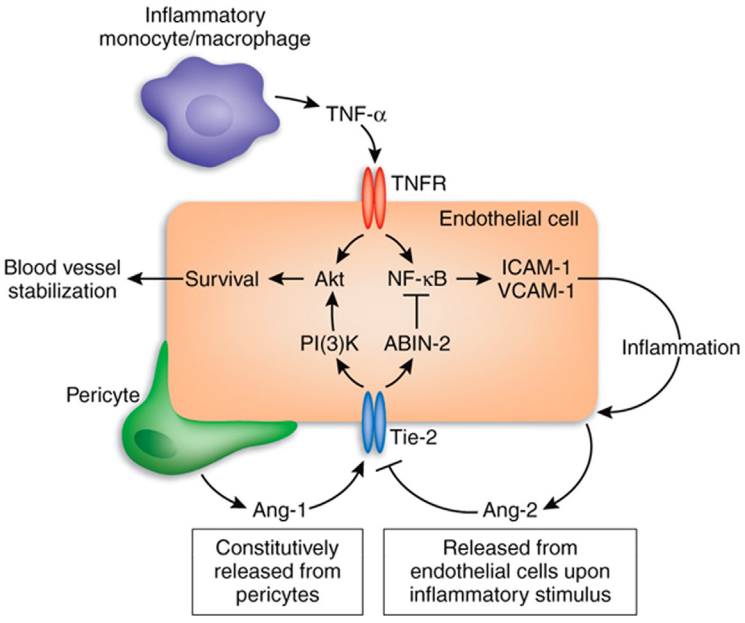


FIGURE 1.4 Cell survival induced by activation of the PI3K/Akt pathway by Ang-1 elaboration by pericytes. The release of Ang-1 enhances survival of the *in situ* endothelial cell by allowing it to resist active inflammation mediated by Ang-2 as well as serving as a chemoattractant for EPC. ABIN-2, A20-binding inhibitor of NF-kappa-B activation 2; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; Tie-2, tyrosine kinase receptor 2. *Source: By permission from Macmillan Publishers Ltd; Ref. [52].*

differentiation, migration, and/or proliferation of either circulating EPC or EPC of tissue origin [54]. Angiogenesis, which constitutes a common component of wound healing as well as tumor growth, is consistently a local phenomenon that extends an already existing vessel either by "sprouting" or by splitting the vessel into two via a poorly understood "intussusceptive" mechanism [55]. The evolving understanding of vasculogenesis in arterial disease appears to suggest that it involves elements of angiogenesis as well. (See Chapter 6).

Maturation of BM-Derived EPC

The maturation process of BM-derived circulating EPC is poorly understood, but clearly involves a number of competing processes. Prime among them is vascular shear stress which has been implicated in an increase in NO production, EPC proliferation, and retention [56]. Adhesion, proliferation, maturation, and a reduction in apoptosis have been noted in circulating CD133+ cells exposed to shear that is mediated by the PI3K/Akt signaling pathway [57]. Similarly, it appears that the PI3K/Akt pathway promotes the maturation of early EPCs [58].

Alternatively, NADPH oxidase (NOX)-derived ROS have the potential to act as redox signaling for the mobilization of BM-derived EPCs as well as their differentiation and maturation [59]. This is in contradistinction to the customary role of ROS as being toxic to EPCs when overly expressed [60]. Recent work in a mouse model has revealed proliferator-activated receptor alpha (PPARα) induced activation of NOX was required for both mobilization and homing of BM-derived EPCs, and that its absence was associated with enhanced recruitment of progenitor cells into the BM [61].

Platelet Interaction

A number of studies have suggested that platelet activation is associated with the recruitment, differentiation, and homing of BM-derived EPCs [62–65]. Conversely, BM-derived CFU EPCs have recently been observed to inhibit platelet activation, aggregation, collagen adhesion, and thrombus formation through upregulation of cyclooxygenase-2 (COX-2) and the secretion of PGI_2 [66]. This has been determined to be a result of the inhibition of P-selectin expression by PGI_2 [67].

PGI_2 as a Primary EPC Paracrine Mediator

Shear stress results in the expression of PGI_2 by both endothelial cells and EPCs [68,69]. Age-related impairment of flow-induced vasodilation in gastrocnemius muscle arterioles is due to the reduction in the availability of PGI_2 [70]. Studies of OEC have demonstrated that they release high levels of PGI_2 in association with high levels of COX-1 expression, and that TXA_2 production is low [38]. Although the classic PGI_2 signaling pathway functions by activation of adenylyl cyclase with a resulting increase in cAMP [71], the angiogenic activity enhanced by late EPCs appears to be mediated via the activation of PPARδ, consistent with prior data demonstrating the induction of transcriptional activation by PPARα and PPARδ by PGI_2 [72]. In addition, early EPCs from adherent cells not only produce PGI_2 in a COX-1 dependent fashion, but the PGI_2 so expressed further enhances the EPC adhesion, migration, and proliferation through binding to a prostacyclin receptor (IP) on these same cells [73]. In a similar fashion, OEC transfected to overexpress PGI_2 not only demonstrated enhanced angiogenesis themselves but also provided favorable paracrine-mediated cellular protection, including the promotion of *in vitro* angiogenesis by EPCs, and the protection of potassium channel activity in vascular smooth muscle cells under conditions of hypoxia [74].

COX-2 expression is increased in rabbit basilar arteries transplanted with early adherent EPCs with a resultant increase in PGI_2 and a decrease in TxA_2 [75]. Despite prior observations that early EPCs are rich in eNOS and iNOS, there was no change in the expression of eNOS- or iNOS-mediated vasodilation in rabbit carotid arteries exposed to early EPCs. COX-2 is induced in activated endothelial and inflammatory cells [76]. As much of the systemic PGI_2 is produced in a COX-2-dependent manner, it is reasonable to conclude that PGI_2 produced through multiple pathways can affect the function of EPCs [77]. Hence, COX-1-dependent PGI_2 released by BM-derived EPCs has the capacity to enhance both arterial function as well as the function of the EPCs themselves, while COX-2 expression from native endothelial cells can function in the same manner. Taken together, it appears that related signaling from both cellular beds serves to modulate the migration and function of the BM-derived reparative system.

CIRCULATING ENDOTHELIAL CELLS AND MICROPARTICLES: THE OTHER SIDE OF THE COIN?

Circulating endothelial cells (CECs) were first reported to be present in peripheral blood and tied to vascular injury in 1970 [78]. Since then, their presence has been described in a number of disease entities that are associated with vascular damage including sickle cell disease [79], ANCA-associated vasculitis [80], Behcet's disease [81], systemic lupus erythematosis (SLE) [82], peripheral arterial disease [83], acute coronary syndrome [84], type 2 diabetes mellitus [85], and, to a lesser extent, type 1 diabetes mellitus [86]. They are considered to be generally

apoptotic or necrotic endothelial cells that have been sloughed from the vascular lining, however, some of these cells may be viable, and some may even be maturing EPC.

The mechanism by which CECs are detached from the endothelial surface is not clearly understood, but has been related to the effects of various cytokines, proteases, and the binding of neutrophils, as well as various drugs such as cyclosporine [87]. The integrity of the endothelium is maintained by shear stress, among other things, which inhibits apoptosis through multiple mechanisms, among them the elaboration of eNOS via the PI3K pathway [88]. Under conditions of inflammation, the glycocalyx that lines the endothelial layer begins to break down, shedding its components, resulting in the production of proteases by the pericytes which attack the endothelial basement membrane leading to cell detachment (Figure 1.5) [89].

These cells have been commonly isolated by means of either immunomagnetic separation or by flow cytometry. In part related to its good reproducibility, a consensus was reached in 2006 that described an immunomagnetic methodology for the isolation of CD146-positive cells with a particular set of criteria for standardization [90]. Notwithstanding its reproducibility, it can be difficult to differentiate necrotic from apoptotic and viable cells by this method, these cells being more easily separated from each other by flow cytometry [91]. Neither method, by itself, is particularly exact for the exclusion of EPCs. Hence, some investigators have attempted to combine these techniques in order to increase the specificity of the assay [92,93].

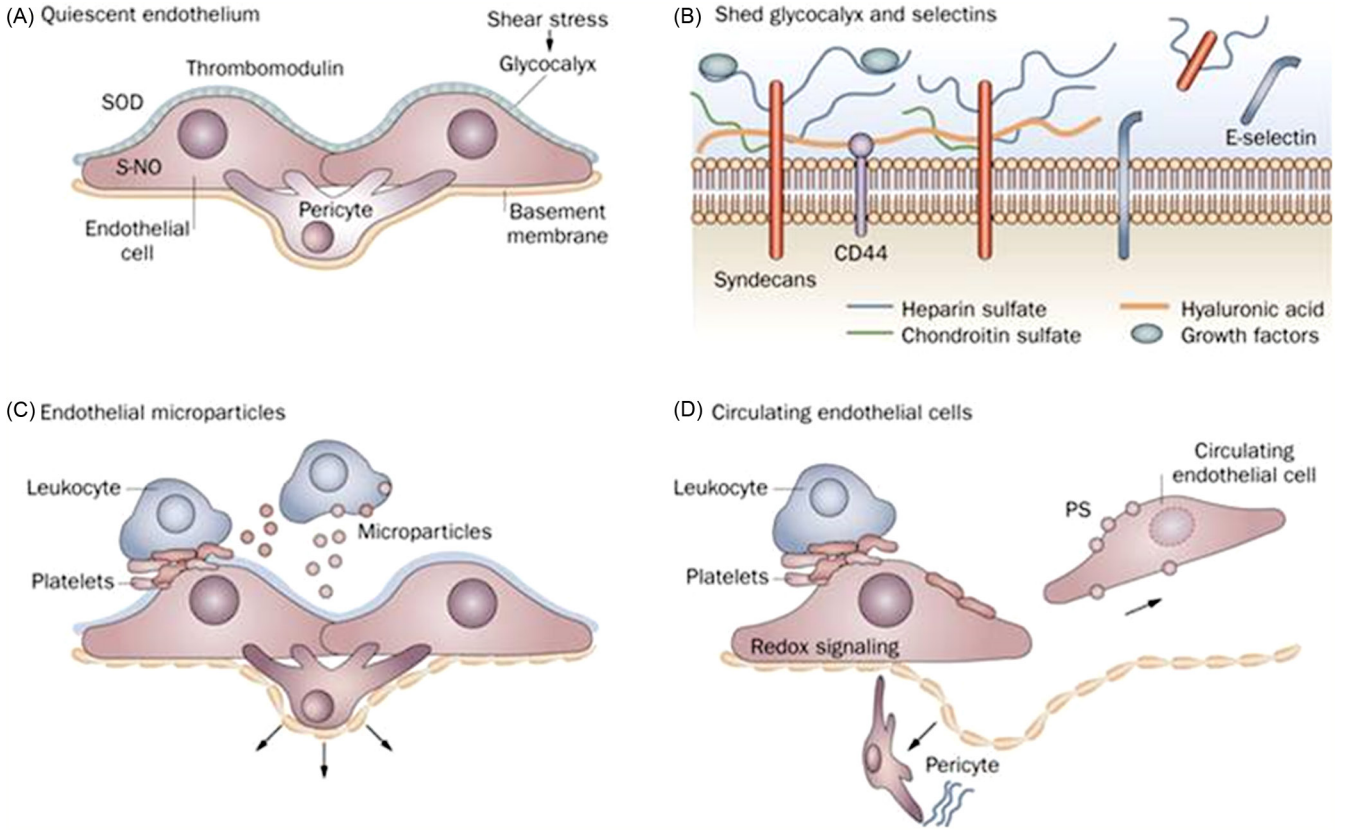


FIGURE 1.5 Disruption of the glycocalyx, leading to shedding of microparticles, detachment of the pericytes, and mobilization of CEC. a) While quiescent, the antithrombotic, anti-inflammatory, and antiproliferative properties of the endothelium are maintained by the dominance of nitric oxide signaling which forms S nitrosylated proteins, shear stress signaling through the glycocalyx which binds thrombomodulin and SOD among other factors, and signaling between pericytes and the endothelium. b) The glycocalyx contains anchoring proteoglycans such as CD44 and members of the syndecan protein family, as well as connecting glycosoaminoglycans such as heparan sulfate, chondroitin sulfate and hyaluronic acid. When activated by inflammation, the glycocalyx is initially modified to allow leukocytes and platelets to interact with the endothelial surface. Glycocalyx components are then released into the circulation. After prolonged inflammatory activation and early apoptotic events, adhesion molecules such as E-selectin are also shed. c) With further sustained endothelial activation, platelets and leukocytes bind to the endothelial surface leading to the formation of proinflammatory factors which can cause further activation of the endothelium and promote the formation of membrane particles (microparticles). Microparticles from endothelial cells, platelets and leukocytes are released into the circulation, at which point the stabilizing interaction of pericytes with the endothelium becomes disrupted and pericytes produce proteases that damage the endothelial basement membrane. d) Sustained redox signaling results in loss of pericyte signaling, which leads to apoptosis or necrosis of the endothelial cell, and the expression of phosphatidylserine residues on the external surface of the plasma membrane. The endothelial cells then detach and are detected in the circulation. PS = phosphatidylserine; S-NO = S-nitrosyl; SOD = superoxide dismutase. *Source: By permission from Macmillan Publishers Ltd; Ref. [89].*

What seems to be clear from the study of CECs is that they are not biologically inert entities. Woywodt et al. found that 86% of CECs isolated from patients with ANCA-associated vasculitis stained positive for tissue factor (TF) associated with a prothrombotic phenotype, and 84% of these cells stained positive for annexin and propidium iodide, consistent with a necrotic phenotype [80]. Previously, Li et al. demonstrated that necrotic, but not apoptotic, dendritic cells induced inflammatory mechanisms via nuclear factor κB (NF-κB) and the Toll-like receptor 2 pathway [94]. Similarly, Barker et al. demonstrated that necrotic, but not apoptotic, neutrophils increased antigen presentation by macrophages [95].

Kirsch et al. subsequently demonstrated that endothelial cells themselves have the capacity to engulf both apoptotic and necrotic endothelial cells, and that engulfment of apoptotic cells was associated with the expression of inflammatory chemokines as well as the enhanced binding of leukocytes [96]. The authors speculated that healthy endothelium might be induced to engulf CEC under conditions of generalized inflammation when the customary, so-called "professional," phagocytes had been overwhelmed by the increased numbers of circulating cells associated with vascular damage, a problem with cell clearance that has been observed in SLE [97]. Other investigators have demonstrated similar endothelial cell activation when confronted with necrotic cells [98].

There is also data to suggest that CECs interfere with the function of EPCs [99]. Decreases in EPC number associated with an increase in the number of CECs have been observed under conditions of mechanical stress [100]. However, recent data from heart transplant patients suggests that patients presenting with cardiac allograft vasculopathy universally present with high numbers of CECs and microparticles, while the number of EPCs remained unchanged from that noted in patients with no evidence of vasculopathy [101]. Hence, the issue of the effect of CECs on EPCs remains an open question at this time.

Microparticles

Endothelial microparticles (EMPs) were reported to be generated following the appearance of blebs on the surface of HUVEC after stimulation with tumor necrosis factor alpha [102]. The EMPs had a procoagulant phenotype *in vitro* mediated via TF, and they expressed E-selectin, intercellular adhesion molecule 1 (ICAM-1), αvβ3, and platelet endothelial cell adhesion molecule 1 (PECAM-1), suggesting that they had adhesive potential as well. Finally, they were discovered to be present *in vivo* in the blood of normal volunteers and significantly increased in number in the blood of patients with the lupus anticoagulant, suggesting a procoagulant role *in vivo*.

In the same manner, the number of EMPs has been reported to be inversely proportional to the amount of shear stress in patients with end-stage renal failure [103]. An increased ratio of EMPs to EPCs has been associated with the presence of atherosclerosis in patients with hyperlipidemia [104]. Similarly, elevated levels of EMPs have been correlated with disturbed flow-mediated vasodilatation, as well as the endothelial dysfunction observed in healthy subjects exposed to secondhand smoking [105,106].

Despite the apparent procoagulant phenotype of EMPs, recent data has suggested that they may also have an opposing anti-inflammatory effect mediated via the protein C receptor, as well as potential fibrinolytic properties that have been described *in vitro* [107,108]. Similarly, *in vitro* data has suggested that EMP uptake by resident endothelial cells can protect them from apoptosis [109]. This is amplified by *in vivo* data that has demonstrated that EMPs isolated from ischemic murine muscle enhance vasculogenesis [110]. Although both eNOS and VEGFR-2 were also found on the surface of the EMP, it is unclear whether they played any role whatever in the vasculogenic mechanism.

Given this contradictory data, it appears that EMPs may serve a number of purposes and have the capability to mediate multiple responses to endothelial damage. The plethora of surface proteins that are expressed by EMPs allows them to act as signaling molecules (Figure 1.6). They have also been observed to be capable of transferring mRNA to target cells [112]. This has led Hoyer et al. to envision a rich therapeutic future for these complex structures once their structure and function are better understood [113].

CLINICAL DATA AND POTENTIAL APPLICATIONS

To date, there are multiple reports that show that the number of CECs and EPCs isolated from peripheral blood can be related to cardiovascular risk factors and can be used to assist with prognosis [114,115]. Attempts to use EPCs in humans in a therapeutically relevant fashion, however, have had a much less propitious history [116,117]. The TOPCARE-AMI trial, which evaluated the effect of intracoronary infusion of early EPCs in patients presenting with acute myocardial infarction without a control group, demonstrated an improvement in ejection fraction at 5 years of follow-up, however this was obtained by an increase in end-diastolic volume, rather than by a decrease in end-systolic volume [118]. A review of all randomized controlled trials of intracoronary stem cell delivery has concluded that intracoronary therapy appears to be safe, but of no genuine clinical benefit [119]. Similarly, a study of

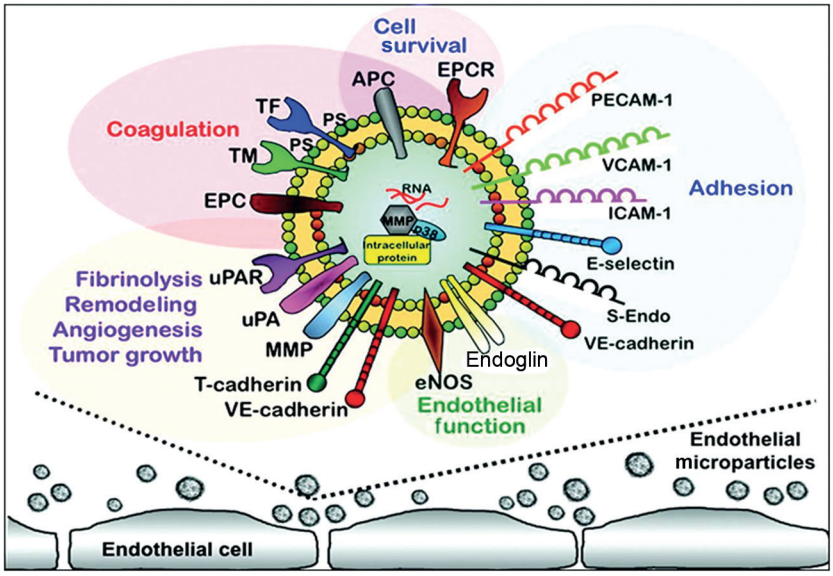


FIGURE 1.6 Surface molecules associated with microparticles and their respective effects. EPCR, endothelial protein C receptor; PECAM-1, platelet endothelial cell adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular cell adhesion molecule-1; S-Endo, CD146/melanoma cell adhesion molecule; VE-cadherin, vascular endothelial cadherin; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; EPC, endothelial protein C; APC, activated protein C; TM, thrombomodulin. *Source: From Ref. [111]. Used by permission.*

repetitive infusion of BM-derived mononuclear cells for the therapy of peripheral arterial disease has demonstrated no significant advantage in a randomized controlled trial [120].

A study of EPC mobilization in patients following acute myocardial infarction found that EPC number peaked some 30 days following the initial event, while CEC number peaked immediately following the event and subsequently fell toward baseline. Cultured cells from both controls and AMI patients demonstrated identical endothelial phenotypic characteristics as well as identical proliferation and vasculogenesis on *in vitro* culture [121]. Observations such as these have led some investigators to speculate whether it is the milieu in which these cells find themselves that inhibits their function under conditions of oxidant stress. This has been demonstrated *in vivo* in patients with type 2 diabetes mellitus in which EPC function was impaired compared to controls, and the addition of a PPAR agonist restored the capability of BM-derived cells to generate new endothelium [122].

The Role of eNOS and NO

Apoptosis of EPCs is induced by incubation with hydrogen peroxide, an effect that is reversed by induction of the PI3K/Akt pathway [123]. Mice deficient in eNOS demonstrate suppression of EPC mobilization as well as angiogenic capability, a process that was improved by cell transfer from wild-type mice [48]. Pretreatment of BM-derived monocytes with eNOS transcription inhibitors, or transplantation of autologous EPCs that overexpress eNOS enhances host neovascularization and vasculoprotection [124,125]. It has been demonstrated in a mouse model of myocardial infarction that at least part of the benefit derived from EPC transplantation is mediated via the PI3K/Akt pathway, a pathway that can be inhibited by conditions of oxidative stress [126]. The addition of nitroglycerin itself to cultured early EPCs from patients with CAD resulted in an increase in cell number and proliferation that peaked at a concentration of 7.5 mg/L [127]. Higher concentrations were associated with an increase in peroxynitrate expression associated with a concurrent reduction in cell number and proliferative capability. Hence, the capability of EPCs to positively affect the coronary vasculature is clearly dependent upon the balance between NO and ROS.

A number of mechanisms exist that can potentially be of use to enhance the effect of cell based therapies. Statin therapy is known to increase both the number of CD34+ cells in patients with stable CAD and the mobilization and incorporation of BM-derived cells at least partly via activation of the PI3K/Akt pathway [128–130]. Among the antioxidant enzymes that are selectively upregulated by shear stress are COX-2, manganese superoxide dismutase, eNOS, and glutathione reductase, any or all of which have the potential to tip the balance away from a hostile environment for the proliferation of endothelial cells [131,132]. Finally, the heme oxygenase (HO) system

has been extensively examined by our group and others as a means of countering oxidant stress. The induction of heme oxygenase-1 (HO-1) (the inducible form of HO) improves vascular recruitment of stem cells, promotes mobilization of circulating EPCs, and improves recovery from myocardial infarction through the enhancement of late EPC vasculogenesis in a mouse model [133–135]. Similarly, the attenuation of HO-1 levels and decreased HO activity corresponds with a reduction in the number and viability of EPCs [136].

Angiotensin converting enzyme (ACE) inhibition has been associated with an increase in mobilization of EPCs from the BM as well as an increase in the number of circulating EPCs in patients with stable angina [137,138]. Resveritrol, an inducer of HO-1, increased the numbers of circulating EPC *in vivo*, and both reduced EPC senescence and enhanced vasculogenesis *in vitro* [139,140]. Our group and others have investigated the somewhat unique effect of $P2Y_{12}$ blockade on patients subjected to inflammatory conditions. Following treatment with clopidogrel for 1 month, CEC numbers fell to normal, while the expression of both Akt and AMPK by EPCs was increased in patients with type 2 diabetes mellitus [141]. Similarly, the level of $P2Y_{12}$ blockade has been positively associated with a reduction in endothelial injury as measured by CECs in patients undergoing percutaneous coronary intervention, and clopidogrel has been associated with improved microvascular endothelial function in patients with stable CAD [142,143].

Finally, aside from the previously described regulatory capacity of PGI-2, multiple other metabolites of arachidonic acid have been observed to potentiate the effect of EPCs on the endothelium. Specifically, the leukotriene LTB4, the epoxyeicosatrienoic acids (EETs), and two of the hydroxyeicosatetraenoic acids (20-HETE and 12-HETrE) have been demonstrated to improve endothelial function, improve EPC adhesion, and promote an angiogenic phenotype *in vitro*, and to promote angiogenesis *in vivo* [144–147]. EETs have also been observed to activate eNOS with the secondary release of NO [148].

Two other possibilities for cell therapy have been investigated in preliminary studies. The observation that transplantation of a combination of EPCs and smooth muscle progenitor cells appears to enhance vasculogenesis suggests that the use of anchoring cells such as smooth muscle precursors or pericyte precursors, perhaps in concert with their chemoattractants, may be more efficacious at rebuilding the vasculature than the methods already tried [149,150]. Also, adult fibroblasts have been converted to what appear to be endothelial cells by means of viral vector transfection in a mouse model [151]. Although clearly in its infancy, this methodology holds promise for the future as the field progresses.

SUMMARY AND CONCLUSIONS

As with most biological systems, the endothelium undergoes a consistent process of generation, senescence, and regeneration. As such, there is no genuine beginning to the process, and no real end until the organism itself dies. Although the study of EPCs has intensified since they were first described, it has become increasingly clear that the generation and regeneration of the endothelium is a much more complex process than originally thought. BM-derived cells clearly play a role, however what kind of role continues to be controversial. It appears clear that so-called early EPCs affect the vasculature largely by their paracrine effects, and some of these early EPCs that have been isolated do not even go on to develop an endothelial phenotype. The precise mechanism by which the late outgrowth BM-derived EPCs exert their paracrine functions, and the degree to which they become incorporated into the resident endothelium remains to be clearly identified. Similarly, the precise steps by which resident EPCs can be induced to proliferate are also not known. Microparticles appear to have both an inflammatory phenotype as well as being capable of facilitating endothelial survival. As such, they constitute a phenotypically diverse population, a single population that behaves differently under different stimuli, or some combination of the above. Given the largely unsatisfactory results of the clinical trials to date, it appears that we need to know much more about these mechanisms if the promise of cell therapy is to be realized in the future.

Until more is known, the most promising therapeutic interventions appear to be those that employ pharmacologic manipulation in order to try to direct the processes that we do know about. Among these are manipulation of the PI3K/Akt pathway by means of statin therapy, further investigation of the influence of arachidonic acid metabolites including the eicosanoids and PGI-2, potential manipulation of the concentration of tetrahydrobiopterin in order to minimize the elaboration of peroxinitrate, pharmacologic stimulation of the HO pathway, and the use of already existent agents such as ACE inhibitors in order to facilitate endothelial regeneration. One of the most important things that has been learned about stem cell biology is the multiplicity of signaling pathways and their interactions, any one of which might be targeted therapeutically in order to enhance vascular health.

When Alice asks the Cheshire Cat to tell her which way that she ought to go from here, she says, “I don’t much care where [I go] so long as I get somewhere.” The Cat responds by saying, “Oh, you’re sure to do that if you only walk long enough.” We have come some distance since the end of the last century and discovered quite a few things that have genuine therapeutic relevance. We do, however, still have a rather long walk ahead of us.

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CHAPTER

2

The Role of Vascular Smooth Muscle Phenotype in Coronary Artery Disease

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INTRODUCTION

Unlike cardiac and skeletal muscle cells, which are terminally differentiated, vascular smooth muscle cells (VSMCs) remain plastic and can modulate their phenotype in response to environmental stimuli by regulating expression of SM-specific genes. The principle function of VSMCs in normal blood vessels of adult animals and humans is contraction and regulation of blood vessel diameter, blood pressure, and blood flow distribution. They are characterized by a quiescent and contractile phenotype [1]. However, in the setting of coronary artery disease (CAD), including atherosclerosis, neointima formation, restenosis after angioplasty, and in aberrant arteriogenesis, in response to signals from growth factors, cytokines, the extracellular matrix (ECM), cell–cell interactions, and mechanical forces, VSMCs can become proliferative, migratory, and synthetic. This altered VSMC phenotype is also characteristically found in conditions which are risk factors for CAD such as obesity, diabetes, hypertension, and metabolic syndrome [2].

The contractile VSMC phenotype is characterized by low proliferation and migration rates, expression of high levels of the co-transcription factor (TF) myocardin and SM-specific contractile proteins: SM myosin heavy chain (SM-MHC), SM-α-actin, calponin, caldesmin, SM22α (transgelin), and smoothelin. In contrast, the VSMC synthetic phenotype exhibits increased proliferation, migration, increased synthesis of ECM proteins, ECM-degrading proteases and growth factors, and decreased expression of the SM-specific contractile proteins [1,3]. Synthetic VSMCs also have impaired ability to respond to contractile stimuli, thus altering their ability to maintain vessel tone [4].

Regulation of VSM Phenotype

VSMC phenotype is determined by integration of signals from the local environment, including mechanical forces, neurohormonal influences, cell and cell–ECM interactions, and circulating growth factors and cytokines. These signals converge on the central regulation of VSMC phenotype which occurs through binding of serum response factor (SRF) to a CArG ($CC(A/T)_6GG$) box in the promoter region of genes encoding SM-specific proteins [5]. SRF expression is increased by factors that are known to stimulate the contractile phenotype, such as transforming growth factor β (TGF-β) [6]. By a positive feedback loop, SRF also increases its own expression [7]. However, SRF is found in many cell types and the presence of SRF alone cannot account for SM-specific gene transcription. Also, the promoters of most SM genes have multiple binding sites for SRF.

Myocardin, Elk-1, and KLF4

This specificity is accomplished through the association of SRF with myocardin [8]. Myocardin, a co-activator of SRF, is a master regulator of SM gene expression and can greatly increase SRF binding to the correct CArG box in the promoters of SM-specific genes [9]. Complexes of myocardin and SRF stimulate the transcription of SM-specific genes, while displacement of myocardin from SRF results in diminished transcription of these genes and a subsequent loss of the contractile phenotype. Thus, regulation of SM phenotype is centrally dependent on the interaction of SRF with other TFs, co-activators, and inhibitors.

Of particular significance in the context of CAD are the co-activators and inhibitors regulated by signaling

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00002-4

pathways of growth factors and cytokines which can direct VSMC away from the normal contractile and toward the pathological synthetic phenotype. Platelet-derived growth factor (PDGF), released in abundance from platelets which accumulate at sites of vascular injury, has been shown to promote multiple aspects of the synthetic phenotype including the reduction of markers of the contractile phenotype, the increase of features of the synthetic phenotype, and the increase of VSMC proliferation and migration [10,11]. Growth factors typically trigger the synthetic phenotype by activating Ras signaling that leads to extracellular signal recognition kinase (ERK)1/2-dependent phosphorylation of a TF, Elk-1. Elk-1 is a member of the E twenty-six (ETS) family of TFs, which, when phosphorylated, stimulates VSMC proliferation [12] and competes for a common SRF binding site to displace myocardin and myocardin-like TFs from SRF, thus preventing transcription of SM-specific genes [13,14].

The TF Kruppel-like factors (KLFs) 4 and 5 have also been shown to promote the synthetic phenotype when upregulated by PDGF signaling by both decreasing myocardin expression and blocking the interaction between SRF and myocardin [15]. Like phospho-Elk1, KLF 4/5 compete with myocardin for binding on SRF [16]. If myocardin is displaced from SRF, SRF will not bind to the CArG box, and the SM-specific contractile proteins will not be transcribed. The activity of KLF 4/5 is contingent on posttranslational modifications that appear to be differentially regulated by the initiating pathway [17]. KLF4 is the more physiologically important regulator of VSMC phenotype. It is not expressed in contractile VSMCs in normal blood vessels, but is rapidly induced in synthetic VSMCs following vascular injury [18]. In addition to displacing myocardin from SRF, KLF4-mediated transcriptional silencing of SM-specific genes is also facilitated by reduced histone 4 (H4) acetylation mediated by recruitment of histone diacetylases (HDACs) 2 and 5, which leads to complete loss of SRF binding to the CArG box [19,20].

Histone 3 and 4 (H3 and H4) acetylation and dimethylation of lysines 4 and 79 on H3 are also necessary for SRF–myocardin binding to the CArG box. In CAD, PDGF and oxidized phospholipids prevent H3 and H4 acetylation reducing transcription of SM-specific contractile proteins and increasing synthetic VSMC content [5].

Signal Transduction Pathways Involved in VSM Phenotype Regulation

Many signal transduction pathways participate in the regulation of VSMC phenotype in different physiological and pathological settings; however, several signaling pathways have been identified as key mediators of VSMC phenotype switching between the contractile and synthetic under a variety of circumstances.

Growth factor and cytokine receptors are the most proximal transducers of signals initiated by PDGF, FGF, EGF, TGF-β, TNF-α, IL-1, IL-6 and IL-8, growth factors, and pro-inflammatory cytokines, which play the most critical roles in driving the VSMC phenotype switch toward the synthetic and proliferative phenotype. Growth factor receptors are classical tyrosine kinase receptors, while cytokine receptors are tyrosine kinase associated receptors. In addition to growth factors and cytokines, classical vasoconstrictors, including angiotensin II (Ang II), endothelin (ET-1), and thromboxane A_2 (TXA_2), activate their respective G-protein-coupled receptors (GPCRs) and downstream signaling, leading to protein kinase C (PKC) activation.

Downstream from both the growth factor receptors and GPCRs, activation of two parallel and synergistic signaling pathways leads to transcription and translation of genes involved in VSMC phenotype switching, as well as their proliferation, migration, and VSMC-dependent ECM synthesis. These are the ERK1/2 mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3-kinase)-Akt (protein kinase B) pathways. While activation of the ERK1/2 MAPK pathway results in transcription of genes involved in cell cycle regulation and some aspects of phenotype switching to the synthetic phenotype (as discussed earlier), activation of the PI3-kianse-Akt pathway regulates protein synthesis, exit of VSMCs from the G_0 phase and re-entry into the cell cycle, increased VSMC survival, proliferation, and migration, and other aspects of phenotype switching. Combined activation of these two pathways accounts for all aspects of the proliferative, synthetic VSMC phenotype. However, another important mediator of increased ECM and protease, specifically matrix metalloproteinase (MMP), synthesis is the Smad2/3 pathway which is activated by TGF-β via its tyrosine kinase associated receptor. Thus TGF-β, although it promotes SM-specific contractile protein expression and thereby promotes the maintenance of the contractile phenotype, also promotes an aspect of the synthetic VSMC phenotype as it relates to increased ECM and MMP synthesis. Inflammatory cytokines generally amplify growth factor signaling and vice versa. Knockdown of IL-1 and an IL-1 receptor antagonist, for example, abolishes PDGF-induced VSMC DNA synthesis, MCP-1 synthesis, and proliferation. PDGF, FGF, and EGF, in turn, increase IL-1 production [21].

Protein kinase G (PKG) has emerged as one of the most important signaling intermediates which maintains the contractile VSMC phenotype. Its high expression in contractile VSMCs is maintained by high nitric oxide (NO) levels. Its expression quickly decreases in response to low NO, increased oxidative stress, treatment with growth factors, like PDGF and FGF, or pro-inflammatory cytokines, like IL-1 and TNF-α, in cultured VSMCs. PKG expression also decreases in response to vascular injury or

in atherosclerosis *in vivo*. Loss of PKG correlates with decreased expression of SM-specific contractile proteins and increased VSMC proliferation [22]. Overexpression of PKG reduces neointima formation [23]. Inflammatory cytokines downregulate PKG by activating protein kinase A (PKA) signaling in VSMCs [23].

MicroRNA Regulation of VSM Phenotype

MicroRNAs (miRs) are short (~22 nucleotide) noncoding RNA molecules that play important regulatory roles by targeting mRNAs either for cleavage or translational repression. miRs have emerged as components of a complex regulatory network that "fine-tunes" gene expression. Studies of the pathogenesis of cardiovascular diseases have demonstrated that deregulation of miRs contributes to disease processes, and they have thus become promising therapeutic targets [24]. Several miRs have been conclusively shown to regulate the phenotype of VSMCs *in vivo*: miR-145, miR-221, and miR-21.

miR-145

miR-145 is a member of the miR-143/145 cluster. miRs-143/145 are transcribed as a bicistronic unit with common regulatory elements. They are encoded within the genes that code for the SM-specific contractile proteins, are under the transcriptional control of myocardin, and are therefore highly enriched in SMCs, with negligible expression in other cell types. Knockout of miR-143 and miR-145 in a mouse model showed these miRs to be clustered in the SM gene compartment [25]. They have been shown to regulate the phenotype of VSMCs [16,26]. miR-143 targets include mRNAs encoding the PDGF receptor [27] and Elk-1 [16]. miR-145 targets include KLF4 [16] and KLF5 [28].

miR-145 has been shown to promote the contractile phenotype by repressing factors that promote proliferation as well as stabilizing factors that promote the contractile phenotype [16]. Also, miR-145 alone has been shown to be sufficient to stimulate the differentiation of multipotent neural crest cells into VSMCs [29]. Upregulation of miR-145 has been shown to be sufficient to induce expression of SM marker genes. Likewise miR-145 inhibition is sufficient to downregulate expression of these markers [28]. These studies strongly suggest that miR-145 alone can regulate VSMC phenotype, and further that miR-145 is the critical regulator of the VSMC contractile phenotype (Figure 2.1).

miR-145 is highly expressed in the vascular wall of normal, healthy blood vessels, but its expression in the vascular wall and VSMCs isolated from vessels of obese, diabetic, or metabolic syndrome animals or patients, from patients with established CAD, and in the neointima of restenotic arteries is low [30]. Studies have shown that miRs can be released into the bloodstream. A significant reduction in miR-145 was found in the serum of patients with CAD versus normal patients [31]. Thus, low miR-145 expression also correlates with synthetic VSMC phenotype in CAD and conditions associated with elevated risk for CAD in humans.

miR-21

miR-21 promotes VSMC proliferation and inhibits apoptosis by downregulating phosphatase and tensin homologue (PTEN), and thus upregulating the PI3-kinase and Akt signaling as well as promoting mitochondrial

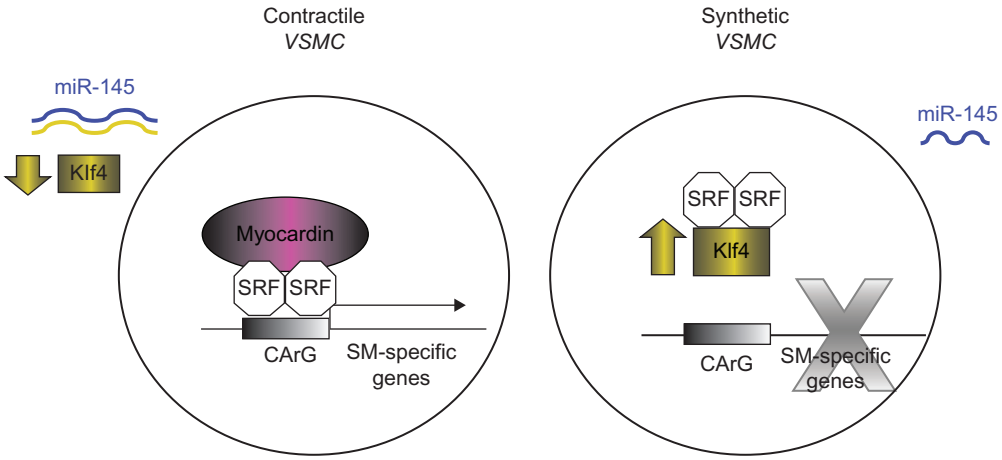

FIGURE 2.1 **miR-145 is a necessary and sufficient determinant of VSMC phenotype regulation.** Co-activator, myocardin, competes with the repressor, KLF4, for SRF binding. If the relative abundance of KLF4 is lower in the cell and myocardin binds SRF, SRF will bind to the CArG box and SM-specific genes will be transcribed; if the relative abundance of KLF4 is higher and KLF4 binds SRF, SRF will not bind to the CArG box and SM-specific genes will not be transcribed. miR-145 directly regulates the abundance of KLF4 by binding to its mRNA and targeting it for degradation, so that in VSMCs where miR-145 levels are high, KLF4 levels will be low and VSMC phenotype will be contractile, whereas if miR-145 levels are low, KLF4 levels will be high and VSMC phenotype will be synthetic.

anti-apoptotic signaling through Bcl-2 [30]. It is highly expressed in neointimal lesions [30].

miR-221

Inhibition of miR-221 prevents PDGF-induced VSMC proliferation and migration, while its overexpression increases basal VSMC proliferation and decreases expression of SM-specific contractile proteins [11,30]. Its effects on proliferation are mediated by repression of the cyclin-dependent kinase inhibitor p27Kip [11]. The precise mechanism by which miR-221 regulates VSMC contractile protein expression is unknown, but miR-221 overexpression is accompanied by dramatic reduction in myocardin expression [11]. Like miR-21, it is highly expressed in neointimal lesions after balloon angioplasty, where synthetic VSMCs are abundant [11,30].

Other miRs, specifically miR-1, miR-24, miR-26a, and miR-146a, have been implicated in the negative regulation of the contractile VSMC phenotype; however results supporting their involvement are confined to isolated studies and the mechanisms of their action have not yet been worked out [11].

ATHEROSCLEROSIS

Atherosclerosis is responsible for more than 40% of all deaths in the United States [5]. The initiating event in the development of atherosclerosis is endothelial dysfunction characterized by decreased production of NO, increased permeability to small lipoproteins (LDL and vLDL), growth factors and blood cells, and increased expression of endothelial adhesion molecules (VCAM-1, ICAM-1, P- and E-selectin). This facilitates adhesion of platelets, monocytes, and T-cells, coupled with monocyte migration into the intima and their consequent activation, proliferation, and differentiation into macrophages. All of this is facilitated by inflammatory interleukins MCP-1, IL-6, and IL-8. Activated macrophages then produce high amounts of reactive oxygen species (ROS), IL-1 and TNF-α, which increase further leukocyte adhesion. Reaction of ROS with LDL forms oxidized LDL (ox-LDL), which cannot be cleared by endothelial cells (ECs) and is ingested by macrophages to form foam cells. Foam cells, along with lipids and cellular debris, form the lipid core of the plaque over which forms the fibrous cap comprised of synthetic VSMCs and ECM, which walls the lesion off from the lumen. The lesion then grows at the shoulders by means of continuous leukocyte adhesion and entry and VSMC conversion to the synthetic phenotype, leading to migration from the media into the intima, and proliferation orchestrated by signaling cascades initiated by PDGF, ROS, and ILs [32].

The synthetic phenotype of VSMCs, causing their proliferation, migration, and apoptosis, plays a key role in all stages of atherosclerosis, from the transition of fatty streaks to atherosclerotic lesions, and from the formation of complex lesions with large lipid cores to plaque rupture. Up to 70% of the mass of an atherosclerotic lesion is estimated to be VSMC-derived [5]. VSMCs synthesize most of the ECM in complex lesions. The PDGF-mediated conversion of medial VSMCs to the synthetic phenotype and PDGF- and TNF-α-induced production of MMPs 2 and 9 to degrade the basement membrane facilitates further contact of VSMCx with atherogenic growth factors and cytokines (PDGF, fibroblast growth factor-2 (FGF-2), ET-1, IL-1, TGF-β, Ang II). They subsequently migrate into the intima, and their arrival on the scene marks the progression from macrophage- and foam cell–dominated simple and slowly developing fatty streaks to complex and rapidly growing atherosclerotic lesions [32]. Although initially macrophages produce the MMPs, growth factors, cytokines, and ROS responsible for VSMC migration, proliferation, and maintenance of the synthetic phenotype, the synthetic VSMCs eventually become the major source of all of these factors in addition to the ECM components of the plaque itself [32].

PDGF promotes VSMC phenotype switch from the contractile to the synthetic. PDGF and oxidized phospholipids together promote their migration and proliferation, which enhances fibrous cap formation. TGF-β is the main culprit behind VSMC-mediated ECM synthesis. IL-1 and TNF-α promote expression of ICAM-1 and secretion of IL-6 and MMPs from VSMCs which promotes continual recruitment of leukocytes and monocytes to the growing plaque. High levels of inorganic phosphate induce VSMCs' ability to promote calcification of the plaque [5]. Thus, VSMCs in atherosclerotic plaques become multifunctional cells with a plethora of phenotypes underlined by the basic loss of SM-specific contractile proteins and increased proliferative, migratory, and synthetic capacity.

The main signaling pathways involved in the progression of atherosclerosis are therefore the classical growth factor–mediated pathways which regulate VSMC conversion to the synthetic phenotype, promoting their proliferation and migration. Activation of the receptor tyrosine kinase PDGF and EGF-2 receptor, tyrosine kinase–associated cytokine receptors, and the Gq-protein-coupled Ang II and ET-1 receptors converges upon activation of the ERK1/2 MAP kinase and the PI3-kianse/Akt/PDK-1 pathways leading to VSMC conversion to the synthetic phenotype with resultant increased proliferation and migration [32]. Decreased NO production leads to decreased PKG activation which further promotes the synthetic VSMC phenotype [33], as described in more detail earlier in this chapter. KLF4 is increased in human atherosclerotic plaques, while myocardin is decreased providing another indication of

the synthetic VSMC phenotype on the mechanistic level [5,34]. It has also been shown that LDL cholesterol loading of VSMCs converts them to macrophage-like cells via downregulation of miR-143/145 and myocardin [35].

Transition to unstable plaque and plaque rupture is the most undesirable outcome of atherosclerosis leading to acute coronary events, i.e., myocardial infarction. The salient features of unstable, rupture-prone plaques are large necrotic, lipid cores with thin fibrous caps and a high apoptotic index. Apoptotic VSMCs are evident in advanced human plaques [32]. Two major apoptotic pathways operate in VSMCs: (i) the "death receptor" pathway, of which the prototypical member is the Fas receptor of the TNF death receptor family, the activation of which leads to Caspase-8 and downstream effector Caspases-3, 6, and 7 activation, and (ii) the mitochondrial apoptotic pathway regulated by Bcl-2-Bad–Bax interaction which determines cytochrome c release from the inner mitochondrial membrane and subsequent Caspase-9 activation, which leads to effector Caspase-3 activation [32]. Within the setting of atherosclerosis, TNF-α and IL-1 increase surface death receptor expression on VSMCs [32]. Also, while miR-21, a major pro-survival miR, is generally associated with the synthetic VSMC phenotype, miR-21 is actually downregulated in patients with advanced CAD and atherosclerosis [36], suggesting a decreased propensity for cell survival under these conditions.

RESTENOSIS

Approximately 1.4 million people in the United States undergo percutaneous coronary intervention (PCI) annually [37]. With bare metal stents, 20–30% of these patients experience post-PCI complications due to restenosis, with a fivefold higher incidence in patients with diabetes or metabolic syndrome [38].

PCI elicits a vascular injury response initiated by the removal of the endothelial layer and stretching of the VSM layer of the vessel wall. Restenosis is a consequence of the healing process, which begins immediately after the injury and occurs in two phases: (i) neointima formation, and (ii) constrictive remodeling of the vessel wall. Once the protective endothelial layer has been removed, the medial VSMCs and the underlying ECM are exposed to the circulating cells and growth factors. Thrombin is a circulating coagulation protein with serine protease activity which cleaves fibrinogen to insoluble strands of fibrin. This recruits platelets to the site of injury and activates them. Platelets are a rich source of various cytokines, chemokines, and growth factors, including PDGF, TGFβ, pro-inflammatory interleukins-1, 6, and 8, and TX A_2. These are potent "dedifferentiation" factors for VSMCs, which promote the VSMC phenotypic switch from the contractile to the synthetic phenotype. Once converted, the VSMCs themselves will secrete additional PDGF and ILs. Additionally, the bordering, activated ECs will also begin to secrete growth factors, such as PDGF and FGF-2 [39–42].

The synthetic, highly proliferative, and migratory VSMCs will begin to mend the wounded area by regenerating cells to replace the damaged ECs. High VSMC proliferation rates are evident for up to 2 weeks postinjury in rodent animal models [43,44]. The VSMCs produce and secrete extracellular proteases to actively degrade the surrounding ECM, thus allowing the cells to cross the internal elastic lamina and migrate into the lumen of the vessel, creating the neointima. VSMCs are the most prominent cell type forming the neointima, but there is still debate as to whether they originate primarily from the underlying medial VSMCs, or if myofibroblasts and circulating SM progenitor cells contribute to this population [41,45,46]. Current dogma suggests that the majority of cells are derived from preexisting VSMCs that have undergone the phenotypic switch, but recent evidence suggests that vascular progenitor cells from circulating blood when exposed to PDGF-BB can differentiate into VSMCs characterized by SM-specific marker expression (SM-MHC and calponin) [47]. These progenitor cells are recruited to the site of injury and thought to display a phenotype similar to synthetic VSMCs. Whether this population is capable of fully differentiating into a functional VSMC is still poorly understood given the difficulty of identifying and tracking such cells *in vivo* and in response to injury. Nevertheless, vascular progenitor cells are mobilized following injury and potentially contribute to the pathology associated with neointima formation [45–47].

The neointima continues to expand due to excessive matrix synthesis and secretion by the synthetic VSMCs, marking the beginning of the second stage of neointima formation: constrictive inward remodeling [48]. Once the endothelial layer has been reestablished, the proliferation and migration of VSMCs stops due to lack of VSMC access to PDGF and other growth factors and cytokines. At this time, the VSMCs begin to re-express SM-specific contractile proteins and assume characteristics of the contractile phenotype, although the cells may not fully respond and function as they did prior to the injury [49]. By 4 weeks postinjury, in part due to excessive ECM deposition and degradation of the elastic components of the vessel wall by proteases synthesized by the VSMCs, and in part due to re-expression of VSMC contractile proteins over the much expanded surface area, the elastic properties of the vessel wall are altered. This contributes to further constriction of the lumen diameter in the second phase of restenosis.

The signaling pathways involved in the development of neointima are primarily the PDGF-induced signaling

pathways which induce VSMC migration and proliferation, previously described in detail earlier in this chapter. They include the ERK1/2 MAPK kinase pathway, which is instrumental in VSMC proliferation, and the PI3-kinase/Akt pathway, which leads to VSMC phenotype switch and migration [38].

Overexpression of miR-145 in rats results in a reduction of neointimal formation resulting from acute vascular injury [16,30]. Since the neointima is highly enriched for synthetic VSMCs and characterized by lack of contractile VSMCs, these findings provide the first evidence that miR-145 can regulate VSMC phenotype *in vivo*. The ability to form neointima following vascular insult is also abrogated in miR-145$^{-/-}$ knockout animals [50]. Thus, the miR-145-KLF4-myocardin-SRF pathway is the major regulator of VSMC phenotype in restenosis *in vivo*. miR-21 and miR-221 are also highly expressed in neointimal lesions and their downregulation prevents restenosis after balloon angioplasty [11,30].

Current Challenges

With the use of drug-eluting stents (DESs), restenosis has been significantly reduced. The mechanical structure of the stent itself prevents constrictive remodeling and the drug coating of mammalian target of rapamycin (mTOR) inhibitors (sirolimus or paclitaxel in first-generation DESs and zotoralimus or everolimus in second-generation DESs) effectively prevents VSMC proliferation, migration, and neointima formation. By 2005, 90% of all PCIs were performed using DESs. However, in-stent thrombosis became a lethal complication occurring in 6.3% of patients (with a fivefold higher incidence in patients with diabetes or metabolic syndrome) despite anti-platelet therapy [38,51]. This is due to the fact that re-endothelialization is a necessary component of successful vascular repair, and mTOR is a signaling intermediate immediately downstream of Akt, the activation of which is required for both VSMC and EC proliferation and migration. Therefore, while DESs effectively inhibit VSMC proliferation and migration, they also inhibit re-endothelialization and promote on-going vascular injury at the stent site [51]. Currently, there are no solutions to this quandary which have been approved for clinical use. However, a signaling pathway involving protein kinase A-dependent activation of the regulatory subunit of PI3-kinase, p85α, has been identified as a possible target because of differential downstream effects in VSMCs versus ECs. Its inhibition represses VSMC but not EC migration and proliferation and, in a rodent model of balloon angioplasty, decreased neointima formation while allowing for re-endothelialization [52].

ARTERIOGENESIS

Arteriogenesis, also known as collateral growth, is an adaptive response to transient, repetitive coronary artery occlusion such as occurs in stable *angina pectoris* [53] and has been associated with lower incidence and severity of myocardial infarction [54,55]. It has been shown that collateral development is impaired in patients suffering from type II diabetes and the metabolic syndrome [53,56,57].

In contrast to angiogenesis, defined as *de novo* vessel (capillary tube) formation, arteriogenesis is defined by enlargement of small arterioles, with very low or no blood flow, to larger conducting arteries. This process begins with endothelial activation, increased expression of VCAM-1 and ICAM-1 and increased endothelial permeability, all of which allow for adhesion of inflammatory cells, mostly monocytes. Next VSMCs switch to the synthetic and proliferative phenotype and migrate across the internal elastic lamina and the basement membrane into the lumen of the preexisting vessel in an inward remodeling process akin to neointima formation following vascular injury. This is followed by reestablishment of the internal elastic lamina and the basement membrane as well as the endothelium, and outward remodeling in which cells migrate across the external elastic lamina into the adventitia and the surrounding myocardium, thus allowing for vessel expansion and significant increases in blood flow. The final phase of remodeling is characterized by the VSMC return to the contractile, nonproliferative phenotype, cessation of ECM remodeling, and pruning of smaller vessels that had originally taken part in the remodeling but eventually are phased out secondary to competitive flow [58,59]. Thus, arteriogeneis is a complex, multi-phase, and temporally carefully regulated process, which requires EC and VSMC phenotype switching, proliferation, and migration, as well as ECM remodeling.

The precise relationship between VSMC phenotype and coronary arteriogenesis has been established only recently. It has been demonstrated that a 10-fold increase in SM-specific contractile proteins, SM-MHC and SM-α-actin, correlates with correlates with excessive VSMC proliferation and successful arteriogenesis in response to coronary artery occlusion in normal, healthy animals, whereas no such increase was observed in metabolic syndrome animals in which coronary arteriogenesis is impaired [60]. Myocardin and miR-145 expression correlates directly, while KLF4 and miR-21 expression correlates inversely with SM-contractile protein expression and collateral artery formation [60]. Moreover, miR-145 delivery to metabolic syndrome animals decreases KLF4 expression, and restores the contractile VSMC phenotype and collateral growth. Inhibition of miR-21 achieves

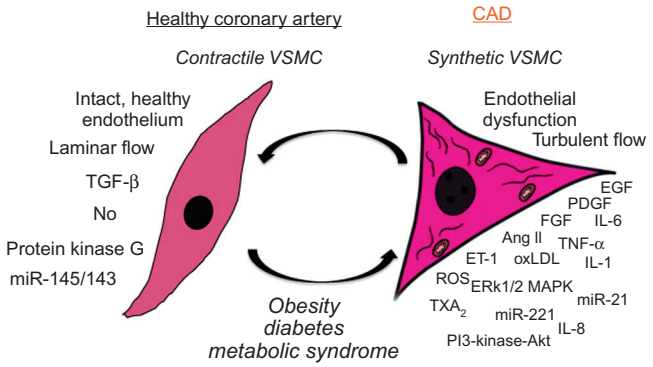

FIGURE 2.2 Positive and negative regulators of the contractile versus synthetic VSMC phenotype. Factors which promote the contractile VSMC phenotype: intact, healthy endothelium, laminar flow, NO, TGF-β, PKG, miR-145/143. Factors which promote the synthetic VSMC phenotype: endothelial dysfunction, turbulent flow, growth factors (PDGF, FGF, EGF), inflammatory cytokines (IL-1, IL-6, IL-8, TNF-α), Ang II, ET-1, TXA_2, ROS, ox-LDL, ERK1/2 MAPK, PI3-kinase-Akt, miR-21, miR-221. Factors which promote the synthetic VSMC phenotype are elevated in CAD in risk factors associated with CAD.

similar, although more modest results [60]. Conversely, downregulation of miR-145 in normal animals decreases arteriogenesis via conversion of coronary VSMCs to the synthetic phenotype [60] (Figure 2.2).

CONCLUSIONS

It is clear that regulation of VSMC phenotype plays a critical role in CAD. Even more telling are studies showing that the effectiveness of several drugs, which are standard therapy for both primary and secondary prevention of CAD, in part depends on their ability to convert the aberrant, synthetic VSMC phenotype to the normal, contractile VSMC phenotype. Insulin has long been known to be required for maintenance of the contractile VSMC phenotype [61]. Synthetic VSMCs express angiotensin converting enzyme and produce Ang II [62], and recent data have shown that ACE inhibitors and angiotensin type I receptor blockers allow for miR-143/145 reexpression and conversion of synthetic VSMCs to contractile VSMCs [63]. Several statins have been shown to affect key signaling pathways involved in VSMC phenotype modulation and to attenuate neointima formation following angioplasty in animal and human studies [64,65].

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CHAPTER

3

Immuno-Inflammatory Basis of Atherosclerotic Coronary Artery Disease

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Atherosclerotic coronary artery disease (CAD) is a major cause of morbidity and mortality worldwide. Recent advances in cellular and molecular mechanisms of atherosclerosis have led to major changes in the therapy of CAD. These advances include knowledge of endothelial dysfunction, macrophage/foam cell formation, and lipid deposition as key events in the formation of coronary atherosclerotic plaque. Further, knowledge on the mechanism of acute coronary events, that is, rupture or erosion of the atherosclerotic plaque followed by clot formation resulting in cessation of blood flow, has led to the development of potent anti-platelet therapies for use in acute coronary syndromes (ACSs).

There is much interest in inflammation in the arterial wall accompanying atherosclerosis. There is also much interest in the role of immune system that can protect the arteries from developing atherosclerosis and also induce injury be activating inflammatory cascade. Inflammation is a well-orchestrated natural protective mechanism in the body that occurs in response to injurious stimuli, but when excessive or uncontrolled can also induce or enhance injury. In this chapter, we review the role of inflammation and immunity in CAD, the current status of inflammatory biomarkers, and potential novel therapies in CAD.

INFLAMMATION AND CAD—MOLECULAR MECHANISMS

The pathogenesis of atherosclerotic CAD has transitioned from being a physical narrowing of the coronary vasculature to a dynamic process with multiple regulators. Inflammation has been recognized to be the cornerstone in the initiation, progression, and rupture of an atherosclerotic plaque.

Figure 3.1 shows various sources of inflammatory mediators that play a variable role in different stages of atherogenesis. The inflammatory signals shown in this figure have formed the basis of their quantitation as index of atherosclerosis and related events. This figure also shows the progression of atherosclerosis from its very beginning to culmination of acute events.

Inflammation in Plaque Initiation

The earliest atherosclerotic lesion is a fatty streak that develops at a young age. Endothelial injury/activation induced by various traditional cardiovascular risk factors, such as hypertension, smoking, and hypercholesterolemia, leads to expression of endothelial adhesion molecules (ICAM-1, VCAM-1) that causes leukocyte adhesion. LDL cholesterol following oxidative modification (ox-LDL) is taken up by lectin-like oxidized lipoprotein-1 (LOX-1) on endothelial cells; this perhaps the most important inciting event for vascular inflammation. LOX-1 is activated by ox-LDL, angiotensin II, advanced glycation end-products, and shear stress. LOX-1 activation initiates a cascade of events that creates a state of oxidative stress, smooth muscle cells migration and proliferation, and fibroblast growth and proliferation. These events perpetuate the atherosclerotic process (Figure 3.2). Adhesion of leukocytes leads to

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00003-6

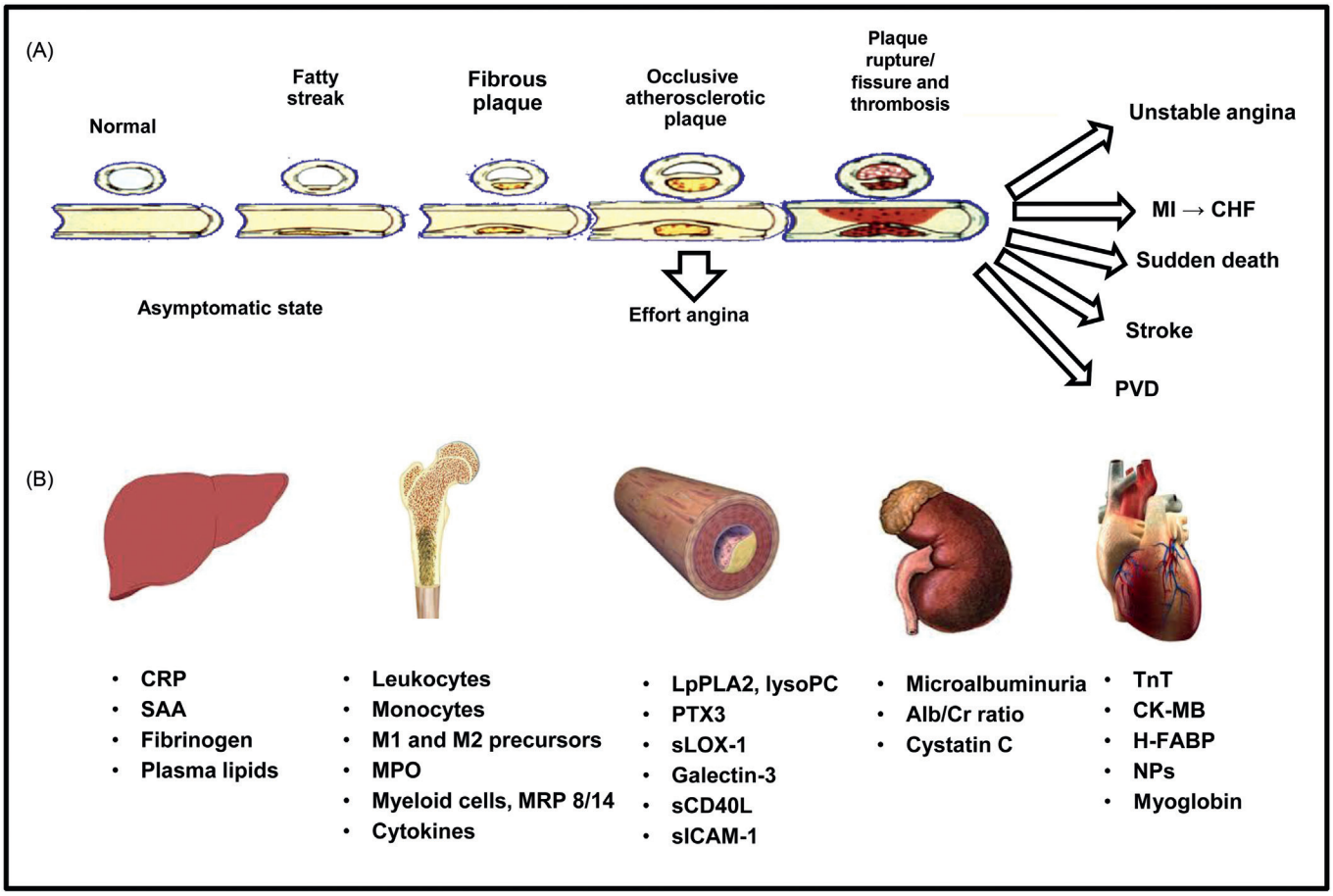

FIGURE 3.1 Panel (A) shows different steps in atherogenesis from its initiation to development of acute events when plaque rupture or erosin induces an occlusive clot formation resulting cessation of blood flow. Panel (B) shows inflammatory markers and their sources.

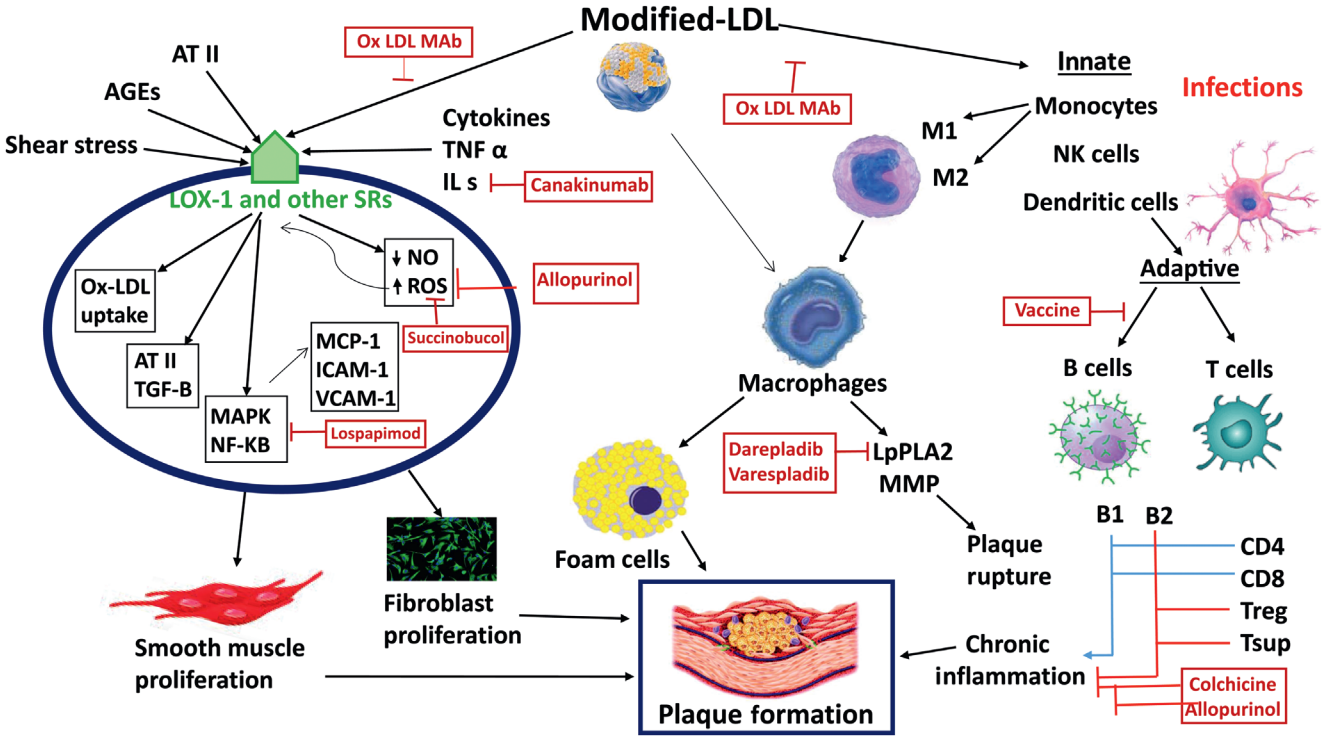

FIGURE 3.2 Role of inflammation and immunity in plaque formation. LOX-1 and other scavenger receptors have an important role in plaque formation by activating a number of critical steps which lead to foam cell formation and smooth muscle cell proliferation and migration. Both innate and adaptive immunity participate in atherogenesis via activation of immune cells. Therapies directed at different steps shown in red boxes are in trial phase.

activation of a series of inflammatory cascades. Monocytes undergo transendothelial migration in response to increased concentrations of monocyte chemoattractant protein-1 and transform into macrophages. Uptake of ox-LDL by macrophages via scavenger receptors such as CD36, MSR1, and LOX-1 transforms them into foam cells, initiating the process of atherosclerosis. Gene deletion of VCAM-1, MCP-1, and other leukocyte adhesion molecules has been shown to attenuate atherosclerosis in mice models of atherosclerosis, despite similar cholesterol and lipoprotein profiles, demonstrating the vital role of inflammation in the initiation of an atheroma [1,2].

Inflammation in Plaque Progression

A fibroproliferative response mediated by various inflammatory mediators aids in the transformation of a fatty streak into a fibrous atherosclerotic plaque. T-lymphocytes that are recruited into the plaque site release pro-inflammatory cytokines like interferon-γ (IFN-γ) and tumor necrosis factor alpha (TNFα) that perpetuate the inflammatory response. Fibrogenic stimuli like the transforming growth factor-beta (TGF-β1) lead to proliferation of fibroblasts and secretion of collagens that form the fibrous cap of the atherosclerotic plaque [3].

As mentioned above, LOX-1, one of the scavenger receptors for ox-LDL, plays a crucial role in signaling pathways involved in the process of oxidative stress and vascular inflammation [4,5]. LOX-1 itself acts as a potent pro-oxidant and pro-inflammatory molecule. Sawamura's group showed that LOX-1 antibody attenuated the inflammatory response to lipopolysaccharide in rats [6]. Mehta et al. [7,8] demonstrated that deletion of LOX-1 gene reduced atherosclerosis in LDLr null mice fed an atherogenic diet, confirming the role of LOX-1 in atherogenesis. LOX-1 is also upregulated in the heart following ischemia and reperfusion injury and is associated with markers of inflammation and oxidative stress. Prior administration of LOX-1 antibody or LOX-1 gene deletion reduced inflammation, oxidative stress and infarct size in experimental myocardial ischemia [9]. LOX-1 is also implicated in myocardial collagen deposition following myocardial ischemia leading to cardiac remodeling and scar formation [10]. Human studies have demonstrated that LOX-1 is released into circulation during acute myocardial ischemia [11]. Circulating LOX-1 particles in the blood (sLOX-1) may arise from activated platelets, endothelial cells, or other components of atheroma. In fact, measurement of sLOX-1 levels has been proposed as a marker of myocardial ischemia as discussed in subsequent sections.

Inflammation in Plaque Rupture

Most acute coronary ischemic events arise because of an anatomic disruption (rupture) or surface erosion of the atherosclerotic plaque. Cytokines and matrix metalloproteinases released by activated macrophages lead to disruption of collagen synthesis that makes the fibrous capsule weak and prone to rupture. Local inflammatory cells also produce tissue factor which activates the extrinsic coagulation pathway that leads to acute thrombosis [12]. In addition, plaque rupture and erosion lead to local platelet activation and accumulation resulting in cessation of blood flow and onset of ACS [13]. Incorporation of leukocytes into the thrombus enlarges thrombus formation [14].

CLINICAL ASSOCIATIONS OF INFLAMMATION AND CAD

Inflammatory Diseases and CAD

The relationship between chronic inflammatory conditions such as rheumatoid arthritis and systemic lupus erythematous and CAD has been appreciated. Degree of systemic inflammation as measured by disease severity in patients with rheumatoid arthritis generally correlates with cardiovascular risk [15]. Large epidemiological studies of rheumatoid arthritis patients in Europe and the United States showed a significantly increased risk of incident myocardial infarction [16]. Similar associations with other chronic inflammatory conditions like psoriasis and atherosclerosis have been reported, supporting the concept of a role for inflammation in CAD [17]. Of note, expression of numerous pro-inflammatory cytokines is common to the pathogenesis of both atherosclerosis and other chronic inflammatory diseases.

Infections and CAD

Chronic infections are associated with an increased risk of CAD. Various pathogens such as *Chlamydia pneumoniae*, *Porphyromonas gingivalis*, *Helicobacter pylori*, *Influenza A*, *Hepatitis C virus*, *Cytomegalovirus*, and *Human immunodeficiency virus* have been shown in epidemiological studies to increase the risk of incident CAD [18–20]. Chronic inflammation induced by infections seems to be the main pathogenic link in this association. Infections lead to an increase in circulating inflammatory cytokines and acute phase reactants that can accelerate atherosclerosis.

Another potential mechanism of infections being pro-atherosclerotic is molecular mimicry. Some bacterial pathogens exhibit cross-reactivity to antigens, such as ox-LDL and heat shock proteins which are potent pro-atherogenic stimuli.

Despite extensive epidemiological association, multiple trials evaluating anti-infective therapies have yielded disappointing results. For example, if eradication of

Chlamydia with azithromycin therapy would reduce CAD events has been studied in large clinical trials [21–23]. These trials showed no benefit of antibiotics in secondary prevention of CAD. Whether chronic infections cause or are mere associations with CAD is still a matter of debate. However, this association underlines the importance of systemic inflammation in CAD.

IMAGING THE INFLAMMATION IN CAD

The ability to visualize, interpret, and quantify the degree of systemic inflammation in patients with CAD is of great interest, as it allows translating preclinical data into meaningful clinical outcomes. Ultra-small superparamagnetic iron oxide (USPIO) nanoparticles have been shown to localize in atherosclerotic plaques and in macrophages in infarct areas in mice [24]. Alam et al. demonstrated increased uptake of USPIO in the infarcted and remote areas of the heart and systemic reticuloendothelial system as detected by cardiac magnetic resonance (CMR) imaging in patients following an acute myocardial infarction [25]. Positron emission tomography (PET) is another imaging modality of interest in detecting inflammation. 18F-fluorodeoxyglucose (FDG) used for PET accumulates in metabolically active tissue. Vascular PET imaging has been shown to correlate with atherosclerotic plaque burden in patients with carotid disease [26]. However, the use of PET imaging of the myocardium is hindered by the fact that even non-infarcted remote myocardium relies on glycolysis and would have a high FDG intake. Superimposing PET data with CMR could potentially overcome this limitation and increase clinical utility. Other imaging modalities such as fluorine-19 perfluorocarbon CMR [27], calcium imaging [28], and leucocyte receptor imaging [29] are currently being investigated. These approaches could help objectively measure the degree of inflammatory response, identify subsets of inflammatory cells that drive injury and repair, and can potentially open up avenues for new targeted therapies in the treatment of CAD.

IMMUNITY AND CAD

The immune system has been designed as the primary protective response of the body against internal and external toxic agents. Most immune responses are protective via induction of inflammation. Thus, it is not surprising to envision the role immune system and mediators play not just in the pathogenesis but also in protection against atherosclerotic CAD. The immune system is broadly composed of innate and adaptive immune mechanisms. Here, we review the current evidence supporting the role of various aspects of the immune system in CAD.

Figure 3.2 provides an overview of the role of immune activation in atherogenesis.

Innate Immunity and CAD

Innate immune system forms the first line of defense of the body. The innate response is nonspecific, immediate, and is mediated through monocytes that transform into macrophages, dendritic cells, natural killer (NK) cells, and mast cells. Toll-like receptors on macrophages recognize danger-associated molecular patterns (DAMPs) on various endogenous antigens. Cholesterol, ox-LDL, and heat shock proteins are the major DAMPs recognized by the innate immune system in the pathogenesis of atherosclerosis.

Genetic modification of the innate immune system has been shown to reduce plaque burden and systemic inflammatory markers in animal models of atherosclerosis. For instance, deletion of macrophage colony-stimulating factor leads to decreased atherosclerosis in mice with severe hypercholesterolemia [30]. Similar studies with selective depletion of NK cells and mast cells appear to reduce atherosclerotic plaque burden in animal models [31,32]. Though considered as part of the innate system, dendritic cells actually serve as the connecting link between the innate and adaptive immune responses in atherosclerosis. The unique ability of dendritic cells to function as antigen-presenting cells allows them to ingest pro-atherosclerotic stimuli such as ox-LDL. In addition, plasmacytoid dendritic cells, a subset of dendritic cells, found in human atherosclerotic plaque have also been shown to elicit a specific T-cell-mediated response, implicating their role in adaptive immunity as well [33]. Though activation of the innate immune system appears to be pro-atherogenic, recent research led to identification of subsets of innate immune system that have atheroprotective properties. Studies in murine models of atherosclerosis led to the identification of two subsets of monocytes, termed M1 and M2 monocytes based on the expression of LY6C. Monocytes with high expression of LY6C (M1 subset) transform into M1 macrophages as a part of the immune-inflammatory response to atherogenic stimuli, such as ox-LDL, that eventually leads to plaque formation. On the other hand, M2 monocytes (low LY6C expression) induce a protective response to endothelial injury and enhance tissue repair [34]. Factors influencing these differentiation pathways are still under investigation and could potentially turn into therapeutic targets.

Adaptive Immunity and CAD

The adaptive immune response is a complex, robust, delayed, and specific response of the immune system mediated by B and T lymphocytes. T cells recognize antigens presented by the antigen-presenting cells

(macrophages and dendritic cells) via the major histocompatibility complex and differentiate into CD4+ and CD8+ T cells. CD8+ T-cell activation leads to mediators that cause a direct cytotoxic effect, whereas proliferation of CD4+ T cells leads to downstream activation of cytokines and B lymphocytes and result in the generation of specific antibodies, which have effector and memory functions. Research in animal models led to the identification of subsets in the adaptive immune response, some with atherogenic and others with atheroprotective properties.

B lymphocytes have a dual effect on atherosclerosis. Previous studies showed that surgical B-cell depletion by splenectomy led to rapid progression of atherosclerosis in mice, and B-cell transfer reduced plaque burden, suggesting B cells to be atheroprotective [35]. However, pharmacologically (anti CD20 antibody) mediated B-cell depletion in fact led to a reduction of atherosclerosis in hypercholesterolemic mice, initiating the concept of differential B-cell activity and recognition of two B-cell subsets, B1 and B2 lymphocytes [36]. Further research has identified B1 lymphocytes (isolated from pleural and peritoneal cavities) to have atheroprotective properties and B2 lymphocytes (derived from bone marrow and follicles) to be atherogenic [37]. A third subset of regulatory B cells has also been identified, the function of which is not clearly known.

T lymphocytes constitute a major component of the adaptive immune response to atherosclerosis. As discussed above, T cells differentiate into CD4+ and CD8+ cells in response to an antigen. CD4+ T cells were initially thought to be pro-atherogenic based on initial experiments which demonstrated that adoptive transfer of CD4+ T cells accentuates atherosclerosis in Apo-E-deficient mice [38]. A subset of CD4+ T cells that selectively express the transcription marker FoxP3, called regulatory CD4+ T cells, were later identified. Selective depletion of these cells led to accelerated atherosclerosis in mice, implicating a potential protective role against atherogenesis [39].

To attribute the differential effect of CD4+ T cells on atherogenesis to variations in cytokine production would be oversimplication of a very complex process. Similar to CD4+ T cells, CD8+ T cells also get activated in response to atherogenic antigens such as modified LDL. However, subsets of CD8+ T cells that are CD25 positive function as suppressor T cells and exhibit atheroprotective properties [40]. The effect of subsets of T cells in mediating atherosclerotic cardiovascular disease has been demonstrated in humans as well. In a long-term prospective cohort study, low levels of regulatory CD4 T cells (CD4+/FoxP3+) and high levels of CD8+ T cells were associated with a significantly higher incidence of CAD events over a 14-year follow-up [41,42].

Thus, the adaptive immune response to atherosclerosis is robust, complex, selective, and intriguing. Identification of further subsets of immune cells and new immune mediators has enhanced our understanding of atherogenesis and at the same time raised further questions.

INFLAMMATORY BIOMARKERS AND CAD

A host of inflammatory biomarkers of CAD have been identified and more are being described every day. However, the predictive power of these biomarkers has been of limited value. It is of note that traditional CAD risk factors, such as lipids and lipoproteins, predict accurately the presence of CAD only to a small extent which, again, suggests a marked degree of variation among patients.

Here, we summarize recent developments in this area and attempt to relate these biomarkers to underlying pathophysiologic mechanisms. Organ and biologic systems from which they originate suggest that the biomarkers that reflect integrated pathobiologic alterations are likely to be more informative.

As shown in Figure 3.1, these markers originate from different organ systems and are released to a variable extent in different stages of the disease process.

Interest in CAD biomarkers is driven by the need for early indicators of drug efficacy in the clinic and the emerging need for targeted drug treatment of different patient populations. In general, two distinct types of biomarkers are sought during drug development, (i) the so-called target engagement biomarkers, designed to inform as to whether a therapy alters (engages) the molecular target under investigation, and (ii) the efficacy biomarkers, designed to inform as to whether target engagement results in changes relevant to disease phenotypes. Interest in CAD predictive biomarkers is further fueled by the recent biomarker qualification process, encouraged by regulatory authorities, to enable early clinical investigations of promising therapeutics.

The concept of disease biomarkers of CAD is not novel and indeed white blood cell counts, serum cholesterol and LDL-cholesterol levels have been used for decades. Initial focus on non-specific inflammatory biomarkers related to acute phase reactants such as fibrinogen, erythrocyte sedimentation rate, and C-reactive protein (CRP) showed that these biomarkers can predict future CAD. However, these relationships appear weak because of the lack of disease specificity and their variability in the acute versus chronic disease. Additionally, such biomarkers predict CAD to a small extent leaving more than 50% of CAD residual risk undetected [43]. Whether more recently described biomarkers, such as metric of the ability of patient derived high-density lipoproteins to efflux cholesterol from cells *ex-vivo*, will

improve prediction of CAD remains to be further evaluated [44].

Biomarkers that are specific to the immuno-inflammatory and oxidative pathways of CAD are also being investigated. It was recently shown that the ratio of oxidized phospholipids to the major protein constituent of LDL, apolipoprotein B (oxPL/apoB), and various enzymes involved in lipoprotein oxidation (LpPLA2, sPLA2, MPO) are strongly associated with future CAD events [45]. Similarly, circulating levels of a soluble portion of the ox-LDL scavenger receptor LOX-1 (sLOX-1) have been found to predict CAD events [46].

A recent PubMed bibliographic analysis of emerging CAD biomarkers identified at least 53 biomarkers relevant to inflammation. These biomarkers can be classified in different families according to their distinct pathophysiologic underpinnings (Table 3.1). However, the overall conclusion was that *individual* inflammatory biomarkers have low predictive power [47]. Since inflammatory genes act in an interactive manner with inflammatory signaling cascades ultimately orchestrating disease associated risk, identification of key inflammatory nodes is not always obvious. A recent network analysis of all inflammatory genes implicated in various inflammatory diseases identified a network with five key hub genes, IL6, VEGF-A, IL-1β, TNFα, and PTGS2, with IL-6 as the central molecule constituting a super-hub of the entire network [48]. Although such network analyses might present an incomplete picture, it does provide a framework to systematize and prioritize the large array of inflammatory biomarkers relevant to CAD. Recent clinical and genetic Mendelian randomization data also point to IL-6 as key "upstream" biomarker for inflammation in CAD [49]. An additional group of evolving biomarkers, the circulating miRNAs, should also be mentioned. The role of miRNAs in regulating multiple target mRNAs could also reflect integrated responses across multiple organ systems and tissues. In fact, circulating levels of several miRNAs have been associated with CAD [50].

The fact that CAD is a complex immuno-inflammatory disease involving multiple organ systems limits the predictive power of individual biomarkers. Dynamic biomarker changes through the clinical spectrum of CAD, confounding influences of medications, lack of standardization of measurement protocols, and low concordance among different studies suggest that more work is needed in this area. Alternative biomarkers that integrate these individual responses into one of a few consistent readouts maybe more informative than individual biomarkers or even collections of such biomarkers into, what is referred to, as biomarker signatures. An ideal biomarker could be a combination of selected markers that could include the status of diverse organ systems such as liver (acute phase response), kidney (tubular reabsorption), bone marrow (HSPC production), and, of course, the vasculature itself and relevant biological systems (Figure 3.1).

TABLE 3.1 Inflammatory Biomarkers in CAD: Origin and Clinical Relevance

CVD biomarker	Clinical relevance
Acute-phase response proteins C-reactive protein (CRP) Pentraxin 3 (PTX3) Homocystein Amyloid A Fibrinogen	Although their predictive power varies, these biomarkers are used to predict atherogenesis, atherosclerosis, unstable angina, acute MI, ACS, heart failure, and myocardial injury. These biomarkers can be used for cardiovascular risk assessment, screening for primary prevention, diagnosis and prognosis of CAD, and MI recurrence.
Blood cells Erythrocyte sedimentation rate (ESR) Monocytes CD40 ligand Leukocytes Neutrophils	These biomarkers are useful for CAD diagnosis, and predict to different degrees STEMI, LVEF, infarct size, myocardial injury, microvascular obstruction, poor functional outcomes, and MI recurrence.
Plaque instability Myeloperoxidase (MPO) Myeloid-related protein 8/14 (MRP-8/14) Pregnancy associated plasma protein A (PAPP-A) sLox-1	These biomarkers can be useful for predicting in-hospital mortality, MI recurrence, death, and for diagnosis of STEMI and NSTEMI.
Lipid metabolism Lp-PLA2 lysoPC Galectin 3	These biomarkers predict atherosclerosis, ACS, ischemic stroke, HF, and CAD severity. These biomarkers can be used for CAD diagnosis and prognosis.
Cytokines IL-6 IL-10 IL-13 IL-17 IL-18 IL-27 IL-33 sST2 (soluble IL-33 receptor) IL-1 receptor antagonist (IL-1Ra) TGF-β	In general, these biomarkers provide a read-out as to the overall inflammation load and, therefore, inform as to the propensity and speed for CAD progression and cardiac damage. For example, ST2 was shown to be independently associated with long-term MACE and IL-1Ra levels in the emergency department correlated directly with CK and CK-MB levels and inversely with LVEF.

ANTI-INFLAMMATORY THERAPIES IN CAD

Although the presence of inflammation in the initiation and progression of CAD has been established beyond doubt, translation of this into clinical practice

has been of great debate. A global so-called pleiotropic anti-inflammatory effect of aspirin and statins may partly be responsible for their beneficial effect in patients with CAD. However, there is growing interest in specific inflammatory pathways as therapeutic targets. Here we review some of the recent trials with agents that specifically targeted these signals. Figure 3.2 shows the precise steps which were modulated by these agents.

Two large-scale randomized trials, STABILITY and SOLID-TIMI 52, studied the effect of the Lp-PLA2 inhibitor darapladib in patients with chronic stable angina in addition to optimal medical therapy. There was no reduction in the composite of cardiovascular death, MI, and urgent coronary revascularization in both studies [51,52]. Randomized trial of another inhibitor of Lp-PLA2 varespladib on atherogenic lipoprotein subclasses in 135 statin-treated patients with CAD showed a modest reduction in total LDL particle concentration (7%) and small LDL particle concentration (11%) without any change in plasma levels of ox-LDL and hs-CRP [53]. The study was stopped because of adverse side-effect profile in varespladib-treated patients [54]. A major randomized double-blind trial was conducted with succinobucol, a potent anti-oxidant, in 6144 patients with ACS. Succinobucol therapy showed no benefit on the primary end point (composite of first occurrence of cardiovascular death, resuscitated cardiac arrest, myocardial infarction, stroke, unstable angina, or coronary revascularization) [55].

Use of the TNFα inhibitor therapy in patients with rheumatoid arthritis has been associated with a reduced risk of future cardiovascular events, raising interest for potential benefit in CAD [56]. A recent meta-analysis showed some benefit of methotrexate therapy in patients with CAD [57]. There are studies showing benefit of allopurinol, a xanthine oxidase inhibitor, in patients with CAD [58]. The potential benefits of colchicine, an anti-inflammatory therapy used for gout, were studied in the prospective LoDoCo trial of 532 patients with stable CAD and a median follow-up of 3 years. Colchicine use led to a significant reduction in the occurrence of a composite end point (ACS, out-of-hospital cardiac arrest, or non-cardioembolic ischemic stroke), but was associated with adverse side-effect profile resulting in withdrawal of a large number of patients [59]. Trials of monoclonal antibodies (MAbs) against P-selectin like inclacumab [60] and leukotriene inhibitors [61] have yielded mixed results in patients with ACS.

MAbs to interleukins have shown clinical benefit in autoimmune disorders like rheumatoid arthritis. Canakinumab (trade name Ilaris) is a human MAb targeted at interleukin-1β that is being investigated currently in the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) in patients with stable CAD and a pro-inflammatory state (elevated CRP) [62]. The LATITUDE trial designed to study the effect of Losmapimod, an inhibitor of p38-mitogen-activated protein kinase, in patients with ACS is currently in the enrollment phase [63]. Results of other anti-inflammatory trials for CAD are currently awaited with cautious optimism.

IMMUNE THERAPIES IN CAD

The understanding of complex immune mechanisms involved in atherogenesis opens doors to various immune targets that can be potentially modified to induce atheroprotection. Potential strategies such as B-cell depletion, cytokine inhibition, regulatory T-cell proliferation, and immunization are being currently studied. Clinical evidence supporting immune suppression as a treatment for atherosclerosis is based on studies done on autoimmune disorders. A meta-analysis of the trials studying the effect of TNFα inhibitors in the management of rheumatoid arthritis has shown a trend toward cardiovascular protection [56]. Similarly, B-cell depletion with agents such as anti-CD20 antibodies is currently approved for the treatment of various immune disorders. The effect of these therapies on atherosclerotic CAD would be of great clinical interest. An antibody against B-cell-activating factor, which plays an important role in B-cell homeostasis, is being investigated.

Immunization appears to be an attractive therapeutic strategy based on the success of immunization strategies in decreasing the global burden of infectious disease. Both passive and active immunization techniques have been under investigation over the past two decades. Induction of immune tolerance by administration of small doses of auto-antigens such as ox-LDL, Apo B-100, and hsp60 has been shown to attenuate atherosclerosis in mice. This protective effect was at least in part secondary to a shift in immune response that leads to an increased production of regulatory CD4+ T cells [64,65].

Passive immunization with administration of antibodies directed at ox-LDL has also been shown to reduce atherosclerosis in mice. The identification of autoantibodies to ox-LDL triggered interest in generating synthetic antibodies that could provide therapeutic benefit. Witztum's group cloned a panel of IgM mAbs, termed "EO" antibodies that bind to both the protein and lipid components of ox-LDL [66]. Horkko et al. [67] later showed that administration of a specific antibody E06 that has affinity to the phosphorylcholine moiety inhibits uptake of ox-LDL by scavenger receptors on macrophages. These antibodies exhibit structural and immune resemblance to anti-Streptococcal antibodies that bind the phosphorylcholine moiety on the bacterial cell wall. This was confirmed by Binder et al. [68] who showed that immunization of $LDLR^{-/-}$ mice with heat killed

Streptococcus pneumoniae led to an increased production of ox-LDL specific (E06) antibodies and attenuated atherosclerosis. Another MAb termed IK17-Fab which binds to modified (oxidized and malonaldehyde) LDL, but not native-LDL and blocks the uptake of ox-LDL by macrophages was studied in atherosclerotic murine models by Tsimikas et al. [69]. Both passive transfer of IK17-Fab via intraperitoneal injection and active induction through gene transfer via a viral vector led to inhibition of ox-LDL uptake and decreased atherosclerosis. However, translating the benefit of passive immunization to clinical practice remains a challenge. A recent phase 2, randomized trial (NCT01258907, GLACIER trial) of a monoclonal anti-ox-LDL antibody (MLDL1278A, aka BI-204) in patients with stable CAD failed to meet the primary end point of reducing vascular inflammation as measured by FDG-PET. Selecting a patient population with low levels of vascular inflammation and inadequacies of imaging techniques are postulated as the reasons for the negative outcome. Larger studies that include appropriate patients with increased vascular inflammation, such as those with ACS, and improvement in imaging specificity can further our quest of an antibody against atherosclerosis.

Vaccine Against Atherosclerosis

The search of a vaccine that can protect against atherosclerosis would be considered the Holy Grail in the prevention of CAD. As with active immunization in other disease states, the first step was the identification of a suitable antigen.

As discussed earlier, various infectious agents, such as *Chlamydia*, *Hepatitis C*, and *Porphyromonas*, have been isolated in atherosclerotic plaques. Witztum and colleagues demonstrated molecular mimicry between the ox-LDL molecule and the cell wall of *S. pneumoniae*. However, the lack of a definite causal relationship between infections and atherosclerosis and the failure of previous antibiotic trials question the efficacy of a vaccine developed against exogenous antigens.

Among endogenous antigens, native or modified-LDL would be an obvious primary choice. Murine studies with native-LDL, ox-LDL, and malonaldehyde-LDL have consistently shown atheroprotective effect [70]. However, the large size and complex structure with multiple antigenic epitopes makes LDL unsuitable for vaccination. Rigorous efforts by multiple research groups led to the isolation of a peptide sequence of the Apo B-100 moiety of the LDL molecule called p210 that has immunogenic potential [71]. A candidate vaccine, CVX-210H (Cardiovax®, Princeton, NJ), combining the p210 sequence with human serum albumin is currently being developed. Studies of this vaccine in mice have been shown to induce a shift in T-cell response and induce atheroprotection [72]. Other vaccine strategies using hsp65 [73] and dendritic cells [74] are currently under investigation. A vaccine that would attenuate atherosclerosis is far from being a reality at this point. It is however and exciting field of research that has the potential to have a huge global impact on health care.

CONCLUSIONS

Here we have discussed the pathogenesis of atherosclerosis, especially the contributory role of inflammation in atherogenesis. This concept has evolved based on the identification of accumulation of inflammatory cells from the beginning of plaque formation to the development of flow restricting disorders resulting in acute myocardial infarction and stroke. Based on this information, a number of trials with anti-inflammatory drugs have been conducted in a variety of patients with CAD. Although clinical trials up till now have not shown significant benefit, it is likely that newer therapies based on new knowledge might reveal benefit of inflammatory signal-targeted drug therapy. Of note, measurement of inflammatory signals in blood as biomarkers also has not proven as effective as previously thought because of multiple sources of inflammatory signals which are released with wide variability at different stages of atherosclerosis. Recent studies also suggest that both innate and adaptive immunity play role in the development of atherosclerosis. This knowledge may yield development of vaccines and immunomodulatory therapies against atherosclerosis.

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CHAPTER

4

Adiponectin: A Mediator of Obesity, Insulin Resistance, Diabetes, and the Metabolic Syndrome

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The worldwide prevalence of obesity has nearly doubled since 1980. More than 42 million children under the age of 5 years were overweight in 2013 according to the World Health Organization's 2014 global status report on noncommunicable diseases. In 2014, 11% of men and 15% of women aged 18 years and older were obese. It is predicted that 3 billion adults are either overweight or obese in 2015 [1] . Obesity increases the risk of developing insulin resistance, type 2 diabetes, dyslipidemia, hypertension, and atherosclerosis, and the coexistence of these diseases has been termed the metabolic syndrome. Once regarded as a relatively inert storage depot for fat, the adipose tissue is now recognized as an important endocrine organ that releases a host of biologically active hormones and cytokines collectively referred to as adipokines. Adiponectin, an adipokine, has emerged as a major player in the pathogenesis of obesity, insulin resistance, and metabolic syndrome. Emerging evidence also indicates the association of obesity and insulin resistance with a state of low-grade chronic inflammation of the adipose tissue as a result of incessant activation of the native immune system which plays a major role in the pathogenesis of obesity-related metabolic complications.

In this chapter, we will review the mechanisms underlying obesity and discuss the role of heme oxygenase and adiponectin in obesity, insulin resistance, and the metabolic syndrome, as well as their relation to the genesis of atherosclerotic heart disease.

ROLE OF OXIDATIVE STRESS AND INFLAMMATION IN OBESITY

Although epidemiological studies have described the association between inflammation and obesity for decades, the underlying molecular mechanisms have only begun to unravel since the dawn of the twenty-first century. Increased oxidative stress in the setting of obesity is now known to play a key role in the initiation of metabolic changes within the adipose tissue.

Reactive oxygen species (ROS) are unstable molecules with potent oxidative effects on cellular proteins, lipids, and DNA, resulting in impaired cellular function. ROS are produced by the electron transport system in mitochondrial respiration and are increased in conditions associated with enhanced oxidation of energy substrate such as glucose and free-fatty acids (FFAs). Increased FFA oxidation in obesity results in mitochondrial overproduction of ROS. Increased release of FFA from adipose tissue also activates nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), an enzyme that converts molecular oxygen to its superoxide radical. Furukawa et al. found increased mRNA expression of NADPH oxidase in adipose tissue of obese mice [2]. Similarly, lipid peroxide levels and hydrogen peroxide generation were found to be elevated in adipose tissue [2]. Obese mice were noted to have increased oxidative stress levels compared to control mice. The extent of fat accumulation has been correlated with markers of systemic oxidative stress

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00004-8

in several human studies [3,4]. Obesity is also associated with a decrease in antioxidant enzymes including catalase and glutathione [2,5,6], thus enhancing oxidative stress in the adipose tissue of obese subjects.

ROS have been shown to increase monocyte chemoattractant protein-1 (MCP-1) expression in adipocytes [2]. MCP-1 is a chemoattractant that attracts and triggers monocyte and macrophage migration into the adipocytes. The adipose tissue is thus infiltrated by macrophages that trigger inflammatory events in obesity. A shift of the pool of macrophages from the alternatively activated M2-type to the classically activated M1-type promotes the secretion of predominantly proinflammatory cytokines in obese subjects. Cytokines, including tumor necrosis factor α (TNFα), interleukin-1 (IL-1), interleukin-6 (IL-6), nuclear factor kappa-B (NF-κB), and c-Jun N-terminal kinase (JNK), released from these activated macrophages mediate inflammation in adipose tissue [7–11]. In addition, transient infiltration of neutrophils and T cells may also contribute to the development of inflammation in adipose tissue [12,13]. There is growing evidence to suggest that oxidative stress in obesity contributes to the establishment of a vicious cycle that promotes elevated oxidative/inflammatory activities in the adipose tissue with a continuum of tissue injury leading to more severe cardiometabolic complications including insulin resistance, diabetes, and ultimately atherosclerosis.

CYTOPROTECTIVE HEME OXYGENASE/ADIPONECTIN AXIS

Heme oxygenase (HO) induction has proven to be a potential therapeutic strategy that modulates both the oxidative stress and the inflammatory aspects of obesity. HO is important in attenuating the overall production of ROS through its ability to degrade heme, to produce carbon monoxide (CO), biliverdin, and bilirubin, and to release free iron [14]. CO and bilirubin are known to suppress apoptosis, necrosis, inflammation, and oxidative stress [15–18], while the iron formed enhances the synthesis of the antioxidant, ferritin [19]. While HO-2 is expressed constitutively, HO-1 is inducible in response to oxidative stress. Inducers of HO-1 have also been reported to cause a robust increase in serum adiponectin levels in diabetic rats [20]. A temporal relationship between HO-1 and adiponectin has been studied. L'Abbate et al. [21] reported that induction of HO-1 decreased superoxide and ROS generation, resulting in a parallel increase in the serum levels of adiponectin. Induction of HO-1 has been shown to slow weight gain and decrease levels of TNF, IL-6, and IL-8 [22–24].

L-4F, an apolipoprotein A1 mimetic peptide and a potent HO-1 inducer, decreases visceral and subcutaneous fat content with limited weight gain, decreases plasma IL-1b and IL-6 levels, increases adiponectin levels, and increases insulin sensitivity and glucose tolerance in a mouse model of obesity and diabetes (ob/ob mice). This has further been demonstrated to result in decreased glucose and insulin levels both in mouse bone marrow and human bone marrow–derived mesenchymal stem cell cultures [25].

Increased HO-1 expression increases Akt and adenosine monophosphate activated protein kinase (AMPK) phosphorylation [26]. Phosphorylated Akt (pAkt) and phosphorylated AMPK (pAMPK) function as fuel sensors in the regulation of energy balance. Impaired pAMPK and pAkt signaling has been implicated in insulin resistance and endothelial dysfunction. HO-1 induction mediates activation of pAMPK and pAkt, increases glucose transport, fatty acid oxidation, mitochondrial function, and NO bioavailability, and improves vascular function [27–30].

Burgess et al. demonstrated that HO-1 induction in adipocyte stem cells not only ameliorates obesity-associated metabolic consequences, including hypertension independent of body weight, but also improves glucose tolerance in both male and female obese mice [31]. These novel findings underscore the importance of HO-1 as a potential therapeutic target in the treatment of metabolic disorders including insulin resistance in obese populations.

ADIPONECTIN

Adiponectin is the most abundant adipokine in the human plasma and accounts for nearly 0.01% of the total plasma protein. The concentration of circulating adiponectin in plasma is between 2 and 30 μg/mL, which is greater than that for most hormones and inflammatory cytokines. Adiponectin is largely secreted by adipocytes, although it can also be secreted by the hepatocytes, cardiomyocytes, skeletal muscle, colon, salivary gland, placenta, and hypophysis at lower concentrations [32]. Adiponectin levels show ethnic and gender differences. Levels are higher in Caucasians than in Indo-Asians [33] and higher in women than in men [34]. Importantly, the serum level of adiponectin is significantly reduced in obese or type 2 diabetic patients [35–37].

STRUCTURE AND SECRETION OF ADIPONECTIN

Reported for the first time by Scherer et al. in 1995 [38], adiponectin is a product of the *APM1* (adipose most abundant gene transcript 1) gene located on the chromosome 3q27. It is a 30 kDa protein composed of 244 amino acids with a C-terminal globular domain and a collagen-like N-terminal domain [39]. Adiponectin is structurally related to proteins of the complement system (C1q) [40] and tumor necrosis factor alpha (TNFα) [41] (Figure 4.1).

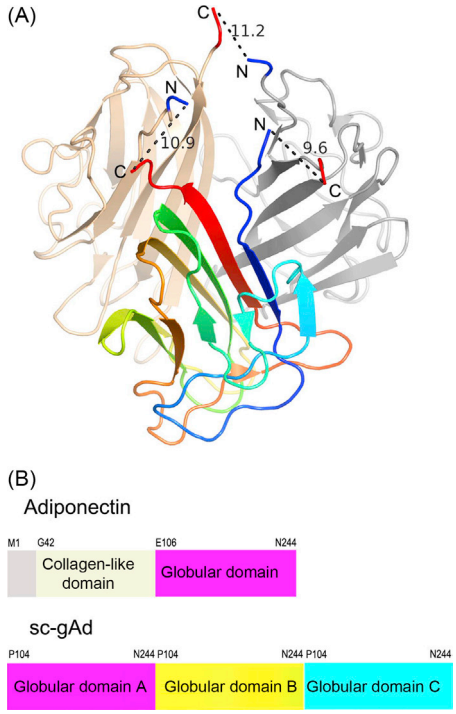

FIGURE 4.1 Structure of single-chain globular domain adiponectin (sc-gAd). (A) Base region of mouse gAd structure. One gAd monomer is shown with blue for the N-terminus of the sequence which transits to red for the C-terminus. The other two gAd monomers are shown in single color with blue and red to denote the N- and C-termini, respectively. (B) Domain organization of human adiponectin. For sc-gAd, the three globular domains (A, B, and C) are shown in magenta, yellow, and cyan, respectively. *Source: Reprinted from Ref. [39], with permission from Elsevier.*

The basic building block of adiponectin is a tightly associated trimer, which is formed by association between three monomers at the globular domains. Adiponectin is found in low-molecular-weight (e.g., trimer and hexamer) and high-molecular-weight (HMW) forms (e.g., dodecamers and octadecamers) in human serum [42]. HMW adiponectin formation requires an intermolecular disulfide bond between cysteine residues that are located in the hypervariable region. A model for assembly of adiponectin complexes is shown in Figure 4.2.

Full-length adiponectin undergoes proteolytic cleavage to form globular adiponectin, which has increased binding in myocytes and skeletal muscle membranes, but reduced binding in hepatocytes and liver membranes.

ADIPONECTIN RECEPTORS: STRUCTURE AND FUNCTION

The metabolic actions of adiponectin are mediated by receptors known as adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) that were identified by expression cloning in 2003. These receptors have seven transmembrane portions, but are functionally different from

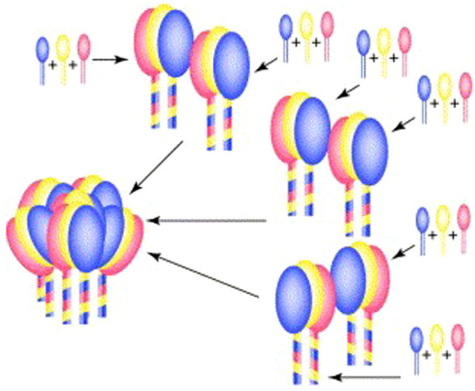

FIGURE 4.2 Model for assembly of adiponectin into complexes of up to 18 monomers. Three monomers form a trimer through the globular domain, and trimers associate through interactions within the collagenous domain. Four to six trimers associate noncovalently through their collagenous domains to form a high-molecular-weight complex. *Source: Reprinted from Ref. [42], with permission from Elsevier.*

the G-protein-coupled receptors, particularly because they have opposite polarity (i.e., internal N-terminus and external C-terminus) [43]. AdipoR1 is abundantly expressed in liver, skeletal muscle, macrophages, and the hypothalamus, while AdipoR2 is expressed in liver, white adipose tissue, and the vasculature.

AdipoR1 and AdipoR2 double knockout mice have been shown to be glucose intolerant and hyperinsulinemic, indicating that AdipoR1 and AdipoR2 help to regulate normal glucose metabolism and insulin sensitivity [44]. Liver AdipoR1 is involved in activating AMPK, while AdipoR2 is involved in activation of peroxisome proliferator-activated receptor alpha (PPARα), leading to increased insulin sensitivity. Full-length adiponectin stimulates the phosphorylation and subsequent activation of AMPK in both skeletal muscle and the liver, compared to globular adiponectin which only exerts its effect in skeletal muscle [45]. Adiponectin, via AdipoR2, activates and increases the expression of PPARα ligands increasing the rate of β oxidation which is a major pathway for lipid metabolism.

In addition to AMPK activation, adiponectin induces carboxylase acetyl-coenzyme A phosphorylation, glucose uptake, nitric oxide (NO) synthesis, lactate production in myocytes, and reduced liver production of molecules involved in gluconeogenesis. Adiponectin also appears to regulate more diverse and complex pathways, such as ceramide and sphingosine1-phosphate (S1P) downstream from AdipoR1 and AdipoR2 [45–49].

AdipoR1 increases the action of a number of genes including those coding for NF-κB, TNFα, IL-1, and IL-4 [50]. AdipoR1 activation decreases vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1 (ICAM1), and IL-18 levels, all of which promote inflammation. AdipoR1 also activates P38 mitogen-activated protein kinase (p38MAPK) which is involved

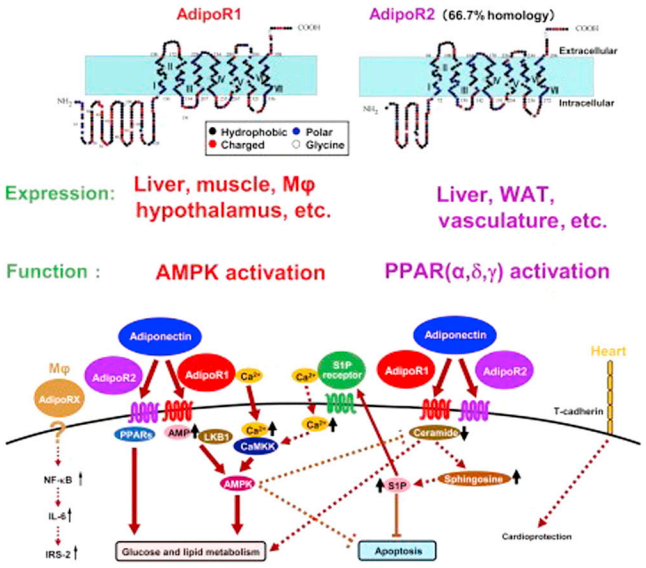

FIGURE 4.3 Functional analysis of adiponectin receptors. *Source: Reprinted from [49], with permission from Elsevier.*

in transcriptional machinery. The action of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) is indirectly regulated by AdipoR1. PI3K acts on heat shock protein 90 (HSP90) which increases the action of endothelial nitric oxide synthase (eNOS). AdipoR2 activates the *APPL1* gene which upregulates AMPK1 which again upregulates eNOS [50,51] and increases the production of NO. Elevated AMPK also increases the action of phosphoenolpyruvate carboxykinase (PEPCK) ultimately increasing gluconeogenesis. *APPL1* transcription also upregulates Akt which increases the glucose transporter type 4 (Glut4) translocation ultimately increasing glucose uptake in the cell [52].

T-cadherin, a membrane-associated adiponectin-binding protein localized in vascular smooth muscle cells and endothelial cells, seems to be another receptor for adiponectin activity [53]. T-cadherin localizes adiponectin to the vascular endothelium. T-cadherin deficiency prevents the ability of adiponectin to promote cellular migration and proliferation [54]. T-cadherin protects against stress-induced pathological cardiac remodeling by activating adiponectin's cardioprotective functions (Figures 4.3 and 4.4) [55].

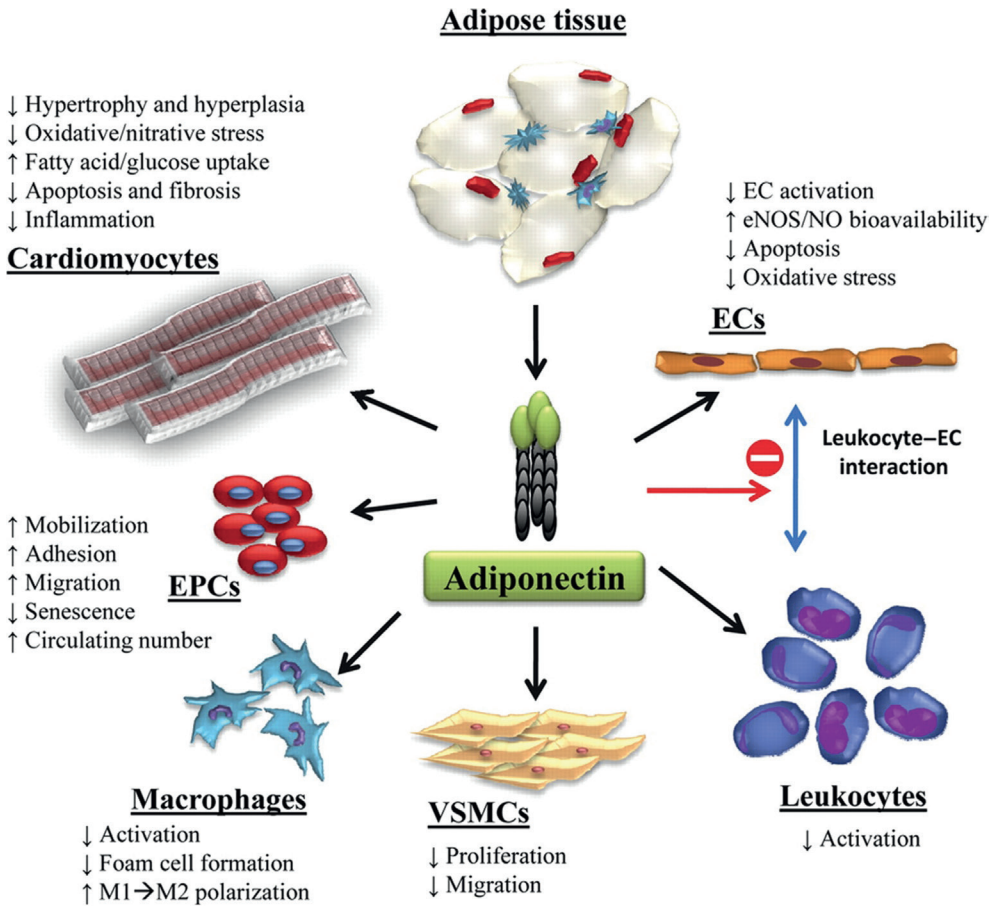

FIGURE 4.4 Actions of adiponectin on different target tissues. EC, endothelial cell; EPCs, endothelial progenitor cells; VSMCs, vascular smooth muscle cells. *Source: Adapted with permission from Ref. [52].*

ADIPONECTIN IN OBESITY

Although adiponectin is secreted only from adipose tissue, its plasma levels are paradoxically lower in obesity. This is in contrast to most other adipokines, whose levels increase in obesity in proportion to the increased total body fat mass. In a similar fashion, adiponectin levels increase with calorie restriction or weight reduction by gastric partition surgery [56]. There is a strong inverse correlation between plasma levels of adiponectin and measures of adiposity, including body mass index (BMI) and total fat mass [57]. Besides total fat mass, intra-abdominal fat is an independent negative predictor of plasma adiponectin [58]. In both lean and obese individuals, adiponectin levels in intra-abdominal fat are much lower than in subcutaneous fat.

ADIPONECTIN AS AN INSULIN-SENSITIZING HORMONE

The role of adiponectin as a key regulator of insulin sensitivity was first described by Fruebis et al. in 2001 when injection of a fragment of C-terminal globular adiponectin into mice decreased plasma glucose levels by increasing fatty acid oxidation in skeletal muscles [59]. Several studies subsequently confirmed this finding using different forms of recombinant adiponectin. Both full-length or globular adiponectin significantly improve insulin resistance and lipid profiles in mouse models of diabetes and obesity [60,61]. An approximately threefold elevation of native adiponectin in a transgenic mouse model significantly increases lipid clearance and lipoprotein lipase activity and enhances insulin-mediated suppression of hepatic glucose production, thereby improving insulin sensitivity [62,63]. Adiponectin knockout mice exhibit severe insulin resistance and dyslipidemia on a high-fat diet, despite having normal glucose tolerance when fed with regular chow. Mild insulin resistance is noted in heterozygous adiponectin knockout mice and moderate insulin resistance is noted in homozygous adiponectin knockout mice even when fed with regular chow.

Mechanistically, the insulin-sensitizing effect of adiponectin appears to be secondary to its direct actions on skeletal muscle and the liver through the activation of AMPK and PPAR [64]. Excessive triglyceride (TG) accumulation has been proposed to be a major causative factor of insulin resistance in skeletal muscle [65]. Activation of AMPK in skeletal muscle by adiponectin causes increased expression of proteins involved in fatty acid transport, fatty acid oxidation (such as acyl-coenzyme A oxidase), and energy dissipation (such as uncoupling protein-2), resulting in enhanced fatty acid oxidation, energy dissipation, and reduced TG accumulation. In the liver, stimulation of AMPK decreases the expression of gluconeogenic enzymes, such as PEPCK and glucose-6-phosphatase, which may account for its glucose-lowering effect *in vivo* [49,62]. In addition, adiponectin acts in an autocrine manner on adipocytes to antagonize the inhibitory effect of TNFα on insulin-stimulated glucose uptake [66] and blocks the release of insulin resistance-inducing factors from adipocytes [67].

Human studies have shown that low plasma adiponectin concentration precedes a decrease in insulin sensitivity [68]. Plasma adiponectin levels are inversely related to basal and insulin-stimulated hepatic glucose production [69], supporting the role of adiponectin as an endogenous insulin sensitizer in humans. Hypoadiponectinemia is more closely associated with the degree of insulin resistance and hyperinsulinemia than with the degree of glucose intolerance and adiposity. Case-controlled studies show that subjects with low concentrations of adiponectin are more likely to develop type 2 diabetes than those with high concentrations [70,71].

Data from human genetic studies further support the causative role of hypoadiponectinemia in the development of insulin resistance and type 2 diabetes [72–75]. The gene coding for adiponectin is located on human chromosome 3q27, a known diabetes susceptibility region, suggesting the association of adiponectin with the development of diabetes [76]. Single-nucleotide polymorphisms (SNPs) at positions 45, 276, and in both the proximal promoter region and exon 3 of the adiponectin gene have been found to be closely associated with insulin resistance and type 2 diabetes in several ethnic groups. *SNP276* is directly linked with lower plasma adiponectin levels, enhancing the risk of type 2 diabetes and insulin resistance [72]. While more adiponectin gene SNPs have been found to cause hypoadiponectinemia and diabetes [73,74,77], healthy levels of adiponectin were associated with decreased risk of diabetes in a systematic analysis of 13 prospective studies [78].

ADIPONECTIN AND ATHEROSCLEROSIS

Adiponectin has been found to have multiple direct antiatherogenic properties [79,80]. While overexpression of adiponectin inhibits atherosclerotic lesion formation in transgenic mouse models, adiponectin deficiency is associated with severe neointimal thickening and increased vascular smooth muscle cell proliferation in mechanically injured arteries [81–83]. Adiponectin improves endothelium-dependent vasodilation by enhancing eNOS activity to increase NO production [84]. Adiponectin restrains plaque formation and atherosclerosis by inhibiting the expression of adhesion molecules and inflammatory cytokines including IL-8, ICAM-1,

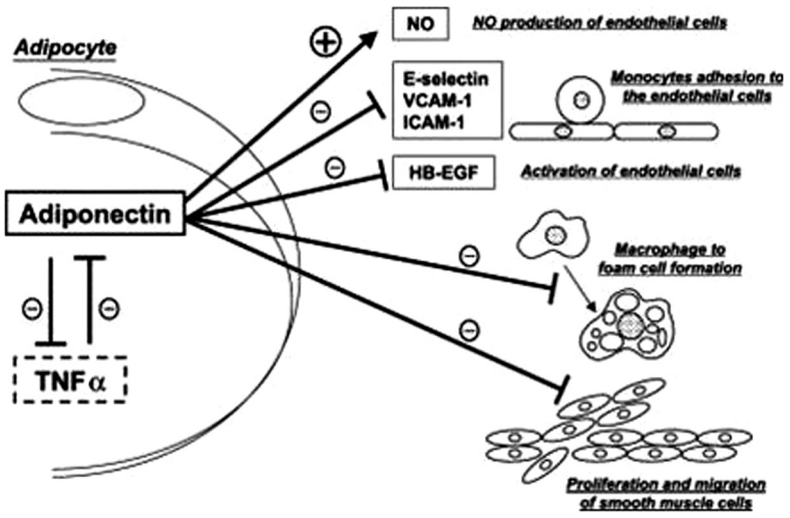

FIGURE 4.5 Protective action of adiponectin in the initiation and progression of atherosclerosis. TNFα, tumor necrosis factor α; NO, nitric oxide; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intracellular adhesion molecule-1; IHB-EGF, heparin-binding epidermal growth factor-like growth factor. *Source: Reprinted from Ref. [85], with permission from Elsevier.*

VCAM-1, and E-selectin, thus suppressing endothelial and smooth muscle cell proliferation and reducing cholesterol uptake via inhibition of class A scavenger receptor expression (Figure 4.5) [86–91].

The protective anti-atherogenic actions of adiponectin have been validated in multiple clinical studies. Low serum adiponectin has been suggested as an independent indicator of the extent of coronary plaque burden [92,93]. Similarly, high plasma levels of adiponectin are associated with a significantly decreased risk of myocardial infarction in a large case control study in which 18,225 male participants were followed over a 6-year period [94].

ADIPONECTIN AND HYPERTENSION

Low serum adiponectin levels are an independent risk factor for essential hypertension [95,96]. Hypertensive individuals have significantly lower concentrations of plasma adiponectin compared with normotensive healthy subjects [97]. In mouse models of obesity, adenovirus-mediated delivery of adiponectin was associated with a significant decrease in blood pressure [98], suggesting that hypoadiponectinemia is also a contributing factor for the development of obesity-related hypertension.

ADIPONECTIN AND DYSLIPIDEMIA

Adiponectin appears to play an important role in the pathogenesis of dyslipidemia by affecting HDL and LDL metabolism. In clinical studies, low levels of plasma adiponectin have been associated with decreased serum HDL-C level, reduced lipoprotein lipase enzyme activity, smaller LDL size, and increased levels of apolipoprotein (Apo) B-100 [99–103]. Adiponectin upregulates ApoA1 protein secretion suggesting that adiponectin may increase HDL assembly in the liver [104]. Adiponectin knockout mice have reduced plasma and hepatic levels of ApoA1 protein when compared with wild-type mice [105].

ADIPONECTIN AND THE METABOLIC SYNDROME

Metabolic syndrome is not merely a single disease but a collection of pathological conditions (i.e., abdominal obesity, insulin resistance, dyslipidemia, hyperglycemia, and hypertension) that increase the risk of developing diabetes and cardiovascular diseases. Low adiponectin levels directly correlate with the development of metabolic syndrome after adjusting for age, sex, and BMI [106,107]. In a study of Japanese adults, an increase in the number of metabolic syndrome components was associated with decreasing adiponectin levels [108]. Hypoadiponectinemia also appears to be a predictor for the future development of metabolic syndrome in obese individuals [109,110].

CURRENT AND FUTURE THERAPEUTIC TARGETS

As discussed above, data from clinical studies identify hypoadiponectinemia as a potential causative agent in the development of a wide spectrum of obesity-related metabolic and cardiovascular disorders. Adiponectin

holds great promise as a potential therapeutic agent for the treatment of insulin resistance, type 2 diabetes, and related atherosclerotic complications. Lifestyle measures including weight loss and aerobic exercise have been shown to increase adiponectin levels [111]. Adiponectin replenishment therapy has not yet been realized, as biologically active recombinant adiponectin proteins are inherently unstable and difficult to synthesize [112]. Certain drug classes such as thiazolidinediones and sulfonylureas, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, nicotinic acid, and omega-3 polyunsaturated fatty acids exert beneficial effects on insulin resistance, in part by increasing plasma adiponectin levels [113,114].

Two agents have been identified as possible pharmacologic mediators of adiponectin metabolism. AdipoRon, an oral AdipoR agonist, activates AMPK and PPARα pathways in muscle and liver, resulting in improvement of insulin resistance and glucose intolerance in mice on a high-fat diet [115], and attenuates post-ischemic myocardial injury in a mouse model [116]. Another potential mediator is CTRP9, an adiponectin paralog, which, in a mouse model, undergoes proteolytic cleavage to generate gCTRP9, the dominant circulatory and actively cardioprotective isoform, which in turn activates cardiac survival kinases, including AMPK, Akt, and eNOS [117].

Further research as well as potential AdipoRon human trials will better delineate the potential of adiponectin to both prevent atherosclerotic disease as well as enhance survival following coronary injury.

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CHAPTER

5

Use of Stem Cells in Ischemic Heart Disease

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INTRODUCTION

The heart is an essential organ which plays a role in supplying blood carrying oxygen and nutrients during fetal and postnatal life. It carries an intrinsic property of regeneration and replacement of lost cardiomyocytes to maintain physiological balance throughout human life.

There has been a significant increase in the prevalence of cardiovascular risk factors in the United States including type II diabetes mellitus and obesity in recent years from which coronary artery disease has emerged as a major health concern. Ischemic heart disease continues to be a main cause of morbidity and mortality in the United States.

Myocardial ischemia results from an imbalance between the myocardial oxygen supply and the myocardial oxygen demand. Coronary artery disease is the main cause of myocardial ischemia. Myocardial infarction leads to loss of cardiomyocytes. Cell death varies according to the therapy given, the site and severity of the coronary artery obstruction, and the time to intervention. There are multiple therapies available for myocardial infarction, but none of these therapies have been shown to reverse the loss of cardiac cells. Quantification of extent of myocardial ischemia can be done by using a number of imaging modalities, which include cardiac magnetic resonance imaging, computed tomography angiography, and positron imaging tomography [1]. Occasionally, the ischemic event results in significant loss of cardiomyocytes which leads to a sequence of events, including fibrosis, scar formation, thinning of the ventricular wall, remodeling of viable cells in the ventricle, and eventually the development of congestive heart failure.

Since the last century, remarkable progress has been made in the treatment of acute myocardial infarction, including pharmacological interventions, percutaneous coronary intervention, and coronary artery bypass graft surgery. In the acute setting, percutaneous coronary intervention with use of bare-metal stents or drug-eluting stents or coronary artery bypass graft surgery helps to restore coronary blood flow in the occluded coronary artery and helps to improve the prognosis. However, a significant number of patients still develop congestive heart failure. Ischemic heart disease continues to be a major cause of congestive heart failure. Even in the presence of these successful treatments, ischemic heart failure or cardiomyopathy is a common chronic consequence of myocardial infarction and carries a poor overall prognosis. After cell death, mechanisms of cell repair are initiated.

Interventions available in the current era, including pharmacological therapy (beta-blockers, angiotensin-converting enzyme inhibitors, and aldosterone antagonists), implantable cardioverter-defibrillators, and mechanical assist devices such as bridge to transplant or destination therapy help in the management of the decompensated heart failure state but do not help to restore the lost cardiac function from an ischemic event. Heart transplantation is an option by which we can replace the damaged heart, but selected patients are candidates for the therapy, and the present paucity of organs available limits its widespread use. Furthermore, rejection complications and close surveillance are drawbacks for this therapy. Survival of transplanted patients has

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00005-X

improved with improvement in the immunosuppressive therapy used to prevent rejection complications.

Inability of the above therapies to help in cardiac repair has led researchers to explore the field of stem cells with an aim to replace the damaged myocardium resulting from myocardial ischemia. Stem cell-based therapy for ischemic heart disease has turned into an evolving and dynamic area of research in the last few years. Initial studies were performed using bone marrow mononuclear cells and were found to be effective in cardiac repair. One of the first nonrandomized trials performed using bone marrow stem cells showed improvement in overall cardiac function after transplanting them in infarcted myocardium. In more recent trials, mesenchymal and cardiac stem cells are being used. Recent meta-analysis have shown that stem cell therapy results in improvement of left ventricular ejection fraction by 3–4% [2–5].

Cardiomyocytes were initially thought to be terminally differentiated cells with no regeneration properties in postnatal life [6]. Recent studies have shown that after an ischemic injury to the myocardium, the circulating bone marrow derived or locally present cells migrate around the infarcted area and differentiate into cardiomyocytes. However, the rate of differentiation is slow to overcome the extent of damage or loss of function from myocardial cell death [7,8]. Another theory for generation of new heart cells is that the preexisiting cardiomyocytes enter into a mitotic cell cycle and differentiate into cardiomyocytes during normal aging and postmyocardial infarction [9]. Cardiomyocytes turn over at a rate of 1% at age 25, decreasing to 0.45% by age 75, reflecting less than 50% of cells being replaced during a life span [10]. Another group reported a much higher turnover up to eight times during the human life span [11].

Cell-based therapy is based on the concept of using embryonic or adult stem cells. Cell transplantation has been receiving attention due to its simplified way of using and a good safety profile. However, even with multiple trials in this field, a specific strategy has not been established yet.

MECHANISM OF ACTION

The mechanism of action of stems cells in improving cardiac function and helping with repair is still controversial. The type of stem cells involved in the process is also not well understood. This is part of the reason why large clinical randomized trials have not been designed and tested in patients. Initial experiments and trials were based on the thought that stem cells differentiate into cardiomyocytes. Intrinsic cardiac cells can also be induced by bone marrow stem cells to enter mitotic cell cycle and proliferate, helping improve cardiac function. They can induce neovascularization via endothelial progenitor cells and prevent cardiac remodeling. Other mechanisms described are release of cytokines by stem cells promoting cardiac repair or via cell fusion of stem cells with intrinsic cardiomyocytes [12,13].

The source of the cells is either from the recipient's bone marrow or the stem cells are mobilized using a growth factor. The most common growth factor used is the granulocyte colony stimulating factor. The initial technique is a time consuming procedure in which cells are obtained from the bone marrow at site of pelvic bone and then separated from the rest of the cells before being infused in the recipient. In the latter method using growth factor, stem cells are obtained from a blood sample.

STEM CELL DELIVERY

Delivery of the stem cells is essential at the site of injury where they can help to restore the cardiac function. There have been multiple methods of cell delivery described in trials over the last decade. The most commonly used methods are intravenous infusion [14], intracoronary injection [15], and intramyocardial or direct epicardial injection [16].

Intravenous infusion appears to be the most simplified noninvasive procedure but very few of the stem cells actually reach the site of injury. Intracoronary delivery of the cells can be done by using a specialized catheter during coronary intervention using a stop flow technique. One of the studies described less than 4% of cells residing in the heart muscle after an intracoronary injection [17]. A meta-analysis showed that intracoronary infusion is effective in patients with acute myocardial infarction [18].

Direct injection in the ventricular wall can be used when patients have chronic ischemic heart disease and occluded coronary vessels prevent the delivery of cells to the target site. Intramyocardial injection can be done either using an angioplasty-like technique with electromechanical mapping (NOGA catheter technique) or during coronary artery bypass graft surgery [19]. It is expected that placing the cells at the site of myocardial injury will have the maximum benefit, but the inflammation provides an unfavorable environment to the stem cells to grow. Poor blood flow and vascular supply at the site of myocardial ischemic injury is another limiting factor in the cells reaching the target area.

It has been described that approximately 90% of the transplanted cells die due to the above described conditions. Therefore, determining the dose of the stem cells is essential to achieve the goal of cardiac repair and improvement in function.

TYPES OF STEM CELLS

Embryonic Stem Cells

Embryonic stem cells are totipotent cells as they can differentiate to any type of cell in the body. Ability to differentiate into cardiomyocytes, endothelial, and smooth muscle cells helps in repair of the damaged myocardium. In animal models, the cardiomyocytes derived from the embryonic stem cells help improve myocardial function [20]. In a mouse model, human embryonic stem cell-derived cardiomyocytes have been shown to improve overall heart function at 4 weeks postmyocardial infarction, but no significant improvement was seen at 12 weeks after myocardial infarction.

There are multiple limitations in applying the concept of embryonic stem cells in humans. An ethical stigma is attached to it as harvesting of these cells involve accessing the embryo. Due to their pluripotent property, they can develop into teratomas, containing cells derived from all three germ layers. These teratomas can also develop in the heart. Another obstacle is immunological incompatibility with the differentiated cells. Cells harvested from animals can be source of viruses which limits its application to humans. Currently, focus is on isolating the cardiac progenitor cell among these embryonic stem cells which can overcome a few of the limitations described above.

Skeletal Myoblasts

Skeletal myoblasts were among one of the first cells injected into the injured myocardium. Skeletal myoblasts have characteristics similar to cardiomyocytes which favor their use. As they can be obtained from the host easily via muscle biopsies, there are no ethical issues or immunological problems. These cells have a higher proliferation rate and also are resistant to myocardial ischemia. Their use in animal models has been shown to improve left ventricular function and prevent cardiac remodeling [21].

A limiting factor of their use is that skeletal myoblasts are unable to form intercalated discs with adjacent cardiomyocytes which result in arrhythmias, mostly sustained ventricular tachycardia. Because of these life-threatening arrhythmias, most of the study designs included implantation of a cardioverter-defibrillator or amiodarone therapy which limited its widespread testing and application. In the myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) trial, implantation of autologous skeletal myoblasts failed to improve heart function [22]. Most of the studies have used open heart surgery for implanting these cells.

Human Adult Bone Marrow-Derived Stem Cells

Adult bone marrow contains multiple stem cell populations. Jackson and coworkers showed regeneration of cardiomyocytes and endothelial cells in a mouse model in which a myocardial infarction was induced by ligating the coronary artery [23]. Orlic et al. also showed that injection of mouse bone marrow cells in the infarcted myocardium led to the formation of cardiomyocytes, endothelial cells, and smooth muscle cells [6].

Based on these animal studies, human adult bone marrow-derived stem cells have been studied in various trials. Bone marrow serves as a source for an intrinsic repair mechanism in the heart which is not enough to overcome the damage caused by myocardial ischemia. In clinical trials, myocardial cell delivery has been performed using intracoronary infusion, an endomyocardial approach, or during open heart surgery. Precursor cells with an ability to differentiate into cardiomyocyte or endothelial cells are present within the mononuclear fraction of the bone marrow cells. Therefore, bone marrow mononuclear cells have been used in multiple clinical trials. In the Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trial, intracoronary infusion of cells improved the left ventricular ejection fraction from 51% to 58%, [24]. In the intracoronary autologous bone marrow cell transfer after myocardial infarction (BOOST) trial, improvement of 6.7% in left ventricular ejection fraction was seen in the treatment group [25]. A few of the studies showed no improvement in heart function, but there was some benefit in reducing the myocardial scar.

Mesenchymal Stem Cells

Mesenchymal stem cells account for 0.001–0.01% of bone marrow cells and have a potential to differentiate into any cell of mesenchymal origin including muscle, bone, ligament, tendon, and adipose tissue. They can be harvested easily from the host bone marrow and do not cause any immunological problems. Mesenchymal stem cells can differentiate into cardiomyocytes and endothelial cells; thereby help improve myocardial function and neovascularization. In animal models, improvement in heart function and regeneration of myocardium has been seen. Chen et al. showed a significant improvement in left ventricular function and reduction in myocardial infarct size after intracoronary injections of mesenchymal stem cells in patients after myocardial infarction [26].

Endothelial Progenitor Cells

Endothelial progenitor cells have been isolated both in blood and bone marrow. They can be attracted to

the site of myocardial infarction and can differentiate into blood vessels. Endothelial progenitor cells can reach the infarcted region within 48 h of intravenous injection. Due to their property of neovascularization, there is reduction in cardiomyocyte apoptosis, scar formation, and left ventricular remodeling along with improvement in cardiac function. Erb et al. [27] mobilized these endothelial progenitor cells from the bone marrow and isolated them in the blood. After intracoronary infusion, improvement in cardiac function and reduction in size of the myocardial infarct was seen [27].

Adipose Tissue-Derived Stem Cells

Adipose tissue has an ability to differentiate into muscle, bone, and cartilage. In animal models, adipose tissue has been shown to differentiate into cardiomyocytes and help in neovascularization [28]. There are limited data in humans so far regarding their use. The cells can be derived from liposuction aspirates.

In the adipose-derived regenerative cells in patients with ischemic cardiomyopathy (PRECISE) trial, after transplantation of adipose-derived regenerative cells, efficacy was measured by using echocardiography, magnetic resonance imaging, single-photon emission computed tomography, metabolic equivalents, and maximal oxygen consumption [29]. In the treatment group, improvements in left ventricular mass measured by magnetic resonance imaging and in a wall motion score index were seen [29]. Single-photon emission computed tomography also showed reduction in inducible myocardial ischemia [29].

Cardiac Stem Cells

As described above, the heart was initially thought to be a postmitotic organ with no properties of differentiation. However, recent studies have shown that heart muscle has a subpopulation of cells which can maintain a homeostatic environment by replacing the dying cells [30]. These cells are called cardiac stem cells. During an ischemic event, release of cytokines or other markers from the inflamed area can cause the differentiation of these resident stem cells into cardiomyocytes, smooth muscle, and endothelial cells. The number of the resident cardiac stem cells continues to decrease with age and at the time of an ischemic event; the turnover of the cells and role in cardiac repair is not significant enough to overcome the amount of damage to the myocardium.

c-kit$^+$ cells are one of the most commonly used cardiac stem cells. Beltrami et al. described the isolation of these cells in adult rat hearts in 2003 [31]. These cells can be found to be present within the atria and ventricles of the heart. These cells have also been isolated in the human heart, and testing these cells in a mouse model showed differentiation of these cells into human cardiomyocytes, resulting in formation of vessels [32].

Sca-1$^+$ progenitor cell is another subtype of cardiac stem cell which has been shown to differentiate into cardiomyocytes. These cells have been tested in the mouse model by intramyocardial injection and have been shown to differentiate into cardiomyocytes and blood vessels [33]. This study showed improvement in left ventricular ejection fraction and reduction in ventricular remodeling at 3 months after myocardial infarction [33].

isl-1$^+$ progenitor cells have been shown to differentiate into endothelial cells, cardiomyocytes, and smooth muscle cells. Side population cells have gained importance as a distinct entity with the capability of differentiating into cardiomyocytes, but they overlap with the Sca-1$^+$ progenitor cells.

Cardiosphere-derived cells have a property to grow in self-adherent clusters and can differentiate into cardiomyocytes. They have been shown to improve left ventricular function in mouse models [34].

Cardiac stem cell therapy has been applied to clinical trials. In the cardiac Stem Cells In Patients with Ischemic Cardiomyopathy (SCIPIO) trial, use of c-kit$^+$ cells in patients after myocardial infarction led to improvement in left ventricle ejection fraction by 8% [35]. In the CADUCEUS (CArdiosphere-Derived aUtologou stem CElls to reverse ventricULar dySfunction) trial, cardiac stems cells were transplanted via an intracoronary method in patient with acute myocardial infarction and reduced left ventricular ejection fraction. The treatment arm showed reduction in scar formation and an increase in the heart muscle mass, but no difference in left ventricular ejection fraction was seen [36]. These trials have shown a few encouraging results but still leave many questions unanswered. We are not up to a point yet where stem cell therapy can help to overcome the epidemic of ischemic heart disease and congestive heart failure, which the United States and other countries are facing.

CONCLUSIONS

Stem cell therapy presents a relatively recent exciting new form of treatment for ischemic heart disease. Even though a number of randomized clinical trials have been performed, the exact clinical role and benefit of this therapy is still being defined. In addition, most of the studies are small to moderate size and differ in types of intervention as well as outcomes studied. Moving forward, we need large randomized, placebo-controlled clinical studies with uniform use of cell lines, delivery methods, and outcome measures to determine whether this form of therapy can be used in clinical practice.

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CHAPTER

6

Vasculogenesis and Angiogenesis

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Vasculogenesis and angiogenesis are the fundamental processes by which new blood vessels are formed. Vasculogenesis is defined as the differentiation of endothelial precursor cells (EPCs), or angioblasts, into endothelial cells (ECs) and the *de novo* formation of a primitive vascular network [1]. Angiogenesis refers to the growth of new capillaries (new blood vessels that lack a fully developed tunica media) from preexisting blood vessels either via "sprouting" or "intussusception" [2] (Figure 6.1).

In contradistinction to these two processes, arteriogenesis involves remodeling of small arterioles, with very low or no blood flow, into larger conducting arteries with a fully developed tunica media (see Chapter 2 for details on arteriogenesis). Substantial progress has been made in our understanding of the cellular and molecular mechanisms underlying these processes of neovascularization. Preclinical studies in animal models have explored the potential use of various growth factors with or without progenitor cells to treat myocardial ischemia with promising results. However, clinical trials of therapeutic coronary angiogenesis for the treatment of ischemic heart disease have been largely disappointing [3,4]. As the field of therapeutic angiogenesis evolves from preclinical investigations to clinical trials, new challenges have emerged [5]. Some of these include selection of appropriate patient populations for clinical studies, choice of therapeutic strategy (gene versus protein versus cell therapy), route of administration, and selection and assessment of therapeutic endpoints. A more in-depth understanding of the biology of vasculogenesis and angiogenesis may help overcome some of these existing challenges.

VASCULOGENESIS

The cardiovascular system is the first functional organ system to develop in the embryo. The differentiation of EPCs or angioblasts from the mesoderm and the formation of a primitive vascular network from angioblasts are the two distinct steps during the onset of vascularization that together constitute embryologic "vasculogenesis" [1]. Blood islands are aggregates of cells that emerge from the splanchnopleuric mesoderm and are the earliest discernible vascular structures in the embryo [6]. EPCs are located at the periphery of the blood islands, whereas the cells in the center of the blood islands are termed hematopoietic stem cells. In addition to this spatial association, hematopoietic stem cells and EPCs share certain antigenic determinants, including vascular endothelial growth factor receptor-2 (VEGFR-2, also known as Flk-1 in mice and KDR in humans), angiopoietin-1 (Ang-1) receptor Tie-2, stem cell antigen-1 (Sca-1), and CD34. This has led to the assumption that EPCs and hematopoietic stem cells may derive from a common precursor called the hemangioblast [7]. Growth and fusion of multiple blood islands in the yolk sac of the embryo ultimately give rise to the primitive capillary network. After the onset of blood circulation, this primitive capillary network differentiates into an arteriovenous vascular system [8].

Members of the fibroblast growth factor (FGF) family are potent inducers of the mesoderm [9]. Data from dominant-negative mutants and knock-out mice provide direct evidence that FGF-receptor tyrosine kinases and FGF-4 are required for the normal development of the posterior and lateral mesoderm, suggesting that the FGF/FGF-receptor system is the crucial signal transduction

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00006-1

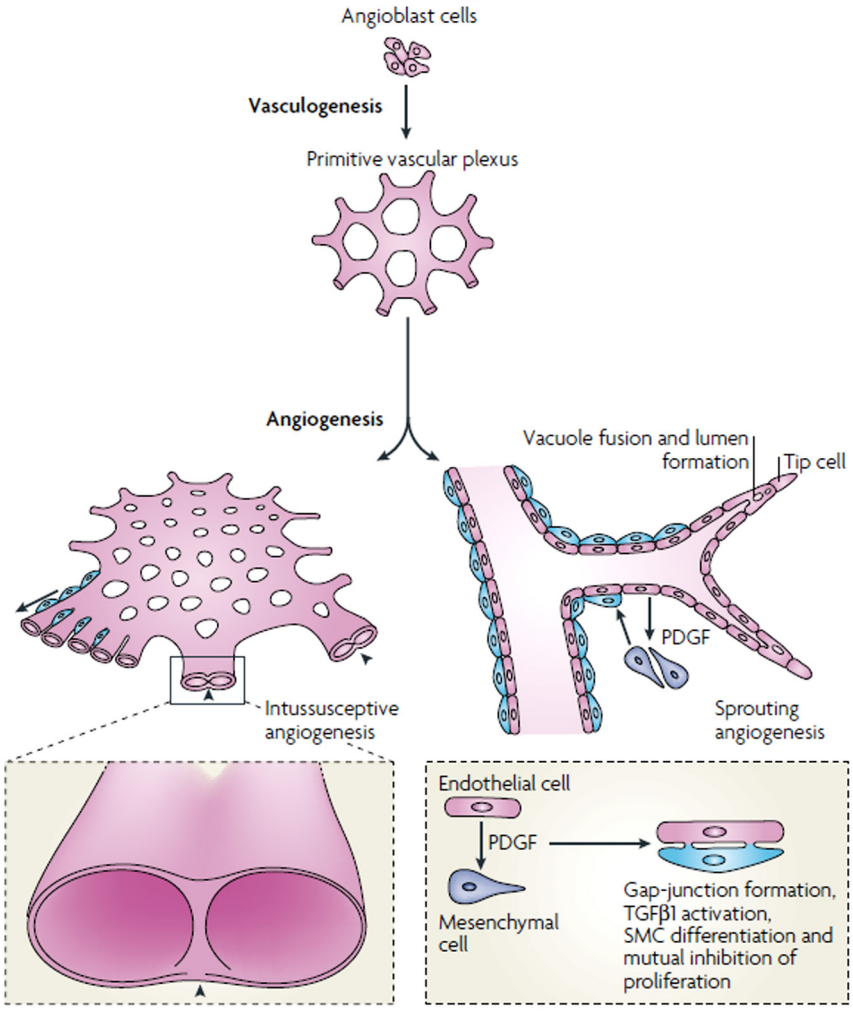

FIGURE 6.1 Vasculogenesis and Angiogenesis. The differentiation of angioblasts, or endothelial precursor cells, from mesoderm and the formation of primitive vascular plexus from angioblasts are the two distinct steps during the onset of vascularization that together constitute vasculogenesis. Angiogenesis refers to the growth of new capillaries from preexisting blood vessels either via sprouting or intussusception. *Source: Reprinted with permission from Macmillan Publishers Ltd; Ref. [173], copyright 2007.*

pathway for mesoderm induction *in vivo* [10,11]. Despite the crucial function of FGF-receptors in mesoderm induction, these receptors do not play a major role in the subsequent morphogenesis of the vascular system [12,13]. On the other hand, VEGF is an important regulator of vasculogenesis, a notion supported by the observation that the first molecule known to be expressed in a population of mesodermal cells giving rise to EPCs is VEGFR-2. VEGF secreted by the endoderm acts in a paracrine manner to support the differentiation of VEGFR-2-expressing mesodermal cells to angioblasts. Gene knock-out experiments have further established the pivotal role of VEGF, VEGFR-2, and VEGFR-1 (also known as Flt-1) in embryonic vasculogenesis. Heterozygous mice lacking one copy of the VEGF gene die *in utero* with aberrant blood vessel formation in the yolk sac and the embryo [14,15]. VEGFR-2 (Flk-1)-deficient mice are both unable to form blood islands and unable to undergo vasculogenesis as a result of defects in angioblastic and hematopoietic lineages [16]. VEGFR-1 plays a role later, as mice lacking Flt-1 can produce angioblasts, but their assembly into functional blood vessels is impaired [17]. Cell adhesion molecules such as vascular endothelial cadherin (VE-cadherin, also known as cadherin 5 or CD144), platelet-endothelial cell adhesion molecule-l (PECAM-1 or CD31), CD34, and fibronectin and its receptor α5β1 integrin also play an important role in vasculogenesis [1,18]. Pathways that regulate coronary vasculogenesis are distinct from those governing vasculogenesis in the rest of the embryo and involve a complex array of growth factors, adhesion molecules, and signaling molecules expressed by the epicardium, the subepicardial mesenchyme, and the myocardium [19].

For many years, the prevailing dogma stated that vasculogenesis occurred only during embryonic development. Recent evidence, however, has shown that EPCs contribute to new blood vessel growth not only in the

embryo but also in ischemic and malignant tissues in the adult, a concept termed "postnatal vasculogenesis" [20]. Asahara et al. [21] first isolated CD34-positive EPCs from human peripheral blood; once adherent, these putative EPCs were shown to differentiate into ECs *in vitro*. In animal models of hindlimb ischemia, heterologous, homologous, and autologous EPCs administered systemically were found to be incorporated into sites of active neovascularization in ischemic muscles of the affected hindlimb providing the first evidence for postnatal vasculogenesis with important therapeutic implications [21]. Subsequent studies demonstrated a bone marrow origin of circulating EPCs responsible for postnatal vasculogenesis [22,23]. Further experimental studies showed that in addition to hindlimb ischemia, EPC-mediated postnatal vasculogenesis also contributes to endogenous neovascularization of developing tumors, wound healing, and myocardial ischemia, as well as physiological neovascularization [22].

ANGIOGENESIS

The term "angiogenesis" was first used in 1787 by British surgeon Dr John Hunter to describe blood vessels growing in the reindeer antler. However, it was the pioneering work of Dr Judah Folkman (who hypothesized that tumor growth is dependent upon angiogenesis) in the 1970s that paved the way for future discoveries in the field of angiogenesis. These studies have enhanced our current understanding of its biology, and have led to the development of novel antiangiogenic and proangiogenic therapies [24].

Sprouting Angiogenesis

The first description of sprouting angiogenesis was reported by Ausprunk and Folkman [25], which included the following stages: (i) degradation of basement membrane, (ii) migration of ECs into the connective tissue, (iii) formation of a solid cord of ECs, (iv) lumen formation, and (v) anastomoses of contiguous tubular sprouts to form functional capillary loops, parallel with the synthesis of the new basement membrane and the recruitment of pericytes. Recent studies have provided further insights into fundamental aspects of sprouting angiogenesis that have led to a mechanistic model of vessel branching [26–29].

Basement Membrane Degradation

ECs and their neighboring mural cells (pericytes and vascular smooth muscle cells) share a common basement membrane composed of extracellular matrix (ECM) proteins such as collagen IV and laminin. This basement membrane and the layer of mural cells prevent resident ECs from leaving their original position. At the onset of sprouting, proteolytic breakdown of the basement membrane and detachment of mural cells is required for ECs to be liberated. Basement membrane degradation is mediated by proteinases, including matrix metalloproteinases (MMPs) such as membrane type (MT)-MMP1, plasminogen activators such as urokinase plasminogen activator (uPA), heparanases, chymases, tryptases, and cathepsins [30,31]. Control of these proteinases is important since excessive degradation of the ECM leaves too little support for the branch to sprout and can inhibit angiogenesis [32]. Activity of these proteinases is regulated by their respective inhibitors such as tissue inhibitor of metalloproteinases (TIMPs) and plasminogen activator inhibitor-1 (PAI-1) [33,34]. Proteinases also facilitate EC sprouting by releasing matrix-bound angiogenic growth factors such as FGF, VEGF, and transforming growth factor-β (TGF-β), and by proteolytically activating angiogenic chemokines such as IL-1β [35]. On the other hand, proteinases also play a role in the termination of angiogenesis by liberating matrix-bound antiangiogenic molecules such as thrombospondin-1 (TSP-1), canstatin, tumstatin, endostatin, and platelet factor-4 (PF-4), and by inactivating angiogenic cytokines such as stromal cell-derived factor 1. The proangiogenic growth factor Ang-2 stimulates detachment of mural cells [36].

Endothelial Tip and Stalk Cells

The concept of "tip" and "stalk" cell in sprouting angiogenesis was first introduced by Gerhardt et al. [37]. Tip and stalk cells have distinct morphologies and functional properties. The tip cell extends numerous filopodia that serve to guide the new vessel branch in a certain direction toward an angiogenic stimulus. Tip cells primarily migrate but proliferate only minimally, in contrast to stalk cells which do proliferate. Thus, tip cells are migratory and polarized, whereas stalk cells produce fewer filopodia, are more proliferative, form tubes and branches, and form the nascent vascular lumen cells [38]. The phenotypic specification of ECs as tip or stalk cells is extremely transient and reversible, depending on the balance between proangiogenic factors such as VEGF and jagged1 (JAG1), and suppressors of EC proliferation such as delta-like ligand 4 (DLL4)/Notch signaling [28].

Role of VEGF and VEGFRs

The first member and the master regulator of angiogenesis, VEGF-A (also known as VEGF), was cloned in 1989 [39]. Subsequently, four other members of the human VEGF family were identified—VEGF-B, VEGF-C (also called VEGF-2), VEGF-D, and placental growth factor (PlGF) [40–43]. VEGFs mediate their cellular effects through interaction with three tyrosine kinase receptors—VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR), and VEGFR-3 (Flt-4) [44].

During sprouting angiogenesis, VEGF stimulates tip cell induction as well as the formation and extension of filopodia via VEGFR-2, which is abundant on filopodia [37]. Blockade of VEGFR-2 is associated with

sprouting defects [45]. The tip cells respond to VEGF only by guided migration; the proliferative response to VEGF occurs in the stalk cells. Whereas tip cell migration depends on the gradient of VEGF, proliferation is regulated by its concentration [37]. VEGFR-3 is expressed in the embryonic vasculature but later becomes confined to the lymphatics. However, tip cells can reexpress VEGFR-3, and genetic targeting of VEGFR-3 or blocking of VEGFR-3 signaling with monoclonal antibodies results in decreased sprouting, vascular density, vessel branching, and EC proliferation [46]. VEGFR-1, on the other hand, is predominantly expressed in stalk cells, and is involved in sprout guidance and the limitation of tip cell formation [47]. VEGFR-1 expression is induced by Notch signaling which results in reduced VEGF availability, thereby preventing tip cell outward migration. Loss of VEGFR-1 increases sprouting and vascularization [47].

Role of Notch and Notch Ligands

The differentiation of ECs into tip and stalk cells is controlled by the Notch pathway [28]. Notch-1, Notch-4, and three Notch ligands (JAG1, DLL1, and DLL4) are expressed in ECs and play an important role in arterial/venous specification as well as the selection of tip/stalk phenotype during sprouting angiogenesis [48]. Notch-1-deficient ECs adopt tip cell characteristics. Notch signaling activity is low in tip cells and high in stalk cells. Conversely, tip cells express higher levels of the Notch ligand, DLL4, as compared to stalk cells. A feedback loop exists between VEGF and Notch/DLL4 which regulates all of the VEGFRs. VEGF/VEGFR-2 enhances DLL4 expression in tip cells [45]. Thus, tip cells with low Notch activity have high VEGFR-2 and low VEGFR-1 expression, which in turn results in higher levels of DLL4 expression. DLL4-mediated activation of Notch in neighboring ECs prevents them from becoming tip cells by downregulating VEGFR-2 and VEGFR-3, while upregulating VEGFR-1. Following VEGF exposure, all cells upregulate DLL4. However, ECs that express DLL4 more rapidly or at higher levels have a competitive advantage to become tip cells, as they activate Notch signaling in neighboring cells more effectively. In contrast to DLL4, the Notch ligand, JAG1, is expressed primarily by stalk cells. Stalk cell JAG-1 antagonizes DLL4 activity, thus reducing the induction of Notch signaling in the adjacent tip cell, hence maintaining its responsiveness to angiogenic stimuli, causing it to migrate outwards. The integrated feedback loop between VEGF and Notch helps to establish a stable pattern of tip and stalk cells [49].

Role of Semaphorins, Netrins, and Ephrins

Developing vessels use tip cells to guide sprouts properly; however, less is known about the molecular mechanisms or cues that control tip cell guidance. Among these are the semaphorins, which are secreted or membrane-bound guidance cues that interact with receptor complexes, formed by neuropilins (NRPs) alone or the NRP/plexin family of proteins [50,51]. Serini et al. [52] demonstrated that during vascular development and experimental angiogenesis, ECs express chemorepulsive signals of class 3 semaphorins (SEMA3 proteins) that localize at nascent adhesive sites in spreading ECs. Disrupting endogenous SEMA3 function in ECs stimulates integrin-mediated adhesion and migration to the ECM, whereas exogenous SEMA3 proteins antagonize integrin activation. SEMA3E-Plexin-D1 negatively regulates the activity of the VEGF-induced DLL4/Notch signaling pathway, which influences EC tip/stalk specification [53]. The class 4 semaphorin SEMA4D induces EC migration and tubulogenesis *in vitro* and stimulates blood vessel formation *in vivo* through plexin-B1 receptors [54].

Netrins are secreted bifunctional guidance cues, which also bind to ECM components. Attraction and repulsion is mediated via binding to the DCC (deleted in colorectal cancer) and UNC (uncoordinated) 5 family of receptors respectively [55]. UNC5B expression is increased in tip cells and its inactivation results in enhanced sprouting, whereas Netrin1 prompts filopodia retraction of ECs, consistent with a suppressive function of netrins and UNC5B on vessel growth [56].

Ephrins and their Eph receptors are regulators of cell contact-dependent signaling [57]. Ephrins generate mostly repulsive signals. Ephrin-B2 is expressed in arterial ECs, whereas EphB4 is a marker of venous ECs. Both regulate vessel morphogenesis, and loss of ephrin-B2 or EphB4 leads to vascular remodeling defects [57]. Ephrin-B2-mediated reverse signaling also controls VEGFR internalization and tip cell behavior. ECs lacking Ephrin-B2 reverse signaling are unable to internalize VEGFR-2 and VEGFR-3 and cannot transmit VEGF signals properly, resulting in impaired filopodial extension and sprouting [58,59].

Lumen Formation and Perfusion

Formation of vascular lumen by ECs is a critical step in the angiogenic process that occurs during invasion and growth of the incipient vascular sprout. Two distinct mechanisms of lumen formation have been described. Observations in intersomitic vessels suggest that ECs form a lumen by coalescence of intracellular (pinocytic) vacuoles, which interconnect with similar vacuoles from neighboring ECs—a process termed "cell hollowing" [60]. On the other hand, recent studies in large axial vessels suggest that ECs adjust their shape and rearrange their cell–cell junctions to open up a lumen, a process called "cord hollowing" [61]. In this model, ECs first acquire a distinct phenotype characterized by an apico-basilar polarity and junction-mediated inhibition. EC polarization starts with the delivery to the apical (luminal) membrane of de-adhesive negatively charged glycoproteins such as CD34 and podocalyxin that confer a repulsive signal, opening

up the lumen [62]. VE-cadherin is required for localizing CD34-sialomucins to the endothelial cell–cell contact [63]. Subsequent changes in EC shape, driven by VEGF and Rho-associated protein kinase (ROCK), are required to expand the lumen [61,63]. Vascular lumen expansion is force-dependent and involves F-actin cytoskeleton and/or blood flow. The onset of blood flow in the new lumen shapes and remodels vessel connections. Upon perfusion, oxygen and nutrient delivery decreases VEGF expression and inactivates endothelial oxygen sensors, thus shifting EC behavior toward a quiescent phenotype [29].

Network Formation

Network formation, or expansion of the complex, interconnected meshwork of crude capillary tubules, results from coalescence of sprouts or intussusceptive microvascular growth (IMG). Tip cells contact other tip cells to add new vessel circuits to the existing capillary network. Tip cell filopodia interact to initiate VE-cadherin-containing junctions to consolidate the connections. Macrophages may also facilitate sprout fusion by accumulating at sites of vessel anastomosis and interacting with filopodia of neighboring tip cells [64]. IMG is described in detail in "Intussusceptive Angiogenesis" section.

Remodeling and Pruning

The initial capillary network generated as a result of vascular sprouting consists of a homogenous web of EC tubes and sacs. This plexus is created in excess and the final adjustment of vascular density involves the regression of unnecessary vessels through a process of vascular remodeling and pruning which creates a more differentiated vascular network [38]. Remodeling determines the formation of large and small blood vessels, the association with mural cells, the establishment of directional flow, and the adjustment of vascular density to meet the nutritional requirements of the tissue supplied. Remodeling involves the growth of new vessels and the regression of others as well as changes in the diameter of vessel lumens and vascular wall thickening. Remodeling is a complex phenomenon that involves various signaling molecules, one of which includes the interaction between the receptor tyrosine kinase Tie-2 and its ligand Ang-1 [65,66]. Mice lacking Tie-2 and Ang-1 die during early embryonic life and show a persisting capillary plexus, suggestive of a defect in vascular remodeling and angiogenesis [66,67].

Pruning, defined as removal of ECs which form redundant channels, was first described by Ashton [68] in retinal vessels. In retinal blood vessels, oxygen supply is regarded as a key factor in pruning. Oxygen suppresses VEGF production and leads to regression of already formed blood vessels via EC apoptosis [69]. Thus, pruning is the consequence of apoptosis of ECs due to the toxic effect of a combination of high oxygen and low VEGF levels at the onset of blood flow through the newly formed vascular system [70]. In addition to EC apoptosis, "intussusceptive vascular pruning" also occurs under low VEGF conditions [71]. Another apoptosis-independent pruning, driven by EC migration or rearrangement, has also been described [72–74]. Regardless of the mechanism, only a subset of vessels is designated for pruning, and the selection of these vessels is highly regulated. However, the factors that target a particular vessel branch for pruning remain ill-defined. Low blood flow and decreased Notch/DLL4 signaling have been shown to play a role, and may be linked, as low blood flow can affect endothelial shear stress and lead to a decrease in Notch activation [73,75].

Vessel Maturation and Stabilization

Maturation of the nascent vasculature involves differentiation and recruitment of mural cells, deposition of ECM, specialization of the vessel wall for structural support, and adaptation of vascular patterning to local tissue needs [76]. ECs also acquire tissue-specific differentiation adapted to meet local homeostatic demands [77].

Recruitment of mural cells is a fundamental feature of vessel maturation and is regulated by platelet-derived growth factor B (PDGFB)/PDGF receptor-β (PDGFR-β) signaling. PDGFB is secreted as a homodimer from the ECs of angiogenic sprouts where it serves as an attractant for comigrating pericytes, which in turn express PDGFR-β [78,79]. PDGFB also stimulates migration and proliferation of both vascular smooth muscle cells (VSMCs) and pericytes, and induces undifferentiated mesenchymal cells to differentiate into mural cells [80,81]. Inactivation of PDGFB or PDGF-β in mice leads to pericyte deficiency, vascular dysfunction, micro-aneurysm formation, bleeding, and perinatal death [79].

The bioactive lipid sphingosine-1-phosphate (S1P) is also indispensable to vessel maturation. EC derived S1P acts on the G protein-couples receptors $S1P_{1–5}$, formerly known as endothelial differentiation gene (Edg) receptors, to regulate cytoskeletal, adhesive, and junctional changes that affect cell migration, proliferation, and survival [82,83]. $S1P_{1–3}$ (Edg1, 5 and 3, respectively) are widely expressed. $S1P_4$ (Edg6) expression is restricted to lymphoid tissue and the lung, while $S1P_5$ (Edg8) is restricted to the central nervous system [84]. S1P signaling in ECs is critical for proper trafficking of N-cadherin to cell adhesions between ECs and mural cells [85]. Disruption of $S1P_1$ or loss of both $S1P_2$ and $S1P_3$ in mice causes defective coverage of VSMCs and pericytes [84].

Ang-1 produced by perivascular and mural cells, acts on its receptor Tie-2 expressed on ECs. Ultrastructural analysis suggests that Tie-2-knockout blood vessels lack mural cells [86]. Similarly, mice deficient in Ang-1 demonstrate poor association between ECs and surrounding matrix and mesenchyme, suggesting that Ang-1

mediates adhesion between these components [67]. However, subsequent reports have challenged the direct role of Tie-2 in pericyte recruitment, and have suggested that the pericyte defects observed in Tie-2 and Ang-1 knockouts may be secondary to EC apoptosis [87,88]. Mural cells also require ephrin-B2 for association around ECs, as mural cell-specific ephrin-B2 deficiency causes mural cell migration and vascular defects [57].

TGF-β signals via two distinct TGF-β type I receptors in vascular cells (activin receptor-like kinase ALK1 and ALK5) triggering discrete intracellular signaling pathways with opposing effects on proliferation, migration, and tube formation. Whereas the TGF-β/ALK5 pathway leads to inhibition of cell migration and proliferation, the TGF-β/ALK1 pathway induces mural cell migration and proliferation [89]. Endoglin, a type III TGF-β coreceptor specifically expressed in ECs, modulates ALK1 and ALK5 signaling [90]. Loss of function of TGF-β1, TGF-β receptor type II, endoglin, or ALK1 in mice results in defective vessel maturation due to impaired mural cell differentiation and development [91–94]. In humans, mutations in endoglin or ALK1 cause hereditary hemorrhagic telangiectasia type I and type II, respectively, an autosomal dominant disease characterized by arteriovenous malformations with abnormally remodeled vessel walls [95,96].

Notch3 signaling also regulates vessel maturation and is required for proper maturation of arterial vascular smooth muscle cell [97]. Mutations in human Notch3 cause CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), a late-onset disorder causing stroke and vascular dementia [98].

Quiescence—Endothelial Phalanx Cells and Survival Signals

During the transition from active sprouting to quiescence, endothelial tip cells adopt a "phalanx" phenotype, resembling the phalanx formation of ancient Greek military soldiers [99] (Figures 6.2 and 6.3).

Quiescent ECs form a barrier between blood and surrounding tissues to control exchange of fluids and solutes and transmigration of immune cells. This barrier function depends on the ability of ECs to regulate cell–cell adhesion between each other and neighboring cells. This involves several adhesion molecules, including VE-cadherin and N-cadherin at adherens junctions, as well as occludins and members of the claudin and junctional adhesion molecule (JAM) family at tight junctions [100]. VE-cadherin is an important component of EC junctions. VE-cadherin maintains EC quiescence through recruitment of phosphatases that dephosphorylate VEGFR-2, thus restraining VEGF signaling [101]. Activation of Tie-2 by Ang-1 protects vessels from VEGF-induced leakage by inhibiting VEGF's ability to induce endocytosis of VE-cadherin.

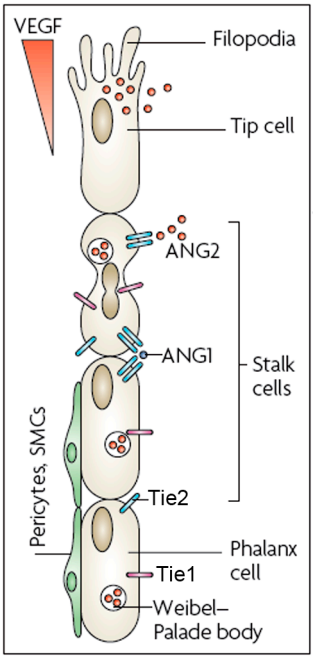

FIGURE 6.2 Endothelial Tip, Stalk, and Phalanx cells. Sprouting angiogenesis is initiated in response to VEGF gradients by invading ECs, known as tip cells. The tip cells extend numerous filopodia that serve to guide the new vessel branch in a certain direction towards an angiogenic stimulus. During sprouting angiogenesis, VEGF stimulates tip cell induction as well as formation and extension of filopodia via VEGFR-2, which is abundant on filopodia. Tip cells are followed by a zone of proliferating and differentiating ECs, known as stalk cells. Below these stalk cells, phalanx cells are in intimate contact with pericytes and smooth muscle cells (SMCs), which keep them in a protected, quiescent state. Tie receptors are expressed by stalk cells and phalanx cells. Angiopoietin 2 (ANG2) is abundantly expressed by angiogenic ECs. Stalk cells and phalanx cells might store ANG2 in Weibel–Palade bodies. *Source: Reprinted with permission from Macmillan Publishers Ltd; Ref. [36], copyright 2009.*

Quiescent ECs must adopt survival properties to maintain the integrity of the vessel lining. Various signaling molecules are involved in EC survival. VEGF, produced by ECs as an intracrine factor, acts as a survival signal preventing EC apoptosis under nonpathological conditions [102]. The survival function of VEGF depends on an interaction among VEGFR-2, β-catenin, and VE-cadherin [103]. FGF has also been implicated in maintaining vascular integrity due to its ability to anneal adherens junctions. Inhibition of FGF signaling results in dissociation of both adherens junctions and tight junctions, subsequent loss of ECs, and vessel disintegration [104]. Ang-1 also promotes, whereas Ang-2 suppresses, EC survival and quiescence [36]. Several endothelial survival factors (VEGF, Ang1, and $\alpha_v\beta_3$) suppress p53, p21, p16, p27, and Bax. Concurrently, they activate the PI3-kinase/Akt, p42/44 mitogen-activated protein kinase, Bcl-2, A1, and survivin survival pathways [105]. Blood flow is another important survival signal for ECs as physiological shear stress

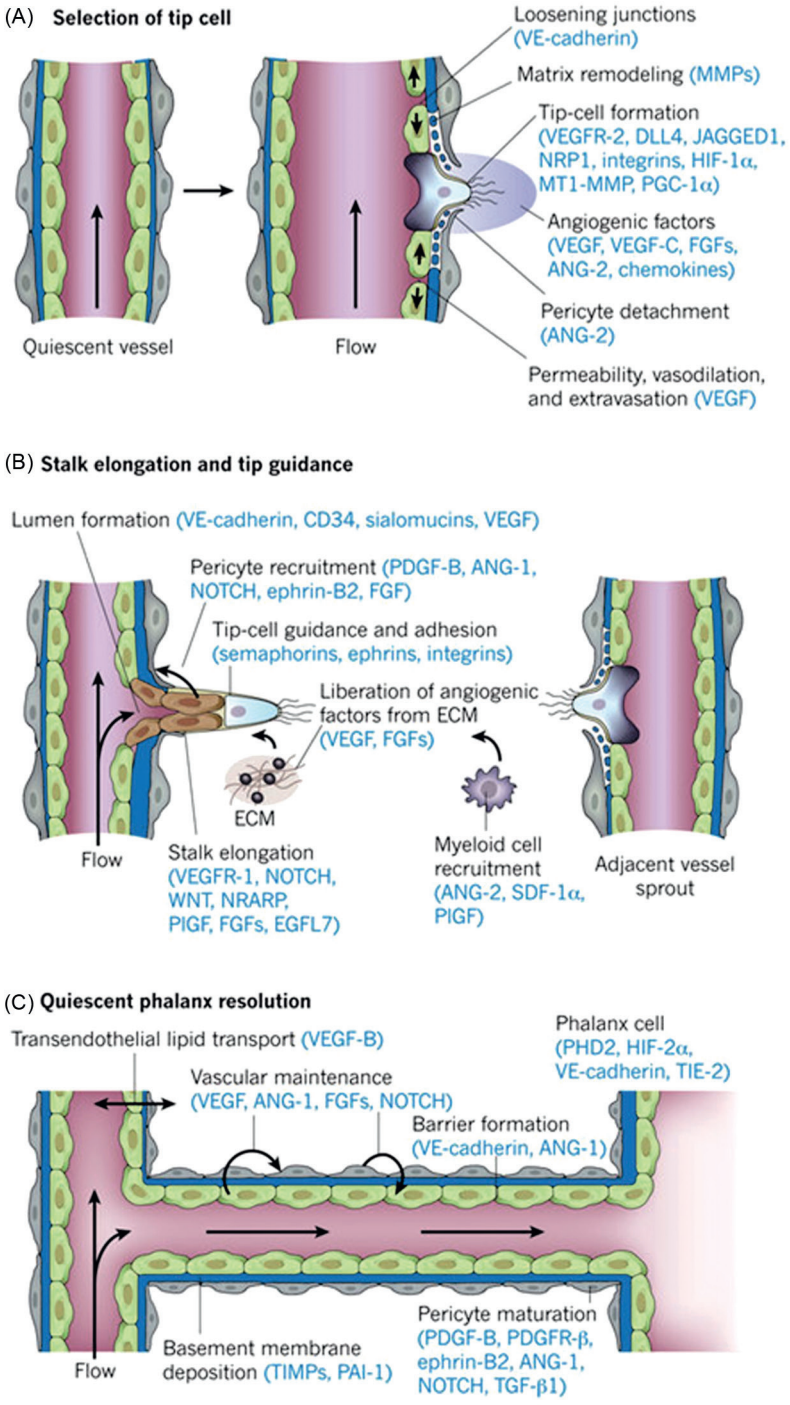

FIGURE 6.3 Molecular Regulation of Sprouting Angiogenesis. (A) Initiation of sprouting angiogenesis requires degradation of the basement membrane, pericyte detachment, and loosening of EC junctions. Basement membrane degradation is mediated by proteinases, including MMPs. Tip cell induction is regulated by the VEGF/VEGFR-2 and Notch/DLL4/JAGGED1 signaling pathways. (B) Tip cells navigate in response to guidance signals (such as semaphorins, netrins, and ephrins) and adhere to the ECM, mediated by integrins, to migrate. Stalk cells proliferate, elongate, and form a lumen. VE-cadherin, CD34-sialomucins and VEGF mediate lumen formation via cord hollowing. Network formation results from coalescence of sprouts or intussusceptive microvascular growth. Vessel maturation and stabilization involves differentiation and recruitment of mural cells (regulated by PDGF-B, Ang-1, and Notch3) and deposition of ECM. (C) During the transition from active sprouting to quiescence, endothelial tip cells adopt a phalanx phenotype. Quiescence is also characterized by re-establishment of cell–cell junctions, deposition of basement membrane, maturation of pericytes, and production of EC survival signals. *Source: Reprinted with permission from Macmillan Publishers Ltd; Ref. [27], copyright 2011.*

reduces endothelial turnover and inhibits TNFα-mediated EC apoptosis. Shear stress also activates the transcription factor Krüppel-like Factor 2 (KLF2), which promotes quiescence both by upregulating endothelial nitric oxide synthase (eNOS) and thrombomodulin, keeping vessels dilated, perfused, and free of clots, and by downregulating VEGFR-2, which prevents tip cell formation [106]. Other EC quiescence factors include bone morphogenic protein 9 (BMP9), cerebral cavernous malformation proteins (CCM1–3), and brain derived neurotrophic factor (BDNF).

Intussusceptive Angiogenesis

In 1986, while investigating the transformation of the capillary network in postnatal lungs of rats, Caduff et al. [107] observed that the lung capillary network could expand by insertion of new transcapillary tissue pillars. This concept represented a new mode of angiogenesis, distinct from capillary sprouting, and was named intussusceptive angiogenesis (IA) or IMG.

Formation of slender transcapillary tissue pillars is the key step in IA, and occurs in four distinct phases—*phase 1*: direct cell contact of ECs located directly opposite to each other in the capillary wall via protrusion of the walls into the vessel lumen, leading to the formation of an interendothelial transluminal bridge; *phase 2*: perforation of the endothelial bilayer and creation of a cylindrical tissue bridge extending across the lumen wrapped by ECs, with elements of interstitial tissue, mainly cytoplasmic extensions of myofibroblasts with their microfilaments inside the core of the cylinder; *phase 3*: framing of the pillar by cytoplasmic processes of pericytes alongside the lateral portions of the capillary walls; and *phase 4*: growth of the slender tissue pillar into a normal intercapillary mesh [108]. Some additional modes of transcapillary pillar formation such as by folding of the capillary wall, and capillary splitting and subsequent fusion have also been described [109,110].

The formation of transcapillary tissue pillars occurs not only in the capillary bed but also in small venules and arteries [111]. The morphological and functional outcomes differ according to the location, timing, and frequency of pillar formation. Disseminated pillar formation and enlargement within a capillary network results in capillary expansion. IMG has been demonstrated in the bone, retina, kidney, heart, and cerebral vasculature, as well as in tumor angiogenesis [112]. Pillars forming in parallel rows in capillary beds can merge and lead to the formation of small precapillary arteries and postcapillary veins, thereby contributing to the formation of the distal vascular trees, a process termed intussusceptive arborization (IAR) [113]. Lastly, when pillars appear at bifurcations of small feeding or collecting vessels, they lead to vascular remodeling and pruning, a process known as intussusceptive branching remodeling (IBR) [114,115].

Regulation of IA

In general, the mechanisms that are involved in the regulation of IA are poorly understood. However, there is sufficient evidence that flow alterations and hemodynamic forces have a major influence in initiating the process of pillar formation [116]. Experiments using the chick chorioallantoic membrane (CAM) have demonstrated an increase in IA in blood vessels in which the blood flow is enhanced by clamping one of the dichotomous arterial branches [113]. It remains unclear whether induction of IA in response to flow alterations is mediated by changes in hydrostatic pressure, shear stress, wall stress, or a combination of all these factors.

Besides blood flow and hemodynamic forces, IA is also regulated by molecular factors. Overexpression of VEGF is associated with sprouting angiogenesis and increased vascular permeability, whereas a drop in VEGF level or its withdrawal has been shown to induce vascular remodeling via IBR [71]. On the other hand, a few studies have shown that local application of VEGF induces IA in the chick CAM [117–119]. Response to VEGF (sprouting versus IA) may depend on the amount of VEGF expression, the release modality (slow versus rapid liberation), and/or differential expression of VEGFRs [120,121]. Besides VEGF, Tie-2, and Ang-1 play an important role in IA. Targeted deletion of Tie-2 expression in mice leads to deficient pillar formation [86]. On the other hand, overexpression of Ang-1, or of Ang-1 in combination with VEGF, leads to the formation of enlarged vessels with abundant small invaginations in the capillary plexus that are reminiscent of transcapillary pillars encountered during IA [122,123]. FGF2 may also regulate IA by inducing PDGFB responsiveness in pericytes through upregulation of PDGF receptors [113]. Other potential regulators of IA include erythropoietin [124], monocyte chemotactic protein 1 [125], and ephrins and Eph-B receptors [126].

THERAPEUTIC VASCULOGENESIS/ ANGIOGENESIS FOR ISCHEMIC HEART DISEASE

Preclinical studies in animal models have explored the potential use of various angiogenic growth factors with or without progenitor cells to treat myocardial ischemia with promising results. Based on the current understanding of the molecular biology of angiogenesis and data from preclinical animal studies, several clinical trials have been performed to date in an effort to stimulate tissue vascularization (i.e., therapeutic angiogenesis) in patients with myocardial ischemia resulting from CAD (Table 6.1). Most clinical trials have involved the introduction of either an angiogenic growth factor or its encoding DNA into the ischemic tissue either via

TABLE 6.1 Clinical Trials of Therapeutic Angiogenesis for Coronary Artery Disease Using Angiogenic Growth Factors

Reference	Agent	Type of therapy	Route of administration	Study design	*N* (Active/ Placebo)	Patient characteristics	Follow-up	Outcome assessment
[127]	FGF-1	Protein	Intramyocardial during CABG	Phase I	20/20	3-vessel CAD with CABG (including LIMA to LAD)	12 weeks	DSA (capillary angiogenesis)
[128]	FGF-2	Protein	Epicardial fat implantation during CABG	Phase I	8/0	Viable but ungraftable myocardium	3 months	Angina, MPI
[129]	FGF-2	Protein	Epicardial fat implantation during CABG	Phase I	16/8	Viable but ungraftable myocardium	16 ± 6.8 months	Angina, MPI, MRI
[130]	FGF-2	Protein	Intracoronary to left main	Phase I	17/8	Stable angina pectoris	1–28 days	AE
[131]	FGF-2	Protein	Intracoronary or intravenous	Phase I	59/0	CAD not amenable to mechanical revascularization	1, 2, 6 months	MPI
[132]	FGF-2	Protein	Intracoronary	Phase I	52/0	CAD not amenable to mechanical revascularization	1, 2, 6 months	ETT, MRI
[133]	FGF-2	Protein	Intracoronary or intravenous	Phase I	66/0	Severe CAD	N/A	PK/PD
[134]	FGF-2	Protein	Intracoronary	Phase II (FIRST)	251/86	CAD not amenable to mechanical revascularization, inducible ischemia ≥15% of LV, LVEF ≥ 30%	90, 180 days	ETT, MPI, CCS angina class, SAQ, SF36
[135]	Ad5-FGF4	Gene	Intracoronary	Phase I/II (AGENT)	60/19	Chronic stable angina pectoris (CCS class 2 or 3), single or multivessel CAD, at least one proximal major vessel with <70% stenosis	4, 12 weeks	AE, ETT
[136]	Ad5-FGF4	Gene	Intracoronary	Phase IIa (AGENT-2)	35/17	Chronic stable angina pectoris (CCS class 2–4), not a candidate for mechanical revascularization, single or multivessel CAD, at least one proximal major vessel with <70% stenosis, LVEF ≥ 30%, LV RPDS > 9%	8 weeks	SPECT MPI
[137]	Ad5-FGF4	Gene	Intracoronary	Phase IIb/III (AGENT-3 and -4)	355/177	Same as AGENT-2	1, 3, 6, 12 months	ETT, angina, QoL, coronary events or deaths
[138] (Inactive)	Ad5-FGF4	Gene	Intracoronary	Phase III (AWARE)	–	Women 18–75 years of age; similar criteria as AGENT-2	3, 6, 12 months	ETT, SPECT MPI, angina frequency, NTG use, CCS angina class, SAQ
[139,140]	Ad5-FGF4	Gene	Modified intracoronary	Phase III (ASPIRE)	Estimated 50/50	Same as AGENT-2	2, 12 months	SPECT MPI, angina frequency, NTG use, CCS angina class, SAQ

(*Continued*)

TABLE 6.1 (Continued)

Reference	Agent	Type of therapy	Route of administration	Study design	*N* (Active/ Placebo)	Patient characteristics	Follow-up	Outcome assessment
[141]	$VEGF_{165}$/FGF-2 plasmid	Gene	Intramyocardial	Phase II (VIF-CAD)	33/19	CCS class 3 or 4 angina, not a candidate for mechanical revascularization, significant ischemia on SPECT, LVEF > 35%	5, 12 months	SPECT MPI, ETT, SF-36, SAQ
[142]	$phVEGF_{165}$	Gene	Intramyocardial via minithoracotomy	Phase I	5/0	CCS class 3 or 4 angina refractory to all therapy, multivessel CAD, viable myocardium, LVEF ≥ 20%	30, 60 days	SPECT MPI, Angiography, angina frequency, NTG use
[143,144]	$AdVEGF_{121}$	Gene	Intramyocardial during CABG or via minithoracotomy	Phase I	31/0	CAD, reversible LV ischemia, LVEF ≥ 25%	30 days and 6 months	Angiography, SPECT MPI, ETT
[145]	$phVEGF_{165}$	Gene	Intramyocardial via minithoracotomy	Phase I	20/0	CCS class 3 or 4 angina, reversible ischemia, inoperable CAD	60, 90 days	Angina, SPECT MPI, angiography
[146]	rhVEGF	Gene	Intracoronary	Phase I	14/0	CAD not amenable to mechanical revascularization	30, 60 days	SPECT MPI
[147]	VEGF-P/L	Gene	Intracoronary during PTCA	Phase I	10/5	Stable CAD	6 months	Angiography
[148]	$phVEGF_{165}$	Gene	Intramyocardial via minithoracotomy	Phase I	13/0	Refractory anginal CCS class 3 or 4, multivessel CAD, viable myocardium	60 days	NOGA LV EMM, SPECT MPI, Angiography
[149]	phVEGF-2	Gene	Intramyocardial	Phase I	3/3	Refractory anginal CCS class 3 or 4, multivessel CAD not amenable to mechanical revascularization, viable myocardium, LVEF ≥ 20%	90 days	NOGA LV EMM, SPECT MPI
[150]	$phVEGF_{165}$	Gene	Intramyocardial via minithoracotomy	Phase I	7/0	Refractory CCS class 3 or 4 angina, multivessel CAD not amenable to mechanical revascularization, > 10% LV ischemia, LVEF > 30%	1, 3, 6, 12 months	SPECT MPI, Stress Echo with TVI, ETT, angiography
[151]	phVEGF-2	Gene	Intramyocardial	Phase I/II	12/7	Same as [149]	2, 4, 8, 12 weeks	CCS angina class, SAQ, ETT, LV EMM, SPECT MPI
[152]	rhVEGF	Gene	Intracoronary + intravenous	Phase II (VIVA)	115/63	CAD not amenable to mechanical revascularization, viable myocardium, LVEF ≥ 25%	60, 120 days	CCS angina class, SAQ, SF-36, DASI, ETT, MPI
[153]	VEGF-Adv or VEGF-P/L	Gene	Intracoronary during PCI	Phase II (KAT)	37/28/38	Stable angina CCS class 2 or 3	6 months	Angiography, ETT, SPECT MPI
[154]	VEGF-2 naked DNA	Gene	Intramyocardial	Phase I	30/0	CCS class 3 or 4 angina refractory to medical therapy, CAD not amenable to mechanical revascularization	1, 2, 3, 8, 12 months	CCS angina class, ETT, MACE

[155]	phVEGF$_{165}$	Gene	Intramyocardial	Phase I	22/0	Undergoing CABG (n=14) or not amenable to revascularization (n=8)	6 months	EF, MPI, angiography, NTG use
[156]	VEGF$_{165}$ naked DNA versus DMR	Gene	Intramyocardial	Phase I	10/12/13	CCS class 3 angina, inoperable CAD, viable myocardium	3 months	PET, ETT, CCS angina class, RAND-36, MFI-20
[157,158]	phVEGF$_{165}$	Gene	Intramyocardial	Phase II (EUROINJECT ONE)	40/40	Stable angina CCS class ≥ 3, inoperable CAD, inducible ischemia, at least one large epicardial vessel with < 70% stenosis, LVEF ≥ 40%	3 months	SPECT MPI, NOGA LV EMM, CCS angina class, angina frequency, NTG use, SAQ, exercise capacity
[159]	Ad-VEGF$_{121}$	Gene	Intramyocardial	Phase II (REVASC)	32/35	CCS class 2–4 angina refractory to medical therapy, CAD not amenable to mechanical revascularization	12, 26 weeks	ETT, SPECT MPI, CCS angina class, SAQ
[160]	VEGF$_{165}$+G-CSF versus VEGF$_{165}$	Gene	Intramyocardial	Phase I/II	16/16/16	Stable angina CCS class ≥ 3, inoperable CAD, reversible ischemia	3 months	SPECT MPI, CCS angina class, angina frequency, NTG use, SAQ, exercise capacity, LV volume
[161]	AdVEGF$_{121}$	Gene	Intramyocardial	Phase I	10/5	CAD not amenable to mechanical revascularization	12 months	AE
[162]	VEGF-Adv or VEGF-P/L	Gene	Intracoronary during PCI	Phase II (KAT)	37/28/38	Stable angina CCS class 2 or 3	8 years	AE, MACE
[163]	VEGF$_{165}$ naked DNA	Gene	Intramyocardial	Phase II/III (NORTHERN)	48/45	CCS class 3 or 4 angina refractory to medical therapy, reversible ischemia or viable myocardium, at least one large epicardial vessel with <70% stenosis, LVEF ≥ 20%	3, 6 months	SPECT MPI, ETT, CCS angina class, SAQ, SF-36
[164]	VEGF$_{165}$ naked DNA	Gene	Intramyocardial	Phase I	13/0	Refractory angina or heart failure, inoperable CAD, LVEF > 25%	1, 3, 6 months	SPECT MPI, ETT, Minnesota QoL questionnaire, CCS class, NYHA functional class
[165]	Ad$_{GV}$VEGF 121.10NH	Gene	Intramyocardial	Phase IIb (NOVA)	12/5	Severe CAD	12, 26, 52 weeks	ETT, symptoms
[166]	Plasmid VEGF$_{165}$	Gene	Intramyocardial	Phase I (GENESIS-I)	10/0	CAD not amenable to mechanical revascularization	2 years	CCS angina class, SPECT MPI, Stress Echo
[167]	AdVEGF$_{121}$	Gene	Intramyocardial during CABG or via minithoracotomy	Phase I	31/0	CAD, reversible LV ischemia, LVEF ≥ 25%	5, 10 years	Survival, CCS angina class, NYHA functional class

(*Continued*)

TABLE 6.1 (Continued)

Reference	Agent	Type of therapy	Route of administration	Study design	*N* (Active/ Placebo)	Patient characteristics	Follow-up	Outcome assessment
[168]	$hVEGF_{165}$	Gene	Intramyocardial via minithoracotomy	Phase II (THEANGIOGEN)	13/0	Refractory angina or heart failure, inoperable CAD, LVEF > 25%	3, 6, 12 months	SPECT MPI, ETT, Minnesota QoL questionnaire, CCS class, NYHA functional class
[169]	AdVEGF-D	Gene	Endocardial	Phase I (KAT301)	Estimated 30 (4:1)	CCS class 2 or 3 angina refractory to medical therapy, inoperable CAD, reversible ischemia, LV wall > 8 mm on Echo (treatment area)	1 year	Safety, MRI, PET, Echo, ETT, QoL
[170]	pHGF	Protein	Intravenous	Phase I	21/28	Sever 3-vessel CAD, LVEF ≥ 35%	2 weeks	ETT, Echo
[171]	Ad-HGF	Gene	Intracoronary	Phase I	18/0	Severe 3-vessel CAD not amenable to revascularization	7, 21, 35 days	Safety, AE
[172]	HGF naked DNA	Gene	Intramyocardial during CABG	Phase I	9/0	Reversible ischemia ≥7% in the inferior wall not amenable to bypass due to narrow vessels	3, 6 months	Echo, SPECT MPI, MRI

Ad, adenoviral; AE, adverse effects; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CCS, Canadian Classification System; DASI, Duke Activity Status Index; DSA, digital subtraction angiography; EF, ejection fraction; EMM, electromechanical mapping; ETT, exercise treadmill test; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; LV, left ventricle; MACE, major cardiac adverse events; MFI-20, Multidimensional Fatigue Inventory; MPI, myocardial perfusion imaging; MRI, magnetic resonance imaging; NTG, nitroglycerin; NYHA, New York Heart Association; P/L, plasmid; PCI, percutaneous coronary intervention; PET, positron emission tomography; PK/PD, pharmacokinetics/pharmacodynamics; PTCA, percutaneous transluminal coronary angioplasty; QoL, quality of life; SAQ, Seattle Angina Questionnaire; SF-36, 36-item Short Form Health Survey; SPECT, single-photon emission computed tomography; TVI, tissue velocity imaging; VEGF, vascular endothelial growth factor.

intracoronary, intramyocardial, and/or intravenous routes. Another approach has been the administration of bone marrow-derived EPCs or mononuclear cells (MNCs) (reviewed in detail in Chapter 1). Unfortunately, results of clinical trials have been largely disappointing.

Most clinical trials have selected patients who have failed or are not candidates for revascularization. These individuals may represent failures of natural angiogenic responses and may be more resistant to stimulation of neovascularization [5]. The optimal therapy (gene versus protein versus cell), dosing schedule (single versus multiple doses), and delivery method (intracoronary versus intramyocardial versus intravenous) continue to pose additional challenges. Lastly, selection of trial end points and methods to evaluate these end points is crucial. Perfusion imaging is necessary for demonstrating angiogenic efficacy and to document that the therapy does indeed enhance blood flow to the ischemic myocardium. Most clinical trials to date have utilized single-photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI). There is a paucity of clinical experience with positron emission tomography (PET) and magnetic resonance (MR) perfusion imaging, which offer important advantages such as better image quality and spatial resolution (particularly with MR) and the ability to assess transmural flow gradients, changes in subendocardial perfusion, and perfusion reserve.

SUMMARY

Vasculogenesis and angiogenesis are the fundamental processes by which new blood vessels are formed. Both processes are regulated via an intricate network of often overlapping signaling pathways that involve pro- and antiangiogenic factors, ECM components, cell–cell adhesion molecules, and apoptosis factors. Despite progress in understanding the molecular mechanisms of angiogenesis, several challenges remain, which need to be overcome to successfully translate the positive results of preclinical studies of therapeutic coronary angiogenesis to clinical trials. A more in-depth understanding of the cellular and molecular biology of vasculogenesis and angiogenesis may help overcome some of the existing challenges and kindle renewed interest in developing novel strategies to revascularize ischemic tissues.

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CHAPTER

7

Lipids in Coronary Heart Disease: From Epidemiology to Therapeutics

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INTRODUCTION

Dyslipidemia is a major contributor to the large burden of coronary heart disease (CHD) in the United States and around the world. A study of acute myocardial infarction (MI) in 12,461 cases and 14,637 age- and sex-matched controls in 52 countries showed that the population-attributable risk was 54% from dyslipidemia as per the apolipoprotein B-100/apolipoprotein A1 ratio and 32% by the total cholesterol/high-density lipoprotein cholesterol (HDL-C) ratio [1]. Considering disability-adjusted life years attributable to ischemic heart disease, ~29% are attributable to elevated total cholesterol levels [2].

Using 2009–2012 National Health and Nutrition Examination Survey data, the American Heart Association (AHA) estimates that ~47% of adults have "ideal" untreated total cholesterol levels of <200 mg/dL, a statistic that has not significantly changed over the past decade [3]. It is estimated that >100 million adult Americans have total cholesterol elevated to 200 mg/dL or higher and 31 billion have levels of 240 mg/dL or higher [3].

While the potential value of primary prevention (preventing a first event) and primordial prevention (preventing risk factors) are becoming increasingly recognized [4], here we focus attention on secondary prevention (i.e., individuals with established CHD). The AHA estimates that 635,000 people in the United States had a first hospitalization for MI or CHD death in 2011 and about 300,000 had a recurrent event [3]. The latter statistic highlights the substantial burden of residual risk despite available evidence-based therapies such as statins.

The number of US adults who may be eligible for consideration of statin therapy based on the 2013 ACC/AHA cholesterol guidelines is estimated at 51 million (49%) [5]. It is not precisely known how many Americans are currently taking statins or lipid-lowering medications, but these medications are among the most commonly prescribed pharmaceuticals and use appears to be growing. Self-reported lipid-lowering medication use rose from 8% in 1999–2000 to 14% in 2005–2006 to 23% in 2007–2010 [3,6,7]. The Agency for Healthcare Research and Quality reported that annual spending on statins in the United States had increased to nearly $20 million by 2005 [8].

The 2013 ACC/AHA cholesterol guidelines [9] identified four "statin benefit" groups wherein the benefits of therapy for atherosclerotic cardiovascular disease (ASCVD) risk reduction were generally found to outweigh potential adverse effects of therapy. The groups were: (i) people with clinical ASCVD, (ii) those with primary elevations of low-density lipoprotein cholesterol (LDL-C) ≥190 mg/dL, (iii) people aged 40–75 years with diabetes mellitus and LDL-C 70–189 mg/dL, and (iv) those without clinical ASCVD or diabetes with LDL-C 70–189 mg/dL and estimated 10-year ASCVD risk ≥7.5%. In estimating 10-year ASCVD risk, traditional risk factors are used, including two lipid variables, total and HDL-C.

Some individuals are born with favorable genes that confer a lower LDL-C over one's lifetime, which

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00007-3

compared to lipid-lowering intervention later in life, appears to confer a significantly greater cardiovascular risk reduction. Examples are individuals carrying mutations in *proprotein* convertase subtilisin/kexin type 9 (PCSK9) or Niemann–Pick C1-Like 1 (NPC1L1) protein, conferring decreased LDL-C levels from birth and reduced coronary risk [10,11]. The relative reduction in CHD risk for those with LDL-lowering polymorphisms has been quantified at 55% per 1 mmol/L (39 mg/dL) decrease in LDL-C, compared with ~20% for use of statins in clinical trials [12].

NONPHARMACOLOGIC MANAGEMENT OF LIPIDS

One of the mainstays of both primary and secondary prevention is lifestyle changes targeted to improve lipid profiles. In lower risk primary prevention, lifestyle changes are first line therapy to combat dyslipidemia. While therapeutic lifestyle changes are also the foundation of therapy in both high-risk primary prevention patients and secondary prevention patients, pharmacological agents (typically statins) are also recommended as concurrent therapy.

Both exercise and weight loss can lower total cholesterol, LDL-C, and triglycerides (TG) [13]. Lifestyle changes to promote improvements in the lipid profile should incorporate a multifactorial approach that includes dietary modifications, regular physical activity, and achievement of a normal BMI (18–25 kg/m^2). Regarding diet, both specific nutritional components (i.e., soluble viscous fiber, phytosterols, nuts, and soy protein) and general dietary patterns (Mediterranean-style, DASH, low-fat, etc.), have been shown to reduce LDL-C and improve an atherogenic dyslipidemic profile.

Soluble fiber is found in plants that resist digestion, mainly nonstarch polysaccharides but also some starches. Sources of soluble fiber include the pulp of fruits, legumes, oats/barley, vegetables like broccoli and carrots, and nuts (almonds). Diets enriched in fiber, particularly viscous fiber, can lower LDL-C [14]. A meta-analysis of 67 controlled studies, found that soluble fiber in a practical dietary range (2–10 g/day) led to a small but statistically significant decrease in both total and LDL-C [15]. The LDL-C-lowering effect is dependent on fiber hydration, formation of a gel that may be fermented by bacteria which increases the viscosity of human digesta and binding bile acids and in turn reduces cholesterol (and sugar) absorption.

Phytosterols, which occur only in plants, have a structure similar to cholesterol and compete for absorption with dietary cholesterol in the gut. A meta-analysis found a dose-dependent LDL-C-lowering effect with increasing intake of phytosterols [16]. However, the cardiovascular benefits of phytosterols are still unclear. A rare disease called sitosterolemia, characterized by hyperabsorption and decreased biliary excretion of dietary sterols, is associated with elevated cholesterol levels and premature ASCVD. However, a systematic review and meta-analysis did not find any association between serum concentrations of plant sterols and risk of ASCVD in the general population [17].

Nuts are another food group with many properties associated with lipid-lowering effects such as soluble fiber, healthy oils, phytosterols, and satiating effects (for weight loss). A meta-analysis from 25 interventional studies found that nut consumption improves blood lipid levels in a dose-related manner, particularly among subjects with higher LDL-C [18]. Replacing meat protein with soy protein in a moderately-high protein diet may also have favorable effects on total and LDL-C [19]. A meta-analysis showed that replacing animal protein with soy conferred significantly decreased serum concentrations of total cholesterol, LDL-C, and TG and nonsignificant slight increase in HDL-C [20]. Finally, a dietary portfolio that incorporates all of these components (plant sterols, soy protein, viscous fibers, and nuts) may result in greater LDL-C reduction [21].

Dietary patterns associated with lower risk of atherogenic dyslipidemia include ones generally lower in saturated fats, but enriched in fruits, vegetables, whole grains, with moderate intake of polyunsaturated and monounsaturated fats. In the landmark Seven Countries Study, higher intakes of dietary saturated fat intake significantly increased serum cholesterol and the risk of ASCVD, whereas countries that followed a Mediterranean-style diet had lower risk [22].

Epidemiology studies have shown that adherence to Mediterranean-style diet is associated with reduced mortality [23]. Furthermore, in the Lyon Diet Heart study (randomized clinical trial (RCT)), assignment to a Mediterranean-type diet in secondary prevention patients also reduced ASCVD events; although traditional risk factors such as high total cholesterol were still associated with increased risk of recurrent events, suggesting this dietary pattern did not alter the usual relationship between lipid risk factors and ASCVD recurrence [24].

In PREDIMED (PREvención con DIeta MEDiterránea), a RCT conducted among persons at high ASCVD risk, a Mediterranean diet supplemented with extra-virgin olive oil or nuts reduced the incidence of major ASCVD events compared to a control diet [25]. Further analysis of PREDIMED found that the Mediterranean diets supplemented with nuts, but not the olive oil, reduced TG and small dense LDL—toward a less atherogenic phenotype [26].

However, in attempting to follow a diet low in saturated fats as recommended by the AHA, unfortunately many individuals end up replacing fats with

carbohydrates, particularly sugars and simple carbs, which can actually lead to a worse metabolic profile of insulin resistance, elevated TG, and low HDL-C. A RCT found that a low-glycemic diet compared to a low-fat diet lowered TG, raised HDL-C more, whereas LDL-C improved more on the low-fat diet [27]. A subsequent meta-analysis of 23 RCTs that compared the effects of a low-carbohydrate diet versus a low-fat diet found that persons on low-carbohydrate diets experience slightly lower decreases in total cholesterol and LDL-C, but a greater increase in HDL-C and greater decrease in TGs [28].

In 2013, the AHA/ACC Guidelines on Lifestyle Management to reduce cardiovascular risk [29] gave the following specific advice for patients who would benefit from LDL-C lowering:

1. Consume a dietary pattern that emphasizes intake fruits, vegetables, whole grains, including low-fat dairy products, poultry, fish, nuts, nontropical vegetable oils and limits intake of sodium, sweets, sweetened beverages, and red meats (Class I, Level of Evidence A)
2. Reduce intake of trans fats (Class I, Level of Evidence A)
3. Reduce intake of saturated fats (aim for 5–6% of calories from saturated fats) (Class I, Level of Evidence A)
4. Engage in aerobic physical activity 3–4 times a week, lasting on average 40 min per session, and involving moderate to vigorous physical activity (Class IIa, Level of Evidence A)
5. Achieve and maintain a healthy weight

Of note, the AHA/ACC guidelines did not recommend an amount of dietary cholesterol, as there was insufficient evidence that lower dietary cholesterol lowers LDL-C. Furthermore, the AHA/ACC guidelines did not specifically endorse a Mediterranean style diet, but stated the above heart-healthy diet pattern can be achieved by following the DASH diet, the USDA food pattern, or the AHA diet.

LDL-C AND LDL-TARGETED THERAPEUTICS

Epidemiology of LDL-C, apoB, and Non-HDL-C with CAD Risk

Dating back to the Framingham Heart Study [30], a rich set of large population-based cohort studies from around the globe have consistently linked hyperlipidemia with risk of CHD. Low-density lipoproteins account for the majority of atherogenic lipoproteins (typically >75%) and severe elevations, as seen in familial hypercholesterolemia, are strongly linked with premature CHD even in the absence of other risk factors. About half of men with heterozygous familial hypercholesterolemia develop coronary disease before age 50, as do ~1/3 of women by age 60, while the homozygous form commonly leads to coronary events in the 1st or 2nd decade of life [31]. Combined with other important lines of evidence, such as animal studies, pathological studies, and clinical trials, the "LDL hypothesis" arose and has become one of the most widely studied and supported hypotheses in the history of medicine.

Other lipid parameters are closely related to LDL, but have the potential to capture different information. For example, non-HDL-C is determined as total minus HDL-C and therefore is equivalent to Friedewald estimated LDL-C plus VLDL-C. It represents the total concentration of cholesterol contained in circulating atherogenic lipoproteins. To determine the total particle concentration of atherogenic lipoproteins, apolipoprotein B (apoB) can be measured as there is a reliable relationship of one apoB per atherogenic lipoprotein particle.

In 2011, a meta-analysis compared risk estimates for LDL-C, non-HDL-C, and apoB in association with fatal or nonfatal ischemic cardiovascular events using data from all published epidemiological studies [32]. A random-effects model was used to summarize the 12 studies including 233,455 individuals and 22,950 events. The standardized relative risk ratio (95% CI) was 1.25 for LDL-C (1.18–1.33), 1.34 (1.24–1.44) for non-HDL-C, and 1.43 (1.35–1.51) for apoB. Head-to-head comparison indicated that apoB-related risk was 5.7% greater than non-HDL-C ($P < 0.001$) and 12.0% greater than LDL-C ($P < 0.0001$). A subsequent meta-analysis related changes in LDL-C, non-HDL-C, and apoB with the relative risk reduction from statin therapy in seven clinical trials. The mean CHD risk reduction (95% CI) per standard deviation lowering in lipid parameter across these seven trials was 20.1% (15.6–24.3%) for LDL-C, 20.0% (15.2–24.7%) for non-HDL-C, and 24.4% (19.2–29.2%) for apoB [33].

Residual Risk

Despite ≥50% LDL-C-lowering with statins, and efficacy of concurrent preventive therapies including aspirin and antihypertensives, individuals with dyslipidemia and CHD remain at high-residual ASCVD risk [34]. From a lipid perspective, residual risk has been tied to the metabolic syndrome, insulin resistance, and diabetes mellitus, which are associated with an atherogenic lipid phenotype not fully captured by LDL-C [35]. Therefore, there is much interest in novel nonstatin LDL-lowering agents.

Residual risk also reaches beyond lipid-related factors to the global risk factor profile. This was shown in an analysis of 9251 secondary prevention patients treated with atorvastatin [36]. Multivariable determinants of increased

residual risk included, beyond lipid-related factors, increased body mass index, smoking, hypertension, and diabetes mellitus. The multifactorial nature of residual risk highlights the need for comprehensive prevention.

Therapies

Bile acid sequestrants

Bile acid sequestrants (BAS) were available prior to statin therapy. They are felt to exert their effect by modifying enterohepatic bile acid exchange, increasing expression of hepatic LDL receptors, and in turn reducing blood LDL-C concentrations by 5–30% in a dose dependent fashion. One attractive aspect of this drug class is that it appears to have no systemic absorption, thereby limiting the possibility of systemic adverse effects. Patients can develop gastrointestinal disturbances, although these commonly are self-limited. In addition, BAS may impair absorption of other medications.

Regarding efficacy, the landmark randomized clinical trial of BAS was the LRC-CPPT trial [37,38]. Compared with the placebo group, individuals allocated to BAS had a lower risk of MI and cardiovascular death. Subsequently, in the statin era, there has not been a trial testing the effect of BAS therapy added to statin monotherapy on ASCVD outcomes, nor is such a trial planned to our knowledge.

Statins

Statins, which inhibit HMG-CoA reductase, the rate limiting step in cholesterol synthesis, are thought to exert much of their LDL-C lowering effect by increasing hepatic availability of LDL receptors. The first statin, lovastatin, was approved by the FDA in 1987. Early statin trials consistently showed efficacy of moderate intensity regimens in higher risk primary and secondary prevention populations, with later trials examining more intensive versus less intensive statin regimens.

The Cholesterol Treatment Trialists landmark meta-analysis in 2010 pooled patient-level data from 170,000 participants included in randomized statin trials [39]. The proportional reduction in the annual rate of major cardiovascular events with statin therapy was just over a fifth for each 1.0 mmol/L (39 mg/dL) LDL-C reduction. Major cardiovascular events included coronary death, nonfatal MI, coronary revascularization, and ischemic stroke. The relative risk reduction in all-cause mortality was 10% per 1.0 mmol/L LDL-C lowering (RR 0.90, 95% CI: 0.87–0.93; $P < 0.0001$) primarily due to a decrease in cardiovascular death (RR 0.80, 99% CI: 0.74–0.87; $P < 0.0001$). Effects were consistent in primary and secondary prevention and across other major clinical subgroups. The data did not indicate a lower limit of LDL-C below which benefit was no longer seen or excessive harms occurred.

Ezetimibe

Ezetimibe impedes LDL-C absorption through inhibition of NPC1L1 protein in the gastrointestinal epithelial brush border. The LDL-C lowering effect is ~20%, which is on the order of about three dose doublings of a statin. Adding ezetimibe to statin therapy is known to increase attainment of target LDL-C goals [40].

Although surrogate endpoint trials produced confusing results, outcomes trials have shown efficacy of ezetimibe in lowering ASCVD risk with effects proportional to statin therapy for the degree of LDL-C lowering. The SHARP (Study of Heart and Renal Protection) trial [41] was the first clinical trial using ezetimibe. It compared the combination of ezetimibe and statin therapy to placebo in chronic kidney disease patients, showing a reduction in ASCVD events. The reduction in events was proportional to the magnitude of LDL-C lowering suggesting that the clinical benefit was not only explained by statin use but also from ezetimibe.

The clinical effect of ezetimibe added to statin therapy was then formally tested in the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT). This large international RCT compared ezetimibe with placebo in acute coronary syndrome patients taking a background of moderate intensity statin therapy (simvastatin) [42]. In intention-to-treat analyses, over a follow-up period of 6 years, the relative risk reduction in the primary composite cardiovascular endpoint was 6.4% (CI: 1.2–11.3%) with events occurring in 32.7% of patients in the statin-ezetimibe group and 34.7% of patients in the simvastatin group ($P = 0.016$). Therefore, the absolute risk difference was 2%, equating to a number needed to treat (NNT) of 50. Regarding safety, there were no significant differences between the study groups in cancer, muscle toxicity, or gallbladder-related events.

PCSK9 Inhibitors

The PCSK9 enzyme binds to the LDL receptor to promote its degradation and thus inhibitors of PCSK9 increase availability of LDL receptors on hepatocytes and removal of LDL from the circulation [43]. Multiple monoclonal antibodies to PCSK9 are in development and are self-administered subcutaneously every 2–4 weeks. In a meta-analysis of 24 clinical trials including 10,159 participants, PCSK9 antibody therapy reduced LDL-C levels by a mean of 47% (95% CI: 25–70%) [44]. Large pivotal trials powered for hard clinical outcomes are ongoing, but early outcome data suggests an ~50% reduction in the rate of MI and all-cause mortality, without an increase in serious adverse events [44].

LDL Apheresis

LDL or lipoprotein apheresis can be indicated in patients with severe familial hypercholesterolemia when medications are insufficient in reducing LDL-C

[45]. Performed every 2 weeks, it can reduce the time averaged LDL-C by 60% and there is also a reduction in Lp(a). Apheresis is also associated with a reduction in inflammatory markers and blood viscosity, and a reduction in the rate of cardiovascular events.

Niacin

Niacin is thought to inhibit lipoprotein synthesis and therefore yield a modest lowering of LDL-C of ~15%. Therefore, it could be considered for additional LDL-C lowering in high-risk patients who are unable to tolerate the maximum recommended statin therapy or have an inadequate initial response. This particular situation, however, has not been formally tested in clinical trials.

Other Novel LDL-Lowering Therapies

Among novel agents to lower LDL-C, PCKS9 inhibitors, as described above, appear to have the greatest potential. Table 7.1 summarizes three other novel agents, two of which have already achieved restricted regulatory approval for patients with familial hypercholesterolemia.

HDL-C AND HDL-TARGETED THERAPEUTICS

HDL Overview

HDL is the smallest class of the major serum lipoproteins and is distinguished from the other classes due to the presence of apolipoprotein A (apoA) rather than apoB as the primary surface protein. Compared with apoB-containing lipoproteins, HDL is significantly more complicated in structure and function [50]. The role of HDL in reverse-cholesterol transport through the ability to accept cholesterol from peripheral macrophages has been the principle function of interest. However, HDL also has well-characterized anti-inflammatory, antioxidative, and antithrombotic capabilities [50].

HDL-C levels are classically inversely related to ASCVD risk. A complicating matter is the extensive interaction of HDL with other lipoproteins in serum, principally through the cholesteryl ester transfer protein (CETP). Low levels of HDL-C may simply be a marker of the atherogenic lipid phenotype (small, dense LDL; increased TGs) rather than a direct mediator of ASCVD.

HDL Epidemiology

In the mid-twentieth century, seminal work by Gofman et al. from the prospective Livermore study based in California demonstrated an inverse association between HDL-C levels and ischemic heart disease [51]. This finding was later confirmed in several prospective studies of both primary and secondary prevention populations [52–55]. HDL-C has consistently qualified as an independent, inverse measure of risk for incident ASCVD in risk calculators such as the Framingham Risk Score, Reynold's Risk Score, and 2013 ACC/AHA Pooled Cohorts ASCVD risk estimator [56–58].

However, sufficient data to verify a causal role for HDL-C in ASCVD risk are lacking and instead suggest that HDL-C levels may only be an ASCVD risk marker. In patients with prior ASCVD who are treated with high-dose statin therapy, there is conflicting evidence over the ability of HDL-C levels to predict residual risk [59–61]. In Mendelian randomization studies, genetically elevated levels of HDL-C from birth did not translate to reduced CHD risk [62,63]. Most critically, there has been a failure to demonstrate ASCVD risk reduction by interventions that increase HDL-C [64–67].

HDL Therapeutics

In addition to lifestyle modifications, several nonstatin therapies have been considered for their ability to increase HDL-C, including niacin, CETP inhibitors, and fibrates.

TABLE 7.1 Other Novel LDL-C-Lowering Therapies

Class of therapy	Agent name	Route of administration	FDA regulatory status	LDL-C lowering results
Antisense oligonucleotide inhibitors of apolipoprotein B	Mipomersen	Subcutaneous	Approved for restricted use (through REMS program)	~20–30% LDL-C lowering over 6–26 weeks in HoFH/HeFH patients [46,47]
Microsomal triglyceride transfer protein inhibitors	Lomitapide	Oral	Approved for restricted use (through REMS program)	~40–50% LDL-C lowering over 26–78 weeks in HoFH patients [48]
Thyromimetics	Eprotiromone	Oral	Not approved	~20–30% LDL-C lowering over 12 weeks in statin treated patients [49]

FDA, Food and Drug Administration; REMS, risk evaluation and mitigation strategies; HoFH, homozygous familial hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia.

Thus far, despite substantial increases in HDL-C, none of these pharmacotherapies (Table 7.2) have reduced adverse cardiovascular outcomes when routinely added to statin therapy. Furthermore, most pharmacotherapies affect both LDL-C and HDL-C with ensuing difficulties in isolating any possible HDL-C related risk reduction.

Niacin

Niacin, which raises HDL-C up to 30% while also lowering LDL-C and TGs, has historically been the most commonly used medication to address HDL-C in clinical practice. In the prestatin era, modest reductions in recurrent MIs were seen with niacin therapy over 6 years in the Coronary Drug Project, which translated to a substantial 6% *absolute* reduction in 15-year mortality [70,71]. While not specifically tested, these reductions in risk were more likely related to effects on apoB containing lipoproteins rather than changes in HDL-C levels. The isolated effect of niacin on HDL-C was tested in two large randomized trials of niacin formulations.

In the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High TG: Impact on Global Health Outcomes (AIM-HIGH) trial, investigators tested the addition of extended-release niacin to intensive statin therapy with or without ezetimibe to achieve equivalent LDL-C levels in both arms for a reduction in CHD deaths, MI, strokes, and revascularization in more than 3400 participants [64]. Both the placebo and niacin study arms achieved LDL-C <70 mg/dL, while the niacin arm had an increase in HDL-C from 35 to 42 mg/dL (20% increase) compared to no change in the placebo arm. Despite these significant changes in HDL-C on top of well-controlled LDL-C levels, the trial was stopped early due to futility, with a hazard ratio of 1.02 (95% CI: 0.87–1.21; $P = 0.80$) for the niacin arm. Critics suggested that the study was small, should not have been halted early, and that the findings warranted confirmation.

The AIM-HIGH findings were validated by the Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) [65]. More than 25,000 participants with well-controlled LDL-C levels (mean 63 ± 17 mg/dL) and average HDL-C levels (44 ± 11 mg/dL) were randomized to placebo versus extended release niacin combined with laropiprant, an agent designed to reduce flushing side effects. After a median follow-up of nearly 4 years, compared with

TABLE 7.2 Clinical Trials of Key Therapies Targeting HDL-C Levels on Background Statin Therapy

Study name	Participants	Drug	Results
NIACIN PREPARATIONS (AVERAGE 15–30% INCREASE IN HDL-C)			
AIM-HIGH [64]	3414 participants with established cardiovascular disease and low HDL-C followed for CHD death, MI, stroke, or revascularization	Niacin/Simvastatin versus Placebo/ Simvastatin	Niacin led to 20% increase in HDL-C, significant reductions in LDL-C and TGs, but no difference in ASCVD events over 2 years (HR 1.02; 95% CI: 0.87–1.21)
HPS2-THRIVE [65]	25,673 participants with vascular disease followed for CHD death, MI, stroke, or revascularization	Niacin/Laropiprant versus matching placebo	Niacin led to 15% increase in HDL-C, significant reduction in LDL-C, but no difference in ASCVD events over 3.9 years (HR 0.96; 95% CI: 0.90–1.03)
CETP INHIBITORS (AVERAGE 30–140% INCREASE IN HDL-C)			
ILLUMINATE [66]	15,067 participants with high cardiovascular risk followed for CHD death, MI, stroke, or hospitalization for unstable angina	Torcetrapib/Atorvastatin versus Placebo/ Atorvastatin	Torcetrapib led to 75% increase in HDL-C and 25% decrease in LDL-C, but increased ASCVD events (HR 1.25; 95% CI: 1.09–1.44) and mortality (HR 1.58; 95% CI: 1.14–2.19) over 1.5 years
Dal-OUTCOMES [67]	15,871 participants with recent acute coronary syndrome followed for CHD death, MI, stroke, unstable angina or resuscitated cardiac arrest	Dalcetrapib/Statin versus Placebo/Statin	Dalcetrapib led to 40% increase in HDL-C, no change in LDL-C, and no difference in ASCVD events over 2.5 years (HR 1.04; 95% CI: 0.93–1.16)
REVEAL [68]	30,624 participants with high cardiovascular risk followed for CHD death, MI, or coronary revascularization	Anacetrapib/Statin versus Placebo/Statin	In prior studies, anacetrapib has led to 140% increases in HDL-C and significant LDL-C reductions. Outcomes study ongoing with anticipated completion in 2017
ACCELERATE [69]	12,000 participants with high cardiovascular risk followed for CVD death, MI, stroke, unstable angina or revascularization	Evacetrapib/Statin versus Placebo/Statin	In prior studies, evacetrapib has led to 130% increases in HDL-C and significant LDL-C reductions. Outcomes study ongoing with anticipated completion in 2016

placebo, the niacin/laropiprant arm had an LDL-C that was 10 mg/dL lower and an HDL-C that was 6 mg/dL higher. Despite these significant differences, there were no differences in cardiovascular events with niacin having a hazard ratio of 0.96 (95% CI: 0.90–1.03; $P = 0.29$).

In the presence of well-controlled LDL-C levels in statin-treated patients, there appears to be no added benefit of niacin therapy to improve cardiovascular risk, despite significant 15–20% increases in HDL-C levels. It should be noted that niacin may still have a role in patients who have not achieved the aggressive LDL-C levels <70 mg/dL on statin ± ezetimibe seen in AIM-HIGH and HPS2-THRIVE [72].

CETP Inhibitors

CETP is responsible for transferring cholesteryl esters from HDL to LDL or VLDL particles in exchange for TGs. Inhibition of CETP in an animal model reduced progression of atherosclerosis [73]. Subsequent phase II and III studies of four CETP inhibitors have generated excitement over impressive increases in HDL-C by as much as 130%, while also lowering LDL-C by as much as 40% [74]. Nevertheless, thus far, two trials of CETP inhibitors on cardiovascular outcomes have disappointed [66,67].

In the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial of 15,000 participants at high ASCVD risk, the CETP inhibitor torcetrapib led to a 72% increase in HDL-C and 25% decrease in LDL-C compared with placebo when added to statin therapy [66]. However, the study was terminated early due to an increase in blood pressure, cardiovascular events and all-cause mortality in the torcetrapib arm. The adverse outcomes were attributed to an off-target effect on aldosterone concentrations, yet there remained concern over an adverse effect of CETP inhibition [75].

Dalcetrapib was the next CETP inhibitor tested in a large-scale clinical trial and was eagerly anticipated due to the absence of the off-target effects seen with torcetrapib. Notably, in contrast to other CETP inhibitors, dalcetrapib has much more modest effects on HDL-C and no effect on LDL-C. The dal-OUTCOMES trial in over 15,000 post-ACS participants tested the effects of dalcetrapib versus placebo when added to statin therapy on cardiovascular outcomes [67]. Dalcetrapib led to 40% increase in HDL-C and no change in LDL-C. However, the trial was stopped prematurely at a prespecified interim analysis due to a lack of efficacy with a HR of 1.04 for dalcetrapib (95% CI: 0.93–1.16; $P = 0.52$). Ongoing pivotal trials of anacetrapib and evacetrapib aim to definitively answer whether CETP inhibition can improve cardiovascular outcomes [68,69]. Assuming there are no off-target effects similar to torcetrapib, each of these therapies yield substantially greater changes in HDL-C and LDL-C levels than dalcetrapib, leaving some hope for benefit [76,77]. In total, the evidence thus far raises the question of whether increasing HDL-C is beneficial through this mechanism.

Other Potential Therapies

Omega-3 fatty acids have been proposed as potential agents to reduce the risk of cardiovascular disease due predominantly through reduction in TGs as well as a very small potential effect on HDL-C levels. Fibrates have also been tested in populations with low HDL-C levels with the hopes of improving cardiovascular outcomes. In the prestatin era VA-HIT trial, gemfibrozil led to a 6% increase in HDL-C [78]. After a median of 5 years of follow-up, there was a 22% relative reduction in risk of MI or CHD death among the gemfibrozil group (95% CI: 7–35%; $P = 0.006$). In a post-hoc analysis, the benefit achieved with gemfibrozil was predicted by the achieved HDL-C more so than changes in other lipids [79]. In the statin era, fibrates have not reduced ASCVD risk, though some post-hoc data suggest there is a benefit in the low HDL-C, high TG group [80].

Other novel therapies directed at HDL are in development. Recombinant apoA1-Milano infusions showed early promise in reducing atherosclerotic burden; however, later phase trials have not been reported with this compound [81]. Additional approaches include autologous delipidated HDL infusions, liver X receptor (LXR) agonists, niacin receptor agonists, lecithin:cholesterol acyltransferase (LCAT) activators, and peroxisome proliferator-activated receptors (PPARs) agonists among others [82,83].

HDL Function

HDL function is becoming an increasingly important focus of HDL-related risk. Cholesterol-efflux capacity measures the ability of HDL particles to extract cholesterol from macrophages, similar to the *in vivo* function of HDL in reverse cholesterol transport when removing cholesterol from plaque. In a multivariable adjusted case-control analysis, investigators found an approximately 25% decreased odds of CHD (HR 0.75; 95% CI: 0.63–0.90; $P = 0.002$) for every standard deviation increase in efflux capacity, after adjustment for HDL-C levels [84]. Importantly, HDL-C levels were the strongest predictor of efflux capacity, but only explained approximately 25% of the variation in efflux capacity.

In a prospective study of nearly 3000 participants from the Dallas Heart Study, efflux capacity was moderately correlated with HDL-C levels (Spearman correlation 0.52, $P < 0.05$) [85]. Efflux capacity at baseline was a strong predictor of events in multivariable analysis such that the highest quartile of efflux capacity carried a 67% decreased risk of ASCVD events over a median of 9.4 years (HR 0.33; 95% CI: 0.19–0.55) after adjustment for

HDL-C levels. Inclusion of cholesterol efflux capacity significantly improved measures of discrimination and risk reclassification as well.

These studies of HDL function may explain why HDL-C levels are markers of risk given the correlations of HDL-C with efflux capacity. These studies also suggest that changing HDL-C through mechanisms such as CETP inhibition would not necessarily improve the efflux capacity of HDL and therefore would not decrease ASCVD. Niacin also does not affect cholesterol efflux capacity, despite increasing HDL-C significantly [86].

In light of the disappointing results of clinical trials aimed at raising HDL-C in the statin era and considering the promising emerging evidence of HDL's diverse functionality, there has been a paradigm shift from an "HDL-Cholesterol" hypothesis to an "HDL-function" approach [87]. Future therapies targeting HDL to address cardiovascular risk should likely focus on improving HDL function, or the ability to remove cholesterol from plaque, rather than solely increasing lipid levels within HDL.

TRIGLYCERIDES

Overview

TGs, a type of blood fat, serve the purpose of storing fat and excess calories in the body. Elevated TGs are frequently found in patients with type 2 diabetes, metabolic syndrome, or insulin resistance and associated with fatty liver disease (hepatosteatosis). Additionally, there are genetic syndromes that manifest with hypertriglyceridemia, including mixed or familial combined hyperlipidemia (FCHL), type III dysbetalipoproteinemia, and familial hypertriglyceridemia (FHTG) [88].

Virtually all plasma lipoproteins contain TGs, but the three main types of TG-rich lipoproteins associated with elevated levels of TGs include chylomicrons, VLDL, and IDL. Measurement of non-HDL-C, apoB, or both, may be useful in patients with elevated TGs levels. If TG levels are ≥150 mg/dL, Friedewald estimation of LDL-C commonly estimates LDL-C as <70 mg/dL despite directly measured levels ≥70 mg/dL, suggesting alternate measures are warranted [89].

Epidemiology

Hypertriglyceridemia (fasting TG levels ≥150 mg/dL, nonfasting of >200 mg/dL) is common among U.S. adults with an estimated prevalence of 33% [90]. Among these, approximately 1.7% (~3.4 million Americans) have severe hypertriglyceridemia (levels 500–2000 mg/dL), placing them at risk for pancreatitis [91]. Surprisingly, despite the epidemic of obesity and diabetes, TG levels have been decreasing over time during the years of 2001–2012 [92], perhaps in part to increasing use of statins.

It has been a long-standing controversy whether hypertriglyceridemia is an independent risk factor for ASCVD, as elevated TG often presents in a triad of atherogenic dyslipidemia that includes low HDL-C and elevated small dense LDL. Early epidemiologic evidence did not suggest a causal role of TG for CHD [93]. Some studies did not find TG to be an independent risk for CHD when total and HDL-C were taken into account [94] but it was a potent risk factor when occurring in conjunction with a high LDL-C/HDL-C ratio [95,96].

More recent data contrasts with this. Pooled data from 29 prospective studies including over 200,000 patients identified elevated TG as a risk factor for incident CHD [97]. A dose response relationship has been noted with a 13% and 12% increased risk of ASCVD and all-cause mortality respectively per 1 mmol/L increase in TGs [98]. Elevated TG has been shown to be a stronger risk factor for CHD in women compared to men [99]. Furthermore, TG levels >150 mg/dL are associated with increased CHD risk in high-risk patients even when the LDL-C is <70 mg/dL on statin therapy [100–102].

Strengthening this argument, Mendelian randomization studies do suggest that hypertriglyceridemia, or an elevation in TG-rich lipoproteins, is a causal factor in ASCVD [103]. Genes encoding apolipoprotein C3 (APO3C) are strongly associated with plasma TG levels. The APOC3 protein is present on the apoB-containing

TABLE 7.3 Factors Associated with Elevated Triglycerides

Diseases that may elevated TG	Diet/Lifestyle that may elevate TG	Drugs that may elevate TG
Chronic kidney disease	Positive energy balance	Oral estrogens
Nephrotic syndrome	High glycemic load	Tamoxifen
Diabetes mellitus	Excess alcohol	Raloxifene
Metabolic syndrome	Weight gain	Retinoids
HIV infection		Immunosuppressive drugs (cyclosporine, sirolimus)
Autoimmune disorders		Interferon
Hypothyroidism		Beta-blockers (especially nonbeta 1-selective)
Pregnancy		Atypical antipsychotic drugs (fluperlapine, clozapine, and olanzapine)
Polycystic ovary syndrome		Protease inhibitors
Menopause transition		Thiazide diuretics
		Glucocorticoids
		Rosiglitazone
		Bile acid sequestrants
		L-asparaginase
		Cyclophosphamide

Source: Adapted from National Lipid Association materials.

TG-rich particles of chylomicrons and VLDL and functions as an inhibitor of lipoprotein lipase (LPL)-mediated lipolysis and facilitates hepatic VLDL secretion. Carriers of mutations disrupting APOC3 function have lower lifetime TG levels and ASCVD risk [104,105].

Treatment (Lifestyle and Pharmacotherapy)

Many factors including chronic diseases, diet/lifestyle changes, and medications may elevate TG levels (Table 7.3). Often these factors are modifiable and should be addressed when evaluating the patient with elevated TGs. Lifestyle changes promote lower TG levels including improving dietary nutrition (reducing intake of sugars and simple carbohydrates, increasing fiber and omega-3 fatty acids), reducing alcohol intake, regular exercise, weight loss, and quitting smoking [106]. A Mediterranean-style diet pattern was associated with lower TG levels in the Framingham Heart Study [107].

Several classes of lipid-lowering medications (with the exception of BAS) can lower TG levels by varying degrees. Reductions in TG can be seen with statins of 7–30%, ezetimibe 9%, niacin 20–50%, fibrates 20–50%, and omega-3 fatty acids 19–44%. TG reduction with statins, particularly higher intensity statins, is even greater in patients with hypertriglyceridemia (20–50%).

Historically, fibrates are the most common class of medications studied to lower TGs. RCTs with hard clinical outcomes evaluating fibrates are reviewed in Table 7.4. While the Helsinki Heart Study [110] of primary prevention patients and the VA-HIT [78] study of secondary prevention patients in the prestatin era did find reduction in ASCVD events with fibrate therapy, other studies have not found an overall benefit, especially on top of statin therapy. Despite this, subgroups of patients may benefit from fibrate therapy. For example, the ACCORD study [80] of diabetic patients found a benefit of fenofibrate in the subgroup with atherogenic dyslipidemia characterized by elevated TG and low HDL-C. Similarly, with niacin, a secondary analysis of the AIM-HIGH

TABLE 7.4 Key Randomized Clinical Trials with Hard Outcomes Evaluating Fibrate Therapy

Study name	Primary versus Secondary prevention	Patients	Drug	Follow-up (years)	Results
BIP [108]	Secondary	3090 patients with previous myocardial infarction or stable angina and low HDL	Bezafibrate	6.2	Bezafibrate reduced TGs by 21%. There was no significant reduction of the primary outcome overall. However in subset with TG ≥200 mg/dL, there was a 40% reduction in primary end point with bezafibrate ($P = 0.02$)
VA-HIT [78]	Secondary	2531 men with CHD, low HDL	Gemfibrozil	5.1	RR reduction of 22% (95% CI, 7–35%; $P = 0.006$) for primary endpoint of nonfatal myocardial infarction or death from coronary causes
ACCORD [80]	Mixed	5518 patients with type 2 diabetes (37% with previous ASCVD) treated with open-label simvastatin randomized to fenofibrate or placebo	Fenofibrate	4.7	Fenofibrate on top of baseline statin therapy did not reduce primary outcome of fatal CVD, nonfatal MI, or nonfatal stroke, as compared with simvastatin alone. There was noted a possible benefit for the subgroup of patients with both high baseline TG and low HDL ($P = 0.057$ for interaction)
FIELD [109] (2005)	Mixed	795 participants with type 2 diabetes mellitus age 50–75 (22% with ASCVD), not taking statin therapy at baseline	Fenofibrate	5	22% reduction in TGs compared to placebo. Fenofibrate did not significantly reduce the risk of the primary outcome of coronary events. It did reduce total ASCVD, due to fewer nonfatal MI and revascularizations. More patients assigned to placebo started statins which might have masked a larger benefit
HHS [110] (1987)	Primary	4081 asymptomatic (age 40–55 years) with primary dyslipidemia (non-HDL ≥ 200 mg/dL)	Gemfibrozil	5	34% reduction in the incidence of CHD (95% CI, 8–53%; $P < 0.02$)

study showed that the addition of niacin in secondary prevention patients with both high TG and low HDL-C conferred a 26% reduction in ASCVD events [111].

Omega-3 fatty acids have also been used for the management of elevated TGs. Observational data and some RCTs have suggested a benefit in cardiovascular risk with "fish-oil" supplementation, while more recent RCTs have suggested that there is no benefit [112–117]. A comprehensive systematic review and meta-analysis of 20 RCTs evaluating the effects of omega-3 supplements on cardiovascular outcomes showed trends for benefit, but none reached statistical significance [118].

Thus, RCT data generally does not support an incremental benefit of these secondary agents when added to a background of statin therapy for lowering ASCVD events. However, recently there has been renewed interest in using omega-3 fatty acids to treat high-risk patients with hypertriglyceridemia. There are two RCTs of omega-3s for patients with elevated TGs currently in progress. STRENGTH [119] plans to enroll ~13,000 patients at high risk for ASCVD who have low LDL-C <100 mg/dL but elevated TG (≥200 but <500 mg/dL) and is powered for major cardiovascular events. REDUCE-IT [120] similarly will enroll about 8000 patients at high ASCVD risk with TG levels ≥150 mg/dL and follow them for hard clinical outcomes. These key RCTs may change current recommendations for prescription omega-3 therapy. In the meantime, increased dietary consumption of marine-based omega 3's in conjunction with a healthy lifestyle is an AHA recommendation.

At this time, the 2013 ACC/AHA cholesterol guidelines [9] advise that an elevated TG level is not a target of therapy per se, except when very high (≥500 or even ≥1000) to minimize risk of pancreatitis. A drug targeting TG reduction (such as a fibrate) could be considered first-line in those with TG ≥500 mg/dL, although a statin is still a reasonable first line agent if TG is ≥500 but <1000 mg/dL in the absence of a history of pancreatitis.

Novel Directions from Translational Research

The recent developments from the Mendelian studies supporting a causal role of remnant lipoproteins has led to renewed interest in developing therapeutic targets against TG, specifically of APOC3. Administration of an antisense oligonucleotide drug that decreases production of APOC3 was shown to lower plasma TG levels in Phase I studies [121]. Phase III clinical trials are now underway, such as the APPOACH study, in patients with familial chylomicronemia, with a primary outcome measure of percent change in fasting TG [122]. For LDL-therapeutics, the rapid progression from bench to bedside for PCSK9 inhibitor therapy is a great success story of translational research; perhaps inhibitors of APOC3 will be another similar success [123].

CONCLUSION

LDL-C is causally associated with ASCVD whereas the causal role of HDL-C and TG remains uncertain. Lifestyle changes targeted at improving the lipid profile form the foundation for primary and secondary prevention whereas in both higher risk primary prevention and secondary prevention, pharmacological treatment with statins is also recommended as concurrent therapy. The current ACC/AHA lipid guidelines recommend matching the intensity of statin treatment with absolute ASCVD risk. In certain cases, additional lipid-lowering medications may be considered for add-on therapy, although their incremental utility on top of maximal statin therapy is still uncertain. The recent IMPROVE-IT trial with ezetimibe and the early success of the PCSK9 inhibitors however support the concept of "lower is better" when it comes to reducing the atherogenic lipoprotein burden of LDL-C and apoB. Based on this, perhaps subsequent versions of the guidelines will consider bringing back lipid targets or encourage even lower LDL-C goals. Furthermore, there are several large RCTs in progress powered for clinical ASCVD outcomes testing the efficacy of some of the more novel lipid therapeutics (including PCSK9 inhibitors, CETP inhibitors, and omega-3 fatty acids); the results of these key trials may shape future iterations of the lipid guidelines.

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CHAPTER

8

Genetics of Coronary Disease

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Coronary artery disease (CAD) results from interplay between genetic and environmental factors and is strongly associated with aging. Few methods of identifying a highly susceptible population are as potent as a simple family history of early-onset coronary disease as a marker of shared genetic, environmental, and behavioral risk among family members. It has been estimated that 30–60% of the interindividual variation in CAD risk is accounted for by heritable factors [1]. Multiple approaches have been used to identify DNA sequence variants responsible for trait and disease variation in humans. Prior to 2005, when the first microarray became available with 500,000 single nucleotide polymorphisms (SNPs) across the genome, candidate gene studies with *a priori* biological hypotheses or identified by family-based linkage analyses were the primary methods used to identify association between genes and disease phenotypes. Segregation and linkage analyses in families have led to discovery of gene variation with Mendelian transmission with large effects. Although Mendelian disorders demonstrate potentially dramatic effects of genetic variation, they are rare and do not explain much of the variability of complex diseases such as CAD at the population level, where common diseases are expected to be associated with commonly expressed genetic variations. Candidate gene approaches based on biologically based hypotheses led to hundreds of published associations of genes and variants with CAD phenotypes, almost none of which could be replicated and confirmed in subsequent studies [2]. The more recent and unbiased genome-wide association studies (GWAS) have identified more than 50 common genetic variants robustly associated with CAD (Table 8.1), consistent with genetic susceptibility in certain individuals, but surprisingly only about 15% of heritability is explained by these common variants [3–5]. Moreover, discovered allelic variants do not necessarily reflect risk in populations of different ancestral origins. Although some of these variants appear to work through known risk factors, including lipid levels and/or blood pressure, most are completely novel with mechanisms yet to be elucidated.

THE GWAS ERA

Classic Mendelian genetics has been successful in identifying rare single gene disorders occurring in less than 1% of the population with high penetrance and large biological effects. In Mendelian disorders, mutations are typically identified in protein coding exons that disrupt the function of the translated protein. These variants can be mapped with family pedigrees of multigenerational affected individuals using genetic linkage techniques. By genotyping a few hundred DNA markers with known chromosomal location it is possible to determine those that co-segregate in subjects affected by the disorder compared to those without the phenotype. Such markers are considered linked to a nearby gene of interest, which can then be sequenced to determine the functional mutation. For example, linkage analysis was used to localize the chromosomal position of causality for hypertrophic cardiomyopathy, followed by the discovery of mutations in the beta-cardiac myosin heavy chain [138,139]. Using a candidate gene approach, Lehrman et al. [140] sequenced the low-density lipoprotein receptor (LDLR) gene in a patient with homozygous familial hypercholesterolemia and discovered the deletion of several exons. The discovery of additional mutations in genes leading to homozygous familial hypercholesterolemia demonstrated an increased risk of early-onset CAD working through the known risk factor of elevated low-density lipoprotein (LDL) cholesterol [141]. Follow-up of candidate gene

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00008-5

TABLE 8.1 Genome-Wide Association Studies Coronary Artery Disease/Myocardial Infarction (MI)

Chromosome	SNP	Closest Genes	DNA Region	Allele Risk Frequency (Allele)	Allelic Odds Ratio (95% CI)	GWAS Population (Candidate)	GWAS Phenotype (Candidate)	Other SNP/ Phenotype Associations	Mechanistic Evidence	References
1p13.3	rs599839	SORT1 CELSR2 PSRC1	IG	0.78 (A)	1.11 (1.08–1.15)	EA South Asian (Japanese) (Korean)	CAD Early MI	LDLC level Lp-PLA_2 activity and mass (TC and HDLC) (AAA) (Hypertension)	• rs599839 is eQTL for PSRC1 in monocytes and SORT1, CELSR2, and PSRC1 in liver	[3,5–18]
1p32.2	rs17114036	PPAP2B	Intron	0.91 (A)	1.17 (1.13–1.22)	EA	CAD	–	• Expression of the PPAP2B protein product LPP3 is present in foam cells from human atherosclerotic plaques • oxLDL exposure up-regulates LPP3 • Inactivation of LPP3 in murine endothelial cells increases vascular inflammation	[3,5,10,19,20]
1p32.3	rs11206510	PCSK9	IG	0.82 (T)	1.08 (1.05–1.11)	EA (Han Chinese)	CAD Early MI	(TC and LDLC)	• LDL metabolism	[3,5,10,21–23]
1q21.3	rs4845625	IL6R	Intron	0.47 (T)	1.04 (1.02–1.07)	EA	CAD	(Atrial fibrillation)	• Inflammatory signaling	[5,24]
1q41	rs17465637	MIA3	Intron	0.74 (C)	1.14 (1.09–1.20)	EA (Meta Asian)	CAD Early MI		• Protein essential for collagen secretion	[3,10,25,26]
2p11.2	rs1561198	VAMP5 VAMP8 GGCX	IG	0.45 (A)	1.05 (1.03–1.07)	EA	CAD		• VAMP8 protein is important for platelet granule secretion	[5,27,28]
2p21	rs4299376	ABCG5-8	Intron	0.32 (G)	1.07 (1.04–1.11)	EA	CAD	(TC and LDLC) (LDLC intestinal absorption and level)	• ABCG5 and ABCG8 important cholesterol efflux and reverse transport proteins	[5,29–31]
2p24.1	rs2123536	TTC32 WDR35	IG	0.39 (T)	1.12 (1.08–1.16)	Han Chinese	CAD			[8]
2p24.1	rs515135	APOB	IG	0.83 (G)	1.08 (1.05–1.11)	EA	CAD	(TC and LDLC)	• Lipid metabolism	[5]
2q22.3	rs2252641	ZEB2-AC074093.1	Intron	0.46 (G)	1.06	EA	CAD			[5]
2q33.1	rs6725887	WDR12 TEX41 NBEAL1	Intron	0.15 (C)	1.14 (1.09–1.19)	EA	CAD Early MI	(LDL receptor expression)	• eQTL for NBEAL1 in aorta media	[3,5,10,17,32]
3q22.3	rs2306374 rs9818870	MRAS	Intron 3′UTR	0.18 (C) 0.11 (C)	1.12 (1.07–1.16) 1.07	EA	CAD	–	• rs9818870 variant alters miRNA-mediated gene expression and is an eQTL for MRAS in aorta media	[5,10,17,33]

4q31.22	rs1878406	EDNRA	IG	0.15 (T)	1.06 (1.02–1.16)	EA	CAD	(Ischemic CVA)		[5,34]
4q32.1	rs7692387 rs1842896	GUCY1A3	Intron IG	0.81 (G) 0.76 (T)	1.06 (1.03–1.09) 1.14 (1.10–1.19)	EA Han Chinese	CAD	(Blood pressure)		[5,8]
5p15.3	rs11748327	IRX1 ADAMTS16	IG	0.18 (T)	1.25 (1.09–1.41)	Japanese	CAD			[35]
5q31.1	rs2706399	IL5	IG	0.52 (G)	1.07 (1.03–1.11)	EA	CAD		• IL5 stimulates B-cells and increases immunoglobulin secretion	[29]
5q31.1	rs273909	SLC22A4 SLC22A5	Intron	0.14 (C)	1.09 (1.05–1.12)	EA	CAD			[5]
6p21.2	rs10947789	KCNK5	Intron	0.76 (T)	1.06 (1.03–1.08)	EA	CAD			[5]
6p21.31	rs17609940	ANKS1A	Intron	0.75 (G)	1.07 (1.05–1.10)	EA	CAD			[5,10]
6p21.32	rs3869109	HCG27-HLA-C	IG	0.55 (G)	1.14	EA (Han Chinese)	CAD (Early CAD)		• MHC class I proteins are putatively involved with vascular inflammation and T-cell responses in atherosclerosis	[36,37]
6p21.32	rs9268402	C6orf10 BTNL2	IG	0.59 (G)	1.16 (1.12–1.20)	Han Chinese	CAD			[8]
6p24.1	rs6903956	ADTRP	Intron	0.07 (A)	1.65 (1.44–1.90)	Han Chinese (Singapore) (Japanese)	CAD (Plaque severity)		• Protein expressed in human macrophages and atherosclerotic lesions in a PPARg-dependent manner	[38–42]
6p24.1	rs12526453 rs9349379	PHACTR1	IG Intron	0.67 (C) 0.61 (A)	1.13 (1.09–1.17) 1.34	EA Han Chinese Lebanese	CAD Early MI Stenosis severity	CAC Cervical artery dissection and large vessel stroke (Hypertension)	• rs12526453 is binding site for myocyte enhancer factor-2 leading to differential expression • rs9349379 is eQTLfor PHACTR1 expression in human coronary endothelium	[3,5,8,10,16, 43–48]

(*Continued*)

TABLE 8.1 (Continued)

Chromosome	SNP	Closest Genes	DNA Region	Allele Risk Frequency (Allele)	Allelic Odds Ratio (95% CI)	GWAS Population (Candidate)	GWAS Phenotype (Candidate)	Other SNP/ Phenotype Associations	Mechanistic Evidence	References
6q23.2	rs12190287	TCF21	3′UTR	0.62 (C)	1.08 (1.06–1.10)	EA (Chinese)	CAD	(CAC) (Hypertension)	• Variant disrupts a miR-224 binding site and modulates gene expression • eQTL for TCF21 transcript in liver and adipose	[5,10,16,49,50]
6q25.1	rs6922269	MTHFD1L		0.25 (A)	1.09 (1.05–1.14)	EA	CAD (Early MI)	(Cardiovascular mortality post MI)		[3,6,51]
6q25.3	rs3798220 rs2048327	LPA	Miss Intron	0.02 (C) 0.35 (G)	1.51 (1.33–1.70) 1.06	EA	CAD (Number of obstructed coronary vessels)	(LDLC and Lp(a)) (Ischemic stroke) (PAD) (AAA)	• Lp(a) strongly implicated in vascular inflammation and thrombosis	[5,52–57]
6q26	rs10455872	LPA	Intron	0.07 (G)	1.68 (1.43–1.98)	EA (Brazilian)	CAD (Coronary plaque on angiography)	(LDLC and Lp(a)) (Aortic valve stenosis)	• Lp(a) strongly implicated in vascular inflammation and thrombosis	[52,53,58–61]
6q26	rs4252120	PLG	Intron	0.73 (T)	1.06 (1.03–1.09)	EA	CAD	(Periodontitis)	• Plasminogen essential to fibrinolysis	[5,62,63]
7p21.1	rs2023938	HDAC9	3′UTR	0.10 (G)	1.07 (1.04–1.11)	EA	CAD	(Ischemic CVA)		[5,34]
7q22.3	rs10953541	BCAP29	Intron	0.75 (C)	1.08 (1.05–1.11)	EA South Asian	CAD			[7]
7q32.2	rs11556924	ZC3HC1	Miss	0.62 (C)	1.09 (1.07–1.12)	EA	CAD	(Carotid IMT)		[5,10,64]
8p21.3	rs264	LPL	Intron	0.86 (G)	1.05 (1.02–1.08)	EA (Japanese)	CAD	(HDLC and TG)	• Lipid and lipoprotein metabolism	[5,13,65]
8q24.13	rs17321515 rs2954029	TRIB1	IG IG	0.53 (A) 0.55 (A)	1.06 (1.03–1.10) 1.04	EA	CAD	(LDLC, HDLC, TG)	• Lipid and lipoprotein metabolism	[5,29,66–71]
9p21.3	rs4977574 rs3217992	CDKN2A CDKN2B ANRIL	IG 3′UTR	0.46 (G) 0.38 (A)	1.29 (1.23–1.36) 1.16	EA Japanese Korean (Korean) (Han Chinese) (Asian Indians)	CAD MI Early MI (Incident CAD) (Stenoses severity)	CAC Glioma AAA Intracranial aneurysm (Ischemic CVA) (Open-angle glaucoma) (Periodontitis)	• p16INK4a expression • ANRIL expression See text	[3,5,13,15,42,44,72–84]

9q34.2	rs579459	ABO	IG	0.21 (C)	1.10 (1.07–1.13)	EA	CAD MI (Recurrent MI) (Ischemic CVA)	(ACE activity and level) (Pancreatic cancer) (Ischemic CVA) (VTE) (LDLC) (Serum E-selectin, P-selectin, ICAM-1)	• Inflammation	[34,85–93]
10p11.23	rs2505083	KIAA1462	Intron	0.42 (C)	1.07 (1.04–1.09)	EA			• Protein involved with cadherin-based endothelial cell–cell junctions	[5,94]
10q11.21	rs1746048 rs2047009	CXCL12	IG IG	0.87 (C) 0.48 (C)	1.09 (1.07–1.13) 1.05	EA (Chinese)	CAD MI	(CXCL12 level)	• Protein is chemoattractant for progenitor cells in ischemic tissue	[5,49,95–97]
10q23.31	rs1412444	LIPA	Intron	0.34 (T)	1.09 (1.07–1.12)	EA (Chinese) (Mexican)	CAD MI		• eQTL for LIPA in monocytes • Protein lipase A catalyzes the hydrolysis of cholesteryl esters and triglycerides	[29,98–100]
10q24.32	rs12413409	CYP17A1 CNNM2	Intron	0.89 (G)	1.12 (1.08–1.16)	EA (Chinese) (Japanese)	CAD MI	(Ischemic CVA) (HTN)		[5,13,34,49]
11q22.3	rs974819	PDGFD	IG	0.29 (T)	1.07 (1.04–1.09)	EA (Korean)	CAD			[5,15]
11q23.3	rs964184	ZPR1 APOA5	IG	0.13 (G)	1.13 (1.10–1.16)	EA	CAD	Metabolic syndrome vitamin E (DM) (LDLC) (TG) (HDLC)	• ApoA5 protein is key regulator of triglycerides	[10,101–105]
12q21.33	rs7136259	ATP2B1	Intron	0.39 (T)	1.11 (1.08–1.17)	Han Chinese	CAD		• ATP2B1 gene is associated with hypertension by GWAS and in animal studies	[8,106,107]
12q24.11	rs3782889	MYL2	Intron	0.21 (C)	1.26 (1.19–1.34)	Korean Japanese	CAD	(Hypertension)		[15,108]
12q24.12	rs3184504	SH2B3	Miss	0.44 (T)	1.07 (1.04–1.10)	EA	CAD MI	sICAM-1 Eosinophil count Autoimmune hepatitis beta-2 microglobulin Hypothyroidism Celiac disease (HTN) (Ischemic CVA) (Multiple sclerosis) (Rheumatoid arthritis)	• Variant is trans regulator of the expression of 6 genes associated with BP (FOS, MYADM, PP1R15A, TAGAP, S100A10, and FGBP2) • Murine model suggestive of SH2B3 as link of immune signaling, inflammation, and hypertension	[5,34,109–116]

(*Continued*)

TABLE 8.1 (Continued)

Chromosome	SNP	Closest Genes	DNA Region	Allele Risk Frequency (Allele)	Allelic Odds Ratio (95% CI)	GWAS Population (Candidate)	GWAS Phenotype (Candidate)	Other SNP/ Phenotype Associations	Mechanistic Evidence	References
12q24.12	rs671	ALDH2	Miss	0.23 (A)	1.43 (1.35–1.51)	Japanese	CAD	(Multiple cancers)	• Regulation of inflammation, endothelial progenitor cells, oxidative stress, dimethylarginine, endothelial nitric oxide synthase	[83,117,118]
13q12.3	rs9319428	FLT1	Intron	0.32 (A)	1.05 (1.03–1.08)	EA (Japanese)	CAD	(CKD)	• VEGFR-1 is a key regulator of inflammation and is expressed by endothelial cells and macrophages in atherosclerosis	[5,13,119,120]
13q34	rs4773144 rs9515203	COL4A1 COL4A2	Intron Intron	0.44 (G) 0.74 (T)	1.07 (1.05–1.09) 1.08	EA	CAD		• Collagen type IV alpha proteins	[5,121]
14q32.2	rs2895811	HHIPL1	Intron	0.43 (C)	1.07 (1.05–1.10)	EA	CAD			[5]
15q26.1	rs17514846	FURIN	Intron	0.44 (A)	1.05 (1.03–1.08)	EA	CAD	Hypertension (Blood pressure)	• Proprotein overexpression in plaque mononuclear cells • Alters lipid metabolism in mice	[5,122–124]
15q25.1	rs3825807 rs7173743	ADAMTS7	Miss IG	0.57 (A) 0.58 (T)	1.08 (1.06–1.10) 1.07	EA	CAD	(CAC)	• Protein accumulates in coronary plaques • In mice protein inhibits re-endothelialization of injured arteries and promotes vascular remodeling through cleavage of thrombospondin-1 • Variant rs3825807 has an effect on ADAMTS7 maturation, thrombospondin-5 cleavage, and VSMC migration	[5,82,125,126]

17p11.2	rs12936587	RASD1 SMCR3 PEMT RAI1	IG	0.56 (G)	1.07 (1.05–1.09)	EA	CAD	(Ischemic CVA)	• PEMT/ApoE KO mice have improved lipoprotein profiles and less atherosclerosis • eQTL for PEMT and RASD1 in monocytes	[5,34,127,128]
17p13.3	rs216172	SMG6 SRR	IG	0.37 (C)	1.07 (1.05–1.09)	EA	CAD			[5]
17q21.32	rs46522	UBE2Z	Intron	0.53 (T)	1.06 (1.04–1.08)	EA	CAD		• eQTL for UBE2Z in blood	[5]
19p13.2	rs1122608	SMARCA4	Intron	0.77 (G)	1.14 (1.09–1.19)	EA	CAD	(Hypertension) (TC) (Ischemic CVA) (ABI)		[5,16,129,130]
19p13.2	rs6511720	LDLR	Intron	0.89 (G)	1.18 (1.11–1.25)	EA	CAD MI Early MI	AAA (LDL) (ApoB/A1)	• Lipid/lipoprotein metabolism	[3,22,29,131, 132]
19q13	rs2075650 rs445925	APOE APOC1	Intron IG	0.14 (G) 0.90 (C)	1.14 (1.09–1.19) 1.13	EA	CAD	Cognitive aging BMI Hippocampal brain volume Alzheimer's (Carotid disease) (LDL particle size)		[5,29,133–137]
21q22.11	rs9982601	MRPS6 SLC5A3 KCNE2	Gene Desert	0.15 (T)	1.18 (1.12–1.24)	EA	CAD Early MI		• eQTL for MRPS6 in blood and for SLC5A3 in aorta media	[3,5,17]

Abbreviations: AAA, abdominal aortic aneurysm; ABI, ankle brachial index; ACE, angiotensin-converting enzyme; CAC, coronary artery calcification; CVA, cerebral vascular accident; DM, diabetes mellitus; EA, European ancestry; HDLC, high-density lipoprotein cholesterol; IG, intergenic; IMT, intima-medial thickness; LDLC, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); Miss, missense; PAD, peripheral arterial disease; TC, total cholesterol; TG, triglycerides; VTE, venous thromboembolism. Table Interpretation: If a finding in the listed GWAS discovery population(s) was replicated in another population using a candidate gene approach, that population is listed in parentheses. Similarly, the GWAS phenotypes are listed, but if additional CAD phenotypes were found to be significant in candidate gene studies, they are listed in parentheses. Other non-CAD phenotypes associations are listed. If they were discovered by GWAS, they remain without parentheses, otherwise they were discovered by a candidate gene approach.

studies have often included transgene animal models where insertion of the mutated gene induces the same or similar phenotype to that observed in humans, thus demonstrating causality and functional relevance.

Classic Mendelian genetics is not possible for common polygenetic diseases like CAD with multiple genes contributing only mild effect sizes. However, the basic premise of allelic linkage underlies the development of GWAS. Kruglyak and Nickerson [142] suggested that case–control association studies could be a useful strategy to discover linked variants of nearby genes but would require hundreds of thousands of markers densely spaced across the entire genome. Prior to the development of the powerful microarray tools required to use this approach, investigators were forced to genotype individual candidate genes in order to compare cases and controls. Hundreds of variants in candidate genes were reportedly associated with atherosclerosis, thrombosis, or clinically manifest CAD but were inconsistently replicated. With the availability of the HapMap project [143] and new dense microarrays, the GWAS era started in 2007 with the discovery of a novel variant on chromosome 9p21 that was strongly associated with CAD [72,73]. Samani et al. [6] identified 142 SNPs in 91 candidate genes previously reported to be associated with CAD or myocardial infarction (MI). Using a GWAS array, in addition to the 9p21 locus, only two SNPs in linkage disequilibrium (LD) with the previously reported SNPs were found to be significantly associated with CAD in both the founder and replication populations. This finding should not dismiss the importance of many identified candidate genes given the stringent criteria for GWAS level of significance. For example, genetic variation in apolipoprotein E had established effects on LDL cholesterol levels and was associated with CAD. However, none of the early GWAS found significant gene variants in the APOE gene until larger sample sizes and newer arrays were utilized more recently [29]. The potential and pitfalls of GWAS discovery are discussed herein.

IMPORTANCE OF DEFINING THE PHENOTYPE

GWAS is a hypothesis free approach to identify mutations that occurred in past generations but remained common in populations. Genetic variants that occur at higher frequency in cases than controls are considered to be associated with disease risk. It should be noted that most GWAS-identified SNP variants have been associated with CAD using an additive risk model, but some were found to be more consistent with either a recessive or dominant mode of inheritance [3]. All major GWAS studies have been case–control studies with CAD defined as MI or angiographically defined stenosis greater than or equal to 50% in severity. However, the ultimate clinical manifestations of CAD, MI, and sudden death, are thrombotic complications of atherosclerosis, which is a separate biological process predating plaque rupture, thrombosis, and acute coronary syndromes. Thus the above definition of the CAD phenotype as a MI or a coronary artery stenosis is ambiguous to two distinct important mechanisms in the natural history of CAD, especially since stable or subclinical atherosclerosis is highly prevalent in adult populations. Since atherosclerosis is a prerequisite for MI, this dual mechanistic conundrum is amplified by the paucity of genetic data associated specifically with acute coronary syndromes, including MI. Reilly et al. [85] performed a GWAS which examined MI cases versus controls with angiographically defined atherosclerosis but no history of MI. They found the first gene variant associated with MI in a population with coronary atherosclerosis, located in the ABO blood group gene. Conversely, the locus at 9p21.3 was originally reported to be associated with both CAD and MI but subsequent studies indicate that it is likely associated with atherosclerosis but not thrombosis.

Other GWAS studies have focused on mechanisms of thrombosis that may contribute to MI. Johnson et al. [144] identified seven loci associated with platelet aggregation in response to agonists, including a novel intronic variant in the PEAR1 gene that was significant in subjects of European and African ancestry. Subsequent studies have robustly replicated these findings and determined that genetic variation in PEAR1 may be a determinant of platelet response to antiplatelet therapy and cardiovascular outcomes [145]. The PEAR1 finding demonstrates another strategy in discovering the genetic architecture of thrombosis in CAD, using GWAS to discover genes associated with intermediate phenotypes with known functional relevance, followed by determination of its association to clinical outcomes. Similarly more than 120 genomic loci have been associated with blood lipid and lipoprotein levels from more than 20 GWAS studies, most of which to date do not overlap with CAD GWAS signals. Thus, both functional and clinical relevance of an identified variant need to be established for a specific phenotype to fully discriminate true molecular risk pathways from false positive findings. Proxy phenotypes for subclinical coronary disease, reflecting atherosclerosis without thrombosis, including coronary calcium, intima-medial thickness, and ankle brachial index have also been reported but do not necessarily reflect atherogenic mechanisms leading to clinically manifest CAD and have not had robust overlap with the identified CAD variants.

SIGNIFICANCE AND REPLICATION

Since CAD is a common disease resulting from variants in multiple genes, each with small effects, large

sample sizes are required to detect allelic risk variants. Moreover, most risk variants discovered to date are located in noncoding DNA sequences, likely representing linkage to the true risk variant and/or conferring risk through regulation of protein coding sequences in nearby or distant genes or even on different chromosomes. A major limitation of GWAS is the potential for false positive results from multiple testing of greater than 1 million SNPs on modern arrays for a given phenotype, resulting in the need for a very stringent GWAS threshold of significance of $P < 5 \times 10^{-8}$, based on the Bonferroni correction of dividing a 5% false positive rate by 1 million tests of association [146]. Moreover, once an allele variant is identified to be in association with a specific phenotype in a discovery population then replication is expected in an independent population to further reduce the false discovery rate. Since the typical effect sizes for common variants of individual SNPs are relatively small, these statistical considerations require GWAS to be performed in large populations in order to provide adequate power for detection of single risk variants. For example, a discovery sample size of 20,000 subjects is required to detect a SNP variant that increases risk by 10%, occurring in 15% of the population, with 80% power [147]. This problem necessarily has led to the formation of large consortia made up of multiple studies with GWAS data, using meta-analysis techniques to combine the results from individual studies. Such consortia, often consisting of tens of thousands of subjects, are not perfect given differences in phenotype definition, subject selection, and population characteristics. Nonetheless, ever-increasing sample sizes have led to new discoveries. For example, the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) consortia confirmed 10 and discovered 13 novel risk variants for CAD with a sample size of greater than 82,000 individuals of European descent. Two other large studies, the Coronary Artery Disease (C4D) Genetics Consortium [7] and the IBC 50K CAD Consortium [29], included South Asians and Europeans and were powered to detect variants that were common to both ancestry populations. When CARDIoGRAM joined with other consortia, including C4D, the sample size expanded to more than 240,000 subjects and led to the confirmation of 46 variants and the discovery of an additional 15 novel genetic variants [5]. Wang et al. [38] reported on the first GWAS in Han Chinese, with the discovery of a genetic variant on 6p21 that appears to be specific to that population. Subsequent GWAS identified additional loci in the Chinese population, some with overlap in European ancestral populations [8].

Most GWAS have focused on individuals of European ancestry; so many discovered variants have been tested in different ancestral populations using a candidate gene approach. One glaring omission in the GWAS findings are reported risk variants in persons of African ancestry. African Americans have a higher lifetime risk of developing CAD than whites [148] and there is evidence for genetic susceptibility for CAD [149]. The few GWAS studies performed in African Americans were relatively small and underpowered compared to the larger consortia in populations of European ancestry [150,151] but nonetheless could not replicate the findings from previous GWAS. Franceschini et al. evaluated published GWAS risk alleles in a multiethnic cohort and were able to replicate associations with CAD in individuals of European ancestry but not in almost 9000 African American subjects [74]. The Genetic Study of Atherosclerosis Risk, a prospective study of early-onset CAD families, identified and replicated a variant in CDKN2B using a candidate gene approach that was protective for incident CAD, but subsequent case–control studies did not reproduce this finding [152]. Many factors likely contribute to the absence of reported genetic susceptibility data in persons of African ancestry, most notably the paucity of studies, differences in LD, less tagging of functional SNPs in GWAS arrays, ancestral differences in allele frequencies, genetic admixture, and environmental interactions. Ironically, the genetic architecture in Africans is more conducive to GWAS than those of Europeans given the smaller LD blocks and likelihood of tagging a functional nearby SNP.

BIOLOGICAL AND FUNCTIONAL RELEVANCE

The most intriguing potential of the GWAS approach is the ability to identify loci which do not associate with known CAD risk factors and are therefore likely to increase risk through mechanisms yet to be considered or discovered. Indeed, most of the identified risk variants are independent of lipid and lipoprotein levels, hypertension, and diabetes. Although the discovery of common allele variants associated with CAD has the potential to better elucidate new and known molecular pathways leading to atherosclerosis or thrombosis, the scientific challenge is significant as most variants reside in regulatory regions, introns, or intergenic DNA rather than in coding regions of known genes. These noncoding variants may regulate one or multiple nearby genes or even distant genes on different chromosomes (called cis- and trans-effects, respectively). Cis-regulatory elements can affect genes millions of base pairs upstream or downstream from its relative location on the chromosome and may be unaffected by meiotic recombination [153]. Therefore it is most difficult to implicate the true gene(s) contributing to CAD. The genes listed in the Table 8.1 for the discovered risk variants must be considered with caution and reflect proximity or assumed biological

relevance. Complicating this already difficult architecture are genetic effects of allelic heterogeneity (multiple variants in the same gene acting independently), copy number variation (variable repeats of genetic code), and pleiotropy (multiple biological and phenotypic effects).

Although some of the discovered susceptibility variants have known biological relevance to CAD, especially those already strongly associated with traditional risk factors, most have mechanisms yet to be elucidated. A prime example is the risk locus at 9p21, the first and still the most strongly associated GWAS finding with CAD as well as other chronic diseases, with multiple replications in a number of ancestral populations. This simultaneous discovery in 2007 by two independent investigative teams [72,73] has led to intense research to unravel the complicated genetics and biology of 9p21 (specifically discussed under the heading The 9P21.3 Risk Locus).

Although the discovery of variants in genes known to be related to lipid metabolism, including ABCG8, APOB, LPA, LPL, APOA5, LDLR, and APOE are not surprisingly associated with CAD, novel genes have been identified in association with CAD that were previously unknown to affect lipids. The SORT1/CELSR2/PSRC1 locus on chromosome 1p13.3 was found to be associated with CAD before it was identified as an extremely strong determinant of plasma LDL cholesterol concentration [9,154]. Variants in PCSK9 in people of African and European ancestry have been studied extensively for significant effects on LDL cholesterol and CAD risk and subsequently led to tangible new treatments for elevated cholesterol, thus demonstrating the reality that genetics can lead to novel pharmacologic therapies to lower CAD risk. Variants can also be mapped as quantitative trait loci (QTLs) to intermediate phenotypes (co-segregation of traits), such as LDL cholesterol or hypertension [155,156], or to expression QTLs (eQTLs) of mRNA or protein in specific tissue [32]. Whereas co-segregation of multiple phenotypes with an allele variant can suggest biological mechanisms leading to disease, eQTLs can identify regulatory roles with cis- and trans-effects [7]. TRIB1 (Drosophila tribbles homologue), was found to be a QTL for LDL cholesterol, HDL cholesterol, and triglyceride levels, consistent with pleiotropic effects [156]. Similarly, CYP17A1 on chromosome 10 was found to be a QTL for systolic blood pressure [157]. The variant on 1p13.3 was found to be an eQTL for SORT1, CELSR2, and PSRC1 in the liver and PSRC1 in monocytes demonstrating the potential of tissue-specific effects.

Vascular inflammation is putatively involved in all stages of atherosclerosis through a chronic interplay of innate and adaptive immunity within the vessel wall [158]. Given the involvement of multiple cell types and their interaction, GWAS should *a priori* be expected to identify genes related to these processes. True to what is understood about the pathophysiology of atherosclerosis, the most represented variants associated with CAD are lipid/lipoprotein and inflammatory loci. IL6R, IL5, CXCL12, and FLT1 are all involved in inflammatory signaling pathways. CXCL12 appears to be atheroprotective through the recruitment of progenitor cells and preventing endothelial apoptosis [159]. Circulating CXCL12 levels have been shown to be lower in CAD subjects compared to controls [160]. The signal near ABO has been associated to serum E-selectin, P-selectin, and ICAM-1 levels. A risk variant at 6p21.3 near the major histocompatibility complex (MHC) locus is of great interest given the importance of MHC molecules for antigen presentation and bridging of the innate and adaptive immune response in CAD [161]. The discovered variant is in LD with a long segment of DNA that spans multiple genes, including HLA-B and HLA-C, illustrating the difficulty of identifying the true causal variant in large LD blocks and finding functional significance of potentially multiple responsible genes.

Recent GWAS results have confirmed the role of many risk factors with genetic underpinnings and have implicated even more biological pathways related to atherosclerosis. Understanding the functional relevance will require years of applied complex bioinformatics solutions and laboratory studies. A few examples highlight the difficulties and promise of GWAS and the effort to understand the molecular mechanisms contributing to CAD: The loci on 9p21.3, 1p13.3, 1p32.3, and 12q24.12.

THE 9P21.3 RISK LOCUS

There has been no better example of the use of GWAS to identify novel genes associated with CAD than the discovery of a 58-kb region on the chromosome 9p21.3 locus that encompasses multiple SNPs in tight LD. This was also the first GWAS association with CAD and now the most robust and reproducible finding in a number of ancestral populations [6,72,73]. This common variant, with a risk allele frequency of close to 50%, has a relatively high effect size with an increased relative risk of approximately 25% for one copy and 50% for two copies, as well as a twofold increased risk of early onset CAD [3]. Importantly, unlike many other variants associated with CAD, the increased risk is independent of known risk factors. Moreover, the identified allele variants for CAD have been associated with a number of additional phenotypes, including ischemic stroke, abdominal aortic aneurysm, intracranial aneurysm, and glioma. Nevertheless, the molecular basis for this association has remained elusive and despite dense targeted resequencing at high coverage and 1000 Genome imputation, the causal variant is unclear [162,163].

The importance of phenotype definition is also part of the 9p21 story. The risk variants were originally associated with both angiographic CAD [72] a substrate for atherothrombotic manifestations, as well as MI [73]. Subsequently, a number of studies have reported associations with the presence, burden, and progression of coronary atherosclerosis but not MI, including using a large meta-analysis of angiographically defined CAD with MI status [164–166]. Furthermore, the 9p21 variants are primarily associated with risk of a first CAD event rather than subsequent events [167]. These later findings suggest that the 9p21 locus is associated with atherosclerosis but not thrombosis. Associations with other atherosclerotic phenotypes also support this hypothesis, including peripheral vascular disease [129], ischemic stroke [168], vascular dementia [169], and abdominal aortic and intracranial aneurysms [170].

Evidence to date has implicated a number of major molecular pathways to help explain the mechanistic importance of the 9p21 risk locus, an intergenic region devoid of protein-coding genes. However, this region overlaps a large antisense nonprotein-coding RNA called ANRIL, and is about 100,000 base pairs downstream of two cyclin-dependent kinase inhibitors, CDKN2A, and CDKN2B tumor suppressor genes [171] whose gene products inhibit important regulators of cell proliferation, senescence, and apoptosis [172,173]. The proteins coded by these genes, $p16^{INK4a}$ and $p15^{INK4b}$, as well as $p14^{ARF}$ from an alternate reading frame of the CDKN2a gene, are expressed in human coronary arteries. In atherosclerotic plaque, these proteins are localized to two primary cell types involved in atherogenesis; smooth muscle and CD68+ macrophages [174]. Targeted deletion of the orthologous 9p21 region in mice markedly decreased expression of these genes and increased the proliferation of aortic smooth muscle cells [175]. Using vascular injury models, Leeper et al. [176] found that CDKN2B knockout mice develop larger aortic aneurysms with increased smooth muscle cell proliferation and apoptosis, attributable to a reduction in MDM2, a protein that is degraded by ARF to inhibit apoptosis, and an increase in p53 signaling. In a recent elegant study, Kojima et al. [177] showed that deletion of CDKN2B in mice promoted advanced development of atherosclerotic plaque composed of large necrotic cores. Through a series of experiments, these investigators showed that human carriers of the 9p21 risk allele had reduced expression of CDKN2B which was associated with impaired expression of calreticulin, a protein required for activation of phagocytic receptors on macrophages. Reduced calreticulin resulted in resistance of apoptotic smooth muscle cells to removal by neighboring macrophages, reduced reverse cholesterol transport, and increased foam cell formation and inflammation. The addition of exogenous calreticulin reversed these defects associated with reduced CDKN2B expression.

The 9p21 locus overlaps exons 13–19 of ANRIL whereas CDKN2B is located within the first intron [178]. ANRIL has multiple splicing variants [179] and is expressed in endothelial, smooth muscle, and immune cells [180]. ANRIL expression levels have been inversely correlated with CDKN2A and/or CDKN2B expression and positively associated with CAD [179,181,182]. Jarinova et al. [179] showed that in white blood cells, homozygotes for the risk allele demonstrated upregulation of gene sets of cellular proliferation, increased expression of short variants of ANRIL, and a reciprocal decrease in expression of CDKN2B, GAPDH, and TDGF1 genes, supporting the hypothesis that the 9p21.3 risk region contains cis-regulatory elements of gene expression. Using a knock-down model of ANRIL expression in human aortic vascular smooth muscle cells and gene expression profiling, Congrains et al. [183] showed that different splicing variants of ANRIL may have distinct roles in regulating genes involved in cell proliferation, apoptosis, extracellular remodeling, and inflammation. Although the mechanism by which ANRIL functions is still under investigation, it has been shown to interact with polycomb repressor complex 1 and 2 that are involved with increased expression of ANRIL and the epigenetic repression of CDKN2A/B [184–186]. Methylation of CDKN2B and elevated expression of ANRIL have been associated with angiographic CAD in Chinese population [187]. These findings together suggest that the 9p21 risk variant alters gene regulation, most notably of CDKN2A/B, possibly through changes in ANRIL and other cis-regulatory elements.

Indeed, Harismendy et al. [188] examined the 9p21.3 region for potential regulatory elements and identified 33 predicted enhancers. The risk alleles of two SNPs, rs10811656 and rs10757278 were found to disrupt a binding site for STAT1. Using lymphoblastoid cell lines homozygous for nonrisk alleles, STAT1 binding inhibited ANRIL expression compared to cell lines homozygous for the risk alleles. Moreover, using chromatin conformation capture, this locus physically interacted with CDKN2A, CDKN2B, and MTAP genes as well as a distant region downstream of the IFNA21 gene. The investigators then demonstrated that IFN-ϒ activation affected chromatin structure and STAT-1 binding, leading to increased expression of CDKN2B and CDKN2BAS in human vascular endothelial cells and HeLa cells. However, Almontashiri et al. [189] showed that IFN-ϒ increased the expression of CDKN2B at the RNA and protein levels, but that this was independent of the 9p21 genotype in multiple cell types. Additionally, Erridge et al. [190] showed no effect of 9p21 genotypes on interferon regulation or in response to IFN regulatory factors.

Other potential mechanisms have been proposed, including altered TGFβ-SMAD signaling by the 9p21 locus leading to changes in TGFβ-dependent gene

activation which includes CDKN2A/2B [184]. The potential for gene–environment interaction is another possibility. For example, smoking and omega-3 fatty acid intake may modify the genetic risk conferred by rs4977574 on chromosome 9p21.3 [191,192]. Clearly it will take an intense integrative approach of molecular genetics and functional biology to fully understand the complex mechanisms underlying CAD risk at 9p21.

SORT1/CELSR2/PSRC1

Multiple CAD risk variants are in or near genes that are well known to affect lipid and lipoprotein levels. The discovery of a CAD risk variant on chromosome 1p13.3 near the SORT1/CELSR2/PSRC1 gene cluster, which also had the strongest association of any locus with LDL cholesterol in separate GWAS [156], represents a novel finding elucidating the complexities of regulatory variants and the promise of discovering biological mechanisms through functional genomics. Interestingly, rs599839 was found to be an eQTL for all three nearby genes; SORT1, CELSR2, and PSRC1 in the liver, as well as PSRC1 in monocytes. This noncoding intergenic polymorphism is part of a transcription factor binding site which alters hepatic expression of sortilin [193]. Murine knockdown studies showed that SORT1 alters plasma LDL cholesterol by regulating very LDL secretion [193]. In humans, homozygosity for the minor allele is associated with a more than 10-fold higher SORT1 expression in the liver, a mean 16 mg/dL lower LDL cholesterol, and a 40% reduced risk for MI [194]. Increased hepatic sortilin 1 expression appears to both reduce hepatic Apo B-100 secretion and increase LDL catabolism, independent of LDLR [195], but results of precise mechanisms have been inconsistent across animal studies [196,197], and additional potential pathways are being discovered, including the identification of sortilin as a high-affinity receptor for PCSK9 that modulates LDLR expression [198].

CELSR2 encodes for a nonclassic type of cadherin, cadherin EGF LAG seven-pass G-type receptor 2, involved in contact-mediated cell adhesion and receptor–ligand interactions [199]. The PSRC1 gene product, proline/serine-rich coiled coil protein 1, plays a role in cytoskeletal stabilization [200]. Using adeno-associated viruses to overexpress PSRC1 in hyperlipidemic LDLR knockout mice did not affect plasma cholesterol levels but overexpression of SORT1 led to a 40% decrease in cholesterol [197]. So far, SORT1 appears to be the gene most strongly associated with CAD, but given that the common variant is an eQTL for all three genes in this cluster, tissue-specific effects may overlap, providing pleiotropic effects.

PCSK9

PCSK9 is an excellent example of the promise of Mendelian randomization, a strategy to use genetic and phenotypic variation to investigate potential causality of a genetic variant to a disease phenotype. Allelic variants are randomly passed to gametes during meiosis and remain free of confounders throughout the life of an individual. If a gene variant associates with an intermediate phenotype, and the intermediate phenotype associates with disease, then the variant should associate with the disease if the intermediate phenotype is putatively contributing to the disease [201]. Although GWAS eventually identified a CAD risk variant near the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene, several years earlier Abifadel et al. [202] first reported a PCSK9 autosomal dominant gain-of-function mutation at chromosome 1p32.3 in a large Utah kindred, which associated with hypercholesterolemia and increased risk of CAD. Conversely, Cohen et al. [203] found that 2.6% of African Americans had a loss-of-function nonsense mutation in PCSK9 that was associated with a 28% lower mean LDL cholesterol level and an 88% lower risk of CAD. In subjects of European ancestry, 3.2% had a sequence variation in PCSK9 which was associated with a 15% lower LDL cholesterol level and a 47% reduction in the risk of CAD. These findings highlight the usefulness of Mendelian randomization, where in the case of PCSK9, the loss-of-function mutation and its effects on LDL cholesterol and CAD events showed that the protein is a viable and a safe target for specific inhibitors. PCSK9 belongs to a family of serine proteases and is expressed particularly in the liver where it binds to and increases the degradation of LDLR, VLDLR, and apolipoprotein receptor-2 (ApoER2) [204,205]. Since the liver primarily removes plasma LDL cholesterol through uptake by the LDL receptor where it is internalized, reduction in available LDLR by PCSK9 increases plasma LDL cholesterol. Given the genetic findings and understanding of the biological mechanisms of PCSK9, monoclonal antibody inhibitors of PCSK9 have been developed and are undergoing clinical trials. Studies so far have shown that these PCSK9 inhibitors cause an increased removal of LDL cholesterol by the liver and a reduction in plasma LDL cholesterol levels [206]. The results are extremely promising and complementary to statin therapy, which works by decreasing LDL cholesterol production [207].

SH2B3

SH2B3 (lymphocyte adaptor protein; Lnk) is a member of the SH2B (Src homology 2-B) family of adaptor proteins, is induced on activation of the Janus kinase

signal transducer-activator of transcription (JAK-STAT) pathway, and is involved in the negative regulation of several tyrosine kinases and cytokine signaling pathways [208]. Like the 9p21 risk locus, gene variants in SH2B3 have been associated with CAD as well as multiple other disease phenotypes. In particular, a common nonsynonymous SNP, rs3184504, has been associated with ischemic stroke [34], hypertension, type 1 diabetes [209], renal dysfunction [109,210], thrombocytosis [211], thrombotic antiphospholipid syndrome [212], myeloproliferative neoplasms [213], rheumatoid arthritis [214], multiple sclerosis [110], celiac disease [214], and generalized vitilgo [215]. The risk variant may, at least in part, contribute to CAD through its association with systolic and diastolic blood pressure [155] and LDL cholesterol [216], but Lnk is involved in a myriad of important cell signaling pathways. For example, rs3184504 has been identified by GWAS for its association with sICAM-1 [111]. Unlike most risk alleles identified by GWAS, rs3184504 is exonic and leads to an R262W amino acid change located in the pleckstrin homology domain of Lnk [217]. Interestingly the mutation is highly conserved in humans suggesting functional relevance, possibly through alterations in immune cell regulation [208,218]. The reason for the joint associations of this variant with multiple disease phenotypes is likely from strong pleiotropic effects of the gene [219]. Lnk is an important negative regulator of hematopoiesis and TNFα signaling in endothelial cells and may play an essential role in integrin signaling and affect platelet function during thrombus formation [208], all biological processes involved in CAD. Thus SH2B2 is a most promising gene for novel discovery of mechanisms in the development of CAD and other chronic diseases.

ADDITIONAL GWAS VARIANTS ASSOCIATED WITH CAD

Other identified risk variants are listed in the Table 8.1 by chromosomal location. The risk allele frequency is listed, although in many cases with high frequency, this could be interpreted as the minor allele conferring protection. Allelic odds ratios are from the study with the largest sample size. If a finding in the listed GWAS discovery population(s) was replicated in another population using a candidate gene approach, that population is listed in parentheses. Similarly, the GWAS phenotypes are listed, but if additional CAD phenotypes were found to be significantly in candidate gene studies, they are listed in parentheses. All listed associations are in reference to the GWAS SNP variant. If additional variants in a given locus were associated with a disease phenotype it is not listed. For example, the 9p21 locus has been associated with diabetes by GWAS but the variants are independent of the CAD variants. Other non-CAD phenotypes associations are listed. If they were discovered by GWAS, they remain without parentheses, otherwise they were discovered by a candidate gene approach. Finally, mechanistic evidence, if any, is summarized and referenced.

ASSESSING GENETIC RISK

The promise of genetics is to personalize risk assessment and develop therapies to reduce risk. Given the small effect sizes of most risk variants, the presence of one would add somewhat to traditional risk assessment or simple family history screening. However, these are common variants and some individuals may have multiple risk variants with possible cumulative or even synergistic risk. Many investigators have tried to use GWAS data to devise genetic risk scores, similar to traditional risk factor screening algorithms. Davies et al. [220] used a panel of 12 GWAS risk variants to significantly predict CAD beyond traditional risk factors. Isaacs et al. [221] found that a genetic risk score of common genetic variants for lipid levels are associated with subclinical and clinical CAD. However, the reality remains that most CAD risk variants do not act through known risk factors and those that do have well-established treatments. For example, if a patient had genetic variants that increase LDL cholesterol, it would be cheaper and easier to measure LDL cholesterol and treat with appropriate agents. Should an individual have multiple CAD risk variants (and likely would since they are common), how should that person be treated? Without a mechanistic understanding and appropriate targeted interventions being available, the only treatment which could be offered at this time would be more aggressive traditional risk factor modification despite independent risk conveyed by the gene. Ethical issues exist as well if the diagnosis of risk has no preventive solution.

Another approach has been to use gene expression profiling to assess risk of clinically relevant CAD. Thomas et al. [222] used a peripheral blood gene expression score based on quantitative real-time PCR in the Personalized Risk Evaluation and Diagnosis in the Coronary Tree (PREDICT) study to show high sensitivity and negative predictive value in detecting obstructive CAD and plaque burden in symptomatic patients referred to myocardial perfusion imaging [223]. This score algorithm was developed from genes representing a diverse set of inflammatory biological processes [224]. The identification of a loss-of-function CYP2C19*2 variant (rs4244285) as a major determinant of clopidogrel response [225] led to point of care testing, although the efficacy has been

questioned [226,227]. Certainly there is future promise to use genetics to risk stratify individuals as personalized medicine continues to develop.

FUTURE DIRECTIONS

There remains a lot of unexplained genetic heritability of CAD. The GWAS era will continue as consortia grow larger and increase their power of detection of common variants. The availability of the 1000 Genomes Project has opened up imputation based analyses particularly useful for less common variants. However, as technology continues to advance rapidly, multiple large studies are now examining associations with CAD on entire exome arrays or moving to whole genome sequencing to explore low frequency or "private" mutations that should lead to the discovery of additional important genes and biological pathways, and/or putative risk variants [228]. Powerful new technologies that sequence using chromatin conformation capture can help decipher short- and long-range interactions of risk loci thought to have cis- or trans-regulatory effects [188]. The ENCODE project has already mapped regulatory features across hundreds of transcription factors and multiple cell lines using ChIP-Seq (chromatin immunoprecipitation followed by high-throughput sequencing) [229]. Ultimately cell culture studies and animal models using knockout and overexpression methods will be needed to prove causality and define precise pathophysiological mechanisms.

Perhaps the greatest challenge will be to understand how genetic variants interact cumulatively to cause CAD. Genes usually interact to maintain homeostasis in a variable biological environment. Genetic variation can disturb this integrated network leading to disease, especially in the setting of environmental pressures [230]. The disease itself can present with high variability. For example, coronary atherosclerosis can exist without ever causing clinical manifestations, so genes that destabilize plaque or promote thrombosis may discriminate stable from unstable disease. Pathway and network analyses will thus be invaluable tools to evaluate overall biological systems in the pathogenesis of CAD [231,232]. Vangala et al. [233] recently showed the promise of an integrative bioinformatics analysis of genomic and proteomic approaches to understand transcriptional regulatory changes in CAD mechanistic pathways. Makinen et al. [234] performed an integrative genomics study for CAD using information from gene–gene and gene–transcript interactions which were then explored within established network and biological pathway data. The results confirmed several known CAD processes such as immune response, cholesterol transport, and metabolism, but also revealed novel pathways of neuroprotection, cell-cycle regulation, and proteolysis as involved in CAD.

Novel genome engineering technologies are now providing opportunities to study genetic variation in much greater detail. The RNA-guided clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPER-associated protein 9 (Cas9) system makes it possible to permanently alter the genome in living cells to study single-base editing, promoter activation, and gene repression or disruption *in vivo* [235,236]. For example, Ding et al. [237] used this system to introduce deletion mutations and disrupt the PCSK9 gene with subsequent transduction to the liver of adult mice via an adenoviral vector. This resulted in increased LDLR expression with a 40% reduction in cholesterol [237].

SUMMARY

The GWAS era has provided a platform for discovery of genes involved in the pathogenesis of CAD. However, all of the genetic variants discovered to date explain only a small portion of the disease. Gene–gene (epistasis) and gene–environment interactions, rare genetic variants, epigenetic influences, alterations in regulatory elements, and posttranslational modification of proteins are all likely important processes which contribute to the unexplained genetic causes of CAD. Most of the discovered risk variants for CAD still need years of bioinformatics and laboratory research to understand the molecular mechanisms involved. A few are showing great promise today as new preventive therapies and treatment strategies emerge based on the identification of specific genes being important in the development of CAD. It has been only 10 years since the first GWAS studies were begun. The "omics" era is just beginning in earnest, as the technology to sequence the entire genome, transcriptome, and proteome has only recently been developed and is being applied to ever increasing numbers of people. Deciphering this overwhelming amount of data and prioritizing which scientific questions to pursue will remain the biggest challenges in understanding the genetics of CAD in the coming years.

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CHAPTER

9

The Role of Nitric Oxide and the Regulation of Cardiac Metabolism

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FUNCTIONAL ANATOMY OF THE HEART

The coronary circulation arises from the aorta, via the ostia of the left main and right coronary arteries [1–7]. These large vessels support the delivery of blood flow to the capillaries for the exchange of oxygen, carbon dioxide, and substrate among other things. The dynamics of this arborization is such that the volume of blood contained within the coronary circulation under resting conditions is 3 mL/25 g of myocardium, with a circulation time from the left main coronary artery (LMCA) back to the LMCA of 9 s, given an aortic blood flow velocity of 92 cm/s. The circulation time shortens considerably during maximal blood flow with exercise [3,5–8]. The ability to vasodilate necessitates the evolution of a number of redundant but specific vasodilator mechanisms. One of the most studied is adenosine whose production is governed by the metabolism of ATP, a remnant of cardiac muscle contraction which converts ATP to ADP and is then rephosphorylated via the consumption of creatinine phosphate [2,9]. This couples the regulation of coronary extraction with metabolic need and allows for a fivefold increase in oxygen delivery (from 1 to 5 mL/min/g). Coronary blood flow is primarily mediated by resistance which is best measured by late diastolic resistance while the heart is at rest. Adenosine is responsible for large increases in blood flow as measured by significant reductions in late diastolic resistance, and is also responsible for dilatation of large coronary arteries which accounts for approximately 10% of total coronary flow. Figure 9.1 shows the waveform for a single-beat recording of coronary diameter (CD).

Coronary reserve is the difference between resting blood flow and historically maximum blood flow demonstrated by using adenosine to increase coronary blood flow by fourfold [2,9]. Oxygen delivery to the cardiac myocytes is also controlled by the amount of oxygen delivered to the arterial end of the capillary minus the amount of substrate which is extracted. Vasodilation in the heart is a two-step process. Initially, there is an increase in extraction at constant blood flow resulting in doubling of oxygen delivery, followed by a fourfold increase in blood flow, for a total of a fivefold increase in oxygen delivery. Thus, the product of the delivery of oxygen, substrates, fatty acids, glucose, and lactate to the heart minus the amount of these that leave the heart via venous blood flow in the coronary sinus is the net amount of the substrate used to support cardiac cell metabolism [10,11].

ROLE OF NITRIC OXIDE AND REACTIVE DILATATION IN CORONARY ARTERY VASOMOTION

In the 1990s, emphasis was focused upon the control of the coronary circulation by means of endothelial shear stress. For many years it was known that the application of acetyl choline or carbachol to blood vessel strips

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00009-7

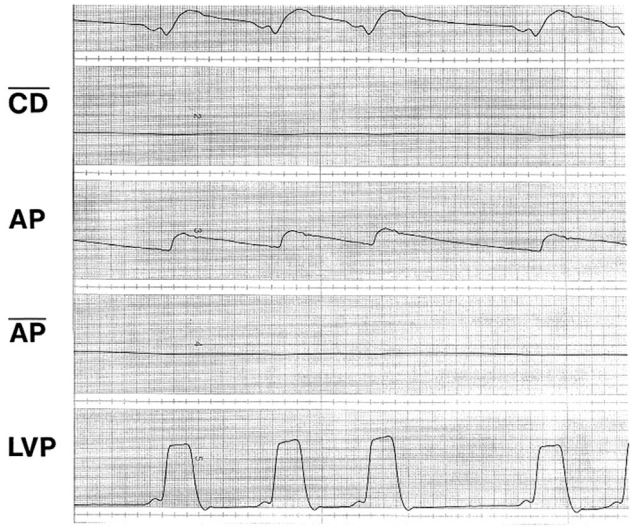

FIGURE 9.1 Waveforms for coronary artery diameter (CD), arterial pressure (AP), and left ventricular pressure (LVP) from a high-fidelity recording, demonstrating the similarity of the CD curve to that of AP. Large coronary artery cross-sectional area can be calculated from CD, and wall stress can be calculated if the wall thickness is also known.

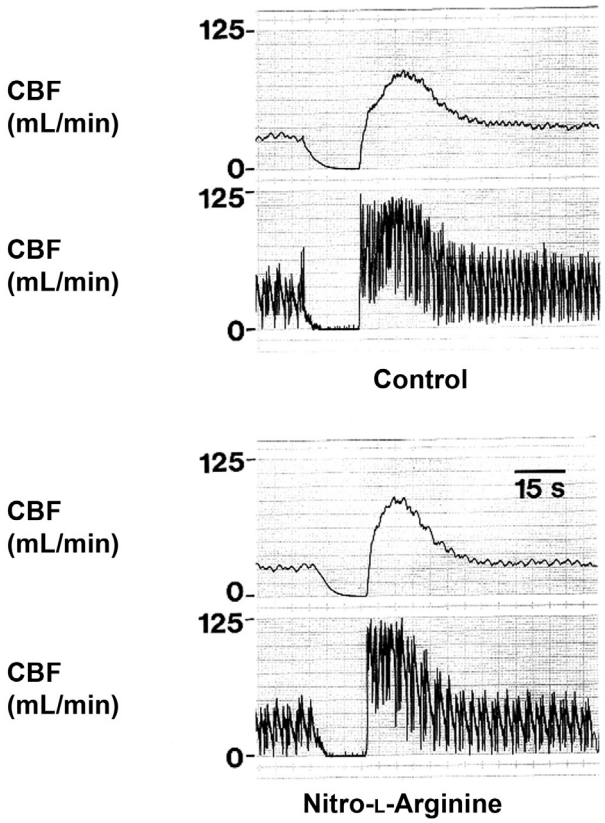

FIGURE 9.2 Reactive hyperemia following release of transient occlusion before and after administration of nitro-L-arginine. A small reduction in reactive hyperemia is observed to be associated with the presence of this substituted arginine molecule.

resulted in relaxation of blood vessels that was variable and unpredictable. Through meticulous recording of data by Furchgott, Ignarro, and Murad, it was found that when the endothelium was damaged or missing, relaxation was either diminished or replaced by overt vasoconstriction in strips of blood vessels from multiple species.

Occlusion of a large epicardial coronary artery in dogs results in characteristic reactive hyperemia similar to that observed in the peripheral circulation [12–20]. Transient occlusion of a large coronary artery for 30 s results in a drop in flow to zero, as calibrated with an electromagnetic blood flow transducer, following which release of the occluder results in marked increase in flow (Figure 9.2) associated with large artery vasodilatation as measured by sonomicrometry.

Reactive dilatation is proportional to the duration of the occlusion, is independent of occlusion proximal or distal to the placement of the diameter sensing crystals, and is not altered by drugs to inhibit the production of adenosine, prostaglandins, or sympathetic receptor blocking agents. In contrast, it is entirely eliminated if blood flow is maintained at a constant velocity (Figure 9.3), and is similarly eliminated by the inhibition of endothelial nitric oxide synthase (eNOS) with a substituted arginine molecule (Figure 9.4), hence confirming that this phenomenon is mediated by the endothelium [16,21].

This demonstrates a precise, sensitive control mechanism which employs shear stress as a stimulus for the

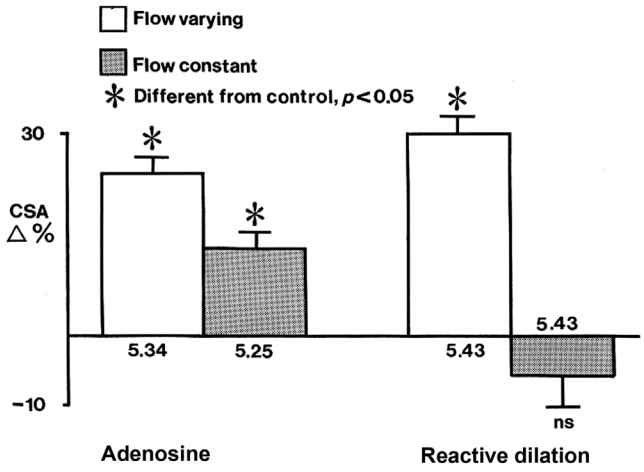

FIGURE 9.3 Large coronary artery dilation to adenosine is reduced by approximately 50% when the blood vessel blood flow is held constant, demonstrating that 50% of adenosine-mediated vasodilation is facilitated by adenosine receptor stimulation, while the remaining 50% results from a flow-dependent mechanism. In contrast, 100% of the large vessel dilation during reactive dilation is flow dependent.

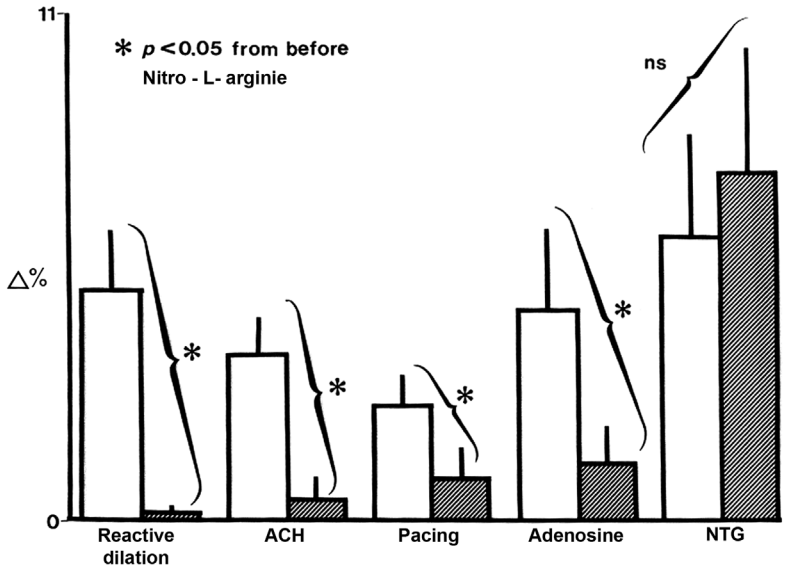

FIGURE 9.4 Change in diameter of the left circumflex coronary artery following 20 s of occlusion, administration of acetyl choline (ACH), cardiac pacing, adenosine infusion, and nitroglycerine (NTG) infusion. The open bars represent response to the stimulus alone, while the striped bars represent the arterial response following blockade of nitric oxide synthase (NOS) with nitro-L-arginine (L-NNA). All or nearly all of the vasodilatory response to arterial occlusion or ACH is obliterated by L-NNA, as is the flow-mediated portion of the vasodilatory response to pacing and adenosine. The addition of exogenous nitrate eliminates the inhibitory effect of L-NNA.

endothelium to make NO from arginine that can be used to quantitatively estimate the damage to the endothelium in various vascular disease states. As there is a fixed relationship between altered reactive dilation in the heart and forearm, studies of peripheral flow-mediated vasodilatation can predict disorders in coronary vasomotion [22]. Feldman et al. [23] have demonstrated that nitroglycerin causes transient dilatation of epicardial coronary arteries which is size related. Hence, dilatation in response to nitroglycerin becomes more and more attenuated as one moves from epicardial to intermediate to small endocardial arteries [23]. The function of this segmental dilation *in toto* is 10% of the capacitance of coronary circulation [1]. Figure 9.5 demonstrates the effect of prostaglandin-mediated dilation of a large coronary artery when exposed to arachidonic acid infusion, as compared to the more robust response to NO in Figure 9.6.

ORGANIC NITRATES, PROSTANOIDS, AND VASOCONSTRICTORS

Exogenous organic nitrates include nitroglycerin, nitroprusside, and CAS 936, which is a nitrate donor with an extremely long half-life [24]. Initially, the mechanism of action of the organic nitrates was thought to be related to vasodilation and its accompanying reduction in afterload. However, careful studies while investigating the effects of nitrates on cardiac dynamics demonstrated a reduction in ventricular filling due to a decrease in venous return resulting from an increase in venous capacitance [24]. Thus, venodilatation reduces both end diastolic volume and diastolic wall stress, resulting in a decrease in oxygen consumption and the overall work load of the heart. This apparently reflects different relaxation kinetics in arterial versus venous smooth muscle.

Zhang et al. [25] demonstrated a 39-fold greater sensitivity to nitroglycerin in venous compared to arterial endothelium. In contrast, other vasodilators such as prostacyclin [26], perhaps due to its activation of cAMP rather than cGMP, have a reduced vasodilatory potency compared to NO. Epicardial coronary dilatation in response to organic nitrates reflects the interaction of the nitrates and cGMP on smooth muscle in both large coronary arteries and in large peripheral veins [22,25,27,28]. Organic nitrates also modify substrate uptake in the hearts of multiple mammalian models including those of mice, rats, pigs, dogs, monkeys, and humans through a NO-cGMP-dependent mechanism [29]. In addition to the cGMP-mediated regulation of oxygen uptake through altered cardiac mechanics, NO also acts in a cGMP independent manner to regulate mitochondrial electron transport, resulting in a net reduction in oxygen consumption per unit oxygen consumed [30–34].

Potential mediators of coronary vasospasm include alpha- and beta-sympathetic agonists, arachidonic acid-related metabolites including thromboxanes,

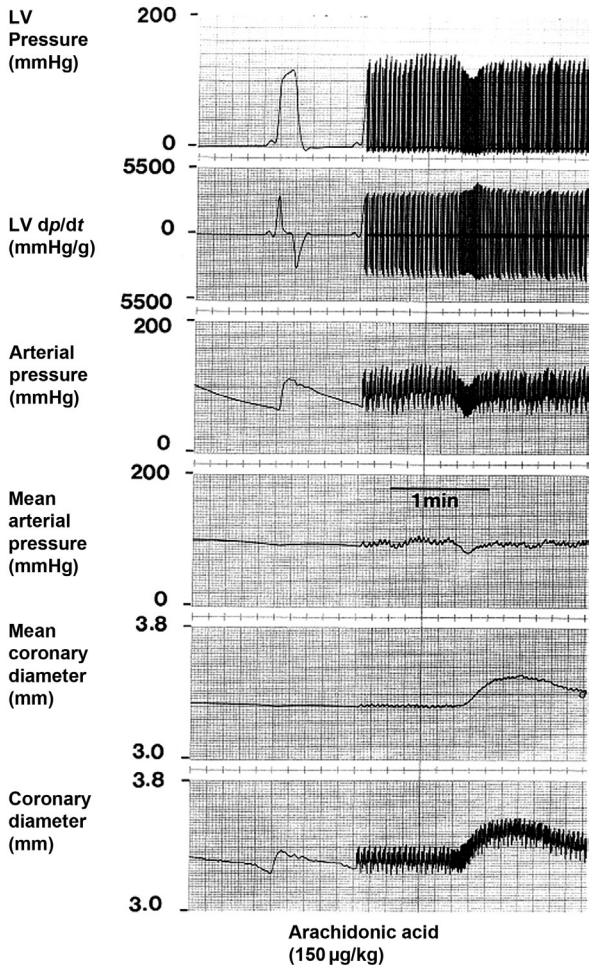

FIGURE 9.5 Changes in large coronary artery diameter when exposed to arachidonic acid (lanes 5 and 6). Arachidonic acid is the fatty acid precursor for eicosanoid vasodilators. Arachidonic acid-mediated vasodilation is blocked by nonsteroidal anti-inflammatory agents (NSAIDS) such as indomethacin indicating that it is prostaglandin dependent.

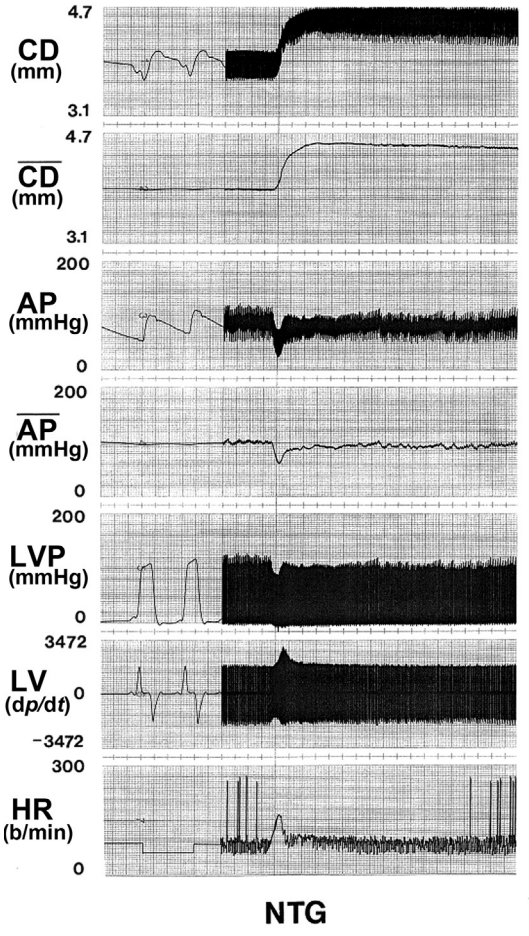

FIGURE 9.6 In contradistinction to Figure 9.5, infusion of nitroglycerin causes profound dilatation (CD), when compared to arachidonic acid. Exogenous NTG donates NO which serves as a more effective vasodilator than prostacyclin.

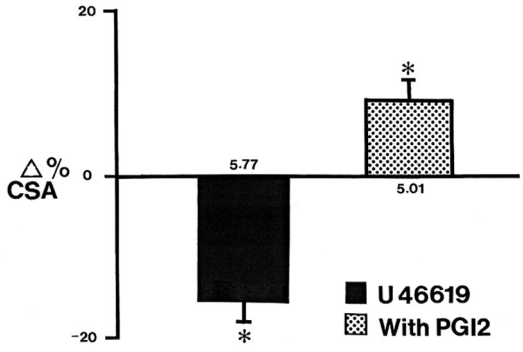

FIGURE 9.7 U46619 causes significant vasoconstriction of the large epicardial coronary arteries (black bar), an effect that is completely reversed and supplanted by vasodilatation following injection of prostacyclin (PGI2). *p < 0.05

hydroxyicosatetraenoic acids (HETEs), and leukotrienes [26]. The most potent of the vasoconstrictors is U46619, a thromboxane mimetic, which can cause profound large epicardial arterial constriction that is both prevented and supplanted by vasodilatation following the administration of prostacyclin (Figure 9.7).

NO AS A MEDIATOR OF PHARMACEUTICAL AGENTS

As NO is a powerful inducer of flow-mediated dilatation and has the ability to regulate venous capacitance, it is now known that it facilitates the activity of many therapeutically active cardiovascular drugs. The first angiotensin converting enzyme (ACE) inhibitor was originally developed as an agent to enhance the activity of bradykinin [35]. Its vasodilatory capacity, originally attributed to the activity of prostaglandins, has since been found to be mediated by NO [36]. Statins block

the inactivation of the mRNA for HMG-CoA reductase, increasing the half-life and production of NO [37]. In a similar fashion, they have been shown to enhance the vasodilatory capacity of ACE inhibitors and amlodipine [38]. The AT1 receptor antagonists reduce the generation of superoxide, thereby enhancing the half-life of NO, and have been demonstrated to reduce the production of nitrotyrosine in diabetic patients independent of their effect on blood pressure [39]. Evidence also suggests that the AT1 receptor activates assembly of NADPH oxidase in the heart and coronary blood vessels, hence reducing NO production [40]. Amlodipine also enhances NO production both by itself and in concert with other agents [41–45]. Dipyridamole has also been demonstrated to dilate large coronary arteries, but in contradistinction to the effect of NO, it also affects the small vessels, hence being less efficacious for relief from, if not responsible for the exacerbation of, ischemia due to fixed arterial stenosis [46,47].

Many drugs used in the treatment of cardiovascular disease may function under conditions such as acute exercise [1–3,5,7,35,48], exercise training, and pregnancy [32,33,49] via their effects on NO generation and metabolism. Furthermore, a great many metabolic disease states including low salt [50,51], hyperhomocysteinemia [52], fructose feeding [53], coronary microvascular stunning [40], and both compensated and decompensated heart failure [31,34,37] result in part or in whole from deranged NO synthesis and degradation. Heart failure, in particular, is mediated by altered gene expression [54–59] and reduced vagal-mediated NO-dependent coronary vasodilatation [28].

CONCLUSION

In this chapter, we have highlighted the potential mechanisms by which the epicardial blood vessels of the heart participate in the regulation of cardiac and vascular function with special emphasis on the role of large coronary arteries, the regulatory effect of which is not consistently appreciated in discussions of the management of blood flow in the heart. This is perhaps a result of the small combined cross-sectional area of the large arteries compared to the sum of the smaller vessels and the resulting relatively small volume of blood that is consistently present within them. Notwithstanding, there are instances in which the large epicardial coronary blood vessels are the primary source of vascular control. This occurs in acute myocardial infarction, vasospastic angina, the enhancement of coronary reserve that generates the typical four- to fivefold increase in substrate and oxygen delivery, and in the control of the coronary circulation by organic nitrates as well and other drugs such as ACE inhibitors, AT1 receptor blockers, statins, and L-type calcium channel blockers, all of whose effects are mediated in part or in whole by NO. The remarkable plasticity of these large coronary arteries, both in size and function, continues to make them fascinating and fruitful entities for mechanistic and therapeutic research.

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CHAPTER

10

Revascularization for Silent Myocardial Ischemia

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"Silent myocardial ischemia" is typically defined as the presence of ischemia without symptoms. Angina pectoris, first described by William Heberden [1], has long been considered the cardinal symptom of myocardial ischemia. However, it is now well established that myocardial ischemia may occur in the absence of overt symptoms. In fact, several studies in the 1970s demonstrated that asymptomatic (or silent) ST-segment depression during ambulatory electrocardiogram (AECG) monitoring occurred more often than symptomatic ST-depression in patients with coronary artery disease (CAD) [2–4]. Subsequently, studies in the 1980s and 1990s showed that silent myocardial ischemia portends a poor prognosis in patients with or without known CAD [5–8]. Randomized clinical trials at that time suggested that revascularization of patients with silent myocardial ischemia might improve clinical outcomes [9]; however, more recent studies have shown similar outcomes in patients with stable ischemic heart disease (SIHD) treated with optimal medical therapy (OMT) alone versus OMT combined with revascularization [10,11]. Thus, over the last few decades, there has been a paradigm shift in our understanding of the concept of silent myocardial ischemia, its prognostic significance, and its role in clinical decision making for the management of patients with and without established CAD.

PATHOPHYSIOLOGY OF SILENT MYOCARDIAL ISCHEMIA: A HISTORICAL PERSPECTIVE

Myocardial ischemia occurs when there is an imbalance between coronary blood flow (i.e., myocardial oxygen supply) and myocardial oxygen demand [12]. Atherosclerotic CAD is the most common underlying disorder responsible for myocardial ischemia. Autopsy studies demonstrating a strong link between coronary atherosclerosis and angina pectoris led to the original concept that, in the presence of a fixed coronary obstruction, myocardial ischemia occurred when myocardial oxygen demand out-stripped the capacity of the diseased coronary artery to deliver oxygen [13]. The point beyond which an increase in myocardial oxygen demand cannot be met by a proportionate increase in blood flow supply, hence resulting in myocardial ischemia, has been commonly referred to as the "ischemic threshold." However, "fixed obstruction" alone could not explain the onset of angina at rest or the variations in the ischemic threshold from patient to patient, by time of the day, or even by the level of mental or physical stress. This led to the concept of "dynamic obstruction"—that is, one in which the resistance offered by the atherosclerotic plaque on the epicardial vessels, and the resistance at the level of the microcirculation, vary as a consequence of coronary

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00010-3

vasoconstriction in large and small coronary blood vessels, respectively. In turn, this would result in myocardial ischemia due to transient reductions in myocardial oxygen supply or modifications in the ischemic threshold [14–18]. The increased coronary vasomotor tone may be a result of decreased production of vasodilator substances by the dysfunctional endothelium, as well as increased release of vasoconstrictor substances from the platelet thrombi and leukocytes.

Earlier studies suggested that a reduction in coronary blood flow played a dominant role in the genesis of silent myocardial ischemia [19,20]. This hypothesis was based primarily on the observations that heart rate at the onset of symptomatic or asymptomatic (silent) ST-depression on daily AECG monitoring was significantly lower than heart rate at the onset of ST-depression during ETT in the same patients. Also, since most episodes of silent myocardial ischemia occur during minimal or no strenuous activity, it was proposed that increased myocardial oxygen demand was unlikely to play a significant role. None of these studies, however, provided a direct evidence of reduction in coronary blood flow during episodes of silent ischemia.

Subsequently, several studies in the late 1980s and 1990s showed that increases in myocardial oxygen demand also play a significant role in the pathophysiology of silent myocardial ischemia [21–25]. The observations that supported this concept were: (i) most episodes of ischemia during daily activities were preceded by significant increases in heart rate and blood pressure; (ii) the morning surge in the frequency of silent ischemic episodes paralleled the morning increases in heart rate and systolic blood pressure; (iii) patients with ischemia during daily activities developed ST-depression earlier and at a lower heart rate and rate-pressure product during exercise stress testing (i.e., had lower ischemic threshold) than did those without ST-depression during ambulatory monitoring; and (iv) patients with a relatively high exercise ischemic threshold developed ST-depression in association with high heart rates during daily activities.

MAGNITUDE OF THE PROBLEM

Prevalence of Silent Myocardial Ischemia in Asymptomatic Subjects

Silent myocardial ischemia, by definition, implies absence of symptoms. Hence, it is difficult to determine the true prevalence of silent ischemia in the general population. Data from two large studies, the United States Air Force School of Aerospace Medicine (USAFSAM) and the Oslow Ischemia Study, showed that in asymptomatic middle-aged men with evidence of myocardial ischemia on exercise stress testing (ETT), approximately 2.5% had angiographic evidence of CAD (≥ 50% stenosis of one or more coronary arteries) [26,27]. In the Baltimore Longitudinal Study of Aging (BLSA), the prevalence of exercise-induced silent ischemia, defined by concordant ST-segment depression on ETT and a perfusion defect on thallium scintigraphy (201Tl), in apparently healthy individuals was 2% in those less than or equal to 59 years of age and increased to 15% in those more than 79 years of age [28].

Another group of patients includes those who suffer from silent myocardial infarction (MI). It is estimated that 155,000 first silent MI occur each year in the United States [29]. In a report from the Framingham study in 5127 asymptomatic individuals undergoing routine ECG evaluation, 28% of men and 35% of women developed ECG evidence of MI during the 30-year follow-up [30]. Similarly, in the Atherosclerosis Risk in Communities (ARIC) study, in 12,843 asymptomatic men and women aged 45–65 years, silent MI occurred in 20% of patients over a 9-year follow-up [31].

Prevalence of Silent Myocardial Ischemia in Known Coronary Artery Disease

Silent ischemia is common in patients with known CAD, which includes those with a prior MI, unstable angina, or chronic stable angina pectoris. Using either AECG or ETT, the reported prevalence of silent ischemia in survivors of MI varies from 30% to 43% [32,33]. Similarly, nearly one-half of patients admitted with unstable angina will have silent ischemia detected during continuous ECG evaluation [34]. In patients with CAD and chronic stable angina pectoris undergoing AECG monitoring, asymptomatic ST-segment depressions have been shown to occur in 41–56% of patients [6,35]. Similarly, pooled data on 1162 patients showed a 27–48% incidence of silent ischemic ST-segment changes during exercise [36].

PROGNOSTIC SIGNIFICANCE OF SILENT MYOCARDIAL ISCHEMIA

Presence of Ischemia as a Prognostic Factor

Several studies have demonstrated that the presence of silent myocardial ischemia is associated with an increased risk of adverse clinical outcomes in asymptomatic patients without a history of CAD as well as in those with various manifestations of CAD. In the Multiple Risk Factor Intervention Trial of 12,866 asymptomatic middle-aged men with two or more coronary risk factors, 12.5% had evidence of silent myocardial ischemia on ETT [37]. The presence of silent ischemia

was associated with a threefold increased risk of CAD death as compared to those without ischemia on ETT. In the Lipid Research Clinics Coronary Primary Prevention Trial of 3806 asymptomatic hypercholesterolemic men, during the 7- to 10-year (mean 7.4) follow-up period, the mortality rate from CAD was 6.7% in men with silent ischemia on ETT and 1.3% in men without ischemia [38]. Similarly, in 2682 men without CAD who participated in the Kuopio Ischemic Heart Disease study, exercise-induced silent ischemia was associated with an increased risk of death and of any acute coronary event (relative risk (RR) of 5.9 and 3.0 in smokers, 3.8 and 1.9 in hypercholesterolemic subjects, and 4.7 and 2.2 in hypertensive patients, respectively) [39]. These associations were weaker in men without these risk factors. In 407 healthy individuals enrolled in the BLSA, 9.8% had cardiac events (angina pectoris, MI, or cardiac death) over a mean follow-up of 4.6 years [40]. The incidence of cardiac events was 48% in patients with concordant abnormal ETT and 201Tl scintigraphy as compared to 7% in those without evidence of ischemia on either ETT or 201Tl scintigraphy.

Silent myocardial ischemia in patients with acute coronary syndromes is also associated with adverse prognosis [32]. In 70 patients with unstable angina managed medically in the coronary care unit, Gottlieb et al. showed that the presence of silent myocardial ischemia on continuous ECG monitoring was associated with poor short- and long-term outcomes, including death, MI, and revascularization [34,41]. In the Thrombin Inhibition in Myocardial Ischemia study, 232 patients with unstable angina underwent measurement of cardiac troponin T (cTnT) at admission and 24-h continuous ST-segment monitoring with vectorcardiography [42]. One or more episodes of ST-segment depression were independently associated with 30-day occurrence of cardiac death or acute MI (RR 7.43), and provided additional prognostic information to that of an early cTnT determination. Studies of patients with non-ST elevation MI and those with ST elevation MI have similarly demonstrated that the presence of transient ischemia, either during the acute or convalescent phase of MI, is predictive of increased mortality and reinfarction during short- and long-term follow-up [32]. Silent ischemia is also predictive of outcomes in patients who have undergone percutaneous coronary intervention (PCI) and stenting. In 356 consecutive patients with successful PCI and stenting and follow-up myocardial perfusion single-photon emission computed tomography (MPS) after 6 months, 23% had evidence of target vessel ischemia, which was silent in 62% [43]. Incidence of adverse events (cardiac death, MI, and target vessel revascularization) was 17%, 32%, and 52% in patients without ischemia, silent ischemia, and symptomatic ischemia, respectively.

The increased risk of coronary events and cardiac mortality associated with silent ischemia has been extensively documented in patients with SIHD [6,44–46]. Analysis of patients with known CAD from the Coronary Artery Surgery Study (CASS) registry showed that the presence of silent ischemia (≥1 mm ST-depression on ETT) was associated with increased risk of death and MI at 7 years as compared to those without ischemia [8,47]. Patients who had angina pectoris with or without ischemic ST-depression on ETT had a similar increased risk of death and MI, except in the three-vessel CAD subgroup, where the risk was greater with silent ischemia. The Total Ischemic Burden Bisoprolol Study, Atenolol and Silent Ischemia Study, and the Asymptomatic Cardiac Ischemia Pilot (ACIP) also reported a significant association between ambulatory ischemia either before or during treatment and adverse outcomes [48–50]. The end points included in these trials were death, MI, revascularization, hospitalization for ischemic event, and aggravation of angina requiring medical therapy. More recently, the Heart and Soul study examined the impact of self-reported angina, inducible ischemia, as determined by treadmill stress echocardiography, or both on outcomes in 937 outpatients with SIHD [51]. Inducible ischemia was present in 24% of patients, of which more than 80% was silent. The primary outcome of CAD death or MI at a mean 3.9 years of follow-up occurred more often in patients with inducible ischemia than in those without inducible ischemia (21% vs 8%). The presence of angina alone was not associated with these adverse outcomes.

Burden of Ischemia as a Prognostic Factor

Whereas the presence of silent myocardial ischemia is linked to poor prognosis, several studies have shown that the extent or severity of ischemia might be a more important predictor of adverse events. In 686 patients with chronic stable angina pectoris included in the Angina Prognosis Study in Stockholm, ischemia on AECG monitoring, especially when present for more than or equal to 30 min during a 24-h period, was independently associated with cardiovascular death only in patients with more than or equal to 2 mm ST-segment depression (i.e., marked ischemia) on ETT [52]. Similarly, studies using nuclear imaging modalities such as MPS to quantify the extent and severity of ischemia have also demonstrated a strong association between the burden of ischemia and the adverse event rate. In 2200 patients without known CAD, Hachamovitch et al. [53] demonstrated that the frequency of hard events (cardiac death or nonfatal MI) as well as the rate of referral to cardiac catheterization or revascularization increased as a function of increasingly abnormal exercise MPS. Similar results were reported in patients undergoing pharmacological

(adenosine) stress MPS, in men and women, in patients with and without diabetes mellitus, and in those managed with medical therapy with or without revascularization [7,54–56]. In another study of 356 consecutive patients with successful PCI and stenting and follow-up MPS after 6 months, summed difference score (SDS) was found to be the only independent predictor of silent ischemia [43]. For patients with an SDS of 0, 1–4, and more than 4, the critical event (cardiac death, MI, and target vessel revascularization) rates were 17%, 29%, and 69%, respectively. Farzaneh-Far et al. [57] demonstrated that in patients with SIHD, ischemia worsening is an important predictor of death and MI. In 1425 patients with angiographically documented CAD who underwent two serial MPS studies, the composite end point of death or MI at 5 years occurred in 43% of patients with more than or equal to 5% ischemia worsening compared with 26% of those with less than 5% ischemia worsening. After adjustment for established predictors, more than or equal to 5% ischemia worsening remained a significant independent predictor of death or MI, irrespective of the treatment strategy.

Disconnect Between Myocardial Ischemia and Adverse Prognosis: The Missing Link?

It is clear from the above studies that the presence and extent of silent myocardial ischemia is a marker of poor prognosis. However, the mechanism by which myocardial ischemia confers an increased risk of adverse cardiac events remains unclear. Data from animal models and human studies have shown that repeated episodes of transient myocardial ischemia are associated with small but distinct areas of subendocardial necrosis, myocardial hypokinesia, and regional wall motion abnormalities [58–60]. Thus, it is possible that recurrent episodes of silent ischemia may lead to progressive myocardial fibrosis and left ventricular dysfunction. Nevertheless, this does not explain the increased risk of cardiac death or MI, which is often a consequence of acute plaque rupture and thrombosis, and/or serious ventricular arrhythmias. A detailed angiographic analysis of 439 patients with ischemia on AECG in the ACIP study showed that 75% had multivessel CAD, 62.2% had proximal stenoses of more than or equal to 50%, and 50.1% had features suggesting complex plaque [61]. These findings may explain, in part, the increased risk for adverse cardiac outcomes associated with ischemia during activities of daily life. However, serial angiographic and stress echocardiographic studies have demonstrated that 54–65% of acute MI results from plaque rupture and thrombus formation in lesions that have luminal stenosis of less than 50% on prior angiography and demonstrate no ischemia on stress testing [62–66]. In 47 patients who underwent MPS, reversible perfusion abnormality was detected in only 28 segments (60%) that were the site of future acute MI [67]. Thus, a causal relationship between silent myocardial ischemia and risk of acute MI remains unclear.

Studies in small groups of patients with chronic stable angina pectoris have shown that potentially life-threatening ventricular arrhythmias are closely associated with severe repetitive episodes of symptomatic or silent ischemia [68]. Ventricular arrhythmias accompany spontaneous ischemic ST-segment depression, particularly when the latter is recurrent, prolonged, and symptomatic [69]. Ventricular arrhythmias are characterized by a greater frequency, duration, and malignancy during the ischemia phase than during the recovery phase of ischemic attacks. These data suggest a potential link between silent ischemia, ventricular arrhythmias, and sudden cardiac death (SCD) [70]. However, the exact mechanism underlying these observations remains unknown.

The pathophysiological underpinning of untoward cardiovascular events (MI and SCD) in patients with CAD is the spontaneous rupture of an atherosclerotic plaque, but this is not the mechanism that leads to myocardial ischemia during daily life or stress testing in these patients. Hence, there is a missing link between the identification of ischemia in patients with stable CAD and the events that confer poor prognosis (Figure 10.1). Further, there is no clear relationship between the location of ischemia during stress testing and the location of the ruptured plaque. Thus, it is possible that the presence and extent of myocardial ischemia (silent or

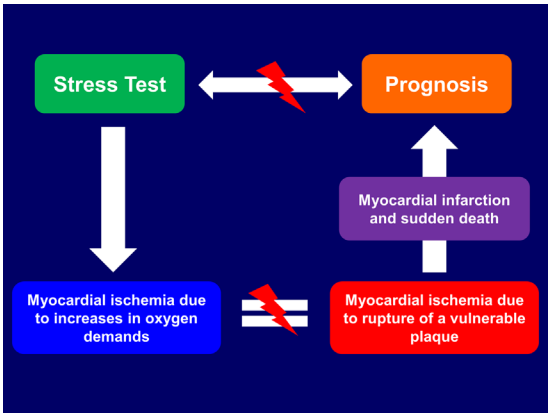

FIGURE 10.1 **Schematic representation of the disconnect between testing for inducible myocardial ischemia and adverse prognosis.** *Counterclockwise from top left:* Stress testing is performed to unveil myocardial ischemia that occurs as a consequence of increases in oxygen demands in the presence of flow-limiting coronary stenoses. This type of ischemia is pathophysiologically different from that which occurs as a consequence of the rupture of a vulnerable coronary plaque. It is the latter (but not the former) mechanism the one that leads to myocardial infarction and sudden death, which in turn determine the prognosis of patients with CAD. Thus, although the presence and extent of ischemia on stress tests are associated with poor prognosis, the mechanism that provides the link for this association is elusive.

otherwise) is only a marker of more extensive CAD, which in turn is associated with increased risk due to an anatomic substrate more prone to the possibility of a ruptured plaque.

REVASCULARIZATION FOR SILENT MYOCARDIAL ISCHEMIA

Revascularization Versus OMT

In the 1970s and 1980s, three landmark clinical trials established the survival benefit of CABG versus medical therapy alone in patients with SIHD—the Veterans Administration (VA) Cooperative Study Group, the CASS, and the European Coronary Surgery Study [71–73]. However, only the VA Study required an objective evidence of ischemia on AECG or ETT in addition to chronic stable angina pectoris as the inclusion criteria. Collectively, these studies demonstrated that CABG is associated with improved long-term survival in high-risk patients with significant (≥50% stenosis) left main (LM) coronary artery disease, significant (≥70% stenosis) 3-vessel disease (3VD), and proximal left anterior descending artery stenosis constituting a component of either 2-vessel disease or 3VD [74,75]. The presence of impaired left ventricular ejection fraction (LVEF) less than 0.50, or more than or equal to 2 high-risk clinical features (ST-segment depression on baseline ECG, history of MI, and history of hypertension) increased the absolute survival benefit of CABG over medical therapy alone [76–79]. These studies also established that CABG is more effective than medical therapy for relieving anginal symptoms. A meta-analysis of 2649 patients treated with either CABG or medical therapy between 1972 and 1984 demonstrated that the CABG group had significantly lower mortality than the medical treatment group at 5, 7, and 10 years [80]. The absolute benefits of CABG were most pronounced in patients in the highest risk categories, as determined by a combination of several prognostically important clinical and angiographic risk factors.

These findings were subsequently reproduced only once in the Medicine, Angioplasty, or Surgery Study II (MASS-II). The MASS-II (1995–2000) compared relative efficacies of medical treatment, PCI, and CABG in 611 patients with angiographically documented proximal multivessel coronary stenosis of more than 70% and myocardial ischemia. Ischemia was documented by either ETT or the typical stable angina assessment of the Canadian Cardiovascular Society (CCS) class II or III [81]. The primary end point was the combined incidence of cardiac death, MI, or refractory angina requiring revascularization. Patients with multivessel CAD who were treated with CABG were less likely than those treated with medical therapy alone to experience cardiac death, MI, or refractory angina requiring revascularization at 5- and 10-year follow-up [81,82]. Additionally, CABG was also superior to medical therapy at eliminating anginal symptoms. On the other hand, compared with PCI, medical therapy was associated with a lower incidence of short-term events and a reduced need for additional revascularization.

The potential benefit of revascularization in suppressing silent myocardial ischemia was examined in the National Heart, Lung, and Blood Institute (NHLBI)-sponsored ACIP study, which randomized 558 patients to one of the three treatment strategies: (i) medical therapy to suppress angina, (ii) medical therapy to suppress both angina and silent ischemia as assessed by AECG monitoring, or (iii) revascularization with CABG or percutaneous transluminal coronary angioplasty (PTCA). Medical therapy included atenolol plus sustained release nifedipine or diltiazem if needed, plus sustained release isosorbide dinitrate if needed. The primary outcome of complete suppression of ischemic episodes on 48-h AECG at 12 weeks occurred in 39%, 41%, and 54% of patients in the three treatment groups, respectively. Mortality at 1 and 2 years was significantly lower in the revascularization group as compared to the ischemia-guided and angina-guided medical therapy groups (0%, 1.6%, and 4.4%, respectively, at 1 year, and 1.1%, 4.4%, and 6.6%, respectively, at 2 years). Rates for the combined end points of death, MI, or recurrent cardiac hospitalization at 1 and 2 years were lower for patients undergoing revascularization compared with those undergoing medical therapy. There was no significant difference between the two medical therapy strategies.

The Swiss Interventional Study on Silent Ischemia Type II (SWISSI II) was a randomized, unblinded, controlled trial conducted from 1991 to 1997 that compared the effect of PCI versus medical therapy alone on the long-term outcome of 201 asymptomatic patients with a recent MI, silent myocardial ischemia verified by stress imaging, and one- or two-vessel CAD [83]. During a mean follow-up of 10.2 ± 2.6 years, rates of major adverse cardiac events (defined as cardiac death, nonfatal MI, and/or symptom-driven revascularization) were significantly lower in the PCI group as compared to the medical therapy group (adjusted hazard ratio, 0.33; 95% confidence interval, 0.20–0.55; $p < 0.001$). Patients in the PCI group also had lower rates of exercise-induced ischemia and had preserved LVEF. Further, compared with medical therapy alone, PCI also reduced the rate of residual myocardial ischemia, recurrent MI, and SCD [84].

Although these earlier studies suggested that revascularization may be beneficial in suppressing ischemia, reducing anginal symptoms, and improving outcomes, these trials were conducted prior to the advent of modern medical, interventional, and surgical advances.

Medical therapies in these studies were clearly less than optimal; particularly, the lack of aggressive lipid lowering with statins, and medication adherence was poor. PCI has also evolved rapidly over the past three decades. PTCA has been replaced since the introduction of bare metal stents (BMSs) in the mid-1990s, followed by the drug-eluting stents (DESs) in 2003, along with improved adjunctive pharmacotherapy and advances in technology other than stenting, such as distal protection devices, thrombectomy and atherectomy catheters, and fractional flow reserve (FFR). Similarly, advances in surgical techniques have resulted in a substantial decrease in the perioperative mortality associated with CABG.

Data on the impact of revascularization on outcomes in the contemporary era of modern medical therapies, and advanced interventional and surgical techniques comes from the following major randomized controlled trials (RCTs)—Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D), Surgical Treatment for Ischemic Heart Failure (STICH), Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE), and Fractional Flow Reserve versus Angiography for Multivessel Evaluation 2 (FAME 2) [10,11,85,86]. The BARI 2D study randomized 2368 patients with type 2 diabetes mellitus, myocardial ischemia, and CAD (≥ 50% stenosis of a major epicardial coronary artery associated with a positive stress test, or ≥ 70% stenosis of a major epicardial coronary artery and classic angina) to prompt revascularization (with PCI or CABG, at the discretion of the treating physician) plus OMT or OMT alone [11]. Randomization was stratified according to the choice of PCI or CABG as the more appropriate intervention. At 5 years, there was no difference in rate of death or major cardiovascular events between the revascularization plus OMT and OMT alone groups. The rate of death did not differ significantly between the two treatment groups in either the CABG or the PCI stratum. However, in the CABG stratum, which included patients with more extensive CAD (more 3VD, proximal LAD disease, and chronic coronary occlusions), rate of major cardiovascular events was significantly lower in patients assigned to revascularization group as compared to those receiving OMT alone (22.4% vs. 30.5%, $p = 0.01$). In the CABG stratum, MI events were significantly less frequent in revascularization plus OMT versus OMT alone groups (10.0% vs. 17.6%; $p = 0.003$), and the composite end points of all-cause death or MI (21.1% vs. 29.2%; $p = 0.010$) and cardiac death or MI ($p = 0.03$) were also less frequent [87].

A common exclusion criterion in all preceding CABG trials was the presence of significant heart failure. The NHLBI-sponsored STICH trial was specifically designed to compare CABG plus intensive medical therapy to intensive medical therapy alone in patients with CAD and LVEF less than or equal to 35% [86]. The primary outcome was the rate of death from any cause. Secondary end points were death from cardiovascular causes and rate of death from any cause plus cardiovascular disease-related hospitalization. At a median 56-month follow-up, there was no significant difference between CABG plus intensive medical therapy and intensive medical therapy alone with respect to the primary end point of death from any cause (36% vs. 41%, $p = 0.12$). However, patients assigned to CABG, as compared with those assigned to medical therapy alone, had lower rates of death from cardiovascular causes and of death from any cause or hospitalization for cardiovascular causes.

The COURAGE trial was designed as an intention-to-treat comparison between PCI + OMT and OMT alone in 2287 patients with SIHD [10]. Patients were included in the study if they had more than or equal to 70% stenosis in at least one proximal epicardial coronary artery and objective evidence of myocardial ischemia or at least one coronary stenosis of more than or equal to 80% and classic angina without provocative testing. Patients with CCS class IV angina, a markedly positive stress test, LVEF less than 30%, revascularization within the previous 6 months, and coronary anatomy not suitable for PCI were excluded from the study. At a median 4.6 years follow-up, the primary outcome of death from any cause and nonfatal MI occurred in 19% of patients in the PCI group versus 18.5% of patients in the OMT alone group ($p = 0.62$). There were no significant differences between the PCI group and the OMT alone group in the prespecified secondary end points (composite of death, MI, or stroke, and hospitalization for acute coronary syndrome). However, there was a statistically significant difference in the rates of freedom from angina throughout most of the follow-up period, in favor of the PCI group.

The COURAGE Nuclear Substudy enrolled 314 (13.7%) of 2287 patients included in the original trial to compare the effectiveness of PCI for ischemia reduction when added to OMT [88]. Patients underwent serial MPS scans before treatment and 6–18 months after randomization. Moderate to severe ischemia was defined as more than or equal to 10% ischemic myocardium on MPS. At baseline, moderate to severe ischemia was present in 34% of PCI + OMT versus 33% of OMT patients ($p = 0.81$). The primary end point, more than or equal to 5% ischemia reduction, occurred in 33% of PCI + OMT patients compared with 19% of OMT patients ($p = 0.0004$). In patients with moderate to severe pretreatment ischemia, event-free survival was 83.8% versus 66.0% for patients with versus without significant ischemia reduction ($p = 0.001$). The rate of death or nonfatal MI ranged from 0% for patients with no ischemia to 39.3% for patients with more than or equal to 10% ischemic

myocardium on their follow-up MPS. However, an updated analysis published in 2012, the COURAGE Nuclear Study 0, showed no difference in rates of primary end point (death or MI) between OMT and PCI + OMT groups for no to mild (18% and 19%, $p = 0.92$), and moderate to severe ischemia (19% and 22%, $p = 0.53$, interaction p value = 0.65) on baseline stress MPS [89]. In this updated analysis, the extent of ischemia was defined based on the number of ischemic segments using a six-segment myocardial model (no to mild: <3 ischemic segments, and moderate to severe ischemia: ≥3 ischemic segments). Similar to the findings of this post hoc analysis, the STICH Myocardial Ischemia Substudy, which included 399 patients with CAD and LEVF less than or equal to 35% who underwent ischemia testing with either MPS or dobutamine stress echocardiography, showed that inducible myocardial ischemia did not identify patients with worse prognosis or those with greater benefit from CABG over OMT alone [90].

Nonetheless, there are other sources of data supporting the prognostic benefits of revascularization in SIHD, particularly revascularization of coronary vessels that have been shown to produce the myocardial ischemia. In a retrospective observational study, Hachamovitch et al. [91] showed that in 10,627 patients with no previous history of MI or revascularization who underwent exercise or adenosine stress MPS, revascularization with either PCI or CABG was associated with lower rates of cardiac death and all-cause mortality as compared to medical therapy alone in patients with moderate to severe ischemia (>10% of ischemic myocardium), whereas in patients with no or mild ischemia, survival was better with medical therapy alone as compared to revascularization. In a subsequent study of 13,555 patients including those with previous history of CAD, MI, or revascularization, Hachamovitch et al. [92] also demonstrated that that the extent of myocardial ischemia identified a survival benefit with early revascularization in patients without prior MI, whereas no such benefit was present in patients with prior MI (interaction $p = 0.021$).

FFR is now considered the gold standard for identifying coronary artery lesions that cause ischemia. FFR is an index of the physiological significance of a coronary stenosis and is defined as the ratio of maximal blood flow in a stenotic artery to normal maximal flow (P_d/P_a) [93]. FFR in a normal coronary artery equals 1.0. An FFR value of less than or equal to 0.80 identifies ischemia-causing coronary stenoses with an accuracy of more than 90% [94]. The FAME trial demonstrated that in patients with multivessel CAD, FFR-guided PCI was associated with significantly lower rate of death, nonfatal MI, and repeat revascularization at 1 year, as compared to PCI guided by angiography alone [95]. The FAME 2 trial randomized 888 patients with multivessel CAD to FFR-guided PCI plus OMT and OMT alone [85]. The prespecified primary end point was a composite of death from any cause, nonfatal MI, or unplanned hospitalization leading to urgent revascularization during the first 2 years. Secondary end points included individual components of the primary end point, cardiac death, nonurgent revascularization, and angina class. The trial was terminated early (mean follow-up of 213 ± 128 days in the PCI + OMT group, and 214 ± 127 days in the OMT alone group) due to significant between-group difference in the rate of the primary end point—4.3% in the PCI + OMT group versus 12.7% in the OMT alone group ($p < 0.001$). The difference was driven mainly by a lower rate of urgent revascularization in the PCI group than in the medical therapy group (1.6% vs. 11.1%, $p < 0.001$). Several factors may explain the differences between results of FAME 2 and those of previous trials, particularly COURAGE, involving patients with SIHD. First, in previous trials, patient enrollment was based primarily on initial anatomic characterization with coronary angiography, with or without noninvasive documentation of ischemia. It is likely that a significant proportion of the patients had only limited ischemia. For instance, even in the COURAGE trial, in which noninvasive testing was performed in 85% of the patients, less than one-third of the patients had more than 10% ischemia on MPS [88]. Second, PCI in FAME 2 was performed only in lesions with an FFR less than or equal to 0.80, which has been shown to improve outcomes significantly as compared to PCI guided by angiography alone [95]. Third, the primary end point in FAME 2 included not only death and MI, but also urgent revascularization, a component that was not included in the primary end point of most previous trials, including COURAGE.

Gada et al. [96] recently performed a meta-analysis of three RCTs (SWISSI II, COURAGE Nuclear Substudy 0, and FAME 2) enrolling a total of 1557 patients with SIHD and an objective evidence of ischemia as assessed by MPS or FFR to compare all-cause mortality with PCI versus medical therapy alone. When compared with medical therapy alone in this population of patients with an objective evidence of ischemia, PCI was associated with significantly lower all-cause mortality (hazard ratio 0.52, 95% confidence interval 0.30–0.92, $p = 0.02$). The authors proposed that the mortality benefit of PCI over medical therapy alone may relate to the prevention of prognostically important spontaneous MI events [97]. This hypothesis is supported by findings of another recent meta-analysis by Bangalore et al. [98], which demonstrated a reduction in spontaneous MI with PCI versus OMT, as well as the landmark analysis of FAME 2 at 7 days (which would essentially exclude periprocedural MI), which showed a substantial reduction in death or MI between 8 days to maximum follow-up with PCI versus OMT alone [85].

CABG Versus PCI

With the routine use of DES in the contemporary era, RCTs comparing CABG with PTCA or BMS are mostly of historical significance. A systematic review of 23 RCTs comparing CABG with PCI (PTCA or BMS) showed that CABG was more effective in relieving angina and was associated with a lower rate of repeat revascularizations, but higher rate of procedural stroke [99]. Survival was similar at 1, 5, and 10 years. Of note, in the ACIP study, CABG was also superior to PTCA in suppressing myocardial ischemia (as demonstrated by AECG and ETT) despite the presence of more severe CAD in patients who underwent CABG [100]. Another collaborative analysis of 7812 patients with multivessel CAD included in 10 RCTs also showed similar all-cause mortality with CABG and PCI (PTCA or BMS) at a median follow-up of 5.9 years [101]. However, CABG was associated with a lower mortality in patients with diabetes mellitus and in those more than 65 years of age.

In the DES era, the most compelling data on outcomes of CABG versus PCI comes from the Synergy between PCI with Taxus and Cardiac Surgery (SYNTAX) and Future Revascularization Evaluation in Patients with Diabetes Mellitus: Optimal Management of Multivessel Disease (FREEDOM) trials [102,103]. The SYNTAX study was a multicenter RCT involving 1800 patients with LM or 3VD randomly assigned to CABG surgery or PCI using a paclitaxel-eluting stent (PES) in a 1:1 ratio [103]. The primary end point was noninferiority of PES–PCI versus CABG in the incidence of major adverse cardiovascular and cerebrovascular event (MACCE defined as death from any cause, stroke, MI, or repeat revascularization) at 12 months. At 1-year follow-up, CABG was associated with significantly lower rates of MACCE compared with PES-PCI (12.4% vs. 17.8%, $p = 0.002$), mainly due to decreased rate of repeat revascularization (5.9% vs. 13.9%, $p < 0.0001$). Similar results were seen at 3- and 5-year follow-up [104,105].

In patients with 3VD included in the SYNTAX trial, at 5-year follow-up, CABG was associated with lower rates of MACCE (24.2% vs. 37.5%; $p < 0.01$), death (9.2% vs. 14.6%; $p < 0.01$), MI (3.3% vs. 10.6%; $p < 0.01$), and repeat revascularization (12.6% vs. 25.4%; $p < 0.01$) compared with PES–PCI [106]. Moreover, no difference in the rate of stroke was observed between the groups (3.4% vs. 3.0%; $p = 0.66$). In contrast to patients with 3VD, no significant differences in MACCE were observed between PES-PCI and CABG in patients with LM disease (36.9% vs. 31%; $p = 0.12$) at 5-year follow-up [107]. However, CABG was associated with an increased rate of stroke (14% vs. 5%; $p = 0.03$), which was counterbalanced by a lower revascularization rate (15.5% vs. 26.7%; $p < 0.01$). When stratified according to the baseline SYNTAX score, there was a survival advantage in LM patients with scores less than or equal to 32 who were treated with PCI. Conversely, in patients with high SYNTAX score of more than or equal to 33, CABG was associated with a significantly lower rate of MACCE. In the Premier of Randomized Comparison of Bypass Surgery versus Angioplasty Using Sirolimus-Eluting Stent in Patients with Left Main Coronary Artery Disease (PRECOMBAT) trial, there was no difference in rates of MACCE at 2- or 5-year follow-up between CABG and PCI overall as well as when stratified according to SYNTAX score [108,109]. The Evaluation of the Xience Everolimus-Eluting Stent Versus Coronary Artery Bypass Surgery for Effectiveness of Left Main Revascularization (EXCEL) trial (NCT01205776), which randomized 1905 patients with LM disease and mild-to-moderate anatomical complexity (SYNTAX score $\leq$32), is currently in the follow-up phase and will provide further insight into the efficacy and safety of PCI in patients with LM disease.

Subgroup analysis of the SYNTAX trial showed that in diabetic patients with complex LM and/or 3VD, 5-year rates were significantly lower with CABG versus PES-PCI for MACCE and repeat revascularization [110]. There was no difference in the composite of all-cause death/stroke/MI or individual components all-cause death, stroke, or MI. Similar results were seen in the FREEDOM trial, which randomized 1900 patients with diabetes mellitus and multivessel CAD to undergo either CABG or PCI with sirolimus-eluting stent or PES [102]. The primary outcome measure was a composite of death from any cause, nonfatal MI, or nonfatal stroke. At 5 years, CABG was superior to PCI in terms of lower rates of primary outcome (18.7% vs. 26.6%; $p = 0.005$), MI (6% vs. 13.9%; $p < 0.001$), and death from any cause (10.9% vs. 16.3%; $p = 0.049$). Stroke rate, however, was higher in CABG versus PCI group (5.2% vs. 2.4%; $p = 0.03$).

The second-generation DESs were introduced in 2008 and have been shown to result in improved clinical outcomes in patients undergoing PCI [111–113]. The recently published Randomized Comparison of Coronary Artery Bypass Surgery and Everolimus-Eluting Stent Implantation in the Treatment of Patients with Multivessel Coronary Artery Disease (BEST) trial randomized 880 patients with multivessel CAD to either CABG or PCI with the second-generation everolimus-eluting stent [114]. The trial, which had originally planned to randomize 1776 patients, was terminated early due to slow enrollment, resulting in reduced statistical power. At 2 years, the primary end point (MACE defined as a composite of death, MI, or target-vessel revascularization) occurred in 11.0% of patients in the PCI group versus 7.9% of those in the CABG group ($p = 0.32$ for noninferiority of PCI vs. CABG). At longer-term follow-up of median 4.6 years, CABG was associated with a lower incidence of MACE, any repeat revascularization, and composite of death, MI, stroke, and

any repeat revascularization. However, further data are needed to compare outcomes of patients with multivessel CAD undergoing CABG versus PCI with newer second- and third-generation DESs.

ROLE OF MYOCARDIAL ISCHEMIA IN DECISION MAKING

Although data from individual RCTs and subsequent meta-analyses constitute the highest form of evidence, direct translation of their results to routine clinical practice has several limitations. All trials comparing revascularization with OMT suffer from significant selection bias, as randomization was usually performed following delineation of coronary anatomy by angiography without prior assessment of ischemia. This is typically not the sequence encountered in routine clinical practice, where patients will present with cardiac symptoms and/or abnormal results on noninvasive testing, but have unknown coronary anatomy. Objective assessment of ischemia, which is often done in clinical practice, has been rather limited in most RCTs. To date, no prospective RCTs have been performed comparing revascularization plus OMT versus OMT alone based on the extent of myocardial ischemia in patients with SIHD. Despite the lack of these data, guidelines and appropriate use criteria statements place great emphasis on the results of tests for inducible ischemia to support the indications for revascularization [115–117]. Thus, current recommendations on revascularization to improve outcomes in asymptomatic SIHD patients with moderate to severe ischemia are based solely on the rational link between inducible ischemia and prognosis (Figure 10.2) and on data from observational studies.

Of note, findings from 9 published reports from 51 sites (N = 5833) indicate that only 35–65% of patients with moderate or severe ischemia on MPS are referred for coronary angiography [118]. Thus, there exists a "clinical equipoise" in the medical community regarding the decision to pursue an invasive strategy with coronary angiography and the value of revascularization in patients with SIHD with moderate to severe ischemia.

The NHLBI-sponsored International Study of Comparative Health Effectiveness with Medical and Invasive Approaches (ISCHEMIA) trial (NCT01471522) may potentially resolve this conundrum. The ISCHEMIA trial will randomize approximately 8000 patients with SIHD and at least moderate ischemia on stress imaging to either an invasive strategy with coronary angiography followed by revascularization, if feasible, plus OMT versus a conservative strategy with OMT alone and coronary angiography reserved for patients who fail medical therapy. Important exclusion criteria include patients with less than 10% of myocardial ischemia, LVEF less than 35%, prior PCI or CABG within 12 months, ACS within

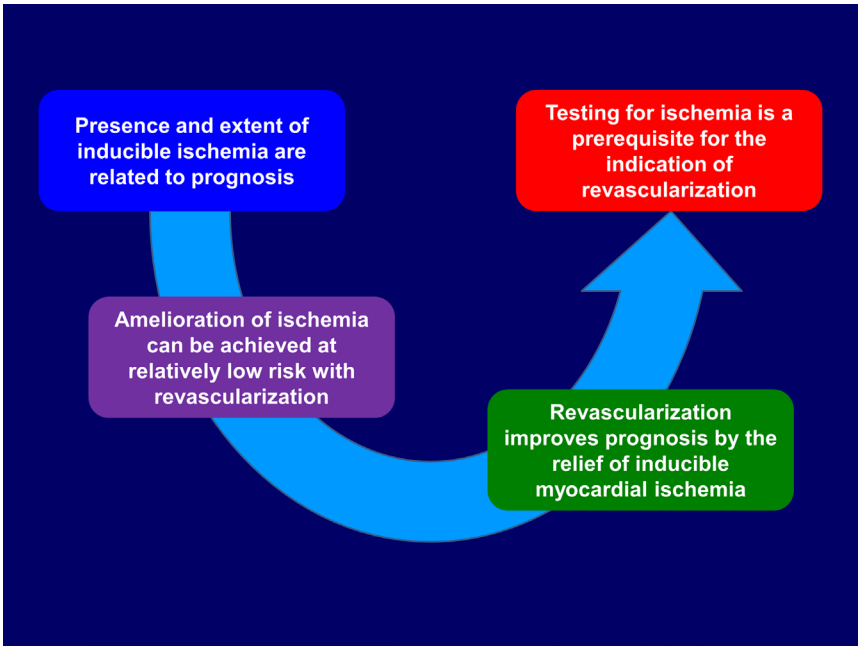

FIGURE 10.2 Paradigm linking testing for inducible myocardial ischemia and the indications for revascularization in stable CAD. *From top left:* The presence and extent of ischemia on stress testing are associated with poor prognosis in patients with CAD. If ischemia can be ameliorated with revascularization at relatively low risk, then it follows that revascularization with either PCI or CABG may improve prognosis through the relief of myocardial ischemia. Hence, society guidelines and appropriate use criteria statements place great emphasis on testing for inducible ischemia to support the indications for revascularization.

previous 2 months, history of unprotected LM more than 50%, and CCS class III or IV angina. The primary end point is a composite of cardiovascular death or nonfatal MI over a 4-year follow-up. Secondary end points will include angina-related quality of life, composite of cardiovascular death, nonfatal MI, resuscitated cardiac arrest, or hospitalization for unstable angina or heart failure, individual components of primary end point, stroke, as well as health resource utilization, costs, and cost effectiveness. The trial is estimated to be completed in May 2019.

CONCLUSION

Myocardial ischemia in patients with SIHD is more often silent than symptomatic, even in patients who report angina. The presence and extent of ischemia have been associated with poor prognosis in CAD patients. However, the mechanism that provides the link for this association is elusive as poor outcomes are related to the rupture of an atherosclerotic plaque whereas ischemia during the stable phases of the disease is largely related to increases in myocardial oxygen demands (Figure 10.1). The association between the magnitude of ischemia and the extent of CAD and the relationship between the latter and poor prognosis suggest that the presence of ischemia during daily life—or unveiled during a stress test—may be a marker of more extensive anatomical disease rather than the causative mechanism triggering the acute cardiovascular events. The benefit of revascularization and the selection of the type of revascularization (PCI or CABG) are clearly influenced by the anatomic location and extent of CAD, as demonstrated in randomized clinical trials. Selection of therapy based on the presence and magnitude of inducible myocardial ischemia is advocated by society guidelines and appropriate use criteria. This concept is founded on the rational link between ischemia and prognosis (Figure 10.2) and is supported by observational studies. However, no trial has been conducted to date in which patients were randomized after the assessment of ischemia with noninvasive methods. The ongoing ISCHEMIA trial may provide a definitive answer to the question of whether myocardial ischemia (silent or not) should be used as the basis for an invasive approach (including revascularization) in the management of patients with stable CAD.

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CHAPTER

11

Noninvasive Diagnostic Modalities for the Evaluation of Coronary Artery Disease

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INTRODUCTION

Coronary artery disease (CAD) remains the leading cause of mortality on a global scale. However, the last few decades have seen a significant decrease in the mortality from CAD (Figure 11.1) [1]. Whereas, dramatic new developments in the therapeutic modalities have contributed largely to this favorable trend, nevertheless, availability of safe, simple, and readily available noninvasive modalities for the detection and risk-stratification of patients with suspected or know CAD have also contributed significantly to this trend. Invasive coronary angiography is considered as the definitive diagnostic modality for CAD. However, its use is limited to only those with a very high likelihood of CAD, who may require a therapeutic interventional procedure. Noninvasive diagnostic modalities now constitute the first line of diagnostic workup in a vast majority of patients with suspected CAD. A wide array of noninvasive diagnostic imaging tests is currently available for diagnosis of CAD. Each of these modalities employs a different imaging technique and has strengths and limitations. These noninvasive imaging modalities can be grouped as follows:

1. Stress electrocardiography
2. Nuclear myocardial perfusion imaging (MPI)
3. Stress echocardiography
4. Computed tomography (CT) imaging
5. Magnetic resonance (MR) imaging

Of these techniques, nuclear MPI is the most well established techniques and widely used in clinical practice for a long time. Following is a description of these techniques:

Stress Electrocardiography

This was the first technique introduced in the 1960s and 1970s for the noninvasive evaluation of CAD in clinical practice [2,3]. This is a relatively simple and inexpensive technique. This requires a treadmill or an upright bicycle and a 12 lead ECG machine. Treadmill is the most commonly used exercise modality used in the United States and the United Kingdom, whereas upright bicycle is used in Europe and in many other countries in the world. The patient performs exercise on the treadmill or exercise bicycle, while heart rate, blood pressure, and 12 lead ECG are closely monitored. The patients are observed for the development of angina, abnormal ST segment depression, arrhythmias, or an abnormal drop in blood pressure (Figure 11.2). Of several treadmill exercise protocols, Bruce Protocol, modified Bruce Protocols or several of their variations are the ones used most extensively. The goal of an exercise protocol is to gradually increase the workload to a maximum age-predicted workload. Several parameters such as the peak heart rate, mets (metabolic equivalent) or peak double product (product of heart rate and systolic blood pressure) are used to determine the adequacy of workload. Development

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00011-5

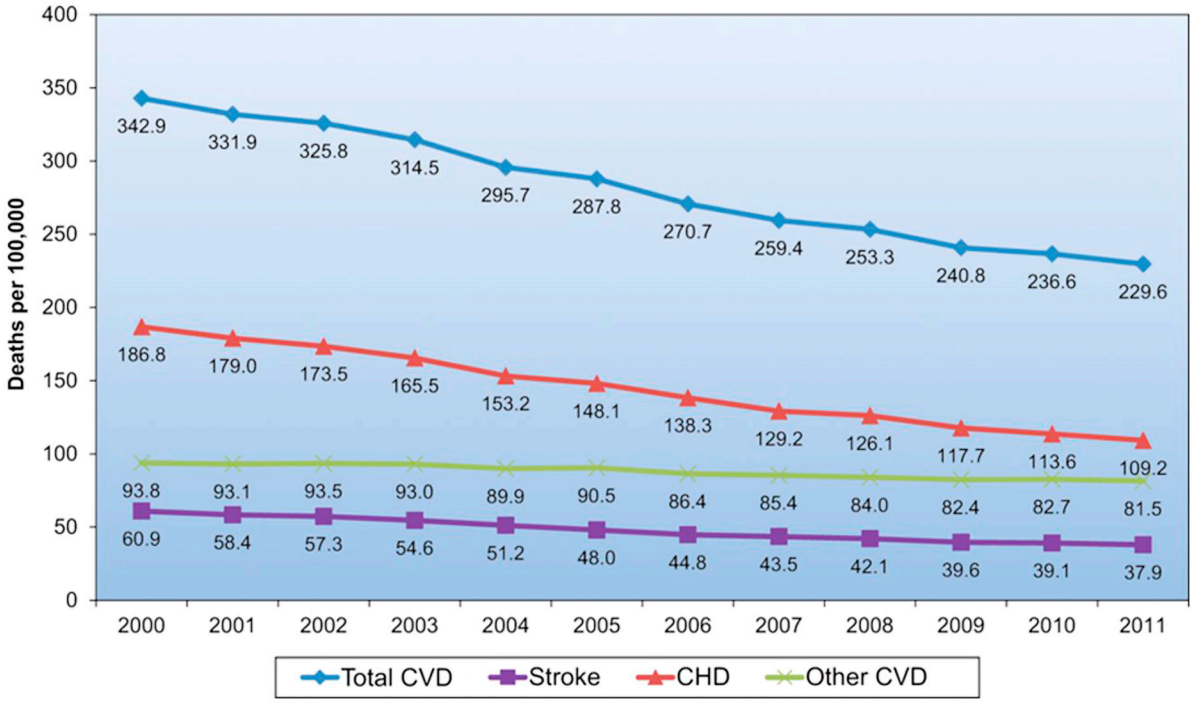

FIGURE 11.1 US age-standardized death rates from cardiovascular diseases, 2000–2012. CHD, coronary heart disease; CVD, cardiovascular disease. *Source: Centers for Disease Control and Prevention, National Center for Health Statistics. Reproduced with permission from Mozaffarian et al. [1].*

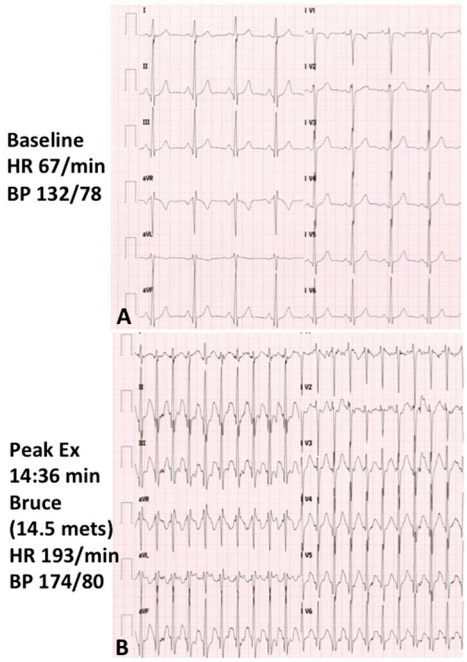

FIGURE 11.2 Rest (A) and peak exercise (B) ECGs of a 27-years-old male with exertional chest pain. He had family history of premature CAD, history of smoking for a few years in college and no other risk factors. His physical exam was normal. On a symptom-limited treadmill exercise, he exercised for 14:36 min using Bruce Protocol (14.5 mets). HR increased from 67 to 193 beats per minute (100% of age predicted max HR), BP increased from 133/78 to 176/80 mm of Hg, with a peak double product of 34 K. He did not develop any chest pain. The resting and exercise ECGs are normal. His chest pain was attributed to be noncardiac based on normal exercise ECG.

of angina, accompanied by 1 mm or greater horizontal or down-sloping ST segment depression constitutes an abnormal stress test. The sensitivity and specificity of the test are modest (sensitivity 60–65%, specificity 55–60%). The test is inconclusive in patients who are unable to reach a target workload due to deconditioning, pulmonary, musculoskeletal, peripheral vascular or neurological limitations. The changes in ST segment are sometimes unreliable or difficult to interpret in patients with ST-T segment abnormalities at baseline. With an aging population with concomitant comorbidities, currently well over half of the patients requiring stress testing are unable to exercise to an adequate workload. Due to all these limitations, stress electrocardiography alone is less frequently performed in the United States. This is often combined with a cardiac imaging modality such as nuclear imaging or echocardiography. However, with increasing emphasis on cost containment, there is resurgence of interest in exercise electrocardiography as the first diagnostic modality, particularly in younger patients, who are otherwise free from other comorbidities, have normal baseline ECG and are able to exercise to a target workload [3].

Nuclear MPI

Nuclear MPI techniques are well established and most extensively used for noninvasive evaluation of patients with established or suspected CAD. MPI provides important diagnostic and prognostic information in a

wide array of patients with known or suspected CAD. The atheromatous luminal narrowing of the coronary arteries evolves slowly over several decades. Symptoms occur relatively late, only after a significant luminal narrowing of coronary arteries has already occurred. Coronary arterial narrowing interferes with myocardial perfusion downstream. With partial narrowing of the lumen, myocardial perfusion may be normal at rest, but fails to increase appropriately during physical exercise or pharmacological vasodilation. This results in regional flow heterogeneity, which can be imaged by scintigraphy using the radiotracers, which are extracted by the myocardium proportionate to its perfusion.

Radiotracers

Thallium-201 (^{201}Tl) is the conventional perfusion tracer [4]. ^{201}Tl behaves like a potassium analog and enters the myocytes through Na^+/K^+ ATPase channels. ^{201}Tl is injected intravenously at peak exercise or during pharmacological stress and imaging is started soon after completion of stress. Myocardial segments perfused by narrowed coronary arteries or with scarring due to prior myocardial infarction show diminished tracer uptake on stress images. ^{201}Tl shows a continuous redistribution after initial myocardial extraction. Stress imaging is followed by redistribution imaging 3–4 h later. Perfusion abnormality due to ischemia reverses on redistribution imaging whereas, that due to scarring remains unchanged. Segments characterized by scar and ischemia show partial reversibility of the perfusion abnormality. Stress ^{201}Tl imaging has a sensitivity of nearly 85–90% and a specificity of 80% or above for the detection of CAD [4]. Limitations of ^{201}Tl include its long physical half-life (72 h) and low-energy photons (69–83 KeV). ^{201}Tl has largely been replaced by technetium-99m (^{99m}Tc) tracers. ^{201}Tl is currently used only for the detection of myocardial viability. Dual-isotope MPI, where ^{201}Tl was used for rest perfusion imaging and a ^{99m}Tc-labeled perfusion tracer for stress perfusion imaging is not in use any more because of unacceptably high radiation exposure from this protocol [5].

^{99m}Tc has a shorter half-life (approximately 6 h) and emits slightly higher energy photons (140 KeV). ^{99m}Tc-labeled agents provide better quality images and lower radiation exposure. Two agents: sestamibi (Cardiolite™, Lantheus, North Billerica, MA) and tetrofosmin (Myoview™, GE Healthcare, Princeton, NJ) are FDA approved agents in current clinical use [6,7]. These are lipophilic cationic agents, which are taken up by the myocardium because of their lipophilicity and positive charge. Their myocardial uptake is not mediated by Na^+/K^+ ATPase pump. In the myocytes, they are localized mainly in the mitochondria. These agents are tightly bound to the myocardium and show no significant redistribution. Two separate injections are required for stress and rest imaging. The perfusion images are gated to the electrocardiogram. This provides information about left and right ventricular function and wall motion. However, both ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin suffer from several limitations: liver and gastrointestinal uptake can degrade image quality and produce artifacts; and relatively low first-pass myocardial extraction can potentially result in an underestimation of myocardial ischemia, particularly in the presence of lower grades of coronary arterial narrowing. An ideal myocardial perfusion tracer should have minimal or no hepatic and gastrointestinal uptake and should have a high first-pass myocardial extraction that linearly tracks the myocardial blood flow over a wide range [8].

Instrumentation

Nuclear imaging is carried out using gamma cameras, which convert gamma rays emitted by the radiotracers into electronic signals to image various organ systems in the body. Conventional gamma cameras consists of a large sodium iodide crystal as a scintillator, which converts gamma rays into specs of light, which are amplified by an array of photomultiplier tubes and then converted into electronic signals. A collimator comprising of a sheet of heavy metals such as lead or tungsten with holes in it covers the imaging surface of the scintillating crystal and permits only gamma rays traveling in a particular direction to pass through to the scintillating crystal. Imaging is carried out using single photon emission computed tomography (SPECT), which provides a series of cross-sectional images of the heart in multiple axes (Figures 11.3–11.5). SPECT images are gated with ECG (gated SPECT) to assess left ventricular (LV) wall motion, thickening, and ejection fraction (EF) from the same study. Myocardial ischemia and LV function are the two most important determinants of optimal therapy and short-term as well as long-term prognosis, gated SPECT MPI is currently the single most powerful diagnostic and prognostic modality in cardiovascular medicine. Soft-tissue attenuation continues to be a major source of artifacts in MPI. Attenuation correction is carried out with the use of a simultaneously acquired transmission map using an external source of radiation such as gadolinium or a low-dose CT imaging [9]. Recently, several new advances have been made in gamma camera design. Newer solid-state cameras using cadmium-zinc telluride crystals have significantly higher efficiency for the detection of gamma rays compared to the traditional gamma cameras using sodium iodide crystal and photomultiplier tubes [10]. These systems directly convert gamma rays into an electronic impulse and have much higher photon flux and efficiency for imaging. These systems are less bulky, improve image quality and cut down the image acquisition time and dose of radiotracers. These solid-state cameras are likely to replace

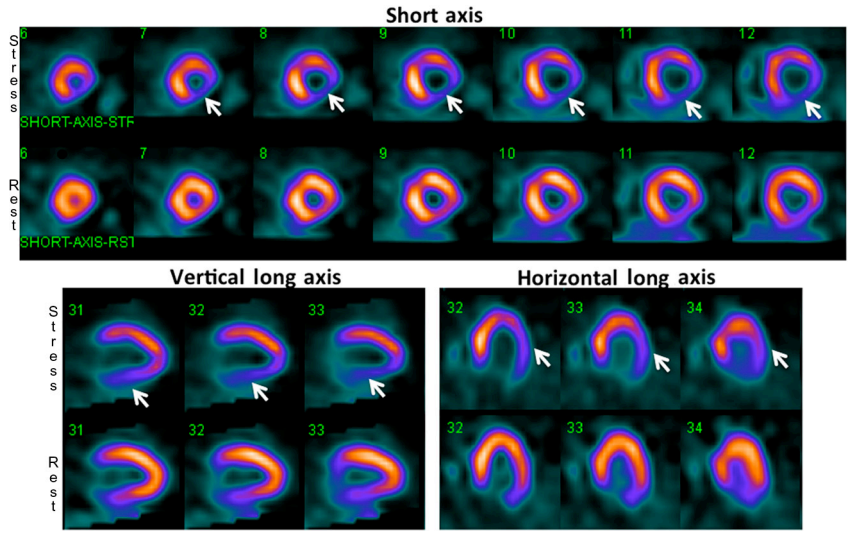

FIGURE 11.3 Exercise (Ex) and rest ^{99m}Tc-sestamibi SPECT images of a 56-year-old man with long-standing poorly controlled diabetes (poor compliance), hypertension, and diabetic neuropathy and nephropathy with stage 4 chronic kidney disease (serum creatinine 5.6 mg/dL). The patient underwent pharmacological stress-rest MPI as a part of cardiac evaluation prior to being listed for renal transplant. He had poor Ex capacity, but denied any symptoms of chest pain. He was on aspirin, metoprolol, atorvastatin, and insulin. He received 0.4 mg of regadenoson i.v. over 10 s. His heart rate changed from 90 to 102 beats per minute and BP from 160/100 to 146/80. He developed chest heaviness following regadenoson, which resolved spontaneously over the next 2 min. There was 2 mm down-sloping ST segment depression in the precordial leads, which persisted for more than 3 min into recovery. Stress and corresponding rest perfusion images in short axis (top two rows), vertical and horizontal long axes (rows 3 and 4) show a large area of perfusion abnormality involving the inferior and lateral walls and contiguous anterior wall, which is reversible on rest imaging (arrows). In addition, transient post stress LV dilation is seen (TID ratio 1.20). On gated SPECT imaging, transient hypokinesia of inferior and lateral walls was noticed on poststress images, with normal wall motion on rest imaging. LVEF was 52% on the poststress images and 57% on the rest images. This is a high-risk abnormal study with a large area of ischemia in multiple vascular territories, transit poststress LV dilation, and wall motion abnormalities and warrants coronary angiography. His impaired renal function necessitated institution of dialysis prior to coronary angiography. He underwent coronary angiography after being started on hemodialysis. This showed severe three vessel CAD: LAD had multiple 60–80% narrowing, with 70% narrowing of D1 branch; LCx has 90% proximal narrowing with 70–80% narrowing of OM1 and OM2; and RCA had multiple 80–90% narrowing. He underwent CABG with left internal mammary artery graft to the LAD and SVG to the LCx and PDA branch of the RCA. He was cleared for renal transplant after he recovered from the CABG.

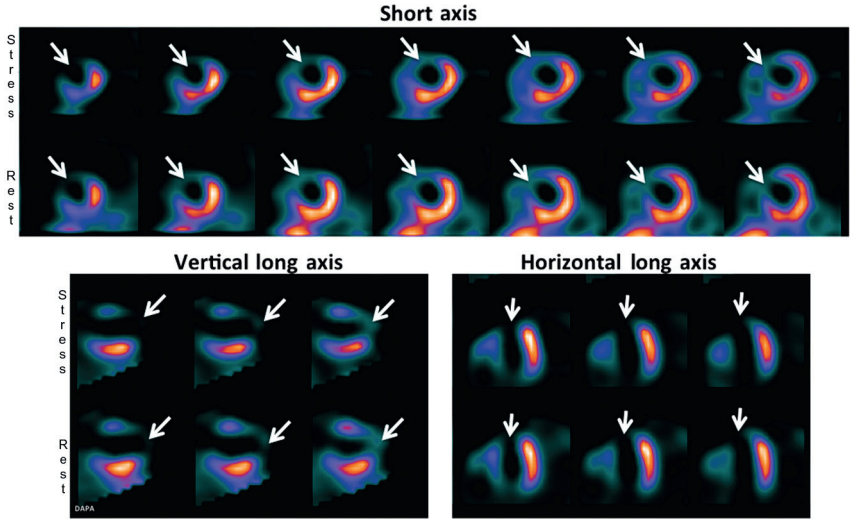

FIGURE 11.4 Regadenoson stress and rest SPECT images of an 83-year-old male with prior anterior wall myocardial infarction 20 years ago. Coronary angiography revealed completely occluded LAD proximally, which could not be revascularized. The RCA and LCX showed 50–60% narrowing, which was not revascularized. He has been complaining of mild exertional shortness of breath and chest heaviness over the last few weeks. There was no chest pain or ST segment depression with regadenoson. There is a large dense scar involving the anterior wall, septum, and apex. There is no ischemia. The apex was dyskinetic and the anterior wall and septum were akinetic and the global LVEF was severely impaired at 37%.

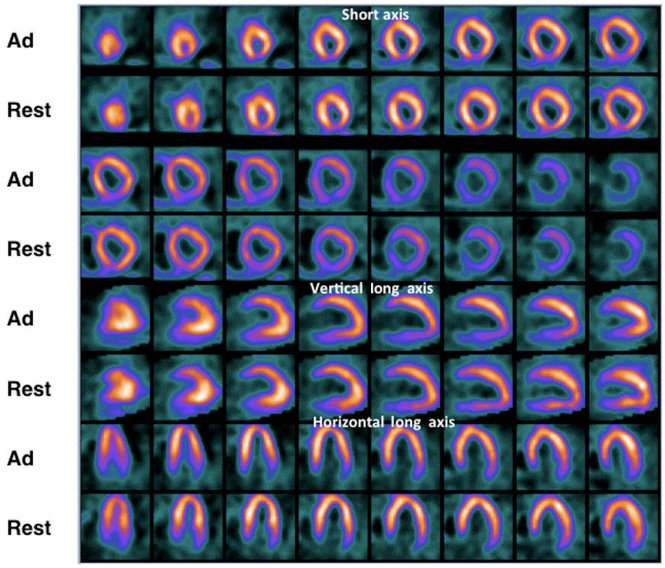

FIGURE 11.5 Adenosine stress and rest perfusion images of a 48-year-old male with long history of hypertension, heavy alcohol abuse, smoking, and atrial fibrillation. He was admitted with worsening heart failure after an alcohol binge. After initial stabilization, he underwent adenosine and rest perfusion imaging. He has no chest pain or ST depression. There is no perfusion abnormality. But the LV is markedly enlarged, hypertrophied, and severely hypokinetic on gated SPECT images with an ejection fraction of 28%. This patient has nonischemic cardiomyopathy.

conventional sodium-iodide crystal-based cameras in the coming years.

Choice of Stress

Exercise is by far the preferred method of stress testing. Information about exercise capacity, changes in heart rate, blood pressure, adverse symptoms such as chest pain, undue fatigue, and the magnitude and duration of ST segment depression and arrhythmias provide important and independent prognostic information. The radiotracer is injected close to the peak exercise and exercise is continued for another 2 min to allow radiotracer extraction by the myocardium at peak exercise. It is important for patients to reach an adequate workload, as already discussed. A peak heart rate above 85% of the age-predicted maximum heart rate, peak workload, or a double product greater than 25K are used to determine the adequacy of the exercise level. However, with changing demographic patterns, an increasing proportion of patients requiring stress testing are unable to exercise to an adequate workload and require pharmacological stress testing either alone or in conjunction with low-level exercise.

In patients who are unable to exercise or unable to reach an adequate workload due to noncardiac limitations (peripheral vascular disease, musculoskeletal disorders, or pulmonary disease) pharmacological stress should be used. Dipyridamole, adenosine (Adenoscan®, Astellas Pharma, Deerfield IL) and regadenoson (Lexiscan™, Astellas Pharma, Deerfield IL) are the most widely used agents for this purpose [11–15]. Following i.v. administration, these agents cause maximum coronary vasodilation and can increase myocardial blood flow 3–4 times the resting flow. However, blood flow increase is blunted in myocardial segments perfused by narrowed coronary arteries. This produces flow heterogeneity and results in apparent perfusion abnormalities. True ischemia is rare and occurs in patients with severe CAD where collateral circulation contributes significantly toward myocardial perfusion and these vasodilators may induce a coronary steal in such cases. At a cellular level, dipyridamole acts by inhibiting the intracellular uptake of adenosine. Thus adenosine is more directly acting than dipyridamole and has more predictable effect on the coronary blood flow. Dipyridamole is a long-acting agent and its pharmacological effects persist for more than 30 min, which poses a challenge in those with significant dipyridamole-induced side effects and often requires longer monitoring and reversal of dipyridamole with iv aminophylline. Due to these limitations, dipyridamole has largely been replaced by adenosine and regadenoson for pharmacological stress. Adenosine has an extremely short half-life of less than 10 s and requires an infusion pump for its administration. This is administered at a dose of 0.14 mg/kg/min for 6 min. The radiotracer is injected at midpoint of adenosine infusion. Side effects are very common with adenosine infusion, but generally are minor and self-limiting. The most common side effects are nausea, headache, flushing of face, and hypotension. Transient high-grade AV block or symptomatic hypotension occurs in 5–7% of patients with adenosine infusion. These side effects are generally self-terminating on completion of infusion. Chest pain occurs in approximately 25% of cases but is not specific for myocardial ischemia. The exact mechanism of dipyridamole- or adenosine-induced chest pain is not clear; perhaps they act directly on the pain receptors. ST segment depression occurs rarely but if it occurs, it is indicative of severe CAD. Adenosine infusion can be combined with low-level exercise [12]. This reduces adverse effects such as hypotension, nausea, and flushing and improves image quality by reducing splanchnic radiotracer uptake and improves the sensitivity and specificity of the test [12]. Theophylline derivatives, including caffeine, act as antagonists of dipyridamole and adenosine at a cellular level and should be stopped prior to performing dipyridamole or adenosine stress MPI. Aminophylline can be given i.v. to reverse the side effects of dipyridamole. Because of the extremely short half-life, side effects of adenosine generally disappear with the discontinuation of its infusion and aminophylline is rarely required.

Apart from coronary vasodilation, adenosine also results in systemic vasodilation and slowing of AV nodal

conduction, which are undesirable side effects for pharmacological stress testing. Adenosine is nonselective for all four adenosine receptor sub-types: A1 receptors in AV node (AV nodal block), A2a receptors in coronary vessels (coronary vasodilation), A2b receptors in systemic vessels (systemic vasodilation), and A3 receptors in lungs, bronchioles, and several other tissues (shortness of breath, atypical chest pain, and bronchospasm). Adenosine can induce bronchospasm in patients with a history of bronchial asthma or COPD. Regadenoson (Lexiscan™, Astellas Pharma, Deerfield IL) is an adenosine analog, which is highly selective for adenosine A2a receptors, and is used for pharmacological stress MPI [13–16]. Unlike, adenosine, regadenoson does not require continuous infusion and is given as a fixed-dose (0.4 mg) bolus injection over 10 s. The diagnostic information provided by regadenoson is similar to that of adenosine but, adverse effects are fewer and less intense compared to adenosine [13]. Because of the ease of administration and fixed dose, regadenoson has almost replaced all other pharmacological stress agents in clinical practice in the United States. Figures 11.3 and 11.4 are examples of regadenoson stress and rest ^{99m}Tc-sestamibi MPI. Regadenoson is well tolerated in patients with history of bronchial asthma, COPD, and renal insufficiency [16,17]. Caffeine containing food and beverages prior to vasodilator stress testing can result in underestimation of ischemia and should be withheld for at least 12 h prior to regadenoson stress testing [18].

Dobutamine can also be used for stress MPI [19,20]. This acts by increasing the heart rate, myocardial contractility, and oxygen demand. Adenosine or regadenoson are preferable over dobutamine because of a greater increase in myocardial blood flow and flow heterogeneity with these agents. Dobutamine was used in patients where dipyridamole or adenosine are contraindicated, such as in patients with severe bronchopulmonary disease. Dobutamine is administered as a continuous infusion starting at 10 µg/kg/min and increased by 10 µg/kg/min every 3 min until a maximum dose of 40 µg/kg/min is reached, target heart rate is achieved or adverse symptoms or evidence of ischemia develops [19,20]. Side effects with dobutamine are common and are of particularly of concern in elderly and sick patients and in those with impaired LV function and with history of significant atrial or ventricular arrhythmias [19,20]. However, with the availability of regadenoson, dobutamine is only rarely used these days, since regadenoson is well tolerated in patients with bronchial asthma and COPD [16].

Interpretation of Perfusion Images

Interpretation of MPI requires experience, skill, and an adequate understanding of the cardiac physiology, pathology, and applied physics, as well as awareness of the possible sources of artifacts. MPI is prone to artifacts due to attenuation from surrounding and overlying structures such as diaphragm and breast. SPECT imaging is also prone to a variety of other artifacts, such as patient motion during imaging and tracer activity in the gut and other subdiaphragmatic structures. Bowel loops with significant radiotracer activity can sometimes overlap the heart and can substantially degrade the image quality and in the worst scenario can render the images uninterpretable. Strict and rigorous quality control of gamma camera is mandatory for avoiding technical artifacts. A meticulous effort is required to prevent false interpretation of the images due to these artifacts. The perfusion images can be interpreted visually. However, quantitative analysis of the images is more reliable. Subtle abnormalities can better be appreciated with quantitative analysis. Quantitative analysis is performed using a normal database. The distribution of counts in the patients' images is compared with a database derived from the normal subjects of the same gender. Quantitative analysis can also provide an estimate of the extent or severity of myocardial ischemia. Quantitative analysis also minimizes the intra- and inter-observer variability of the image interpretation.

A systematic approach is required for a comprehensive interpretation of MPI studies [21,22]. The raw images should be examined for possible sources of artifacts and overall image quality. Important extra-cardiac abnormalities, such as tumors in lungs, mediastinum or breasts, pleural effusion, gastrointestinal abnormalities such as hiatal hernias or ascites may be detected incidentally for the first time on raw images [23]. The patient's gender, body weight, and body habitus, the radiopharmaceutical used and its dose, the interval between tracer injection and imaging, and the type of stress used should be taken into consideration. The lung fields should be examined for increased lung tracer uptake on stress images. The processed images should be interpreted qualitatively as well as quantitatively. Clinical history, pretest likelihood of CAD, details of stress testing, and electrocardiographic changes should be taken into consideration while performing the final interpretation. The report should be comprehensive, succinct, and clearly concluded whether abnormal or normal and if abnormal, the extent, location, and nature of abnormality should be adequately described [22].

Clinical Applications of MPI

1. **Detection of CAD**
   MPI is useful for establishing the diagnosis of CAD in patients with symptoms of chest pain or in those with a high clinical suspicion of CAD because of the presence of risk factors for CAD. This serves as an important and cost-effective gatekeeper for identifying patients who should be considered for further invasive studies. Addition

of MPI to exercise ECG increases the sensitivity as well as specificity of the test for the detection of CAD. MPI has a particular advantage over exercise ECG in patients with LV hypertrophy, left bundle branch block (LBBB), therapy with digoxin, and other abnormalities interfering with proper interpretation of ST-segment changes on exercise. In a study involving more than 4000 patients who underwent stress MPI for the evaluation of CAD, the subsequent cardiac catheterization rates over a mean follow-up period of 9 months were 32% in those with reversible perfusion abnormality and only 3.5% in those without reversible perfusion abnormality [24]. Furthermore, in the reversible perfusion abnormality group, cardiac catheterization rate was 60% in those with high-risk studies (reversible perfusion abnormality of the left anterior descending coronary artery territory, multiple areas of ischemia or increased lung tracer uptake), compared with 9% in the remaining patients with reversible perfusion abnormalities. The findings on MPI were far more predictive of subsequent cardiac catheterization than clinical and treadmill exercise ECG variables alone or in combination, indicating the important role of stress MPI for initial evaluation of patients with suspected CAD.

2. **Risk Stratification of Patients with CAD**
   The severity, location, and extent of myocardial ischemia are useful for risk stratification of patients with known CAD [25,26]. Large areas or multiple areas of perfusion abnormality identify patients at high risk for cardiovascular events on follow-up (Figure 11.3). Increased lung tracer uptake and transient LV dilation on stress images are indicative of severe CAD and are predictive of poor prognosis. The quantitative extent and severity of myocardial ischemia determined from MPI correlates with the subsequent occurrence of unstable angina, acute myocardial infarction, and cardiac death. Of various clinical and laboratory variables including ECG and coronary angiographic variables, MPI provides the most powerful prognostic information in all groups of patients with CAD. Based on the quantitative extent, severity, and reversibility of abnormalities on MPI, CAD patients can be categorized into low- to high-risk categories for the occurrence of adverse cardiac events on follow-up [26,27]. Patients with normal MPI have an excellent prognosis; several large clinical studies have shown these patients to have a less than 0.6% annual cardiac event rate [28,29]. A normal MPI study, even in the presence of angiographically documented CAD, is associated with an excellent long-term prognosis and a very low incidence of cardiac events on follow-up [30].
3. **Postmyocardial Infarction Evaluation**
   Sub-maximum MPI was used routinely for risk stratification of patients with uncomplicated myocardial infarction prior to hospital discharge from the hospital in the prethrombolytic era. Patients with fixed defects had a low incidence of adverse cardiac events, whereas those with reversible defects have a higher incidence of adverse cardiac events. This test was used to identify patients with recent myocardial infarction who could benefit from cardiac catheterization and revascularization. With the current practice of routine revascularization with percutaneous interventions in patients with acute myocardial infarction and unstable angina, predischarge stress MPI is rarely needed these days. However, MPI plays an important role in determining the need of revascularization in the presence of additional coronary lesions of unclear significance in noninfarct-related vessels [30]. MPI can be used in the postinfarction patients with recurrent symptoms of chest pain.
4. **Triaging of Patients with Chest Pain of Uncertain Origin**
   With greater public education and awareness for presenting immediately to the nearest emergency department in case of chest pain or symptoms suspicious of an acute myocardial infarction, a large number of patients with chest pain are seen in the emergency departments these days. Acute chest pain is the second most common condition seen in the emergency departments in the United States [31–35]. In the United States, 6–8 million patient visits for chest pain occur annually to the emergency department. Fewer than 15% of these patients turn out to have acute coronary syndrome. On the other hand, a small but significant proportion of patients with acute coronary syndrome have only atypical symptoms with no overt electrocardiographic or biochemical abnormalities at presentation, which may prompt an early but inappropriate discharge from the emergency department with a mistaken conclusion of noncardiac chest pain. Most large emergency departments now have a dedicated chest pain center or institutional protocols for an effective, reliable, and prompt triage of patients with chest pain. A combination of serial electrocardiograms, cardiac enzymes, and noninvasive cardiac imaging is used for triaging these patients [32–34]. Resting and stress MPI can be used quite effectively in chest pain centers. Despite an upfront cost associated with instrumentation and training of personnel and recurring cost associated with each study, an appropriate use of MPI in emergency departments is associated with significant reductions in the

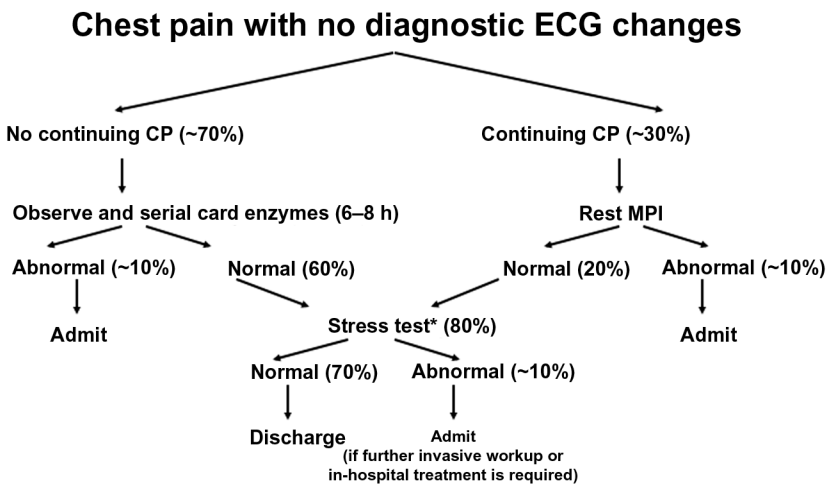

FIGURE 11.6 A schematic representation of the proposed algorithm for early triaging of patients presenting with acute chest pain using myocardial perfusion imaging.

time required to arrive at a definitive diagnosis, hospital admission rate, average hospital stay, and overall per-patient cost [35]. The presence of resting perfusion abnormalities in patients with chest pain in the absence of prior myocardial infarction is indicative of an acute coronary syndrome and warrants admission to the hospital and appropriate treatment. Absence of perfusion abnormalities points more toward a noncardiac cause of chest pain. Patients with negative resting perfusion images can further undergo stress MPI right away to detect the presence of exercise-induced myocardial ischemia [31]. Figure 11.6 shows a proposed scheme for the utilization of MPI in chest pain centers [31]. Currently, there is a great interest in rapid triaging of patients presenting to the emergency department with chest pain but no overt evidence of acute coronary syndrome. Whereas, several different imaging modalities are available and have shown some promise for this role, nuclear MPI using a judicious combination of rest imaging, rest-stress imaging or stress imaging alone remains the most readily available, cost-effective, and relatively simple technique for this purpose [36,37]. The information provided by MPI is not only useful for safely discharging patients from the emergency department, but is also critically important for arriving at a definitive diagnosis and for guiding long-term management and follow-up plan and for providing long-term prognostication of these patients.

5. **Risk-Stratification Prior to Noncardiac Surgery**
Adverse cardiac events are important cause of morbidity and mortality following noncardiac surgery, particularly in the elderly patients and in those with known CAD or with risk factors for CAD [38,39]. Appropriate use of MPI can significantly lower the incidence of this complication. The frequency of occurrence of adverse cardiac events in the perioperative period depends on a number of factors: the prevalence and severity of CAD and LV dysfunction and the nature and severity of hemodynamic stress during the perioperative period. Patients with a high prevalence of CAD, either symptomatic or occult, and with LV dysfunction, are particularly vulnerable to cardiac events. Prolonged vascular surgery involving cross-clamping of the aorta, major shifts between intravascular and extravascular fluid compartments, and hypotension impose significant stress on the cardiovascular system and can result in arrhythmias, pulmonary edema, or myocardial infarction in the perioperative period in patients with CAD. Patients with peripheral vascular disease have a high prevalence of CAD and are at a high risk of perioperative cardiac events. Even after peripheral vascular surgery, these patients continue to have very high morbidity and mortality due to cardiac events. A number of studies have established the role of pharmacological stress MPI for identifying patients at a high risk for perioperative cardiac events [38,39]. Adenosine and regadenoson are particularly suitable because of the inability of these patients to exercise. Abnormalities on MPI are predictive not only of perioperative morbidity and mortality but also of long-term mortality and morbidity [39]. The current ACC/AHA guidelines for preoperative risk-stratification take into consideration the presence of CAD or CAD risk factors, functional capacity and the nature

and risk of the surgical procedure [39]. MPI is useful in patients with risk factors for CAD, impaired functional capacity and in those undergoing relative high-risk surgery. Patients with no risk factors of CAD, good functional capacity and those undergoing relative low to intermediate risk surgical procedure do not warrant routine preoperative MPI. Similarly patients undergoing emergent or life-saving surgery also do not warrant preoperative MPI. Optimization of their hemodynamic status in the perioperative period is probably the most effective approach in these patients. However, following recovery from successful surgery many of these patients may require consideration for MPI for long-term management if they have multiple CAD risk factors and impaired functional capacity.

6. **Detection of Myocardial Viability**

The impairment in LV function and regional wall motion abnormalities in many patients with CAD may be reversible to varying extents with a proper utilization of revascularization procedures [40–44]. This can result in improvement in LV function and symptoms of heart failure, as well as prognosis, and can potentially avoid or delay the need for cardiac transplantation in some patients with advanced heart failure. However, identification and differentiation of this viable but dysfunctional myocardium with a potential for recovery in contractility and LV dynamics from the irreversibly scarred myocardium with no potential for recovery in function poses a major challenge in the current practice of cardiology. Symptoms, clinical examination, electrocardiogram, and conventional techniques for functional assessment are often not helpful. A number of techniques such as dobutamine echocardiography and MR imaging have been employed with varying success, but nuclear imaging techniques, conventional perfusion imaging as well as positron emission tomography (PET), have played a crucial role in this field. A brief episode of myocardial ischemia may result in regional wall motion abnormalities, which may persist for a variable period of time even after the restoration of perfusion. This is called as stunned myocardium. This myocardium is characterized by wall motion abnormalities, but preserved perfusion and metabolism. Wall motion abnormality recovers spontaneously over time without any intervention. The myocardium with chronic low flow, without necrosis is called as hibernating myocardium. This myocardium is characterized by reduced perfusion, impaired resting wall motion, but preserved metabolic activity, improvement in wall motion with low-dose dobutamine administration, and restoration of contractility after revascularization. This myocardium needs to be differentiated from scarred myocardium, which also has impaired perfusion and wall motion at rest, but shows no improvement in contractility with low-dose dobutamine, has no evidence of metabolic activity and shows no change in wall motion with revascularization. Myocardial uptake and retention or washout of perfusion tracers is dependent on the structural and metabolic integrity of the myocytes. Rest-redistribution ^{201}Tl imaging can be used for detecting myocardial viability. Presence of significant myocardial uptake on quantitative analysis (greater than or equal to 50% uptake compared to the normal myocardial segments) and or redistribution on delayed imaging are indicative of myocardial viability and predictive of functional improvement after revascularization in abnormal myocardial segments [43]. Resting ^{99m}Tc-sestamibi or ^{99m}Tc-tetrofosmin can also provide information about myocardial viability using a quantitative approach. Similar to ^{201}Tl, greater than or equal to 50% uptake of ^{99m}Tc-sestamibi or ^{99m}Tc-tetrofosmin in abnormal myocardial segments is predictive of myocardial viability [42]. Nitrate administration prior to the injection of perfusion tracers has also been used for the detection of myocardial viability. Viable myocardial segments with resting hypoperfusion show an improvement in perfusion following nitrate administration and is predictive of functional improvement after revascularization [42]. PET imaging techniques for myocardial viability are based on the demonstration of metabolic activity in the dysfunctional myocardial segments. PET permits imaging of myocardial metabolism at rest and under different physiological conditions. Myocardium has high energy requirement, which is derived from the metabolism of free fatty acids and glucose. Free fatty acids are the preferred substrate in normally perfused myocardium. However, in the presence of ischemia, such as in hibernating myocardium, anaerobic glycolysis is the predominant source of energy production. Hibernating myocardium is characterized by a profound increase in glycolytic activity despite a reduction in perfusion. Glucose uptake and its phosphorylation in the cells can be imaged by ^{18}F-Fluorodeoxyglucose (^{18}FDG). Myocardial flow-metabolic mismatch is the hallmark of myocardial viability on PET imaging. A proper evaluation of myocardial viability requires an adequate understanding of coronary physiology, pathology and metabolism, and a thoughtful integration of pertinent clinical information and angiographic data with the nuclear imaging data [44].

7. **Evaluation of Patients with Congestive Heart Failure (CHF)**
   Currently, there are more than 4.5 million patients with CHF in the United States, and more than 500,000 new patients are diagnosed with CHF every year. A detailed and often expensive workup is required to determine its etiology, assess its severity, and optimize its treatment. MPI can be used to differentiate between ischemic and nonischemic cardiomyopathy and for the assessment of left and right ventricular function (Figures 11.4 and 11.5). This is highly cost-effective and can potentially avoid invasive tests in a substantial proportion of patients with CHF. In patients with ischemic cardiomyopathy, MPI can be used quite effectively for the detection of myocardial viability, which may warrant a consideration for revascularization. Furthermore, nuclear imaging techniques can be used for an accurate, reliable, and highly reproducible serial assessment of LV and right ventricular function in these patients. This is critical because appropriate use of angiotensin-converting enzyme inhibitors and β-blockers can result in an improvement in LV function in a significant proportion of patients with CHF.

Positron Emission Tomography

PET is an integral part of nuclear cardiac imaging. However, because of different instrumentation used and unique properties of radiotracers used, PET imaging warrants a separate discussion. PET involves the use of positron-emitting isotopes (^{11}C, ^{18}F, ^{13}N, ^{15}O, ^{82}Rb, ^{68}Ga, ^{67}Cu). These radiotracers decay by converting a proton into a neutron while ejecting a positron from their nucleus. Upon coming in contact with an electron, positron disintegrates into two γ rays each with 511 KeV energy released at 180°, which can be detected as coincident photons by an array of detectors placed around the patient. These detectors employ different scintillators, which are much denser than sodium iodide scintillator used by regular gamma cameras and are more efficient in absorbing high-energy photons. PET images are of higher technical quality and are less vulnerable to artifacts, inherent to SPECT imaging. PET tracers have a relatively short half-life and are produced by a generator or a cyclotron. ^{82}Rb, used for MPI is produced from an ^{82}Sr/^{82}Rb generator. PET traces can readily be incorporated into a number of metabolic substrates such as deoxyglucose, free fatty acids, acetate, sympathomimetic amines, peptides, and proteins and are useful for studying the perfusion, metabolism, adrenergic neuronal activity, various cell membrane receptors of the myocardium, and other organs [45–53]. ^{15}O-water, ^{13}N-ammonia, and ^{82}Rb are used for MPI. A major advantage of PET perfusion imaging is that, apart from a qualitative assessment, an accurate quantitative assessment of the regional myocardial blood flow per gram of myocardial tissue at rest and under various physiological conditions can be carried out [51,52]. Thus myocardial flow reserve can be studied by PET imaging. This is particularly useful in detecting patients with multivessel CAD and diffuse endothelial dysfunction and for studying microvascular function. Currently ^{18}F-flurpiridaz, a new ^{18}F-based perfusion tracer is undergoing clinical evaluation for MPI. The preliminary results are encouraging [53]. Availability of an ^{18}F-based myocardial perfusion tracer would permit PET MPI without the need for on-site cyclotron or generator. ^{18}FDG imaging is useful for studying myocardial viability.

Direct Myocardial Ischemia Imaging

Myocardial ischemia results in an immediate and profound metabolic shift: suppression of fatty acid uptake and a substantial increase in glucose uptake. Therefore myocardial ischemia results in an immediate and profound increase in myocardial glucose uptake. This provides a strong rationale for the use of ^{18}FDG for direct imaging of myocardial ischemia [54–56]. Preliminary studies have shown a remarkable potential of exercise ^{18}FDG imaging for the detection and quantification of CAD. The sensitivity of exercise ^{18}FDG imaging is significantly higher than that of exercise-rest MPI for the detection of individual vessels with greater than or equal to 50% luminal narrowing. Figure 11.7 shows exercise ^{18}FDG and exercise-rest MPI of a patient with CAD. These data indicate the potential superiority of direct ischemia imaging over conventional MPI for the detection and risk stratification of patients with CAD.

Stress Echocardiography

Stress echocardiography can also be used for the detection of patients with CAD. The general principles of stress testing are similar to that for exercise electrocardiography or pharmacological stress testing as described above, except that echocardiography is combined with exercise or pharmacological stress with dobutamine [57–69]. Exercise or dobutamine administration results in an increase in myocardial wall motion and thickening, a reduction in end-systolic volume, and an increase in LVEF in normal hearts. Myocardial ischemia by exercise or pharmacological stress results in an impairment of regional wall motion, wall thickening, and relaxation of myocardial segments perfused by the narrowed coronary arteries. Furthermore, myocardial ischemia may also result in LV dilation, an increase in end-systolic volume and a decrease in LVEF depending upon the severity and extent of myocardial ischemia. All of these changes can be tracked by performing echocardiography at rest and during exercise or pharmacological stress. Addition

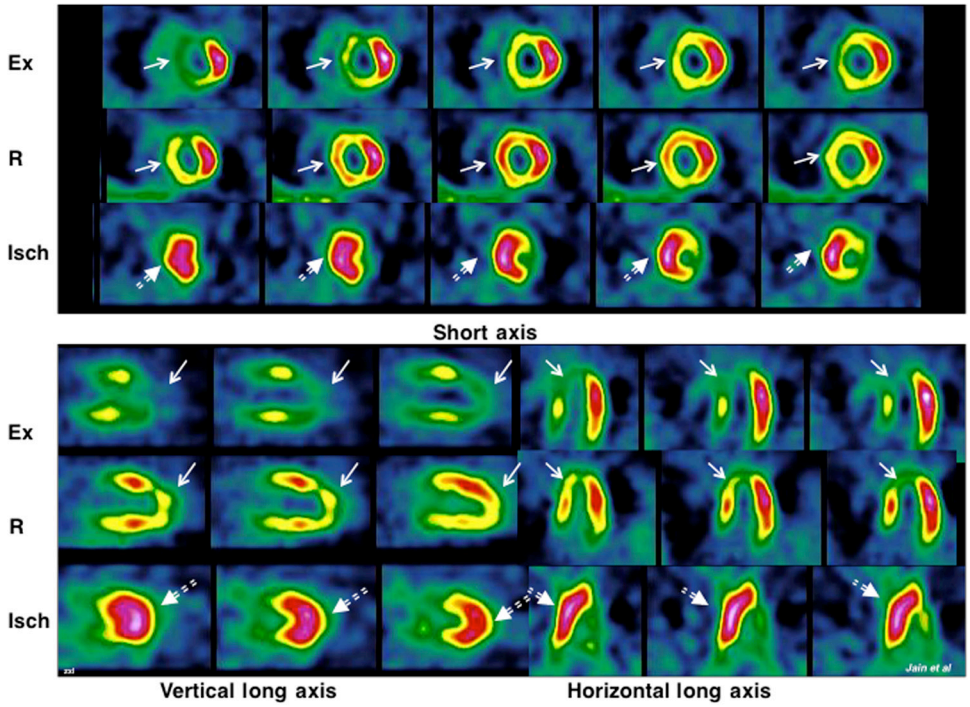

FIGURE 11.7 Exercise (Ex) and rest (R) ^{99}mTc-sestamibi and exercise ^{18}FDG (Isch) images of an 67-year-old man with angina and no prior myocardial infarction. There is a large area of partially reversible perfusion abnormality involving the septum, anterior wall, and apex (small arrows). Intense ^{18}FDG uptake is present in these areas (solid arrowheads). Coronary angiography showed 90% stenosis of the left anterior descending coronary artery and a 60% stenosis of the left circumflex artery. *Source: Reproduced with permission from Ref. [54].*

of stress echocardiography improves the sensitivity and specificity of exercise ECG [57,58]. The clinical indications for the use of this technique are similar to that of nuclear MPI. The potential advantages of this technique include the use of relatively ubiquitously available cardiac imaging technique and lack of radiation exposure to the patient. The sensitivity of stress echocardiography for the detection of CAD is in the range of 80–90% and is similar to that of nuclear MPI, but significantly better than that of exercise ECG alone. The specificity is in the range of 65–85%, again similar to nuclear MPI but significantly better than stress electrocardiography.

Unfortunately, this technique does suffer from several significant limitations: such as technical challenges in obtaining high-quality images during exercise, a lack of objective and quantitative interpretation of images for wall motion abnormalities, and a poor specificity of wall motion abnormalities in patients with baseline wall motion abnormalities due to prior myocardial infarctions or cardiomyopathy [57]. Patients with poor echogenic windows due to chronic obstructive pulmonary disease, obesity or thoracic deformities are not suitable for this technique. Significant degrees of operator experience and skill are required for obtaining good quality cardiac images during exercise or within 1 min of completion of exercise. It is easier to obtain exercise images with the use of exercise supine or upright bicycle rather than treadmill. It is also critically important to attain an adequate workload on exercise. Inadequate workload at peak exercise lowers the sensitivity of the test. For patients, who cannot exercise, or are unable to exercise to an inadequate workload due to noncardiac limitations, pharmacological stress is required. Unlike nuclear MPI, dobutamine is the pharmacological stressor of choice. Coronary vasodilators such as dipyridamole, adenosine, or regadenoson result in a significant flow heterogeneity in myocardial perfusion, but wall motion abnormalities are less common and less impressive compared to the perfusion abnormalities seen on MPI. Picano and colleagues proposed a high-dose dipyridamole infusion (0.84 mg/kg over 6 min) for dipyridamole stress echocardiography [58]. But adverse effects of this high-dose dipyridamole infusion are unacceptably high and this protocol is not in clinical use in the United States. For dobutamine stress echocardiography, dobutamine infusion is started at a dose of 10 µg/kg/min and the dose is increased by 10 µg/kg/min till the symptoms of angina, ST segment depression, age-predicted maximum heart rate is reached, or new myocardial wall motion abnormalities appear or a max dobutamine dose of 40 µg/kg/min is reached. Atropine can be given at a dose of 0.5–1.0 mg if the age predicted maximum heart rate is not reached at max dose of dobutamine and the study is otherwise negative. Additional increase in heart rate

induced by atropine in such cases increases the sensitivity of the test. Whereas, dobutamine infusion is safe in younger patients, particularly those with no comorbidities, the side effects are very common and can be serious in elderly patients and in those with comorbidities such as hypertension, ventricular, and atrial arrhythmias [63]. Dobutamine can result in hypertensive blood response and sustained ventricular and atrial arrhythmias. Beta-blockers attenuate the effects of dobutamine and need to be withheld before dobutamine stress echocardiography.

For stress echocardiography, baseline images are obtained in M-mode and 2-D mode with limited Doppler examination prior to starting the stress. Digital imaging with multiple screen display is required for stress echocardiography for side-by-side display and comparison of rest and stress images. The standard approach for interpretation of the images is qualitative interpretation of regional wall motion and myocardial thickening at baseline and during exercise and recovery. Since, myocardial wall motion is also influenced by translational, torsional, and tethering variables, regional myocardial thickening is the primary variable used for interpretation. Quantitative approaches based on longitudinal and radial excursion, displacement and strain are proposed, but are not used for routine interpretation [62]. This introduces the possibility of interobserver as well as intraobserver variability in the interpretation of images. As described above, a lack of objective technique for the quantitative evaluation of the extent and severity of regional wall motion abnormalities remains a significant limitation of this technique from the data interpretation perspective.

A particular use of dobutamine stress echocardiography is for the detection of myocardial viability [57]. A dual-phase response where segments with abnormal wall motion at rest show an improvement in wall motion and thickening at low-dose dobutamine, but the wall motion worsens again at high-dose dobutamine is highly specific for viable myocardium that will improve with revascularization. This represents hibernating but viable myocardium with chronic low flow at rest. These segments are akinetic or hypokinetic at rest. With low-dose dobutamine, regional wall motion and thickening improves, but at high-dose dobutamine, these segments become ischemic and develop regional wall motion and thickening abnormalities. The sensitivity of dobutamine echocardiography for predicting improvements of wall motion and thickening after revascularization varies from 70% to 85% and specificity varies from 60% to 95%.

Another use of stress echocardiography is for the evaluation of complex patients with valvular heart disease and impaired LV function [67,68]. In patients with severe aortic stenosis, impaired cardiac function and a low cardiac output, a lack of improvement in cardiac output with low-dose dobutamine is predictive of a lack of improvement after valve surgery, whereas an improvement with low-dose dobutamine is predictive of an improvement of cardiac function and cardiac output after surgery.

Echo contrast agents have been developed for better visualization of the chambers of the heart and for studying myocardial perfusion [64–66]. These agents comprise of perfluranes, which are liquid at room temperature, but change into gaseous phase at body temperature after intravenous administration. They are enclosed in microspheres with protein coverings with 2–5 micron diameter. After i.v. administration, they transit through the right heart, lungs, and left heart chambers. This improves the visualization of these chambers and is used routinely in patients with poor echogenic windows or with difficulty in visualizing the endocardial borders. After reaching aorta, they also transit through the myocardium with the coronary flow and result in myocardial blush. Thus myocardial perfusion can be imaged at rest and with exercise or pharmacological stress. Whereas, their use is now routine for LV imaging in patients with poor echogenic windows and suboptimal endocardial definition, study of myocardial perfusion remains technically challenging and unreliable. These agents did not get FDA approval for imaging myocardial perfusion.

CT Imaging

Dramatic advances in the technology of CT imaging now permit noninvasive coronary angiography using intravenous injection of the contrast [70–75]. This has been made possible by simultaneous acquisition of 64–320 transaxial slices of 0.5–2.0 mm thickness using X-ray tubes and detectors mounted on a gantry capable of rotating at extremely fast speeds in a spiral fashion so as to be able to image the entire heart in only a few seconds. Iodinated contrast agents are injected intravenously as a short infusion. As soon as the contrast reaches the ascending aorta, CT imaging is started and completed within the next few seconds. The images are processed off-line to visualize the coronary arteries. Highly sophisticated and technically complex software are used to process the raw data to extract the 3-dimensional coronary anatomy from these images. Some of the limitations of earlier imaging such as high radiation exposure have now been addressed by the use of prospective gating of the heart, which allows imaging to occur only during diastole. This has significantly reduced the radiation exposure to the patient. Additional CT images can be acquired prior to the injection of the contrast agent to quantify the extent and severity of coronary arterial calcification. Apart from the coronary arteries, the anatomy of cardiac chambers, aortic root, and pulmonary veins can also be studied in great detail.

Undoubtedly, CT imaging of the heart is a great technical marvel. However, the indications for its use remain

somewhat unclear at this point. Whereas, the resolution of cardiac CT images is good, it is not anywhere close to that of standard invasive coronary angiography. Therefore, CT coronary angiography remains as a screening tool to detect high-grade CAD, which would require invasive coronary angiography. If a patient is found to have severe multivessel CAD on CT angiography and warrants consideration for coronary bypass surgery, one cannot proceed with surgery based on CT angiographic information alone. A standard invasive coronary angiography is still required.

The indications for CT coronary angiography remains inadequately defined. Patients with equivocal stress MPI or stress echocardiography can perhaps be considered for CT coronary angiography. Another group of patients where CT coronary angiography has been studied is the group of patients presenting with chest pain to the emergency department, with no definite evidence of acute myocardial infarction based on initial clinical evaluation, electrocardiography, and cardiac enzymes. Only a small percentage of these patients do turn out to have acute coronary syndrome. A rapid triage of these patients is required to identify those who would warrant hospitalization and further invasive coronary angiography, whereas the remaining patients with no acute coronary syndrome can be safely discharged from the emergency department. Several studies have shown CT coronary angiography to be effective in triaging these patients in a shorter time compared to the more conventional tests such as stress MPI or stress echocardiography. However, patient enrollment in these studies was limited to the standard working hours during week days when expertize to perform and interpret CT coronary angiography is readily available. Furthermore, it remains unclear whether patient outcome was any better in patients undergoing CT coronary angiography compared to standard stress testing approach. However, there was a greater utilization of invasive coronary angiography and revascularization procedures compared to the patients undergoing stress testing.

Recently cardiac CT has also been utilized to study myocardial perfusion by performing cardiac CT at baseline and following administration of adenosine or regadenoson [74,75]. Transient opacification of myocardium following contrast injection at rest and with pharmacological coronary vasodilation provides information about the functional significance of coronary luminal narrowing. However, this technique is still investigational at this stage.

Further studies are required to establish the indication for CT coronary angiography in clinical practice.

MR Imaging

MR imaging provides a very high-quality images of the chambers of the heart, myocardium, cardiac valves, and vascular structures. MR contrast images can also provide information about the presence of myocardial scar, viability as well as myocardial perfusion [76,77]. This technique is described in detail in a separate chapter.

CONCLUSION

Noninvasive cardiac imaging techniques have played a crucial role in the evaluation of patients with definite or suspected CAD and for optimal and cost-effective utilization of various therapeutic options. Nuclear MPI is the most important and most widely used technique. This provides important diagnostic and very powerful prognostic information in males as well as in females and in all subsets of the patient population: suspected CAD, known CAD, and following acute myocardial infarction. Newer imaging modalities including stress echocardiography, cardiac CT, and MR are slowly making inroads into this field and their use is likely to increase in the coming years.

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CHAPTER

12

Current Approaches to Treatment of Ventricular Arrhythmias in Patients with Coronary Artery Disease

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INTRODUCTION

Patients with coronary artery disease (CAD) can be at increased risk for sustained ventricular arrhythmias (VAs) that may cause significant morbidity and mortality. While not all VA in this population will lead to sudden cardiac death (SCD), they may have prognostic value in predicting those at risk. While the preponderance of VA management decisions will take place in the setting of chronic ischemic cardiomyopathy (ICM), VA may also occur in the setting of ischemia and acute coronary syndromes. The development of the implantable cardiac defibrillator (ICD) has revolutionized the prevention of SCD, but to believe that the management of VA in this patient population starts and ends with implantation is a disservice to the patient. This chapter will discuss management of VA in both the setting of acute ischemia/infarction and in chronic ICM. A detailed discussion of the primary prevention of VA and SCD in CAD is beyond the scope of this chapter.

VAs IN THE SETTING OF ACUTE ISCHEMIA AND INFARCTION

Incidence

Ventricular tachyarrhythmias occurring during acute ischemia or infarction are associated with a poor prognosis. Data from GUSTO-1 [1] indicate that, *in the thrombolytic era*, VA occurred during acute ischemia or infarction in 10.2% of patients, with 3.5% presenting with sustained ventricular tachycardia (VT), 4.1% with ventricular fibrillation (VF), and 2.7% with both. The vast majority occurred within the first 48 h (80–85%). Risk factors for developing VT or VF acutely included older age, hypertension, prior myocardial infarction (MI), higher Killip class, anterior infarct, and depressed ejection fraction. Interestingly, these data indicate that both early (<2 days) and late (>2 days) occurrences of sustained VT or VF were associated with a higher risk of subsequent mortality, and having both VT and VF was a worse prognostic indicator than having either alone.

In the era of primary percutaneous coronary intervention (PCI), data from the APEX AMI trial [2] in high-risk ST-elevation MI patients indicated that 329 out of 5745 patients (5.7%) presenting for primary PCI had VT and/or VF, with 90% occurring within 48 h of presentation (and 64% before the end of their cardiac catheterization). Patients with VT/VF had a higher 90-day mortality compared with those without VT/VF (23.2% vs 3.6%; unadjusted hazard ratio 7.33, adjusted hazard ratio 3.63), with most of the excess in mortality occurring within the first 30 days. In addition, outcomes were worse if VT/VF occurred late (i.e., after the cardiac catheterization) instead of early (90-day mortality of 33.3% vs 17.2%).

In perhaps a lower risk ST-elevation MI patient population, the PAMI investigators [3] reported occurrence of VT/VF in 133 or 3065 patients (4.3%) during PCI. In contrast to the APEX AMI data, when comparing patients

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00012-7

with and without VT/VF there was no difference in in-hospital mortality (3.0% vs 2.9%, respectively; $p = 0.79$) or mortality at 1 year (4.5% vs 5.5%; $p = 0.72$).

In non-ST-segment-elevation acute coronary syndromes, data from the EARLY ACS trial [4], which enrolled 9211 patients, indicate that the cumulative incidence of VT/VF was 1.5%, with 0.6% occurring within 48 h, and 0.9% occurring after 48 h. Predictors of VT/VF included prior heart failure, ejection fraction less than 30%, and 3-vessel CAD. Patients with VT/VF within 48 h had a higher 30-day mortality than those who did not have VT/VF within 48 h (13.0% vs 2.2%; adjusted odds ratio 6.73), and this risk persisted at 1 year. However, the risk of mortality in patients with VT/VF after 48 h was greater than for those having VT/VF within 48 h (hazard ratio 20.70 vs 7.45, respectively; compared with patients without VT/VF).

Substrate and Mechanism

Sustained VA in the setting of MI or injury occur due to the interplay of many different factors, including necrosis, reperfusion, the healing process, scar formation, electrolyte abnormalities, and autonomic changes. Animal studies have shown that the amount of ischemic injury and myocardial hypoperfusion is correlated with the rate of free radical formation with reperfusion, and reperfusion arrhythmias [5]. In addition, an increase in heart rate exacerbates the ischemic injury and increases the incidence of reperfusion arrhythmias [6].

With respect to VA associated with RCA infarct, one possible mechanism includes increased vagal tone via the Bezold–Jarisch reflex leading to a compensatory increase in sympathetic tone [7].

Treatment

For VA arising due to acute ischemia or injury, therapy for the underlying cause of ischemia should be initiated, including primary PCI, as indicated. In addition, correction of electrolyte disturbances, especially hypomagnesemia and hypokalemia, is recommended due to their potential contribution to VF. The use of β-blockers is associated with lower mortality in acute MI complicated by VA. In the VALIANT Registry, in patients with sustained VT/VF, β-blocker therapy in the first 24 h after acute MI was associated with decreased early mortality without worsening heart failure (relative risk 0.28; $p = 0.013$) [8].

VF should be immediately defibrillated using a non-synchronized mode. For sustained monomorphic VT that is hemodyamically unstable, synchronized cardioversion should be immediately performed. If VT is hemodynamically tolerated, a 12-lead electrocardiogram should be performed and analyzed, followed by intravenous antiarrhythmic therapy. Specifically, intravenous procainamide or amiodarone is recommended for patients with recurrent or incessant polymorphic VT due to acute myocardial ischemia, following revascularization and β-blockade (Class I indication). Intravenous procainamide is also reasonable for initial treatment of patients with stable sustained monomorphic VT (Class IIa indication), as is intravenous amiodarone for patients with hemodynamically unstable monomorphic VT refractory to conversion with countershock (Class IIa indication). Lidocaine may also be useful for treatment of stable monomorphic VT or polymorphic VT specifically associated with acute myocardial ischemia or infarction (Class IIb indication) [9]. Catheter ablation (CA) may be considered for patients with recurrent polymorphic VT or VF when there is a consistent PVC morphology that triggers these arrhythmias, or for recurring or incessant monomorphic VT [10].

VAs IN THE SETTING OF ICM

Incidence

The risk of VAs remains after the initial phases of acute MI and can present in variable ways. Patients can experience sustained, nonfatal VT post MI, but more concerning is the risk of SCD, presumably due to hemodynamically significant VT or VF. While the exact incidence of these arrhythmias as the cause of SCD is not clear, the advent of the ICD for primary prevention of SCD does allow for some estimate of the frequency of these arrhythmias in the ICM population. Two trials showed a mortality benefit of the ICD in patients with ICM, decreased EF with nonsustained VT and inducible sustained VT at electrophysiology study (EPS) [11,12]. The Multicenter Automatic Defibrillator Implantation Trial (MADIT) [11] randomized 196 patients with ICM and EF less than or equal to 35% to receive standard medical therapy or an ICD. The ICD arm experienced a 0.46 hazard ratio for all-cause mortality (95% confidence interval, 0.26–0.82; $p = 0.009$) and a numerically decreased rate of arrhythmic death (13 in control group, 3 in ICD group) [11]. There was also a decrease in non-arrhythmic cardiac death in the ICD group (13 vs 7), felt by the investigators to reflect the difficulty in classifying cause of death [11]. As such, assuming six of these deaths were actually arrhythmic, approximately 20% of this population would experience arrhythmic SCD at 5 years. While EPS identifies a higher risk primary prevention population, patients with NSVT without inducible VA still have a cardiac arrest or arrhythmic death rate of 12% at 2 years and 24% at 5 years [13].

The MADIT II did not require NSVT or EPS, randomizing patients with ICM and EF of 30% or less to standard

FIGURE 12.1 Critical isthmus conduction. Each panel displays an area of myocardial scar composed of dense fibrosis (blue shapes) flanking a zone of surviving myocardium displaying slow conduction (red stippled area), within normal myocardium (red). Left Panel: Conduction during sinus rhythm enters the isthmus from both sides. These wavefronts (yellow arrows) collide inside the isthmus. Middle Panel: A premature ventricular beat (yellow sun) occurs during the isthmus refractory period and conduction is blocked into the near side of the isthmus (black bar), but the wavefront (yellow arcs) conducts around the scar allowing time for the far side of the isthmus to recover excitability. Right Panel: As the wavefront (dashed arrow) enters the far side of the isthmus, it conducts slowly, allowing for the near side of the isthmus to recover excitability, therefore enabling reentry.

therapy or primary prophylaxis ICD. A hazard ratio of 0.69 (95% confidence interval 0.51–0.93; $p = 0.016$) for all-cause mortality was seen in the ICD group [14]. In a follow-up study of the MADIT II, the 720 subjects that received an ICD were evaluated for device-treated VA. An analysis of first-treated arrhythmia revealed that by 3 years, 19% of subjects received therapy for VT as their first VA while only 4% received therapy for VF as the first VA. Of the 701 treated VA episodes, 84% were for VT, the remainder being for VF [15].

Substrate and Mechanism of VAs

After an acute MI, the infarcted territory undergoes fibrosis. Scar deposition is rarely a homogeneous process; usually, surviving myocardial fibers are interspersed in areas of fibrosis [16]. While these surviving fibers do not participate in systolic function, they are capable of impulse conduction and do so during sinus rhythm [16–18]. Conduction in infarcted areas is generally slow due to decreased gap junctions at the intercalated disks and also due to more frequent conduction between parallel myocytes, leading to nonlinear, "zigzag" conduction [19,20]. Action potential characteristics in chronically infarcted areas have been shown to be normal in animal studies [21–23]. These areas of surviving myocardium within scar display longer refractory periods and thus encounter transient unidirectional block due to premature ventricular contractions (PVCs) or changes in heart rate (such as supraventricular tachycardia) [24]. The mechanism of these PVCs that initiate reentry is not completely understood, but likely result from abnormal automaticity of poorly coupled myocytes [25,26]. Due to slow conduction in this "critical isthmus" of tissue, the site of unidirectional block has time to recover conduction. This allows for a reentrant circuit to be established within the scar (Figure 12.1). The initiating PVC may originate from the area of scar that connects the critical isthmus to the normal myocardium; the exit site [27]. The mechanistic underpinning for VF initiation is heterogeneity in ventricular refractoriness, especially in regions of myocardial scar, leading to unidirectional conduction block and the formation of multiple reentrant wavelets [28,29]. The initiation of VF in ICM can be due to VT or PVC [30,31].

Prevention of Recurrence

The remainder of this chapter will discuss the management of VA in chronic ICM (secondary prevention). Much of the literature on this subject includes patients with ICM as well as those with other forms of cardiomyopathy (CMP). This management is rife with paradox. It is important to note that VA prevention and SCD prevention are not one and the same. The prevention of the latter rests solely in the hands of the ICD, which does not actually prevent, merely terminates, VA. While lifesaving, the delivery of high-energy shocks from the ICD is associated with much morbidity and mortality and paradoxically requires interventions to prevent them altogether.

MEDICAL THERAPY

Antiarrhythmic Drugs

Antiarrhythmic drugs that act directly on the ion channels subtending the cardiac action potential can be effective in decreasing the burden of VA, but not enough to impart protection from SCD, and therefore, their use is largely adjuvant to prevent ICD therapies.

Proarrhythmia can increase the risk of SCD in patients with CMP [32,33]. Sotalol has been shown to decrease ICD shocks or death from any cause by 44% compared to placebo in patients with ICD for secondary prevention [34]. The OPTIC trial randomized patients receiving ICD shocks for VA to three AAD arms: Amiodarone plus β-blockers, sotalol, and β-blockers alone. At 1 year, the percentage of patients receiving ICD therapy for VA (ATP or shocks) was as follows: Amiodarone plus β-blockers 13%, followed by sotalol 38.9%, and then β-blockers alone 45% [35]. Of note, the rate of drug discontinuation was 18.2% for amiodarone and 23.5% for sotalol (vs 5.3% for β-blocker alone), due to side effects [35], highlighting a significant issue with AAD treatment. Additionally, an 11-year follow-up of the Canadian Implantable Defibrillator Study (CIDS) trial showed a 5.5% per year mortality and a 50% discontinuation rate with amiodarone therapy [36]. One other Class III AAD has been suggested to decrease ICD therapy for VA; dofetilide. In one brief observational report, 50% of patients had a decrease in ICD therapies after starting treatment with dofetilide [37]. Additionally, Baquero et al. found a significant reduction in monthly VT/VF episodes (1.8 ± 4.5/month before vs 1.0 ± 3.5/month on dofetilide, $p = 0.006$) in 30 patients with ICDs that had failed at least one other AAD (63% amiodarone) [38].

β-Blocker

β-Blockers are considered a Class II AAD in the Vaughan-Williams classification. In addition to positive effects on mortality in patients with prior MI, data suggest a decrease in recurrent VA as well. Observational studies in patients with prior SCD and hemodynamically relevant VT have reported a decrease in recurrence [33,39,40]. Hallstrom et al. [33] reported an adjusted relative risk reduction of 38% related to β-blocker therapy in survivors of cardiac arrest. In this same study, a higher mortality was seen with AAD use. In the Antiarrhythmics Versus Implantable Defibrillators (AVID) registry [39], patients on β-blocker but not treated with AAD or ICD showed an approximately 50% reduction in adjusted relative risk of mortality due to β-blocker therapy. Hreybe et al. also showed an increase in time to first ICD shock for VA in patients treated with β-blocker compared to those that were not [40]. In a randomized comparison to sotalol, metoprolol [41] showed a greater survival free of ventricular tachyarrhythmias in patients with implantable defibrillators. This is in contrast to the OPTIC trial, which showed greater efficacy for sotalol [35].

Nontraditional Agents

While many agents lacking direct ion channel effects have been shown to prevent SCD, few have been shown to prevent the recurrence of VA. It is important to remember that most of these agents may act in preventing the formation/progression of the substrate that leads to VA, thus enacting an indirect "antiarrhythmic" effect. Agents such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, aldosterone inhibitors, and HMG-CoA reductase inhibitors have not been shown to prevent recurrence of VA.

Fish Oil

Poly-unsaturated fatty acids (PUFAs) commonly found in the oil of certain fish may have antiarrhythmic effects to prevent recurrences of VA. The data on these compounds is somewhat contradictory. While some studies suggest a benefit [42–44], others have shown an effect [45] or an actual increase in VA in patients with ICD [46], leading to increased therapies. A recent meta-analysis of over 30,000 patients in nine trials showed no significant effect on VA [47]. At this time, PUFA cannot be recommended as a viable treatment for VA suppression.

Anti-Anginal Agents

The recent addition of ranolazine to the anti-anginal armamentarium has led to discovery of antiarrhythmic properties of this drug, following in the footsteps of amiodarone. Ranolazine is a late sodium channel blocker that has antiarrhythmic properties [48]. While no randomized trials have shown definitive efficacy for VA, case reports and series do suggest this compound may have promise [49–51]. These reports have mostly been in patients with recurrent PVCs or ICD therapies for VA who were also on traditional AAD that were ineffective (usually amiodarone). The addition of ranolazine coincided with an interruption of VT storm or suppression of further ICD therapies [49–51].

IMPLANTABLE CARDIAC DEFIBRILLATOR

The utility of the ICD in primary and secondary prevention of SCD in patients at risk is supported by multiple randomized, multicenter trials, establishing this device as the first-line therapy in patients with ICM and VA. It is important to note that while the ICD does prevent SCD, it does NOT prevent VA. Sustained VAs are terminated either with a high-energy shock or, if monomorphic VT of a rate slow enough to respond, with anti-tachycardia pacing (ATP).

Secondary Prevention Trials

There are three randomized trials of ICD for secondary prevention of SCD from VA. Two of these trials, the CIDS [52] and the CASH [53], showed a nonsignificant

decrease in total mortality. CIDS also showed a non-significant decrease in arrhythmic death, while CASH did show a significant reduction in recurrent SCD. Of note, CIDS also included patients with syncope from VT, VT greater than 150 bpm with presyncope or angina and an EF less than 35%, or unmonitored syncope with subsequent VT greater than 10 s or sustained VT induced at electrophysiologic study. Both trials compared the ICD to amiodarone. Additionally, CASH included treatment arms consisting of metoprolol and propafenone. While the metoprolol arm showed similar results to amiodarone, the propafenone arm was discontinued early after showing an unacceptably high mortality rate.

The Antiarrhythmics Versus Implantable Defibrillators (AVID) randomized over 1000 subjects to initial treatment with an ICD or treatment with an AAD (amiodarone or sotalol) in patients with VF or VT with syncope, or VT with EF less than 40% and symptoms of hemodynamic compromise [54]. As opposed to CIDS and CASH, AVID showed a clear mortality benefit at 1, 2, and 3 years of follow-up as compared to AAD (89.3% vs 82.3%, 81.6% vs 74.7%, and 75.4% vs 64.1%, respectively, $p < 0.02$). These three trials were instrumental in establishing the ICD as first-line therapy for hemodynamically significant VA in patients with CMP. It is, however, important to note that these trials do not address those patients with hemodynamically tolerated VT, or those with ICM and normal/near-normal ventricular function, these data have been generalized to these patient populations in the guidelines [55].

While the ICD has been a life-saving therapy for many patients with VA and CMP, it is by no means infallible. Sudden death can still occur at a rate of about 1.3–4.5% [56–59] with 16–38% due to refractory VA [60,61].

Cardiac Resynchronization

Cardiac resynchronization therapy (CRT) has been a great advance in device therapy for patients with CHF. The primary effect of CRT is on clinical CHF symptoms, but many have reported a decrease in VA as well [62,63]. The mechanism for this is likely multifactorial, including improvement in clinical CHF class, reverse remodeling, and direct electrophysiologic effects [62,64,65]. That said, it must be noted that a number of case reports and series have described the initiation of VT storm in some patients after institution of CRT [66–69], likely due to direct stimulation of the substrate responsible (infarcted area) for reentrant VA [70] and increased dispersion of repolarization [71]. As no randomized trials have been designed to specifically determine if CRT is antiarrhythmic, VA alone is not an indication for CRT and patients should meet traditional criteria for implantation as well.

Shock Morbidity and Mortality

Additionally, ICD therapies can cause significant psychological morbidity. There is a dose-dependent effect of shock frequency on the development of anxiety-related disorders (panic disorder, agoraphobia, and generalized anxiety) and depression [72,73]. Ironically, depression can lead to an increase in ICD therapies [74] as well.

In a seeming paradox, recent data has shown an association between ICD shocks and increased mortality. In an analysis of the Sudden Cardiac Death in Heart Failure (SCD HeFT) trial, patients that experienced appropriate shocks had a hazard ratio of 5.68 for mortality [75]. It is unclear if shocks contribute to, or are simply a marker for, risk of death. Those with a high burden of VA may simply have more advanced disease. One would expect that those being treated with ATP (see below) would have the same mortality if this were so. Data regarding this are conflicting [76–78]. Most recently, an analysis of the Multicenter Automatic Defibrillator Implantation Trial-Reduce Inappropriate Therapy (MADIT-RIT) trial [79] showed that appropriate shocks increased mortality, while appropriate ATP did not; however, any inappropriate therapies (shocks or ATP for supraventricular tachyarrhythmias) were also associated with higher mortality [80]. The authors concluded that this study does provide evidence of the link between ICD shocks for VA and mortality.

Shock Prevention

Due to the clear morbidity and possible mortality associated with ICD shocks, it is imperative to prevent recurrence. While AAD can be effective in preventing recurrent shocks for VA as discussed above, evidence-supported ICD programming is crucial in preventing the delivery of shocks that may not be necessary in the first instance.

Modern ICDs are not only capable of delivering shocks to treat VA, but can also attempt to terminate VT by overdrive pacing, or ATP. ATP terminates VT by causing conduction block within the VT circuit [81], without the discomfort and potential morbidity of ICD shocks. While originally applied only to slower VT due to the fear of inefficacy, syncope, and acceleration to VF when utilized for VT greater than 200 bpm, subsequent studies largely dispelled these concerns [82,83]. The PainFree Rx II trial randomized patients to receive ATP versus shocks for VT of rates 188–250 bpm [83]. The investigators found that ATP effectively terminated fast VTs in 81% of episodes without any difference in mortality, syncope, or acceleration of VT to VF [83]. Additionally, the investigators detected an improvement in quality of life in the ATP arm over that seen in the shock arm [83].

Since the results of the PainFree Rx II trial, many trials have evaluated overall programming protocols to reduce the incidence of ICD shocks. These trials have utilized ATP as well as delaying the delivery of therapies in the hopes that VA may actually prove to be nonsustained and terminate spontaneously without any detriment to the patient. In addition, therapies for slower VT have been minimized in the belief that they pose little risk for sudden death.

The aforementioned MADIT-RIT trial compared three ICD programming protocols in a primary prevention population: A "standard" protocol that would treat VT of 170–200 bpm within 2.5 s and greater than 200 bpm after 1 s; a "delayed therapy" protocol that would treat VT 170–200 bpm after 60 s of tachycardia, 200–249 bpm after 12 s, and greater than 250 bpm after 2.5 s; and a "high-rate" protocol that would treat only VA greater than 200 bpm after 2.5 s [79]. ATP was utilized in all arms of the trial. This trial showed a significant reduction in mortality, appropriate therapies (mostly ATP) and inappropriate therapies in both the delayed therapy and high-rate protocols compared with the standard programming, thus proving that more conservative ICD programming is preferable in primary prevention ICD recipients [79].

The Effect of Long-Detection Interval versus Standard-Detection Interval for Implantable Cardioverter-Defibrillators on Antitachycardia Pacing and Shock Delivery (ADVANCE III) trial [84] also evaluated delaying ICD therapy delivery, but in both primary and secondary prevention ICD recipients. In this trial, prolonged detection time was defined by number of intervals in the tachycardia zone (30 out of 40 in the prolonged detection group; 18 out of 24 for the control group). For primary prevention patients, VA greater than 188 bpm were treated, whereas in the secondary prevention group, VA detection was based on the patients clinical tachycardia [84]. This trial also showed a decrease in both appropriate and inappropriate ICD therapies with prolonged detection time, but without any difference in mortality [84]. In a subgroup analysis of the secondary prevention patients in ADVANCE III, the prolonged detection arm experienced the same results [85].

Ablation

CA for the treatment of VA has evolved substantially over the past few decades, vastly aided by greater understanding of the arrhythmogenic substrate, as well as technological advances in computer mapping systems and delivery of ablation energy. Initially only an option in the minority of patients with hemodynamically stable VT, CA can now be offered to most patients with VT and VF in the setting of ICM.

Ablation of monomorphic VT in the setting of remote MI requires detailed mapping of the substrate area responsible for the arrhythmia. The critical isthmus of the VT circuit (see above) is located within scar utilizing electrode-tipped catheters and rendered electrically inert with ablation energy (most frequently radiofrequency electrical energy). This technique is based on the early experience of surgical subendocardial resection of infarcted myocardium pioneered at the University of Pennsylvania [86,87]. CA offers a minimally invasive approach with less morbidity and has largely supplanted surgical resection.

Contemporary VT ablation utilizes modern tools to enhance both the mapping and ablation processes. Computer mapping systems are able to follow catheter movements in three-dimensional (3D) space and collect data points that denote location and electrical parameters such as activation timing and tissue voltage [88]. These "electroanatomic mapping systems" allow for the reproduction of the endocardial and/or epicardial surface in the computer space, which can be used to guide catheter manipulation with minimal fluoroscopy [89]. Locations with signals of interest can be tagged on the reconstructed geometry and revisited for ablation later in the procedure (Figure 12.2). Signals showing low voltage consistent with scar/fibrosis, especially those that display multicomponent fractionation, can be involved in VT circuits [17,18,90,91].

Mapping can be performed during sinus rhythm and VT. A voltage map is often constructed during sinus rhythm to locate areas of scar [92]. A mapping catheter is moved throughout the ventricle and placed in contact with the myocardium over many cardiac cycles. A 3D geometry of the chamber is created on the mapping system. Confluent areas of low voltage are color-coded and fractionated signals and late potentials (occurring after the end of the QRS complex) are tagged on the map to be revisited after VT is induced. If the VT is hemodynamically tolerated, the circuit can be mapped by annotating the activation timing at sites throughout the chamber in reference to the QRS complex and performing pacing maneuvers to prove the site is a part of the circuit [93–95]. The critical isthmus will display activation during diastole (between QRS complexes), as this low-voltage activity in the scar cannot be seen on the surface ECG. Once this critical isthmus is defined, ablation can terminate the VT and prevent recurrence [95,96]. In addition, patients that exhibit frequent PVC may benefit from their ablation by better targeting reentrant VT and allowing for some improvement in LV function [27,97,98].

Unfortunately, most VT (~90% [99]) are not hemodynamically tolerated long enough for traditional mapping. Modern techniques for VT mapping and ablation

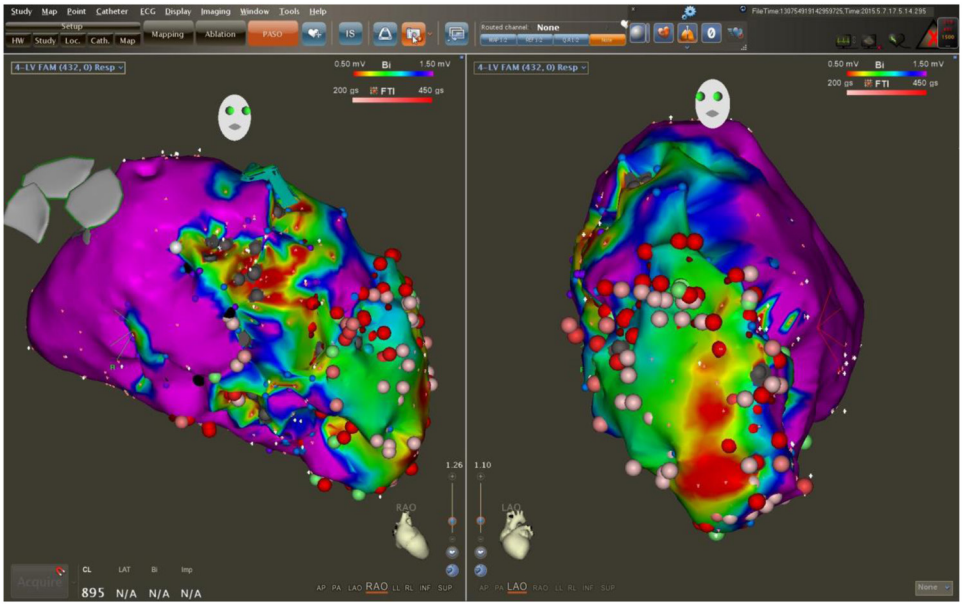

FIGURE 12.2 Electroanatomic map of endocardial scar in a patient with ischemic cardiomyopathy due to a large anteroseptal infarction. A large anteroseptal scar area is shown (dense scar in read, normal tissue in purple, border zone in yellow and green). The gray spheres indicate fibrotic areas without any voltage. Black, blue, and green spheres indicate areas with fractionated signals consistent with slow conduction zones. Red and pink spheres denote ablation lesion placement. A right anterior oblique view is on the left and a left anterior oblique view is on the right.

focus on modification of the scar ("substrate modification") guided by induced VT that is quickly terminated. The potential critical isthmus of an observed VT can be located by pacing inside the scar area ("pacemapping") during a stable rhythm (sinus or right ventricular paced rhythm). If the resultant paced QRS matches the VT QRS, the site is likely at or near the isthmus and is ablated [100,101]. During most VT ablation procedures, a combination of mapping during VT that is tolerated, and pacemapping those that are not, is utilized to maximize outcomes. It is important to note that multiple VT can often be induced during a single procedure and that outcomes are best if all are targeted [102,103]. Observational studies of this approach in ICM patients [103] and both ICM and NICM patients [100] experiencing frequent ICD shocks for VT showed a significant reduction in episodes after ablation.

The most current ablation techniques consist of extensive ablation targeting all signals that display evidence of slow conduction in the hope of interrupting all possible VT circuits. The complete elimination of all these sites, both endocardially and epicardially, does show promise and does predict long-term success independent of noninducibility of VT at the end of a procedure [104,105].

Two randomized trials of VT ablation in patients with ICM have been published. The Prophylactic Catheter Ablation for the Prevention of Defibrillator Therapy (SMASH-VT) trial randomized 128 patients with a recent implantation of ICD for VA or had a recent ICD therapy for VA after a recent implant, to ablation (substrate modification) or standard medical therapy (patients on Class I or III AAD were excluded). There was a significant decrease in ICD therapies between the standard medical therapy and ablation groups (33% vs 12%, $p = 0.007$) [106].

The Catheter Ablation of Stable Ventricular Tachycardia Before Defibrillator Implantation in Patients with Coronary Heart Disease (VTACH) trial randomized 107 patients with hemodynamically tolerated monomorphic VT to ablation prior to ICD implantation or ICD and standard medical therapy (without AAD) [107]. The ablation approach included traditional mapping of the stable VT and substrate modification as needed for other induced VT. The intention-to-treat analysis showed that the ablation group enjoyed a longer ICD therapy-free interval (18.6 vs 5.9 months) and greater VA-free survival at 2 years (47% vs 29%), with the greatest benefit seen in patients with EF greater than 30% [107]. A follow-up on treatment analysis (13% of the ablation group did not undergo the procedure, 19% of the control group were ablated) did show a greater effect of ablation when compared to the intention-to-treat analysis: Relative risk reduction of VA 49% versus 39% and relative risk reduction in cardiac hospitalization 52% versus 45% [108].

While the majority of VT circuits in ICM can be ablated endocardially, some patients have extension of their substrate to the deeper myocardium and

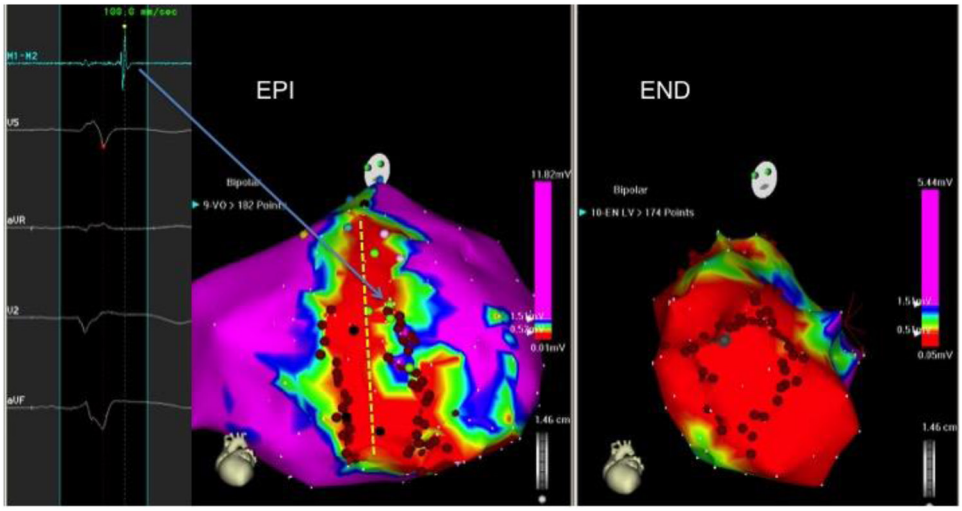

FIGURE 12.3 Transmural infarction with epicardial scar. A large anterior infarction displays low endocardial voltage (END) encompassing the entire anterior/anteroseptal wall. This substrate extends to the epicardium (EPI) surrounding the left anterior descending artery (yellow dashed line). The left panel displays an electrogram (M1-M2) with a very delayed component epicardially (blue line) that occurs after the end of the sinus rhythm QRS complex, as displayed on the ECG leads under this signal. Color coding and spherical markers are displayed as in Figure 12.2.

epicardium (Figure 12.3). The advent of a percutaneous subxiphoid approach to accessing the pericardial space for VT ablation has allowed for targeting of many VT previously unaccessible [109,110]. The ability to identify and ablate mid-myocardial circuits remains a challenge. In refractory cases, selective intracoronary alcohol ablation can be effective, but needs to be used cautiously [111]. New ablation technologies able to deliver deeper lesions will hopefully address this issue in the near future [112,113].

Once considered an arrhythmia not amenable to ablation, techniques to address VF have been pioneered over the past few years. As SMASH-VT suggests, ablation of VT has the additional effect of decreasing ICD therapies for VF as well [106], likely explained by the fact that many VF episodes begin as VT, but also likely due to the extensive substrate ablation. Specifically, surviving Purkinje fibers located in areas of scar can induce VF in ICM [114]. These areas can be identified as displaying early signals that precede the QRS during sinus rhythm and during ectopic beats. Ablation of these areas can prevent further VF episodes, even if the exact site of ectopic beat origination is not identified [114].

Despite the extensive body of literature on ablation and the expanding application to multiple VA substrates and areas of origin, a definitive mortality benefit has not been proven. Additionally, randomized comparisons with AAD or ICD have not been published. For these reasons, at this writing ablation of VA in SHD is largely adjunctive to the ICD in patients that fail, do not tolerate or do not wish to take AAD.

Autonomic Modulation

Based on the known benefits of β-adrenergic blockade previously discussed, and the success of left cardiac sympathetic denervation for long QT syndrome patients [115], invasive approaches to autonomic modulation have generated increasing interest for the treatment of VA in CMP. The stellate ganglia provide direct sympathetic innervation to the heart. A number of case reports and series have investigated the surgical destruction of the lower third to half of the stellate ganglia for arrhythmia control [116,117]. In 41 patients refractory to AAD and ablation for VA, Vaseghi et al. found bilateral stellate ganglia destruction more effective than left-sided ganglion destruction alone (ICD shock free survival 48% vs 30%, $p = 0.04$) [117].

The development of renal artery denervation for the treatment of hypertension has generated much interest in the effects on arrhythmia prevention. A number of case reports and series have displayed potential benefit in CMP patients with refractory VA [118–120]. These data must be tempered by uncertain efficacy of renal denervation for the treatment of hypertension [121]. Lastly, direct spinal cord stimulation, which can help prevent angina by suppressing sympathetic effects on the heart, has been utilized in two patients with refractory VA with good effect [122]. These invasive approaches to cardiac sympathetic blockade offer unique insights into arrhythmogenesis and offer potential future therapies, but have yet to be proven in large-scale trials.

CONCLUSIONS

The treatment of VA in patients with ICM has evolved over the decades from a primarily medical approach to one dominated by technology and invasive interventions. While the ICD has offered extended life to many patients, recurrent VAs are the cause of significant morbidity still. A complex, multi-modality approach by expert clinicians is necessary for successful management of these patients. Future technological advances will allow for the effective treatment of more patients.

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CHAPTER

13

Magnetic Resonance Imaging for Clinical Use and Research Investigation in Coronary Artery Disease

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INTRODUCTION

Invasive coronary angiography (ICA) and cardiac computed tomographic angiography (CCTA) are currently used as the diagnostic tools to identify and characterize coronary artery disease in the epicardial coronary arteries. Coronary magnetic resonance angiography (MRA) is an emerging noninvasive imaging tool to evaluate the epicardial coronary arteries. In this chapter, we will discuss the advantages of coronary MRA relative to ICA and CCTA, techniques involved in performing coronary MRA, indications for the use of coronary MRA, and clinical applications, clinical trial results, and future developments.

TECHNIQUE

Radiation and Contrast

Magnetic resonance imaging (MRI) has two advantages compared to ICA and CCTA. MRI does not require the use of ionizing radiation and can be performed without iodine-based contrast agents. MRI allows clinically reliable and validated information about the anatomy of coronary arteries to be obtained in conjunction with evaluation of function and wall motion in a single setting without radiation or contrast agents. In certain scenarios, coronary MRA can be performed with stress agents to evaluate myocardial function and wall motion abnormalities, allowing for a comprehensive cardiac evaluation without radiation or contrast.

Radiation

Medically related radiation exposure has increased in the United States. A New England Journal of Medicine report showed an increase in computed tomography (CT) imaging tests from 3 million in 1980 to 62 million in 2006 [1]. The growth in the use of CT imaging tests that use ionizing radiation has resulted in an increase in the overall radiation exposure to the population of the United States as well as the medically related radiation exposure [2,3]. The biological risks of radiation include deterministic effects and stochastic effects. While the precise dose relationship between ionizing radiation and malignancy is controversial, radiation remains a potential concern for both ICA and CCTA [4,5]. Coronary MRI does not use radiation in order to visualize the vessels. The technique, which will be discussed in subsequent sections, uses signal generated via a magnetic field to visualize the coronary arteries. The lack of use of radiation removes both the stochastic and deterministic effects of radiation from the imaging modality.

Contrast Agents

ICA and CCTA require the use of contrast agents to visualize the blood vessels. While ICA requires a smaller volume of contrast due to its intra-arterial administration compared to the intravenous administration of contrast for CCTA, the use of contrast is not without risks. The risks include contrast induced nephropathy, urticaria, throat closure, and/or full blown anaphylaxis [6]. The risk of

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00013-9

contrast in patients with prior reactions increases and can require premedication using a combination of steroid and diphenhydramine, which may also potentially complicate the safety and efficiency of obtaining CCTA or ICA [6].

Coronary MRA does not necessarily require the use of a contrast agent. While visualization of the epicardial coronary arteries can be done using signal from blood flow within the epicardial coronary arteries, intravenous contrast can be given to enhance visualization. When gadolinium is administered as part of the imaging protocol, the signal-to-noise ratio (SNR) can be significantly improved [7]. Gadolinium has a favorable safety profile when used in cardiac imaging. Data from the EuroCMR registry show a favorable profile with a low risk of events (0.05–0.42%), depending on the type of agent used (linear nonionic gadodiamide vs linear ionic gadobenate dimeglumine) [8].

CORONARY MRA TECHNIQUE

The utilization of cardiac MRI is growing in the practice of cardiac imaging. Medical centers are increasingly incorporating cardiac MRI in the evaluation of ischemia and cardiomyopathy. However, use of coronary MRA is not as widespread. The use of coronary MRA is less than CCTA and ICA due to the technical requirements to obtain images. The requirements include an MRI scanner with suitable field strength, special sequences to acquire the images, and expertise in interpretation.

FIELD STRENGTH

MRI scanners use a magnetic field to generate signal from flowing blood. The strength of the magnetic field contributes to the strength of the signal used to generate images. There are two clinically available field strengths to perform coronary MRA: 1.5 Tesla (1.5 T) and 3.0 Tesla (3.0 T). The use of a higher field strength scanner has the potential to improve the signal, thereby increasing the SNR, and hence generating higher quality images. However, while high field imaging has the opportunity to improve coronary imaging using MRI, challenges in high field imaging such as field inhomogeneity and signal absorption rate remain an area of continued investigation [9].

ACQUISITION TECHNIQUES AVAILABLE FOR CORONARY MRA

Three techniques have been described to perform coronary MRA: Electrocardiography-triggered, breath-hold, two-dimensional (2D) coronary MRA, electrocardiography-triggered, free-breathing, targeted (thin slab), three-dimensional (3D) coronary MRA, and whole-heart 3D coronary MRA acquisitions [9]. Coronary MRA techniques have evolved since the early 1990s with three generations of techniques available.

First/Early Generation Techniques (Breath-Hold, 2D Coronary MRA)

The 2D breath-hold technique was first described in 1991, and allowed for respiration motion to be minimized and imaging time to be shortened. The reduced imaging time was achieved by filling k-space (where data is stored and used to generate an image) over fewer heart beats to generate a single image. This modality was further enhanced by techniques to both improve vessel signal through imaging in diastole and minimize fat signal (fat suppression), and served as the prototype for coronary MRA in clinical use [10].

Second Generation Techniques (3D Coronary MRA with Respiratory Gating)

Three-dimensional imaging allowed longer segments of the coronary arteries to be visualized with shorter breath-holds. In 3D imaging, instead of a single slice of a vessel being imaged over a single breath-hold, multiple slices are obtained over a single breath-hold. This is further enhanced by the use of respiratory gating which can be accomplished by one of two techniques. Respiratory bellows gating is an early technique that utilizes a belt containing a displacement transducer placed around the upper abdomen to assess respiratory motion and appropriately gate the images. Navigator gating (NAV) is a newer modality that utilizes an initial 30 ms pulse delivered about 50 ms prior to acquisition that excites the right hemi-diaphragm tissue to track the lung–liver interface. The location of the interface provides a criterion to accept or reject the corresponding data segment over multiple breaths and hence allows for images to be obtained without breath-holding [10,11]. Respiratory bellows gating or navigator echo gating are used to identify the position of the diaphragm or heart to obtain a free-breathing sequence [11]. The technique allows for high resolution data with high SNRs. Contrast agents may used in this technique to improve differentiation between the coronary blood flow and the myocardium [12].

Third Generation Coronary MRA (3D Acquisitions with Breath-Hold)

Whole-heart 3D coronary MRA represents a way to obtain multiple slices of the coronary arterial tree with a single breath-hold. This modality combines complex

TABLE 13.1 CCTA Versus Coronary MRA

Characteristic	CCTA	Coronary MRA
Ionizing radiation	Yes	No
Contrast media	Yes	Can be performed without
Temporal resolution	75–200 ms	Can be <75 ms
Calcification	Reduces accuracy	Less impacted by accuracy
Spatial resolution	0.6 mm	At least 1.0 mm
Imaging time	<10–15 s	>10–15 s
Operator experience and cost	Lower	Higher

Source: Adapted from Ref. [13].

techniques to improve the spatial resolution and temporal resolution [10].

CORONARY MRA COMPARED TO CCTA

In addition to the absence of ionizing radiation and the potential to avoid contrast use, coronary MRA has several advantages compared to CCTA when evaluating the epicardial vessels. Coronary MRA allows heavily calcified vessels to be evaluated (in contrast to CCTA) and provides improved temporal resolution compared to CCTA (30 ms). The spatial resolution of coronary MRA, however, is higher compared to CCTA and the imaging time is longer, requiring greater operator expertise compared to CCTA (Table 13.1).

CURRENT APPLICATIONS

Coronary Artery Disease

Coronary MRA has successfully been used to evaluate the coronary arteries for the presence of atheromatous plaque and to evaluate the extent of stenosis within the epicardial coronary arteries. Given the recent developments in the evaluation of the coronary artery disease it is helpful to understand how coronary MRA compares with ICA, an established technique and CCTA, a competing emerging technology to which coronary MRA is compared.

Coronary MRA Compared to ICA

Multiple studies have been performed comparing the sensitivity and specificity of Coronary MRA to ICA with each generation of coronary MRA techniques. First generation coronary MRA (2D breath-hold) had up to 90% sensitivity and 92% specificity in a study by Manning in the evaluable vessels [14]. Second-generation coronary MRA with retrospective respiratory gating had up to 83% sensitivity and 94% specificity in a study by Kessler with 219 evaluable vessel segments [15]. Second-generation coronary MRA with prospective respiratory gating had up to 93% sensitivity and 42% specificity in a study by Kim with 109 vessel segments [16]. Third-generation Coronary MRA with 3D breath-holding had up to 86% sensitivity and 91% specificity in a study by Regenfus evaluating up to 82 vessel segments [17]. The data suggest that, while coronary MRA is not as sensitive or specific as ICA, coronary MRA could still play a role in the evaluation of coronary artery disease as MRA imaging techniques become more refined.

CCTA COMPARED TO CORONARY MRA

A meta-analysis comparing CCTA to Coronary MRA on a per-patient basis shows that coronary MRA has an sensitivity of 87.1% and specificity of 70.3% compared to CCTA which itself has a sensitivity of 97.2% and specificity of 87.4%. This suggests a greater sensitivity and specificity for CCTA in the diagnosis of coronary artery disease [13,18]. The data, however, include a much larger pool of CCTA studies compared with coronary MRA which may partially account for the differences between modalities [13,18]. Though CCTA has a negative predictive value in excess of 98%, coronary MRA may play a larger role in patients who are unable to undergo a CCTA due to severe CT contrast allergy or who wish to avoid ionizing radiation [19].

Plaque Characterization and Coronary MRA

The evaluation of atheromatous plaque in the coronary arteries is challenging as a result of the cardiac and respiratory motion, the non-linear course of the vessels, and the small vessel size [20]. While assessment of plaque burden has been successfully performed, plaque characterization has proved to be more difficult [19]. Atherosclerotic lesions in rabbit models have been successfully monitored for both atherosclerotic burden and plaque characterization [20]. Characterization of plaque in human subjects along with identification of high-risk plaque remains the subject of ongoing research involving intravascular MRI and pulse sequences [21]. Intravascular MRI involves two approaches, placing an intravascular coil to detect signal in an external magnetic field or a system design where the magnet coil and detector are combined into a signal catheter [22]. *Ex vivo* analysis of plaque type has been performed, while *in vivo* assessment remains the subject of continued investigation [21].

Wall Characterization and Coronary MRA

The wall of the epicardial coronary arteries can be evaluated to examine for changes related to remodeling of the wall or changes in the mediators involved with inflammatory injury to the wall [23]. One marker that has been reported involves patients with Type 1 diabetes or abnormal renal function who have increased wall thickness on black blood imaging [23,24].

Animal studies investigating new contrast agents that reflect increased endothelial permeability (albumin bound gadolinium agent) and/or increased neo-vascularization are being tested and show early promise [23,25]. Increased accumulation of iron-oxide particles reflects increased endothelial permeability and vessel wall inflammation from intraplaque macrophages in the carotid system, and may be able to be used as a marker in the coronary system as coronary MRA matures and imaging technology advances [26]. Some molecules are being examined as molecular targets of inflammation, such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), or matrix metalloproteinase (MMP), and these may allow for early detection of endothelial inflammation [27]. Thrombus labeling using a fibrin-specific contrast agent, and the targeting of elastin for the evaluation of extracellular matrix remodeling are new and promising techniques that may also contribute to the evaluation of plaque vulnerability [23].

Calcified Plaque and Coronary MRA

Patients with large amounts of calcified plaque are not ideal candidates to undergo a CCTA secondary to blooming artifact which reduces the sensitivity and accuracy of the study. In patients with calcium scores greater than 100, coronary MRA has a better diagnostic performance and improved image quality compared to 64-slice CCTA [28]. Specifically, coronary segments with nodal calcification are of higher image quality compared to those segments with diffuse calcification due to limits of spatial resolution in diffusely calcified vessel segments [28]. In addition the area under the curve in the receiver operating characteristic curves was higher in coronary MRA compared to 64-slice CCTA and the specificity of coronary MRA was higher compared to 64-slice CCTA [28].

Prognostic Value of Coronary MRA

The value of coronary MRA extends beyond the diagnosis of coronary artery disease and detection of vessel stenosis and can provide prognostic value. In a study by Yoon, 207 patients who underwent whole-heart coronary MRA scanning were followed over a 25-month period [29]. The presence of significant stenosis on coronary MRA was associated with a greater than 20-fold hazard increased risk of a cardiac event (death, non-fatal myocardial infarction, unstable angina and late revascularization), and a higher severe cardiac event rate and overall cardiac event rate [29]. This work indicates that a larger study is needed to better understand how coronary MRA may yield prognostic information and where it might fit in the diagnosis and management of coronary artery disease.

ROLE OF CORONARY MRA IN SPECIFIC PATIENT POPULATIONS

Congenital Heart Disease

Congenital heart disease has an estimated prevalence of 0.4% in the population [30]. Coronary MRA, using free-breathing techniques has a role in evaluating coronary artery anomalies, particularly in the pediatric population in an effort to minimize radiation exposure. Most recently, free-breathing, 3D coronary MRA has been investigated as a tool to evaluate for coronary artery anomalies [31]. Monney showed that coronary MRA was a robust tool that accurately assessed the segmental cardiac anatomy in 93–96% of patients and successfully visualized the left anterior descending, left circumflex, and right coronary arteries in 93%, 87%, and 98% of all patients [31].

Kawasaki Disease

Coronary MRA can have a role in the evaluation of patients with Kawasaki disease [32]. Whole-heart coronary MRA can reveal not only the dilated lumen, but show the wall thickening of the vessel, thereby allowing for risk stratification and therapeutic monitoring [33,34]. Grell has shown that there was complete agreement between ICA and coronary MRA in detecting aneurysms with excellent agreement in determining maximal diameter, length from the ostium, and length of the aneurysms [33,34]. This technique may reduce the need for serial ICA and allow for better monitoring of response to treatment and progression of disease [33,34].

Coronary Artery Bypass Grafts

White described the role of coronary MRA in evaluating bypass grafts in 1988 [35]. MRA can be reasonably easily used to evaluate graft lesions due to the relative immobility of the bypass grafts throughout the cardiac cycle [36]. Coronary MRA has been shown to have a high specificity (93.8%) and sensitivity (89.9%) both for the detection and quantification of graft occlusion and for delineation of the path of the graft. Flow velocity

mapping can be used as a tool to quantify the severity of stenosis in bypass grafts [37,38]. An evolving challenge to evaluation of bypass grafts is artifact from metal clips which can obscure both the anastomotic sites and various segments of the graft [23].

CONCLUSIONS

Coronary MRA is a technically complex imaging tool that is early in its life cycle. It continues to mature as an imaging tool that can be used not only for the evaluation of coronary artery disease, bypass graft disease, and congenital anomalies, but also as a tool whose findings may have prognostic value. It remains an area of ongoing research which seeks to combine molecular imaging and structural imaging, not only to examine stenotic lesions, but to identify markers of inflammation that can be used to determine areas at risk in the vascular wall.

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CHAPTER

14

Invasive Diagnostic Assessment of Coronary Artery Disease

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Coronary artery disease (CAD) is the leading cause of mortality internationally. Traditionally, CAD was assessed using coronary angiographic guidance. Significant lesions were determined by the coronary angiographer. However, interobserver variability demonstrated a significant limitation of interpretation of coronary angiography [1]. In addition, stent deployment and apposition were evaluated based on angiographic appearance only. Risk factors for stent thrombosis and in-stent restenosis such as incomplete stent apposition, edge dissection, and thrombus presence were often not detected [2]. However, in the last 10 years, the field of interventional cardiology has benefitted from the use of more objective measures of the severity of CAD. Fractional flow reserve (FFR), intravascular ultrasound (IVUS), and optical coherence tomography (OCT) have assisted in determining the severity and exact composition of lesions. IVUS and OCT have also assisted in lesion characterization and have helped guide the performance of percutaneous coronary interventions (PCI). This chapter describes these different technologies and discusses overlap and their impact on future clinical practice.

FRACTIONAL FLOW RESERVE

Over the last 10 years, FFR has become a reference standard for the invasive assessment of CAD. Its measurement assesses the functional severity of coronary artery stenoses and the need for coronary revascularization [3,4]. FFR is calculated by dividing the distal coronary pressure by the proximal coronary pressure during maximal hyperemia [3]. In an ideal situation with no limitation to coronary flow, these pressures should be equal. The hemodynamic significance of a coronary lesion is determined by the ratio of these pressures. Pijils et al. showed in follow-up of patients in the Deferral of Percutaneous Coronary Intervention (DEFER) study that an FFR cutoff of 0.75 was excellent in predicting 5-year outcome after deferral of PCI of an intermediate coronary stenosis [5]. This study showed that PCI of a functionally nonsignificant stenosis indicated by a FFR of $\geq$0.75 is no benefit to the patient [5]. This study also showed that the coronary lesions at greatest risk of causing cardiovascular death or myocardial infarction are those that are functionally significant as indicated by a FFR < 0.75 [5].

The FFR versus Angiography for Guiding Percutaneous Coronary Intervention (FAME) trials further solidified the role of FFR in the treatment of patients with multivessel CAD [6,7]. In the FAME 1 trial, FFR-guided PCI with drug-eluting stents was shown to have a lower composite of death, myocardial infarction, and repeat revascularization as compared to interventions done based on an angiographic assessment alone [6]. The 1-year rate of death, nonfatal myocardial infarction, and repeat revascularization in the 1005 patients with multivessel CAD randomized to PCI with drug-eluting stents guided by coronary angiography alone or to FFR measurements plus coronary angiography was 18.3% (91 patients) in the coronary angiography alone group versus 13.2% (67 patients) in the FFR group ($p = 0.02$) [6]. At 1-year follow-up 78% of the coronary angiography alone group and 81% of the FFR group were free from angina pectoris ($p = 0.20$) [6].

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00014-0

The utility of FFR was again shown in the FAME 2 trial, which demonstrated that PCI in addition to maximal medical therapy was superior to maximal medical therapy alone in 888 randomized patients with hemodynamically significant lesions as demonstrated by a FFR value of <0.80 [7]. The primary endpoint of death, myocardial infarction, or urgent revascularization was 4.3% in the PCI group versus 12.7% in the maximal medical therapy alone group (hazard ratio = 0.32, $p < 0.001$) [7]. This was driven primarily by a decrease in the amount of urgent coronary revascularization in the PCI group (1.6%) than in the maximal medical therapy alone group (11.1%) (hazard ratio = 0.13, $p < 0.001$) [7]. In patients without myocardial ischemia, the outcome was favorable with maximal medical therapy alone [7].

The implications of these findings are of importance. The Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial demonstrated that PCI offered no mortality benefit in patients with stable CAD as compared to optimal medical therapy [8]. However, in patients with significant myocardial ischemia, there may be a reduction in major cardiovascular events with PCI. Therefore, FFR, when used as a surrogate marker for ischemia, allows for a direct intervention to an ischemia-causing lesion, which may result in a reduction in major cardiovascular events. In addition, FFR assessment of CAD can also cause deferral of revascularization of lesions that are not hemodynamically significant. The International Study of Comparative Health Effectiveness with Medical and Invasive Approaches (ISCHEMIA) trial is enrolling stable ischemic heart disease patients with at least moderate ischemia who are asymptomatic or symptomatic with or without previous revascularization to investigate whether an invasive strategy with revascularization will improve prognosis compared with optimal medical therapy [9].

Sant'Anna et al. showed that FFR assessment in multivessel CAD has shown a reduction in the number of significant lesions [10]. In this study of 250 patients with 471 coronary stenoses scheduled for PCI, 32% of the coronary stenoses and 48% of the patients would have received a different treatment if a FFR measurement was not made [10]. Other studies have similarly shown that in multivessel CAD, FFR-guided PCI leads to fewer arteries being treated than angiographically treated vessels [11].

Trials have mostly suggested that the risk of major adverse cardiovascular outcomes after FFR-based deferral of PCI is low. However, studies have been variable with respect to the rate of future intervention on these vessels, with the majority suggesting a rate of <10% at 1 year. Depta et al. recently showed that deferral of PCI based on FFR resulted in delayed intervention of 5.3% at 1 year and 18% within 4 years [12]. A clinical prediction model including age, history of tobacco use, history of CAD, higher creatinine, and multivessel CAD plus the FFR value can help predict the risk of deferred lesion intervention in the first year after FFR assessment [12].

Left main coronary artery stenosis is also an important dilemma. Angiographically, the severity of left main coronary artery lesions can vary based on the view selected. A hemodynamic assessment of the left main coronary artery stenosis may help with this ambiguity in light of the potential clinical implications. Courtis et al. showed that an FFR-guided assessment of left main coronary artery stenosis resulted in similar outcomes in patients treated with revascularization versus medical therapy [13]. This study investigated 142 patients with ambiguous or intermediate LM lesions and decided on coronary revascularization based on whether the FFR was <0.75 or >0.80. Those lesions which were <0.75 were treated with coronary revascularization therapy (60 patients) and those with FFR values >0.80 were treated with medical therapy (82 patients). At 14-months follow-up, the incidence of major adverse cardiac events was 13% in the medical therapy group versus 7% with coronary revascularization ($p = 0.27$) [13].

INTRAVASCULAR ULTRASOUND

Intracoronary imaging is increasingly becoming a useful tool in the assessment of CAD [14]. Its value lies in the ability to view the three layers of an artery using ultrasound technology. IVUS is based on the emission, attenuation, and backscattering of ultrasonic waves. The amplitude of this signal is used to form a gray-scale image. The IVUS catheter is placed over a guidewire, which has been placed down the coronary artery of interest. The tip of the catheter is placed beyond the lesion of interest. While the guidewire is kept stationary, the IVUS catheter is retracted, usually under motorized control at a speed of 0.5 mm/s. Theoretically, IVUS was thought to have promise in its ability to identify vulnerable plaque. While ruptured plaque can be identified by IVUS, vulnerable plaque is more difficult to identify. Vulnerable plaque is more likely to exhibit ultrasound attenuation, which can indicate fibrolipidic composition and a necrotic core. Thrombi, eccentric patterns, and an echolucent zone are further characteristics of vulnerable plaque [14]. The effectiveness of IVUS in assessing vulnerable plaque is yet to be studied in a prospective trial.

The role of IVUS in determining whether a lesion is significant has been studied. A study of 226 patients randomized to PCI based on angiography with an IVUS minimal lumen area of <4.0 mm^2 versus an FFR value of <0.8 showed that outcomes were similar among all groups although there was a trend toward more revascularizations when an IVUS minimal lumen area of

<4.0 mm^2 was used [15]. More recent literature indicates that perhaps a smaller minimum lumen area correlates to a lesion that should be revascularized. In a study of 881 lesions assessed by IVUS and FFR, a minimal lumen area of 2.75 mm^2 was best seen to correlate with an already established FFR value of <0.8 [16].

Perhaps the major benefit of IVUS lies in its ability to guide angioplasty. However, studies to date have been mixed with regard to the effectiveness of IVUS to guide angioplasty. The use of IVUS was hypothesized to result in less stent thrombosis and restenosis through larger post procedure lumen diameters, recognition of dissection and thrombus, and avoidance of stent under expansion. All of these factors have been IVUS-guided predictors of stent restenosis and thrombosis [17–19].

Schiele et al. showed in the Restenosis After IVUS-Guided Stenting (RESIST) trial that there was a non-significant decrease in the restenosis rate of vessels treated with IVUS-guided PCI. This was possibly due to the small sample size of the study (n = 164) [20]. Restenosis was looked again in the Optimization With IVUS to Reduce Stent Restenosis (OPTICUS) study [21]. This study also showed that IVUS-guided PCI was not superior to angiographically guided PCI in terms of in-stent restenosis, lumen diameter or major adverse cardiac events [21]. This may have been related to newer techniques including high-pressure insufflation to prevent inadequate stent expansion, which is a primary mechanism of in-stent restenosis.

There is a significant amount of literature that supports the use of IVUS-guided PCI, not because of a reduction in major adverse clinical events, but rather because of a significant decrease in the need for repeat coronary revascularization. A meta-analysis of eight randomized controlled trials performed by Neto et al. showed a 27% reduction (95% CI, 3–46%) in angiographic restenosis with use of IVUS compared to coronary angiography alone [22]. This meta-analysis also showed a significant 12% reduction in percutaneous revascularization and a 27% significant reduction in overall revascularization in the IVUS-treated group, with no significant difference in surgical revascularization between the two treated groups [22].

Multiple studies have also demonstrated that IVUS-guided interventions result in larger post-stent lumen diameters [23]. The Angiography Versus IVUS-Directed Stent Placement (AVID) trial showed that IVUS-directed bare-metal stent placement resulted in larger acute stent dimensions without increased complications and a significantly lower 1-year target lesion revascularization for vessels ≥2.5 mm by coronary angiography and for coronary arteries with high-grade prestent stenosis [23]. However, for the entire study group analyzed on an intention-to-treat basis, IVUS-directed bare-metal stent placement did not significantly reduce the 12-month rate of target lesion revascularization when compared with bare-metal stent placement guided by coronary angiography alone [23]. A study of 550 patients with 670 native lesions demonstrated that angiographic restenosis was highest in cases where the stent lumen area was <5.5 mm^2 and the stent length was >40 mm, indicating that larger stented lumen diameters have clinical benefit [24].

A reduction in major adverse cardiac events has been seen in some studies using IVUS, primarily driven by a reduction in subacute stent thrombosis [25]. Roy et al. showed in 884 patients undergoing IVUS-guided drug-eluting stent implantation that use of IVUS resulted in significantly lower rates of in-stent thrombosis and a trend toward lower target lesion revascularization at 12 months compared with the outcomes of a propensity-score matched population undergoing implantation of drug-eluting stents [25]. At 30 days, the primary endpoint of definite stent thrombosis was 0.5% in the IVUS group versus 1.4% in the no IVUS group (p = 0.046). At 12 months, the primary endpoint of definite stent thrombosis was 0.7% in the IVUS group versus 2.0% in the no IVUS group (p = 0.014). At 12 months, the incidence of target lesion revascularization was 5.1% in the IVUS group versus 7.2% in the no IVUS group (p = 0.07) [25]. The incidence of major adverse cardiac events at 1 year was not significantly different between both groups [25]. Increased stent thrombosis was primarily seen within the first 30 days after stent deployment. The decreased rate of stent thrombosis with IVUS use was primarily attributed to more postdilation, greater cutting balloon use, and greater use of rotational atherectomy. Being able to characterize the content of the plaque, such as the degree of calcification with the use of IVUS allowed for improved preparation prior to stent deployment such as the use of rotational atherectomy.

The use of IVUS in patients with acute myocardial infarction is more controversial. While stent thrombosis and restenosis is a known complication of primary PCI in patients with acute myocardial infarction, studies have not demonstrated a clear clinical benefit to the use of IVUS in this situation [26]. In a study of 909 consecutive patients who underwent primary PCI for acute myocardial infarction discharged alive, 382 patients underwent IVUS-guided PCI [26]. The 1-year incidence of death, myocardial infarction, and target lesion revascularization study did not support the routine use of IVUS-guided PCI in patients with acute myocardial infarction [26]. The utility of IVUS in patients with acute myocardial infarction may lie in ensuring proper lesion coverage and stent expansion in cases that are questionable for the operator. Overall, while growing as an important tool for experienced operators, IVUS has not demonstrated clear reproducible benefit. This may change as operators become more comfortable with interpretation, and the exact indications are understood. Coronary artery

calcium scores determined by computer tomography do not accurately predict significant obstructive CAD determined by IVUS [27].

OPTICAL COHERENCE TOMOGRAPHY

Optical coherence tomography (OCT) is a technology that uses near infrared light to produce images. It can be technically more challenging than IVUS to perform. Older OCT systems utilized an over the wire low-pressure occlusion balloon catheter with distal flush ports. Saline or lactated ringers were infused to clear the lumen of blood in order to limit signal attenuation. Newer systems with accelerated pullback utilize a high-speed bolus dose of contrast to clear the vessel lumen of blood [28]. Images are generated by measuring the echo time delay and intensity of light that is reflected from the arterial wall. Higher band widths used in OCT result in higher image resolution as compared to IVUS. It also provides greatly improved contrast between the vessel wall and lumen. This allows for potentially better assessment of incomplete stent apposition, edge dissection, and thrombus presence. However, visualization of residual plaque behind the stent is viewed better with IVUS, which exhibits better tissue penetration [29]. While it may be easier with OCT to identify complications such as edge dissection, it is unclear if this has a clinical benefit. In a study of 73 patients who underwent OCT after stent implantation, there was a 25% incidence of edge dissection [29]. Despite being able to identify the edge dissection, this finding did not lead to an increase in intrahospital events [30].

OCT has also demonstrated promise in its ability to assess and treat in-stent restenosis [31]. Typically, in-stent stenosis with bare-metal stents demonstrates a homogenous tissue band with OCT, while in-stent stenosis with drug-eluting stents shows a layered heterogenous band. OCT can also allow for the detection of unstable features such as intracoronary thrombus or ruptured plaque, which can influence management [31]. However, prospective trials are needed to determine whether more detection by OCT leads to improved outcomes.

The major value of intracoronary imaging with OCT comes with its ability to guide PCI. In an observational study of 670 patients undergoing PCI, 335 patients had angiographic plus OCT guidance and 335 matched patients had angiographic guidance only [32]. The primary endpoint was the 1-year incidence of cardiac death or myocardial infarction. Angiographic plus OCT guidance was associated with a significantly reduced risk of cardiac death or myocardial infarction at extensive multivariable analysis adjusting for baseline and procedural differences between the two groups (odds ratio = 0.49; 95% CI, 0.25–0.96; $p = 0.037$) and at propensity-score adjusted analyses. Because of the improved resolution with OCT, OCT was proposed to be a better alternative to IVUS for the guidance of PCI. This observational study suggested that use of OCT can improve clinical outcomes of patients undergoing PCI [32].

However, Habara et al. showed that the use of OCT was associated with smaller stent expansion and more frequent residual reference segment stenosis when compared with IVUS guidance [33]. The inferiority of OCT in this case was hypothesized to be due to difficulty in viewing the vessel border. OCT has demonstrated a lesser degree of interobserver variability, however, when compared with IVUS [34]. Despite these observations, OCT has not been extensively studied prospectively. In addition, its use of more contrast and requirement for total vessel ischemia has likely contributed to operator reluctance to use OCT.

IVUS AND FFR IN ASSESSMENT OF LEFT MAIN DISEASE

Perhaps one of the most clinically important applications of these methods is the assessment of the degree of left main CAD. Traditionally, visual assessment of left main CAD has been variable and can be clinically important. Diffuseness of the atherosclerotic process seems to be the major reason for angiographic underestimation of narrowing of coronary arteries [35].

Bech et al. determined FFR in 54 consecutive patients with angiographically equivalent left main CAD [36]. The FFR was ≥0.75 in 24 of 54 patients (44%) who were treated medically. Coronary artery bypass graft surgery was performed in the 30 of 54 patients (56%) who had a FFR <0.75 [36]. Survival at 3 years was 100% for the medical group and 97% for the surgical group [36]. Event-free survival at 3 years was 76% for the medical group and 83% for the surgical group [36].

In 213 patients with an angiographically equivocal left main coronary artery stenosis, quantitative coronary angiography, and measurements of FFR were performed [37]. The FFR was <0.80 in 75 patients and ≥0.80 in 138 patients. When the FFR was < 0.80, coronary artery bypass surgery was performed. When the FFR was ≥0.80, the patients were treated medically or another coronary artery stenosis was treated by coronary angioplasty (the nonsurgical group). The 5-year survival estimates were 85.4% in the surgical group versus 89.8% in the nonsurgical group (p not significant). The 5-year event-free survival estimates were 82.8% in the surgical group versus 74.2% in the nonsurgical group (p not significant). Percent diameter stenosis measured by quantitative coronary angiography correlated with FFR ($p < 0.001$), but a very large scatter was present. In 23% of patients with a diameter left main coronary artery stenosis, the left main coronary artery stenosis was

hemodynamically significant by measurement of FFR [37]. These data suggest that FFR should be measured in patients with equivocal stenosis of the left main coronary artery before making a decision about the need for coronary revascularization [37].

Still, there is some controversy as to which FFR value should be used as the value for coronary revascularization and how distal left main coronary artery stenosis should be approached. Perhaps, IVUS is most useful in cases where borderline FFR readings are attained. Ostial and mid-shaft left main CAD may be able to be reliably assessed with FFR, but distal left main CAD can be more cumbersome as the disease often extends into the daughter vessels. Some coronary interventionalists advocate a pressure wire pullback method from the daughter vessels into the left main coronary artery to localize the most significant disease [38].

Multiple studies have investigated the role of IVUS in left main coronary artery stenosis assessment and intervention. Often, FFR has been used as the gold standard for the assessment for functional stenosis. Jasti et al. showed that a minimal lumen diameter of 2.8 and a minimum lumen area of 5.9 strongly correlate with a significant functional stenosis in ambiguous left main coronary artery stenoses with FFR as the gold standard [39]. Using FFR as the reference standard for the severity of left main coronary artery stenoses, Park et al. showed in 112 patients with isolated ostial and shaft intermediate left main coronary artery stenoses that a minimum lumen area of $\leq 4.5\,mm^2$ could be the cutoff for functionally significant stenosis and a useful index of a FFR of ≤ 0.80 [40].

Subsequent studies have investigated different IVUS minimal lumen areas such as $\geq 7.5\,mm^2$ [41], and more recently $\geq 6.0\,mm^2$ [42], with each measure exhibiting promise as a cutoff for coronary revascularization. In a prospective study of 354 patients from 22 centers, left main coronary artery revascularization was performed in 152 of 168 patients (90.5%) with a minimal lumen area of $<6\,mm^2$ and was deferred in 179 of 186 patients with a minimal lumen area of $\geq 6.0\,mm^2$ [42]. At 2-year follow-up, cardiac death-free survival was 94.5% in the coronary revascularization group and 97.7% in the deferred group (p not significant) [42]. Event-free survival was 80.6% in the coronary revascularized group versus 87.3% in the deferred group (p not significant) [42]. Only eight patients (4.4%) in the deferred group needed coronary revascularization during the 2-year follow-up [42].

While assessing the severity of a left main coronary artery stenosis is one of the applications of IVUS, its ability to guide left main coronary artery PCI may be more valuable. In the revascularization for unprotected left main coronary artery stenosis: Comparison of Percutaneous Coronary Angioplasty Versus Surgical Revascularization (MAIN-COMPARE) registry, left main coronary artery intervention with IVUS guidance was shown to potentially reduce long-term mortality when drug-eluting stents were used [43]. In 145 matched pairs of patients treated with drug-eluting stents, the 3-year incidence of mortality was 4.7% with IVUS guidance versus 16.0% with coronary angiography guidance (log-rank $p = 0.048$; hazard ratio = 0.39; 95% CI, 0.15–1.02; Cox model $p = 0.055$) [43]. The use of IVUS guidance did not reduce mortality in 47 matched pairs of patients treated with bare-metal stents in this study [43]. The risk of myocardial infarction or target vessel revascularization was not associated with use of IVUS guidance [43].

With diffuse circumferential left main coronary artery stenosis, IVUS can be important in ensuring adequate stent coverage and the degree and presence of calcification. In addition, for distal left main coronary artery stenosis, IVUS can be used to ensure adequate stent expansion at the origin of the left anterior descending and/or left circumflex coronary arteries. Therefore, IVUS is generally recommended to guide left main coronary artery PCI [43].

NEAR INFRARED SPECTROSCOPY

Near infrared (NIR) spectroscopy assess lipid content of coronary plaques and thus identify lipid-rich plaques is a novel application of a technology well established in the field of physical sciences. The addition of NIR spectroscopy technology to IVUS helps to further characterize the plaques identified by IVUS [44].

Oemrawsingh et al. performed NIR spectroscopy in a prospective observational study in a nonculprit coronary artery in 203 patients referred for coronary angiography because of stable angina pectoris or an acute coronary syndrome [45]. The primary endpoint was all-cause mortality, nonfatal acute coronary syndrome, stroke, and unplanned coronary revascularization. The 1-year cumulative incidence of the primary endpoint was 10.4% [45]. The cumulative 1-year rate in patients with a lipid core burden index greater than or equal to the median of 43.0 was 16.7% versus 4.0% in patients with a lipid core burden index <43.0 (adjusted hazard ratio = 4.04; 95% CI, 1.33–12.29; $p = 0.01$) [45]. The association between the lipid core burden index and the primary endpoint was similar in the patients with stable angina pectoris or an acute coronary syndrome [45].

The Chronometric Observations of Lipid Core Containing Plaques of Interest in Native Coronary Arteries (COLOR) registry is an ongoing prospective observational study of patients undergoing NIR spectroscopy prior to PCI [46]. This study showed in 62 patients undergoing PCI with stenting that periprocedural myocardial infarction occurred in 7 of 14 patients (50%) with a maximal lipid core burden index (4 mm) measured by NIR spectroscopy of ≥500 versus in two of 48 patients (4.2%) with a maximal lipid core burden index (4 mm)

<500 (p = 0.0002) [46]. The proposed mechanism is by a higher rate of distal embolization of lipid-core plaque constituents during PCI, thus suggesting that the use of embolic protection devices in patients with extensive lipid-core plaques might have better outcomes [46]. The addition of NIR spectroscopy to the armamentarium of the available intravascular imaging modalities has the potential to help in identifying vulnerable plaques and the clinical benefit of an intervention.

CONCLUSIONS

The invasive assessment of CAD with the use of FFR, IVUS, OCT, and NIR spectroscopy is a growing and exciting development in the treatment of patients with CAD. However, despite the obvious benefits of being able to objectively assess the severity and components of a lesion, hard clinical benefit through prospective studies have not been uniformly demonstrated. This fact can be used to question its cost effectiveness. However, the use of imaging such as IVUS and OCT in the guidance of complex PCI has demonstrated slightly more benefit, and it is frequently used in specific cases such as in patients with left main coronary artery stenosis-guided PCI. Perhaps, with the improved comfort and training of operators, their use will be more clearly established.

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CHAPTER

15

Drug Treatment of Stable Coronary Artery Disease

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RISK FACTOR REDUCTION

Coronary artery disease (CAD) is the leading cause of death. Modifiable risk factors should be treated. Smokers should be strongly encouraged to stop smoking because it will reduce cardiovascular mortality and all-cause mortality in patients with CAD. The American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) 2011 guidelines recommend that patients should be asked about tobacco use at every office visit [1]. A smoking cessation program should be recommended to smokers. Patients should be advised at every office visit to avoid exposure to environmental tobacco smoke at work, at home, and at public places [1]. Nicotine replacement therapy [2], bupropion [3], and varenicline [4] are approved pharmacologic therapies for promoting smoking cessation.

Hypertension should be treated initially with sodium restriction not to exceed 1.5 g daily, weight reduction if necessary, cessation of drugs that increase blood pressure, avoidance of caffeine and tobacco, limiting alcohol intake to no more than two drinks per day in men and one drink per day in women and light weight men, an increase in physical activity, a reduction of dietary saturated fat and cholesterol, and maintenance of adequate dietary potassium, calcium, and magnesium intake [5].

Antihypertensive drugs have been demonstrated to decrease new coronary events in men and women with hypertension and CAD [5]. Hypertension is present in 69% of patients with a first myocardial infarction (MI) [6]. A meta-analysis of 147 randomized trials including 464,000 persons with hypertension showed that beta-adrenergic blockers were the best drugs to use in patients after MI [7].

Patients with prior MI and hypertension should be treated with beta-blockers and angiotensin-converting enzyme (ACE) inhibitors [1,5,7–12]. Atenolol should be avoided [12]. If a third drug is needed, aldosterone antagonists may be used based on the EPHESUS trial [13]. Patients treated with aldosterone antagonists should not have significant renal dysfunction or hyperkalemia. The 2014 American Society of Hypertension/International Society of Hypertension guidelines state If the patient with hypertension has CAD, the first drug should be a beta-blocker plus an ACE inhibitor or angiotensin receptor blocker (ARB) [14].

In addition to beta-blockers, patients with hypertension and congestive heart failure (CHF) should be treated with diuretics and ACE inhibitors or ARBs and patients with persistent severe symptoms with aldosterone antagonists [5,11,14,15]. ACE inhibitors or ARBs should also be administered to patients with diabetes mellitus or chronic kidney disease [5,11,14,16,17]. The blood pressure should be lowered to 130–139/80–89 mm Hg in patients younger than 80 years [5,11,14]. The systolic blood pressure should be reduced to 140–145 mm Hg if tolerated in patients aged 80 years and older [5] or to less than 150/90 mm Hg unless these patients have diabetes mellitus or chronic kidney disease when a goal of less than 140/90 mm Hg can be considered [14].

Patients with CAD should be treated with a Step II AHA diet. They should achieve and maintain an ideal body weight. Cholesterol intake should be less than 200 mg/day.

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00015-2

Less than 30% of total caloric intake should be fatty acids. Saturated fatty acids should comprise less than 7% of total calories, polyunsaturated fatty acids should account for up to 10% of total calories, and monounsaturated fatty acids should comprise 10–15% of total calories. Protein intake should account for 10–20% of total calories. Carbohydrates should comprise 50–60% of total calories.

Numerous double-blind, randomized, and placebo-controlled trials have shown that patients with CAD treated with statins have a reduction in cardiovascular events and in mortality [18–22]. The lower the serum low-density lipoprotein (LDL) cholesterol reduced by statins, the greater the reduction in cardiovascular events and mortality. In the Heart Protection Study, where 5806 of the 20,536 men and women at increased risk for cardiovascular events randomized to simvastatin or to double-blind placebo were 70–80 years of age at study entry and were 75–85 years of age at follow-up, 5 years of simvastatin therapy prevented myocardial infarction, stroke, and revascularization in 70–100 persons per 1000 treated patients regardless of age, gender, or initial levels of serum lipids [21]. In this study, reduction of serum LDL cholesterol in patients with a baseline serum LDL cholesterol of less than 100 mg/dL (2.6 mmol/L) was as effective in reducing cardiovascular events and mortality as reducing serum LDL cholesterol in patients with higher serum LDL cholesterol levels [21].

The 2013 ACC/AHA lipid guidelines recommend the use of high-dose statins to adults aged 75 years and younger with atherosclerotic cardiovascular disease (ASCVD) (CAD, stroke, transient ischemic attack, or peripheral arterial disease) unless contraindicated with a class I indication [23]. Moderate-dose or high-dose statins are reasonable to administer to patients with ASCVD older than 75 years with a class IIa indication [23].

High-dose statins (rosuvastatin 20–40 mg daily and atorvastatin 40–80 mg daily) reduce LDL cholesterol 50% or more [23]. Moderate-dose statins (rosuvastatin 5–10 mg daily, atorvastatin 10–20 mg daily, simvastatin 20–40 mg daily, pravastatin 40–80 mg daily, lovastatin 40 mg daily, fluvastatin XL 80 mg daily, fluvastatin 40 mg twice daily, and pitavastatin 2–4 mg daily) reduce LDL cholesterol 30–49% [23].

These guidelines also state that there is no additional ASCVD reduction from adding nonstatin therapy to further lower nonhigh-density lipoprotein (HDL) cholesterol once an LDL cholesterol goal has been reached. Clinical trials have demonstrated no reduction in cardiovascular events or mortality in persons treated with statins by addition of nicotinic acid, fibric acid derivatives, ezetemibe, or drugs that raise HDL cholesterol. We are awaiting the results from ongoing clinical trials to see if addition of an inhibitor of pro-protein convertase subtilisin kexin (PCSK)-9 to treatment with high-dose statins will further reduce cardiovascular events and mortality in patients with CAD [24].

Diabetic patients are more often obese and have higher serum LDL cholesterol and triglycerides levels and lower serum HDL cholesterol levels than do nondiabetics. Diabetics also have a higher prevalence of hypertension and left ventricular hypertrophy than do nondiabetics. These risk factors contribute to the higher incidence of new coronary events in diabetics than in nondiabetics. Diabetics with microalbuminuria have more severe angiographic CAD than diabetics without microalbuminuria [25]. Diabetics also have a significant increasing trend of hemoglobin A_{1c} levels over the increasing number of vessels with CAD [26].

Diabetics with CAD should be treated with dietary therapy, weight reduction if necessary, and appropriate drugs if needed to control hyperglycemia. Other coronary risk factors such as smoking, hypertension, dyslipidemia, obesity, and physical inactivity should be controlled. Hypertension should be treated with an ACE inhibitor or ARB [5,11,14,16]. High-dose statins should be administered. Because there are data showing an increased incidence of coronary events and of mortality in diabetics with CAD treated with sulfonylureas [27–29], these drugs should be avoided if possible in these patients. Metformin should be the initial drug to treat hyperglycemia in most patients [16,30]. The hemoglobin A_{1c} level should be reduced to <7% in patients with diabetes mellitus [16]. Hypoglycemia must be avoided in patients with CAD. In 10, 251 high-risk diabetics in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Study, patients randomized to a hemoglobin A_{1c} of 6.4% rather than 7.5% had at 3.5-year follow-up a 22% increase in all-cause mortality from 4.0% to 5.9% [31].

Obese patients with CAD must undergo weight reduction [1]. Weight reduction is also a first approach to controlling hyperglycemia, mild hypertension, and dyslipidemia. Regular aerobic exercise should be added to diet in treating obesity. The body mass index should be reduced to 18.5–24.9 kg/m^2 [1].

Physical inactivity is associated with obesity, dyslipidemia, hyperglycemia, and hypertension. Exercise training programs are not only beneficial in preventing CAD [32] but also have been shown to improve endurance and functional capacity in patients with CAD [33,34]. The goal to be achieved is at least 30 min of exercise daily for 7 days per week with a minimum of 5 days of physical exercise per week [1].

ASPIRIN

Aspirin decreases the aggregation of platelets exposed to thrombogenic stimuli by inhibiting the cyclooxygenase enzyme reaction within the platelet and thereby blocking synthesis of thromboxane A_2, a powerful stimulus to platelet aggregation and vasoconstriction [35].

Randomized trials involving 20,006 patients showed that aspirin and other antiplatelet drugs administered to patients after MI decreased the incidence of recurrent MI, stroke, or vascular death by 36 events per 1000 patients treated for 2 years [36]. The benefit of aspirin in decreasing MI, stroke, or vascular death in patients after MI was irrespective of age, sex, blood pressure, and diabetes mellitus [36]. Aspirin and other antiplatelet drugs administered to 2920 patients with stable angina pectoris in seven randomized trials reduced the incidence of MI, stroke, or vascular death by 33% [36].

Data from the Multicenter Study of Myocardial Ischemia in 936 patients enrolled 1–6 months after an acute MI (70% of patients) or unstable angina pectoris (30% of patients) showed at 23-month follow-up that the cardiac mortality rate was 1.6% for aspirin users and 5.4% for nonusers of aspirin [37]. Cardiac mortality was reduced 90% in aspirin users who underwent thrombolytic therapy compared with nonusers of aspirin who underwent thrombolytic therapy [37].

Of 5490 survivors of acute MI aged 65 years and older with no contraindications to aspirin, 4149 patients (76%) received aspirin at the time of hospital discharge [38]. At the 6-month follow-up evaluation, aspirin users had a significant 23% reduction in mortality [38].

In an observational prospective study of 1410 patients, mean age 81 years, with prior MI and a serum LDL cholesterol of 125 mg/dL or higher, 832 patients (59%) were treated with aspirin [39]. At 3-year follow-up, the use of aspirin caused a 52% significant independent reduction in new coronary events (95% CI, 0.41–0.55) [39]. The use of statins caused a 54% significant independent reduction in the incidence of new coronary events (95% CI, 0.40–0.53) in this study [39].

On the basis of the available data, all patients with CAD should receive aspirin in a dose of 160–325 mg on the first day of acute MI and continue aspirin in a dose of 75–162 mg daily for an indefinite period unless there is a specific contraindication to its use [1].

CLOPIDOGREL

Clopidogrelis also an excellent antiplatelet drug which is effective in reducing MI, ischemic stroke, and vascular death in postinfarction patients [40]. The ACC/AHA guidelines recommend the use of clopidogrel in postinfarction patients who cannot tolerate aspirin for an indefinite period unless there is a specific contraindication to its use [1].

VORAPAXAR

Vorapaxar is a protease-activated receptor 1 antagonist. At 3-year follow-up of 26,449 patients with a history of MI, peripheral arterial disease, or ischemic stroke, compared with placebo, patients randomized to vorapaxar reduced the primary endpoint of cardiovascular death, MI, or stroke 12% from 10.5% to 9.3% ($p < 0.001$) but increased moderate or severe bleeding 66% from 2.5% to 4.2% ($p < 0.001$) and intracranial hemorrhage from 0.5% to 1.0% ($p < 0.001$) [41]. A US Food and Drug Administration Cardiovascular and Renal Drugs Advisory Committee Meeting in 2014 recommended approval of this drug for the secondary prevention of atherothrombotic events in stable patients at least 2 weeks after MI or those with a history of peripheral arterial disease in addition to standard antiplatelet therapy for the given condition [42]. Vorapaxar is not a substitute for aspirin or $P2Y_{12}$ inhibitors [42]. Vorapaxar should not be given to patients with a history of stroke or transient ischemic attack. Further investigation is needed to determine the benefit of voraxapar in patients weighing less than 60 kg [42]. No data are available to comment on the safety or efficacy of voraxapar with antiplatelet drugs other than aspirin and clopidogrel [42].

ANTICOAGULANTS

The routine use of warfarin after MI is controversial [43]. However, three well-controlled studies have shown a reduction in mortality and/or morbidity in patients receiving long-term oral anticoagulation therapy after MI [44–46]. The Sixty Plus Reinfarction Study Group reported at 2-year follow-up after MI that compared with placebo, acenocoumarin, or phenprocoumon caused a 26% nonsignificant decrease in mortality, a 55% significant reduction in recurrent MI, and a 40% nonsignificant decrease in stroke [44]. The Warfarin Reinfarction Study Group showed at 37-month follow-up after MI of patients that compared with placebo, warfarin caused significant reductions in mortality (24%), recurrent MI (34%), and stroke (55%) [45]. The anticoagulation in the secondary prevention of events in Coronary Thrombosis Research Group reported at 37-month follow-up after MI of patients that compared with placebo, nicoumalone, or phenprocoumon caused a 10% nonsignificant decrease in mortality, a 53% significant reduction in recurrent MI, and a 42% significant decrease in stroke [46].

The ACCF/AHA guidelines recommend as Class I indications for long-term oral anticoagulant therapy after MI (i) secondary prevention of MI in post-MI patients unable to tolerate daily aspirin or clopidogrel; (ii) in post-MI patients with persistent atrial fibrillation; and (iii) in post-MI patients with left ventricular thrombus [1]. Long-term warfarin should be administered in a dose to achieve an INR between 2.0 and 3.0 [1]. If there is a compelling reason for use of oral anticoagulant therapy in patients with CAD such as atrial fibrillation,

prosthetic heart valve, left ventricular thrombus, or concomitant venous thromboembolic disease, warfarin should be administered in addition to aspirin 75–81 mg daily [1]. Use of warfarin in combination with aspirin or clopidogrel is associated with an increased risk of bleeding and should be monitored closely [1].

BETA-ADRENERGIC BLOCKERS

Beta-blockers are very effective antianginal and antiischemic agents and should be administered to all patients with angina pectoris or silent myocardial ischemia due to CAD unless there are specific contraindications to their use [11,47]. Teo et al. [48] analyzed 55 randomized controlled trials comprising 53,268 patients that investigated the use of beta-blockers after MI. Beta-blockers significantly decreased mortality by 19% in these studies [48]. A randomized, double-blind, placebo-controlled study of propranolol in high-risk survivors of acute MI at 12 Norwegian hospitals demonstrated a 52% reduction in sudden cardiac death in patients treated with propranolol for 1 year [49].

Metoprolol [50], timolol [51,52], and propranolol [53] caused a greater decrease in mortality after MI in older patients than in younger patients. The reduction in mortality after MI in patients treated with beta-blockers was due both to a reduction in sudden cardiac death and recurrent MI [51–53]. In patients with a left ventricular ejection fraction (LVEF) ≤40% after MI, compared with placebo, patients aged 25–90 years randomized to carvedilol had a 23% significant reduction in mortality at 1.3-year follow-up [54]. A retrospective cohort study also showed that MI patients aged 60–89 years treated with metoprolol had an age-adjusted mortality decrease of 76% [55].

In the Beta Blocker Heart Attack Trial, propranolol caused a 27% decrease in mortality in patients with a history of CHF and a 25% decrease in mortality in patients without CHF [56]. In this study, propranolol caused a 47% reduction in sudden cardiac death in patients with a history of CHF and a 13% reduction in sudden cardiac death in patients without CHF [56].

In the Beta-Blocker Pooling Project, results from nine studies involving 3519 patients with CHF at the time of acute MI demonstrated that beta-blockers caused a 25% decrease in mortality [57]. In the Multicenter Diltiazem Post-Infarction Trial, the 2.5-year risk of total mortality in patients with a LVEF <30% was 24% for patients receiving beta-blockers (relative risk = 0.53) versus 45% for patients not receiving beta-blockers [58]. Beta-blockers have also been found to reduce mortality in patients with CAD and CHF associated with a LVEF ≤35% [59–62] or ≥40% [62,63].

An observational prospective study was performed in 477 patients, mean age 79 years, with prior MI and a LVEF <40% (mean LVEF 31%) [9]. At 34-month follow-up, patients treated with beta-blockers without ACE inhibitors had a 25% significant reduction in new coronary events and a 41% significant reduction in CHF [9]. At 41-month follow-up, patients treated with both beta-blockers and ACE inhibitors had a significant 37% reduction in new coronary events and a significant 60% reduction in CHF [9].

A retrospective analysis of the use of beta-blockers after MI in a New Jersey Medicare population from 1987 to 1992 showed that only 21% of older patients after MI without contraindications to beta-blockers were treated with beta-blockers [61]. Older patients who were treated with beta-blockers after MI had a 43% decrease in 2-year mortality and a 22% decrease in 2-year cardiac hospital readmissions than older patients who were not treated with beta-blockers [64]. Use of a calcium channel blocker instead of a beta-blocker after MI doubled the risk of mortality [64].

Beta-blockers have also been demonstrated to reduce mortality in patients with complex ventricular arrhythmias after MI and a LVEF ≥40% [65] or ≤40% [66]. The decrease in mortality in patients with heart disease and complex ventricular arrhythmias caused by propranolol is due more to an antiischemic effect than to an antiarrhythmic effect [67]. In these patients, propranolol also markedly decreased the circadian variation of ventricular arrhythmias [68], abolished the circadian variation of myocardial ischemia [69], and abolished the circadian variation of sudden cardiac death or fatal MI [70].

A meta-analysis of trials also showed that the use of beta-blockers after non-ST-elevation-MI is likely to reduce mortality and recurrent MI by 25% [71]. Therefore, patients with Q-wave MI or non-Q-ST-elevation MI without contraindications to beta-blockers should be treated with beta-blockers after MI. Beta-blockers with intrinsic sympathomimetic activity should not be used. The ACCF/AHA guidelines recommend that patients without a clear contraindication to beta-blocker therapy should receive beta-blockers within a few days of MI (if not initiated acutely) and continue them indefinitely if there is abnormal LVEF [1]. Carvedilol, metoprolol succinate, and bisoprolol are recommended [1]. Beta-blocker therapy should be administered for 3 years or longer in patients with an MI or an acute coronary syndrome and a normal LVEF [1]. Beta-blockers should be administered to patients with a LVEF ≤40% without prior MI or CHF [1].

NITRATES

Long-acting nitrates are effective antianginal and antiischemic drugs [72]. These drugs should be administered along with beta-blockers to patients after MI who have angina pectoris. The dose of oral isosorbide dinitrate prescribed should be gradually increased to a dose

of 30–40 mg administered three times daily if tolerated. Isosorbide-5-mononitrate in a dose of 60 mg may also be administered once daily. To avoid nitrate tolerance, there should be a nitrate-free interval of 12 h each day [73]. Beta-blockers should be used to prevent angina pectoris and rebound myocardial ischemia during the nitrate-free interval.

OTHER ANTIANGINAL DRUGS

If patients with CAD have persistent angina pectoris despite treatment with beta-blockers and long-acting nitrates, a nondihydropyridine CCB such as verapamil 40–120 mg three times daily or diltiazem 30–90 mg three times daily should be added to the therapeutic regimen if the LVEF is normal [47]. If the LVEF is abnormal, amlodipine or felodipine should be added to the therapeutic regimen [47]. If angina pectoris persists despite treatment with beta-blockers, long-acting nitrates, and CCBs, ranolazine should be added to the therapeutic regimen in the management of patients with stable angina pectoris [47,74]. The recommended dose of sustained release ranolazine is 750 mg or 1000 mg twice daily.

Other drugs under investigation for the management of stable angina pectoris and being considered for USA Food and Drug Administration approval include nicorandil and ivabradine. Nicorandil is a coronary vasodilator with a unique dual mechanism of action that involves a nitrate-like effect and a potassium ion channel opening action [75]. The Impact of Nicorandil in Angina (IONA) study showed at 1.6-year follow-up in patients with stable angina pectoris that 2565 patients randomized to nicorandil 20 mg twice daily had a 17% significant reduction in CAD death, nonfatal MI, or unplanned hospital admission for cardiac chest pain compared with 2561 patients randomized to placebo [76].

Ivabradine is a heart rate lowering drug that acts specifically on the sinoatrial node [77]. Ivabradine caused dose-dependent improvements in exercise tolerance and time to development of ischemia during exercise [78]. In a subgroup of patients with heart rate of 70 beats per minute or higher, ivabradine did not affect the primary endpoint of cardiovascular death or hospital admission for fatal and nonfatal MI [79]. However, ivabradine did reduce the secondary endpoint of hospital admission for fatal and nonfatal MI by 36% ($p = 0.001$) and coronary revascularization by 30% ($p = 0.016$) [79].

ANGIOTENSIN-CONVERTING-ENZYME INHIBITORS

An overview of 32 randomized trials comprising 7105 patients with CHF showed that ACE inhibitors reduced mortality by 23% and mortality or hospitalization for CHF by 35% [80]. Patients with CAD who develop CHF should be treated with ACE inhibitors unless there are specific contraindications to their use [1].

ACE inhibitors reduce mortality in patients after MI [8,81–85]. In the Survival and Ventricular Enlargement Trial, asymptomatic patients with a LVEF ≤40% treated with captopril 3–16 days after MI had at 42-month follow-up compared with placebo, a 19% reduction in mortality, a 21% decrease in death from cardiovascular causes, a 37% reduction in development of severe CHF, a 22% decrease in development of CHF requiring hospitalization, and a 25% reduction in recurrent MI [81]. Captopril decreased mortality independent of age, sex, blood pressure, LVEF, and use of thrombolytic therapy, aspirin, or beta-blockers [81].

In the Heart Outcomes Prevention Evaluation Study, 9217 patients aged ≥55 years with MI (53%), cardiovascular disease (88%), or diabetes mellitus (38%) but no CHF or abnormal LVEF were randomized to ramipril 10 mg daily or placebo [8]. At 4.5-year follow-up, compared with placebo, ramipril significantly reduced the incidence of MI, stroke, and cardiovascular death by 22% (95% CI, 0.70–0.86) [8]. At 4.2-year follow-up of 13,655 patients with prior MI and stable CAD in the European trial on reduction of cardiac events with perindopril in stable CAD, compared with placebo, patients randomized to perindopril had a 20% significant reduction in cardiovascular death, recurrent MI, or cardiac arrest [85].

On the basis of the available data, ACE inhibitors should be administered to all patients with CAD and a LVEF ≤40% and in those with hypertension, diabetes mellitus, or chronic kidney disease and continued indefinitely unless there are specific contraindications to their use [1]. It is reasonable to use ACE inhibitors in all other patients with CAD [1]. The use of ARBs is recommended in patients with CAD who have CHF or a prior MI with a LVEF ≤40% and who are ACE-inhibitor intolerant [1]. It is reasonable to use ARBs in other patients with CAD who are ACE-inhibitor intolerant [1].

ALDOSTERONE ANTAGONISTS

At 16-month follow-up of 6632 patients after MI with a LVEF ≤40% and either CHF or diabetes mellitus treated with ACE inhibitors or ARBs and 75% with beta-blockers, compared with placebo, patients randomized to eplerenone 50 mg daily had a significant 15% reduction in mortality and a 13% significant reduction in death from cardiovascular causes or hospitalization for cardiovascular events. [86]. The ACCF/AHA guidelines recommend an aldosterone antagonist in patients

after MI treated with ACE inhibitors plus beta-blockers if they have a LVEF ≤40% with either CHF or diabetes mellitus if they do not have significant renal dysfunction or hyperkalemia [1].

CALCIUM CHANNEL BLOCKERS

Teo et al. [48] analyzed randomized controlled trials comprising 20,342 patients that investigated the use of CCBs after MI. Mortality was insignificantly higher (relative risk = 1.04) in patients treated with CCBs [48]. A meta-analysis of randomized, clinical trials of the use of CCBs in patients with MI, unstable angina pectoris, and stable angina pectoris showed that the relative risk for mortality in the trials using dihydropyridines such as nifedipine that increase heart rate was 1.16 [87]. The CCBs diltiazem and verapamil which reduce heart rate had no effect on survival [87].

Furberg et al. [88] performed a meta-analysis of the effect of nifedipine on mortality in 16 randomized secondary prevention clinical trials in patients with CAD. In this study, the relative risk for mortality was 1.06 for patients treated with nifedipine 30–50 mg daily, 1.18 for patients treated with nifedipine 60 mg daily, and 2.83 for patients treated with nifedipine 80 mg daily [88].

The Multicenter Diltiazem Postinfarction Trial demonstrated at 25-month follow-up in patients after MI that compared with placebo, diltiazem caused no significant effect on mortality or recurrent MI [89]. However, in patients with pulmonary congestion at baseline or a LVEF <40%, diltiazem caused a significant increase in new cardiac events (hazard ratios = 1.41 and 1.31, respectively) [89]. In this study, diltiazem also increased the incidence of late-onset CHF in patients with a LVEF <40% [90]. Use of a CCB instead of a beta-blocker after MI in a New Jersey Medicare population also doubled the risk of mortality [64].

Since no CCB has been shown to improve survival after MI except for the subgroup of patients with normal LVEF treated with verapamil in the Danish Verapamil Infarction Trial II [91], CCBs should not be used in the treatment of patients after MI. However, if patients after MI have persistent angina pectoris despite treatment with beta-blockers and nitrates, a nondihydropyridine calcium channel blocker such as verapamil or diltiazem should be added to the therapeutic regimen if the LVEF is normal. If the LVEF is abnormal, amlodipine or felodipine should be added to the therapeutic regimen. The ACC/AHA guidelines state that there are no Class I indications for the use of calcium channel blockers after MI [92].

ANTIARRHYTHMIC THERAPY

Class I Drugs

A meta-analysis of 59 randomized controlled trials comprising 23,229 patients that investigated the use of quinidine, procainamide, disopyramide, imipramine, moricizine, lidocaine, tocainide, phenytoin, mexiletine, aprindine, encainide, and flecainide after MI demonstrated that mortality was significantly higher in patients receiving class I antiarrhythmic drugs than in patients receiving no antiarrhythmic drugs (odds ratio = 1.14) [45]. None of the 59 studies showed a decrease in mortality by class I antiarrhythmic drugs [48].

In the Cardiac Arrhythmia Suppression Trials I and II, older age also increased the likelihood of adverse effects including death in patients after MI receiving encainide, flecainide, or moricizine [93]. Compared with no antiarrhythmic drug, quinidine or procainamide did not decrease mortality in patients with CAD, normal or abnormal LVEF, and presence versus absence of ventricular tachycardia (VT) [94]. On the basis of the available data, patients with CAD should not receive class I antiarrhythmic drugs.

D, L-Sotalol and D-Sotalol

Studies comparing the effect of D, L-sotalol with placebo on mortality in patients with complex ventricular arrhythmias have not been performed. Compared with placebo, D, L-sotalol did not reduce mortality in post-MI patients followed for 1 year [95]. In the Survival with Oral d-Sotalol (SWORD) Trial, 3121 survivors of MI with a LVEF ≤40% were randomized to D-sotalol or placebo [96]. Mortality was significantly higher at 148-day follow-up in patients treated with D-sotalol (5.0%) than in patients treated with placebo (3.1%) [96]. On the basis of the available data, D, L-sotalol and D-sotalol should not be used to treat patients after MI.

Amiodarone

In the European Myocardial Infarction Amiodarone Trial, 1486 survivors of MI with a LVEF ≤ 40% were randomized to amiodarone (743 patients) or to placebo (743 patients) [97]. At 2-year follow-up, 103 patients treated with amiodarone and 102 patients treated with placebo had died [97]. In the Canadian Amiodarone Myocardial Infarction Arrhythmia Trial, 1202 survivors of MI with nonsustained VT or complex ventricular arrhythmias were randomized to amiodarone or to placebo [98]. Amiodarone was very effective in suppressing VT and complex ventricular arrhythmias. However, the

mortality rate at 1.8-year follow-up was not significantly different in the patients treated with amiodarone or placebo [98]. In addition, early permanent discontinuation of drug for reasons other than outcome events occurred in 36% of patients taking amiodarone [98].

In the Sudden Cardiac Death in Heart Failure Trial (SCD-HEFT), 2521 patients, mean age 60 years, with Class II or III CHF, a LVEF of ≤35%, and a mean QRS duration on the resting ECG of 120 ms, were randomized to placebo, amiodarone or an automatic implantable cardioverter-defibrillator (AICD) [99]. At 46-month median follow-up, compared with placebo, amiodarone insignificantly increased mortality by 6% [99]. At 46-month median follow-up, compared with placebo, ICD therapy significantly reduced all-cause mortality by 23% [99].

In the Cardiac Arrest in Seattle: Conventional Versus Amiodarone Drug Evaluation Study, the incidence of pulmonary toxicity was 10% at 2 years in patients receiving amiodarone in a mean dose of 158 mg daily [100]. The incidence of adverse effects for amiodarone also approaches 90% after 5 years of therapy [101]. On the basis of the available data, amiodarone should not be used in the treatment of patients with CAD.

Beta-Adrenergic Blockers

However, beta-blockers have been demonstrated to reduce mortality in patients with nonsustained VT or complex ventricular arrhythmias after MI in patients with normal or abnormal LVEF [65,66,102,103]. On the basis of the available data, beta-blockers should be used in the treatment of patients with CAD, especially if nonsustained VT or complex ventricular arrhythmias are present, unless there are specific contraindications to their use [1].

Automatic Implantable Cardioverter-Defibrillator

The ACCF/AHA guidelines recommend in patients with a LVEF less than 35% due to prior MI at least 40 days previously and New York Heart Association (NYHA) class II or III symptoms implantation of an AICD with a Class I indication [104]. These guidelines recommend in patients with a LVEF less than 30% due to prior MI at least 40 days previously and NYHA class I symptoms implantation of an AICD with a Class I indication [104]. These guidelines also recommend with a Class I indication implantation of an AICD in patients with nonsustained VT due to MI, a LVEF less than 40%, and inducible ventricular fibrillation (VF) or sustained VT at electrophysiologic study [104]. Other Class I indications for implantation of an AICD in patients with CAD are cardiac arrest due to VF or VT not due to a transient or reversible cause, spontaneous sustained VT, and syncope of undetermined origin with clinically relevant, hemodynamically significant sustained VT or VF induced at electrophysiologic study when drug therapy is ineffective, not tolerated, or not preferred [104].

HORMONE REPLACEMENT THERAPY

The Heart Estrogen/Progestin Replacement Study (HERS) investigated in 2763 women with documented CAD the effect of hormonal replacement therapy versus double-blind placebo on coronary events [105]. At 4.1-year follow-up, there were no significant differences between hormonal replacement therapy and placebo in the primary outcome (nonfatal MI or CAD death) or in any of the secondary cardiovascular outcomes. However, there was a 52% significantly higher incidence of nonfatal MI or death from CAD in the first year in patients treated with hormonal replacement therapy (relative hazard = 1.52; 95% CI, 1.01–2.29) than in patients treated with placebo [105]. Women on hormonal replacement therapy had a significantly higher incidence of venous thromboembolic events (relative hazard = 2.89; 95% CI, 1.50–5.58) and a significantly higher incidence of gallbladder disease requiring surgery (relative hazard = 1.38; 95% CI, 1.00–1.92) than women on placebo [105].

The Estrogen Replacement and Atherosclerosis Trial randomized 309 postmenopausal women, mean age 66 years, with coronary angiographic evidence of significant CAD to estrogen plus progestin, estrogen alone, or double-blind placebo [106]. At 3.2-year follow-up, quantitative coronary angiography showed no between-group differences in progression of coronary atherosclerosis [106].

At 6.8-year follow-up in the HERS Trial, hormonal replacement therapy did not reduce the risk of cardiovascular events in women with CAD [107]. The investigators concluded that hormonal replacement therapy should not be used to reduce the risk of coronary events in women with CAD [107]. At 6.8-year follow-up in the HERS trial, all-cause mortality was insignificantly increased 10% by hormonal replacement therapy (relative hazard = 1.10; 95% CI, 0.92–1.31) [104]. The overall incidence of venous thromboembolism at 6.8-year follow-up was significantly increased 208% by hormonal replacement therapy (relative hazard = 2.08; 95% CI, 1.28–3.40) [108]. At 6.8-year follow-up, the overall incidence of biliary tract surgery was significantly increased 48% (relative hazard = 1.48; 95% CI, 1.12–1.95), the overall incidence for any cancer was insignificantly increased 19% (relative hazard = 1.19; 95% CI, 0.95–1.50),

and the overall incidence for any fracture was insignificantly increased 4% (relative hazard = 1.04; 95% CI, 0.92–1.31) [108].

The estrogen plus progestin component of the Women's Health Initiative (WHI) Study included 16,608 healthy postmenopausal women aged 50–79 years with an intact uterus who were randomized to estrogen plus progestin or to placebo [109]. At 5.2-year follow-up, this component of the WHI study was prematurely discontinued because the excess risk of events included in the global index was 19 per 10,000 person-years [109]. Absolute excess risks per 10,000 person-years included 7 more coronary events, 8 more strokes, 8 more episodes of pulmonary embolism, and 8 more invasive breast cancers, while absolute risk reductions per 10,000 person-years were 6 fewer colorectal cancers and 5 fewer hip fractures [109]. On the basis of the available data, hormonal replacement therapy should not be used in postmenopausal women with CAD [92].

INFLUENZA VACCINATION

Evidence from cohort studies and a randomized clinical trial indicate that annual vaccination against seasonal influenza prevents cardiovascular morbidity and mortality in patients with cardiovascular disease [110]. The ACCF/AHA guidelines recommend influenza immunization with inactivated vaccine administered intramuscularly as part of secondary prevention in persons with CAD or other atherosclerotic vascular disease with a Class I indication [1,110].

DEPRESSION

For patients with recent MI or coronary artery bypass graft surgery, it is reasonable to screen for depression if patients have access to a mental health specialist [1]. Treatment of depression has not been found to improve cardiovascular outcomes in patients with CAD but may be reasonable for its other clinical benefits [1].

CARDIAC REHABILITATION

A cardiac rehabilitation program is useful in treating patients with stable CAD [1]. A home-based cardiac rehabilitation program can be substituted for a supervised, center-based program for low-risk patients [1]. Cardiac rehabilitation training programs have been shown to be safe and to improve aerobic fitness capacity, muscular strength, mental depression, and cardiovascular risk factors in patients with stable CAD [111]. Cardiac rehabilitation programs also increase survival in patients with stable CAD and may reserve and prevent cardiac disability [111]. However, cardiac rehabilitation participation is low in patients with stable CAD due largely to low referral rates [111].

CORONARY REVASCULARIZATION

Medical therapy alone is the preferred treatment in patients with stable CAD. The two indications for coronary revascularization in patients with CAD are prolongation of life and relief of unacceptable symptoms despite optimal medical management [112]. However, a randomized trial of 2287 patients with stable CAD and myocardial ischemia treated with optimal medical therapy alone or optimal medical therapy plus percutaneous coronary intervention showed at 4.6-year follow-up no significant difference in death, MI, or other major cardiovascular events [113]. If coronary revascularization is performed, aggressive medical therapy must be continued. The management of patients with unstable angina pectoris [47] and of acute MI [114] is discussed extensively elsewhere.

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CHAPTER

16

Percutaneous Coronary Intervention

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HISTORY

No other field in medicine has witnessed such a tremendous rate of innovation and development in the last four decades, as the field of interventional cardiology. Charles T. Dotter is credited with the first percutaneous transluminal treatment of obstructive peripheral arterial lesions using guide and dilating catheters [1]. The Zurich-born doctor Andreas Gruntzig performed the first percutaneous transluminal coronary angioplasty in 1977 using catheter mounted dilatation balloons [2,3]. Balloon angioplasty showed immense promise in obstructive discrete coronary lesions and obstructed bypass grafts; however, it was associated with early and chronic recoil and adverse remodeling leading to restenosis in up to 40–60% of patients. It was in 1986 that the first vascular scaffolds were inserted in coronary arteries to avoid these limitations of balloon angioplasty [4]. From the first percutaneous coronary angioplasty, the field of interventional cardiology has witnessed immense improvement in guiding catheters, guidewires, intra-coronary stents and devices, and interventional techniques. Percutaneous coronary intervention (PCI) is now available at most hospitals in the United States and is continuously utilized to manage sick patients with acute coronary syndromes (ACSs) and symptomatic stable coronary artery disease (CAD) [5,6]. This chapter discusses the indications for PCI, vascular access, intracoronary devices, recent data on antiplatelet therapy, controversies, and future directions.

INDICATIONS

Broadly, PCI is indicated for either survival benefit or improvement of symptoms in patients with CAD. PCI offers survival benefit in patients with ACS and in cardiac arrest patients in whom ischemia-mediated arrhythmia due to significant stenosis (≥70%) of an epicardial coronary artery is likely [7]. PCI is recommended to improve symptoms in patients with chronic stable CAD who are symptomatic (despite maximal tolerated goal-directed medical therapy) and have significant stenosis of the epicardial coronary artery (class I, level of evidence A) [7]. In the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial, PCI added to optimal medical therapy offered no survival benefit over optimal medical therapy alone during a median follow-up of 4.6 years; however, PCI was associated with higher rates of angina relief compared to optimal medical therapy alone [7a]. Patients with more severe angina are likely to benefit more from PCI combined with goal-directed medical therapy [8]. Interestingly, a large Bayesian network meta-analysis of 100 randomized controlled trials reported a survival benefit of the newer generation of drug-eluting stents (DESs) (everolimus and zotarolimus) over medical therapy in patients with stable CAD. This benefit was not observed for bare metal stents (BMSs) or older DES (sirolimus and paclitaxel) [9].

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00016-4

Multivessel and Left Main Disease

Clinically relevant multivessel disease is defined as presence of significant obstructive disease in more than one epicardial vessel (≥70% in one major epicardial vessel and at least ≥50% in another major epicardial vessel). The improvements in surgical technique for Coronary Artery Bypass Graft (CABG) surgery, increased operator experience, and procedural advancements in PCI have led to greater options for revascularization in this subset of patients. The Synergy Between PCI With Taxus and Cardiac Surgery (SYNTAX) trial was a landmark trial that randomized untreated 3-vessel disease and left main disease to PCI (paclitaxel-eluting stent) or CABG. The composite major adverse cardiovascular and cerebrovascular events (MACCEs) were higher in the PCI group secondary to increased repeat revascularization rates at 1 year. Stroke, however, was higher in the CABG group. The SYNTAX score was a predictive model of adverse events and patients with higher SYNTAX scores did better with CABG [10]. Selected nondiabetic patients with low SYNTAX scores and normal ejection fraction are ideal candidates for multivessel PCI with DESs.

Obstructive left main disease is a particularly high-risk scenario for the patient as it has the potential to jeopardize a significant amount of myocardium. It is associated with significant morbidity and mortality. CABG surgery continues to be utilized for significant left main disease (stenosis ≥50%) irrespective of other significant stenosis. However, there has been a recent trend toward utilizing PCI for management of "unprotected" left main disease (absence of open surgical graft) especially at high-volume centers after the safety and acceptable intermediate outcomes were reported in nonrandomized studies [11,12]. The Revascularization for Unprotected Left Main Coronary Artery Stenosis: Comparison of Percutaneous Coronary Angioplasty versus Surgical Revascularization (MAIN-COMPARE) registry reported similar long-term hard outcomes (death, myocardial infarction (MI), and stroke) between PCI of the left main group and CABG. As expected the surgical groups had lower rates of target-lesion revascularization compared with PCI for unprotected left main disease [13]. The Premier of Randomized Comparison of Bypass Surgery versus Angioplasty Using Sirolimus-Eluting Stent in Patients with Left Main Coronary Artery Disease (PRECOMBAT) trial reported noninferiority of PCI with sirolimus-eluting stents to CABG in patients with unprotected left main stenosis [14]. Recently reported 5-year outcome data of the SYNTAX trial showed similar MACCEs between PCI with paclitaxel-eluting stents and CABG in patients with left main disease and low/intermediate SYNTAX scores. Patients with CABG had higher stroke rates and lower repeat revascularization rates when compared with the PCI group. The PCI group had higher MACCE when utilized for patients with higher SYNTAX scores (≥33) [15,16]. Although trials using newer generation DESs are ongoing, both CABG and contemporary PCI are viable strategies for patients with low/intermediate SYNTAX scores, but patients with diffuse CAD or high SYNTAX scores should undergo CABG if feasible. The current guidelines have a class IIa recommendation for PCI in culprit lesion-unprotected left main stenosis in patients with ACSs [7] (Figure 16.1). PCI for left main stenosis can also be considered in symptomatic patients who are high-risk surgical candidates or symptomatic patients with PCI-amenable left main disease and low SYNTAX scores (class IIa) [7].

Chronic Total Occlusions

Chronic total occlusions (CTOs) are defined as coronary lesions with thrombolysis in myocardial infarction (TIMI) grade flow of 0 (true CTO) or TIMI grade flow 1 (functional CTO) and present for more than or equal to 3 months [17]. They are present in up to 15% of diagnostic angiograms and often challenge operators with decision making regarding percutaneous intervention, technical issues, and lack of randomized data. Registry data have suggested that intervening on CTOs is associated with improvement in quality of life and symptoms, left ventricular ejection fraction, and reduced need for CABG [18–21]. A recent meta-analysis also suggested lower associated long-term mortality with successful CTO revascularization (RR 0.54; 95% confidence interval (CI) 0.45–0.65; $p < 0.001$) as well as a lower need for CABG (RR 0.25; 95% CI 0.21–0.30; $p < 0.001$) [22]. No large randomized data sets are available for comparison of percutaneous treatment of CTO versus medical therapy alone. CTO recanalization should be considered in patients with exercise limiting cardiac symptoms and moderate- to high-risk reversible ischemia on stress testing in the CTO territory. Myocardial viability using dobutamine echocardiography or cardiac magnetic resonance imaging may be useful prior to CTO-PCI. CTO recanalization should be attempted in high-volume centers with experienced operators preferably using DES as CTO interventions are associated with higher restenosis, procedural complexity, and higher complications and failure rates compared with non-CTO interventions.

Saphenous Venous Graft Interventions

Saphenous venous grafts (SVGs) are often utilized as conduits during CABG. They are, unfortunately, associated with higher restenosis rates and atheromatous degeneration. SVGs have a patency rate of 40–50%

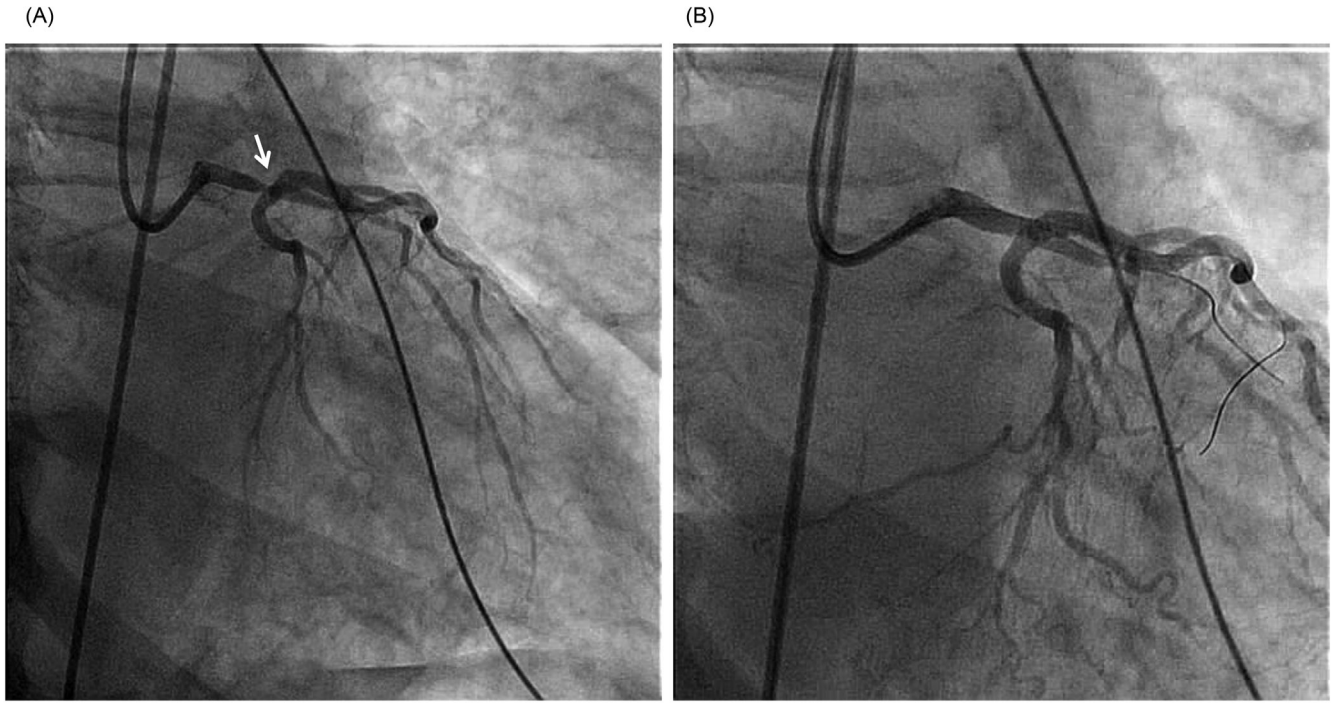

FIGURE 16.1 Seventy-five-year-old male with type II diabetes mellitus, hypertension, and a 40-pack year smoking history presented with non-ST elevation myocardial infarction and incessant ventricular tachycardia on admission. Emergent cardiac catheterization revealed a critical distal left main stenosis (A, white arrow). Patient was treated with a drug-eluting stent with TIMI grade 3 flow immediately post stent placement (B).

compared with 95% patency rates for internal mammary conduits at 10 years. Distal embolization is very common during PCI of SVG and is associated with no reflow, periprocedural angina, MI, or even death. Hence, distal embolization protection devices (discussed later in the chapter) are given a class I indication for SVG-PCI. Predictors of worse 30-day outcomes for SVG-PCI are lesion length, higher estimated plaque volume, and angiographic degeneration of SVGs [23,24]. SVG intervention should be considered in symptomatic patients with angiographic evidence of significant stenosis in the presence of ischemia on noninvasive testing in the territory of the graft. It might be prudent at times to intervene on the native coronary artery rather than the SVG to improve overall outcomes and procedural success. Is Drug-Eluting-Stenting Associated with Improved Results in Coronary Artery Bypass Grafts (ISAR-CABG) demonstrated the superiority of DESs over BMSs in SVG-PCI in terms of the primary outcome (composite of death, MI, or target lesion revascularization at 1 year; 0.64; 95% CI 0.44–0.94; $p = 0.02$) [25]. Numerous meta-analyses have also demonstrated better outcomes and safety of DES compared with BMS for SVG-PCI [26–28]. DES should be used in SVG-PCI whenever feasible, though stent sizing issues do not always allow DES use. Sometimes, pre-dilatation with an undersized balloon to facilitate passage of embolic protection filters prior to stenting the lesion is needed. Although the best outcomes after CABG can be achieved by minimizing SVG utilization either in the form of pan-arterial revascularization or hybrid coronary revascularization using DES to the non-LAD vessels, SVGs continue to be utilized extensively around the globe and efforts should be made by proceduralists to minimize periprocedural complications associated with SVG-PCI.

VASCULAR ACCESS

Femoral artery puncture is the most commonly employed access route for coronary interventions across the world. However, femoral access is associated with complications in the form of pseudo-aneurysm, hematoma formation (both local and retroperitoneal), prolonged immobilization, and longer hospital stays. Femoral artery access site complications are associated with significant morbidity and even mortality. The trend is now shifting toward increased utilization of the radial artery for access [29]. One out of six PCIs are performed in the United States using radial access [29]. The radial artery offers fewer bleeding complications and shorter hospital stays and is the preferred route for obese

patients and those with previous femoral graft surgeries. The RadIal Vs femorAL access for coronary intervention (RIVAL) randomized controlled trial demonstrated that radial access (compared with femoral access) was associated with fewer vascular complications in the form of large hematomas (hazard ratio (HR) 0.40; 95% CI 0.28–0.57; $p < 0.0001$) and pseudo-aneurysms requiring surgical attention (HR 0.30; 95% CI 0.13–0.71; $p = 0{\cdot}006$) in patients presenting with ACSs [30]. In a subgroup analysis of the RIVAL trial, Mehta et al. reported that radial access was associated with lower death/MI/stroke and all-cause mortality when utilized in patients presenting with ST-elevation MI [31]. These findings were also reproduced in the Radial Versus Femoral Randomized Investigation in ST-Elevation Acute Coronary Syndrome (RIFLE-STEACS) randomized controlled trial [32]. Superiority of radial access over femoral access (primarily driven by lower bleeding rates and hence net adverse clinical events) was also demonstrated in patients presenting with ACS in the recently concluded Minimizing Adverse Haemorrhagic Events by TRansradial Access Site and Systemic Implementation of angioX (MATRIX) trial [33].

Radial access is associated with slightly longer door-to-balloon times and has a steeper learning curve. In STEMI, it should be used primarily in high-volume centers by experienced "radial" operators. The most recent guidelines give a class IIa recommendation for radial artery access (Level of Evidence: A) [7].

INTRACORONARY DEVICES

Balloon Angioplasty and Intracoronary Stenting

Plain old balloon angioplasty is associated with very high restenosis rates due to elastic recoil and is seldom used today other than for vessels too small to stent. BMS scaffold systems, offering radial support and prevention of elastic recoil, were first used in man in 1986 and received the Food and Drug Administration (FDA) approval in 1994. Unfortunately, they are also associated with high restenosis rates due to neointimal hyperplasia. Currently, balloon angioplasty and BMS are recommended for use in patients who are deemed high risk for bleeding, and who will be unable to tolerate prolonged dual antiplatelet therapy (DAPT) [7]. DESs were developed to inhibit neointimal hyperplasia and were first FDA approved in 2003. DESs are made up of three components—stent platform (stainless steel in older generation stents and cobalt–chromium and platinum–chromium in newer generation stents), polymer coating for drug delivery, and antiproliferative agents (sirolimus and paclitaxel in older generation stents and everolimus and zotarolimus in newer generation stents). First-generation DESs are associated with increased risk of stent thrombosis (due to incomplete endothelization and impaired arterial healing) and require longer DAPT compared with BMS [34,35]. Second-generation DESs appear to have a lower rate of stent thrombosis than first-generation DESs and even than BMSs [36–38]. DESs with a biodegradable polymer (polylactic acid) are also available outside of the United States. A recent network meta-analysis reported superiority of biodegradable polymer based biolimus-eluting stents over BMSs and first-generation DESs and similar rates of cardiac events as newer generation DES [39].

Fully biodegradable stents may bring a new era in stent technology. They are made up of a biodegradable scaffold, a biodegradable polymer, and an antiproliferative agent (everolimus in the ABSORB bioresorbable vascular scaffold system, Abbott Vascular, USA). The stent is fully biodegradable except small platinum markings at the end for fluoroscopy detection. Theoretically, they offer the advantage of vessel restoration after revascularization. Data from the ABSORB II trial comparing an everolimus-eluting bioresorbable scaffold with an everolimus-eluting metallic stent reported similar recoil between both groups and a slightly lower luminal gain in the bioabsorbable stent group at 1 year. Cumulative rates of recurrent angina were lower in the bioabsorbable stent group [40]. The interventional community awaits further long-term data on hard end points, including stent thrombosis, before widespread acceptance of fully bioabsorbable stents.

Mechanical Thrombectomy Devices

Thrombus burden is associated with suboptimal or no TIMI flow post-intervention and worse clinical outcomes. Almost all patients with ACS have varying degrees of thrombus burden and it is associated with worse short-, medium-, and long-term outcomes and increased risk of stent thrombosis. Thrombectomy devices are available to decrease the thrombus burden and improve procedural outcomes during primary PCI. Thrombectomy devices are of two types—manual or mechanical extraction devices.

Mechanical thrombectomy devices (e.g., Ultra Angiojet Rheolytic Thrombectomy device, Bayer Interventional/Boston Scientific, USA) are more cumbersome to use and require longer preparation times. They work by the principle of disrupting the thrombus with simultaneous extraction. Platelet disruption and adenosine release were associated with advanced heart block and necessitated temporary pacemaker insertion in older generation systems; however, newer generation AngioJet systems may not require routine temporary pacemaker insertion. The AngioJet Rheolytic Thrombectomy Before Direct Infarct Artery Stenting With Direct Stenting

Alone in Patients With Acute Myocardial Infarction (JETSTENT) trial demonstrated better ST-resolution in patients undergoing rheolytic thrombectomy prior to primary PCI [41]. Larger clinical trials and further data are needed for recommending routine use during primary PCI as they are cumbersome to use and may not be a feasible choice during emergency situations after work-hours with minimal healthcare personnel on call.

Manual or aspiration thrombectomy devices are the easiest to use and have two lumens—one for the guidewire and advancement of the device, and the other for manual aspiration by the operator. The most commonly used aspiration devices are Export (Medtronic Inc., USA) and Pronto (Vascular Solutions, Inc., USA). The Pronto extraction catheter was studied in the Dethrombosis to Enhance Acute Reperfusion in Myocardial Infarction (DEAR-MI) trial and resulted in better ST-segment resolution and myocardial blush grade in patients with STEMI undergoing primary PCI [42]. The Thrombus Aspiration during Percutaneous Coronary Intervention in Acute Myocardial Infarction Study (TAPAS) trial randomized patients with STEMI to either thrombus aspiration using the 6-French Export catheter or conventional PCI. The patients undergoing aspiration thrombectomy showed better ST-resolution, myocardial blush grade, and 30-day mortality compared with the patients undergoing conventional PCI [42a]. Meta-analyses confirmed these benefits [43,44]. However, larger studies such as the Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia (TASTE) trial and the recently published Trial of Routine Aspiration Thrombectomy with PCI versus PCI Alone in Patients with STEMI (TOTAL) have demonstrated no improved short- or long-term outcomes with manual thrombectomy [45,46]. Data from the TOTAL trial also provide evidence of a possible increased risk of strokes with manual aspiration thrombectomy prior to primary PCI at 30 days [46]. Adding to these data is the registry analysis of greater than 10,000 patients from the United Kingdom that reported no mortality benefit of routine aspiration thrombectomy prior to primary PCI at a median 3-year follow-up [47]. The recent guidelines (published before the larger trials) have a class IIa recommendation for manual thrombectomy prior to primary PCI [7]. The data regarding a possible increased risk of stroke without a mortality benefit should preclude the routine use of manual thrombectomy in the majority of patients with STEMI.

Distal Embolization Protection Devices

SVG interventions are prone to complications secondary to distal embolization of friable atherothrombotic debris. Major adverse cardiac events are higher with SVG-PCI compared with PCI in the native vessel. The most current guidelines have a class I recommendation for the use of distal embolization protection devices during SVG-PCI when feasible [7]. Two types of distal protection devices are available—distal occlusion devices and distal filter devices. The Saphenous vein graft Angioplasty Free of Emboli Randomized (SAFER) trial demonstrated a 42% reduction in major adverse cardiovascular events with a distal occlusion device during PCI compared with conventional PCI alone [48]. The FilterWire EX Randomized Evaluation (FIRE) trial demonstrated the noninferiority of the distal filter devices to the distal occlusion devices for SVG-PCI with a similar major adverse cardiac event rate at 30 days [49]. The exact choice of device depends on operator preference, though vein graft anatomy is not always suitable to use these devices.

Fractional Flow Reserve

Interventional cardiologists are often faced with coronary lesions that are equivocal and in vessels supplying viable myocardium in patients who are referred for symptomatic stable angina pectoris. Anatomic assessment is sometimes misleading secondary to its two-dimensional nature and lack of data on the eccentricity and length of lesions especially in patients with diffuse multivessel disease. Fractional flow reserve (FFR) is a physiological assessment of these lesions in the cardiac catheterization lab. FFR is essentially an indirect flow measurement. It is calculated using ratio of mean pressure distal to the lesion (P_d) and mean aortic pressure (P_a) under hyperemic conditions (flow = pressure/resistance, flow approximates pressure in maximal hyperemia; using intravenous or intracoronary adenosine). A 0.014-in. pressure-wire system is placed distal to the stenosis to get the mean P_d and the mean P_a is obtained simultaneously from the guiding catheter. Under ideal conditions FFR is 1 and FFR less than or equal to 0.80 has an accuracy of more than 90% for detecting ischemia-causing stenoses. The Fractional Flow Reserve versus Angiography for Multivessel Evaluation (FAME) trial reported that in patients with chronic stable angina, FFR guided PCI with DES reduced the composite end point of death, nonfatal MI, or revascularization at 1-year follow-up versus PCI based on angiography alone [50]. In the subsequent FAME II trial, FFR guided PCI with DES plus optimal medical therapy reduced the need for urgent revascularization when compared with optimal medical therapy alone in patients with chronic stable angina and multivessel disease [51]. Integration of angiographic and physiologic data can improve patient outcomes in multivessel disease, intermediate lesions, diffuse disease, and selected cases of left main disease. Current guidelines have a class IIa recommendation for FFR guided PCI in intermediate coronary lesions (50–70% stenosis) in patients with stable ischemic heart disease [7].

ANTIPLATELET AGENTS

Aspirin

Historically, the first antiplatelet agent to show benefit in ACS was aspirin. Aspirin irreversibly inhibits cyclooxygenase-1 and thus blocks the production of thromboxane A_2 thereby decreasing platelet activation and aggregation. Several trials have consistently demonstrated reduction in cardiovascular events with aspirin treatment in patients with ACS [52]. Although the minimum effective aspirin dosage in the setting of PCI has not been established, patients already on chronic daily aspirin therapy should receive 81–325 mg aspirin before PCI [7]. Patients not on aspirin therapy should be given nonenteric aspirin 325 mg at least 2 h, and preferably 24 h, before PCI. After PCI, aspirin should be continued indefinitely at a daily dose of 75–100 mg since there is no additional demonstrated benefit from higher doses, but a reduction in gastrointestinal bleeding with the lower doses [53–55].

$P2Y_{12}$ Receptor Antagonists

Adenosine diphosphate released from platelets acts on G_i-coupled $P2Y_{12}$ receptors to inhibit the adenylate cyclase–mediated signaling pathway resulting in decreased intracellular cAMP levels, and reduced phosphorylation of the vasodilator-stimulated phosphoprotein, thus inducing activation of the Gp IIb/IIIa receptor and platelet aggregation. The thienopyridines (ticlopidine, clopidogrel, and prasugrel) and non-thienopyridines (ticagrelor, cangrelor, and elinogrel) cause irreversible and reversible antagonism of $P2Y_{12}$ receptors, respectively, thus inhibiting platelet aggregation. The use of a $P2Y_{12}$ receptor antagonist, in combination with aspirin, is recommended for the prevention of ischemic events during both the acute and the long-term phases after ACS.

Ticlopidine, a first-generation thienopyridine, was the first FDA-approved $P2Y_{12}$ receptor antagonist [56]. However, ticlopidine is now rarely used due to its adverse effect profile, including an increased risk of life-threatening hematological disorders. Clopidogrel is a second-generation thienopyridine with a more favorable safety profile than ticlopidine [57,58]. The Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial was the landmark trial that established the benefits of addition of clopidogrel (300 mg loading dose followed by 75 mg daily maintenance dose) to aspirin in patients with non ST-elevation ACS, showing a 20% relative risk reduction in the composite outcome of cardiovascular death, nonfatal MI, or stroke, compared with placebo over 12 months [59]. There was an increase in CURE major bleeding, but no increase in life-threatening bleeding or hemorrhagic strokes with the combination of clopidogrel and aspirin. The benefits of addition of clopidogrel to aspirin were also seen in patients less than or equal to 75 years of age with STEMI who were treated with fibrinolysis in the Clopidogrel as Adjunctive Reperfusion Therapy–Thrombolysis in Myocardial Infarction (CLARITY–TIMI) 28 trial [60]. There was an absolute reduction of 6.7% in the rate of primary efficacy end point (composite of an occluded infarct-related artery defined by a TIMI flow grade of 0 or 1 on angiography, death, or recurrent MI before angiography) with clopidogrel compared with placebo. The rates of major bleeding and intracranial hemorrhage were similar in the two groups. Several studies have shown that a double-loading dose (600 mg rather than 300 mg) of clopidogrel results in faster and greater platelet inhibition, as well as improved clinical outcomes. In the prespecified analysis of 17,263 patients with ACS who underwent PCI in the Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events–Seventh Organization to Assess Strategies in Ischemic Syndromes (CURRENT–OASIS 7) trial, a 7-day double-dose clopidogrel regimen was associated with a reduced rate of the primary outcome (cardiovascular death, MI, or stroke at 30 days) as well as stent thrombosis, although at the expense of an increase in major bleeding [61]. Current guidelines recommend a 600 mg loading dose of clopidogrel in patients undergoing primary PCI [7]. The loading dose of clopidogrel for patients undergoing PCI after fibrinolytic therapy should be 300 mg within 24 h and 600 mg more than 24 h after receiving fibrinolytic therapy. All post-PCI patients should receive clopidogrel 75 mg daily for at least 12 months after DES implantation, and for a minimum of 1 month and ideally up to 12 months for BMS implantation (unless the patient is at increased risk of bleeding in which case it should be given for a minimum of 2 weeks for BMS).

Prasugrel is a third-generation thienopyridine that, similar to clopidogrel, requires conversion from an inactive form to an active metabolite via cytochrome P450 (CYP) enzymes. However, prasugrel is a more potent $P2Y_{12}$ receptor antagonist with a more rapid onset of action and higher levels of platelet inhibition than clopidogrel [62–64]. The clinical efficacy of prasugrel was investigated in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction (TRITON–TIMI) 38 trial, which randomly assigned 13,608 patients with moderate-to-high-risk ACS with scheduled PCI to receive prasugrel (60 mg loading dose and 10 mg daily maintenance dose) or clopidogrel (300 mg loading dose and a 75 mg daily maintenance dose), for 6–15 months [65]. The primary efficacy end point (composite of cardiovascular death, nonfatal MI, or nonfatal stroke) occurred in 12.1% of

patients receiving clopidogrel and 9.9% of patients receiving prasugrel (HR for prasugrel vs. clopidogrel, 0.81; 95% CI 0.73–0.90; $p < 0.001$). Prasugrel was also associated with significant reductions in the rates of MI, urgent target-vessel revascularization, and stent thrombosis. However, major bleeding was observed in 2.4% of patients receiving prasugrel and in 1.8% of patients receiving clopidogrel ($p = 0.03$), and prasugrel was also associated with increased rates of life-threatening bleeding (1.4% vs. 0.9%; $p = 0.01$), including fatal bleeding (0.4% vs. 0.1%; $p = 0.002$). The net benefit of prasugrel over clopidogrel was particularly notable in patients with STEMI [66], those with diabetes mellitus [67], and in those experiencing recurrent cardiovascular events [68]. In contrast, prasugrel was associated with a net harm in patients with a prior history of stroke or transient ischemic attack and is therefore contraindicated in these patients [65]. In patients with ACS undergoing PCI who are at low risk of bleeding, prasugrel may be used as a 60 mg loading dose followed by 10 mg daily maintenance dose after stent placement.

Ticagrelor is a non-thienopyridine, direct-acting, reversible $P2Y_{12}$ receptor antagonist. The drug acts rapidly and has more potent and consistent antiplatelet effects than clopidogrel. The Study of Platelet Inhibition and Patient Outcomes (PLATO) trial was a multicenter, double-blind trial of 18,624 patients with ACS, with or without ST-segment elevation, randomized to ticagrelor (180 mg loading dose, 90 mg twice daily thereafter) or clopidogrel (300–600 mg loading dose, 75 mg daily thereafter) [69]. At 12 months, the primary end point (a composite of death from vascular causes, MI, or stroke) occurred in 9.8% of patients receiving ticagrelor as compared with 11.7% of those receiving clopidogrel (HR 0.84; 95% CI 0.77–0.92; $p < 0.001$). Ticagrelor was also associated with lower rates of MI and death from vascular causes, but not stroke. These benefits were consistent across all subgroups. No significant difference in the rate of overall major bleeding was found between the ticagrelor and clopidogrel groups (11.6% vs. 11.2%; $p = 0.43$), but ticagrelor was associated with a higher rate of major bleeding not related to CABG (4.5% vs. 3.8%; $p = 0.03$). In the PLATO trial, there was a significant interaction ($p = 0.045$) between treatment effect and enrollment region of the trial, with no benefit from ticagrelor in patients enrolled in North America. Although these findings could be caused by chance, a *post hoc* analysis attributed this treatment-by-region interaction to the use of high-dose (≥300 mg daily) aspirin, which was more common in North America than in the rest of the world [70]. Despite subsequent work demonstrating that aspirin dosing does not alter the pharmacokinetic and pharmacodynamic properties of ticagrelor, low-dose aspirin (≤100 mg) is currently recommended in patients treated with ticagrelor [71].

Cangrelor is a potent, intravenous, direct-acting inhibitor that reversibly binds to $P2Y_{12}$ receptors. Platelet inhibition occurs immediately after administration of a bolus of cangrelor and can be maintained with a continuous infusion. The plasma half-life of cangrelor is approximately 3–5 min, and platelet function is restored within 1 h of cessation of the infusion [72]. The Clinical Trial Comparing Cangrelor to Clopidogrel Standard of Care Therapy in Subjects Who Require Percutaneous Coronary Intervention (CHAMPION PHOENIX) study randomly assigned 11,145 patients who were undergoing either urgent or elective PCI to receive either bolus cangrelor (30 μg/kg) followed by infusion (4 μg/kg/min for 2–4 h), or clopidogrel (loading dose 300 or 600 mg, before or immediately after PCI, as per the institution's standard protocol) [73]. Patients in the cangrelor arm also received a 600 mg loading dose of clopidogrel at the end of the infusion. The rate of the primary efficacy end point (composite of death from any cause, MI, ischemia-driven revascularization, or stent thrombosis in the 48 h after randomization) was 4.7% in the cangrelor group and 5.9% in the clopidogrel group (adjusted OR 0.78; 95% CI 0.66–0.93; $p = 0.005$). The key secondary end point of stent thrombosis at 48 h developed in 0.8% of the patients in the cangrelor group and in 1.4% in the clopidogrel group (OR 0.62; 95% CI 0.43–0.90; $p = 0.01$). There was no difference in rates of severe bleeding at 48 h between the two treatment groups. Cangrelor may have an important role across the full spectrum of patients undergoing PCI.

Elinogrel is another reversible, direct-acting $P2Y_{12}$ receptor antagonist [74]. The safety, efficacy, and tolerability of oral and intravenous elinogrel compared with clopidogrel in patients undergoing nonurgent PCI was determined in the INtraveNous and Oral administration of elinogrel to eVAluate Tolerability and Efficacy in nonurgent PCI patients (INNOVATE-PCI) phase 2b study [75]. Elinogrel achieved greater platelet inhibition than clopidogrel, without significant increase in TIMI major or minor bleeding, although elinogrel treatment was associated with an increased frequency of elevations in liver enzyme levels and dyspnea. Currently, no phase III trials of elinogrel are ongoing or planned.

Glycoprotein IIb/IIIa Inhibitors

The intravenous Gp IIb/IIIa inhibitors (abciximab, eptifibatide, and tirofiban) target the final common pathway of platelet aggregation by competing with fibrinogen and von Willebrand factor for binding to Gp IIb/IIIa receptors. Most studies supporting the use of these agents during PCI were performed in the era before routine stenting, DAPT, and use of novel $P2Y_{12}$ receptor antagonists [76]. Nonetheless, Gp IIb/IIIa inhibitors have been shown to improve clinical outcomes in patients with

ACS undergoing PCI, primarily by reducing ischemic complications, including periprocedural MI and recurrent ischemia. Use of intravenous GP IIb/IIIa inhibitors is, therefore, a reasonable treatment option in high-risk patients with ACS undergoing PCI, but their use is limited owing to high rates of bleeding complications.

Abciximab is a chimeric human-murine monoclonal antibody that irreversibly binds to the Gp IIb/IIIa receptor on human platelets. The recommended dosage of abciximab is 0.25 mg/kg bolus, followed by 0.125 mcg/kg/min (maximum 10 mcg/min) continuous infusion for 12 h. It may be reasonable to administer intracoronary abciximab in patients with STEMI undergoing primary PCI, though there is no demonstrated benefit over intravenous administration [77,78]. Eptifibatide is a small-molecule cyclic peptide derivative that reversibly binds to the Gp IIb/IIIa receptor. It is administered as a double bolus (180 mcg/kg boluses 10 min apart) and 2 mcg/kg/min continuous infusion for 18–24 h. The eptifibatide infusion must be reduced by 50% in patients with creatinine clearance less than 50 mL/min, and this drug should be avoided in patients on hemodialysis. Tirofiban is a small-molecule peptide inhibitor of the Gp IIb/IIIa receptor that is administered as a high-bolus dose of 25 mcg/kg followed by 0.15 mcg/kg/min infusion. Patients with creatinine clearance less than or equal to 60 mL/min should receive half the usual rate of infusion. Although Gp IIb/IIIa inhibitors differ in their structure, reversibility with platelet transfusion, and duration, several meta-analyses have shown no difference between their clinical efficacy in patients undergoing primary PCI [79].

Protease Activated Receptor-1 Antagonists

The thrombin receptor protease-activated receptor-1 (PAR-1) mediates platelet activation at low thrombin concentrations and contributes to the formation of platelet-rich occlusive thrombi. Vorapaxar is a selective, potent, competitive antagonist of PAR-1 that blocks thrombin-mediated platelet activation without interfering with thrombin-mediated cleavage of fibrinogen [80]. The Thrombin Receptor Antagonist for Clinical Event Reduction in Acute Coronary Syndrome (TRACER) trial was a multinational, double-blind, randomized trial that enrolled 12,994 patients with NSTE-ACS and compared vorapaxar with placebo, in addition to standard therapy, which included aspirin and clopidogrel in 92% of patients [81]. PCI was performed in 57.8% of patients, of whom 94.6% underwent stent placement (BMS or DES). Overall, vorapaxar was associated with a nonsignificant decrease in the primary efficacy end point at 2 years (a composite of cardiovascular death, MI, stroke, recurrent ischemia with rehospitalization, or urgent coronary revascularization; 18.5% vs. 19.9%; HR 0.92; 95% CI 0.85–1.01; $p = 0.07$), at the expense of a significant increase in the rate of GUSTO moderate or severe bleeding (7.2% vs. 5.2%; $p < 0.001$), and a threefold increase in intracranial bleeding (1.1% vs. 0.2%; $p < 0.001$). The excess incidence of intracranial hemorrhage in patients with a history of stroke led to an unplanned safety review, which recommended early termination of the TRACER trial. However, data from TRA2P-TIMI 50 support the benefit of vorapaxar in stabilized post-MI patients [82]. In this population, some patients underwent PCI and the drug appeared to be safe in this context.

ANTICOAGULANTS

Anticoagulation is recommended in all patients undergoing PCI. Unfractionated heparin (UFH) is the most commonly used anticoagulant during PCI with the dose and target activated clotted time (ACT) based on whether or not a Gp IIb/IIIa inhibitor is administered. The recommended dose of UFH is 50–70 U/kg intravenous bolus to achieve a target ACT of 200–250 s if a Gp IIb/IIIa inhibitor is given, and 70–100 U/kg to achieve a target ACT of 250–300 s (HemoTec device) or 300–350 s (Hemochron device) if no Gp IIb/IIIa inhibitor is given [7]. Although prior studies [83] in the balloon angioplasty era demonstrated a strong relationship between ACT and ischemic complications, more recent studies from the coronary stent era have shown a more inconsistent relationship [84,85]. If no closure device has been used, early sheath removal is recommended when the ACT falls to less than 150–180 s. Full-dose anticoagulation after successful PCI is not indicated unless there is a specific reason (such as atrial fibrillation).

Low-molecular-weight heparin or enoxaparin is a reasonable alternative to UFH in patients with NSTE-ACS undergoing PCI and does not require monitoring. In the Superior Yield of the New Strategy of Enoxaparin, Revascularization and Glycoprotein IIb/IIIa Inhibitors (SYNERGY) trial of 10,027 high-risk patients with NSTE-ACS to be treated with an early invasive strategy, there was no difference in the primary end point (composite of all-cause death or nonfatal MI during the first 30 days after randomization) between patients treated with enoxaparin or UFH (14.0% vs. 14.5%; HR 0.96; 95% CI 0.86–1.06) [86]. More TIMI major bleeding was seen in patients treated with enoxaparin (9.1% vs. 7.6%; $p = 0.008$), particularly in those patients treated with enoxaparin who also received UFH at the time of PCI. Similar results were seen in the 4687 SYNERGY patients who underwent PCI [87]. The recommended dose of enoxaparin is 1 mg/kg subcutaneously (SC) every 12 h until PCI is performed, with an additional 0.3 mg/kg intravenous dose at the time of PCI in patients who have received less than two therapeutic SC doses or received

the last SC enoxaparin dose 8–12 h before PCI. Patients who undergo PCI more than 12 h after the last SC dose should be treated with full-dose *de novo* anticoagulation using an established regimen.

Bivalirudin is a direct thrombin inhibitor that has been used as an alternative to UFH in patients undergoing PCI. Several randomized controlled trials and meta-analyses have shown that bivalirudin, as compared to UFH with or without Gp IIb/IIIa inhibitor, is associated with a reduction in major bleeding, but an increase in acute stent thrombosis (at least in primary PCI for STEMI) [88–90]. In 13,819 moderate-to-high risk ACS patients in the Acute Catheterization and Urgent Intervention Triage Strategy (ACUITY) trial, bivalirudin was noninferior to UFH plus Gp IIb/IIIa inhibitor in reducing the composite ischemic end point (death, MI, or unplanned revascularization for ischemia), and significantly reduced rates of major bleeding and the net clinical outcome end point [91]. Similarly, in the Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction (HORIZONS-AMI) [92], the European Ambulance Acute Coronary Syndrome Angiography (EUROMAX) [93], and the Bivalirudin in Acute Myocardial Infarction vs Heparin and GPI Plus Heparin Trial (BRIGHT) [94] trials, in patients undergoing primary PCI, bivalirudin as compared with heparin plus Gp IIb/IIIa inhibitor was associated with a decrease in net adverse clinical events, primarily due to a significant reduction in major bleeding. In contrast, in the recently conducted, modestly sized, How Effective are Antithrombotic Therapies in Primary Percutaneous Coronary Intervention (HEAT-PPCI) trial, heparin alone was found to be superior to bivalirudin in reducing major adverse ischemic events, with no difference in major bleeding [95]. Despite heparin's lower cost, bivalirudin continues to predominate in PCI centers throughout the United States due to the lower rate of bleeding, especially with femoral access.

Fondaparinux is a synthetic polysaccharide inhibitor of factor Xa. In a prespecified analysis of 6238 patients enrolled in the Fifth Organization to Assess Strategies in Ischemic Syndromes (OASIS-5) trial who underwent PCI, fondaparinux compared with enoxaparin reduced major bleeding (2.4% vs. 5.1%; HR, 0.46; $p < 0.00001$) at day 9, with similar rates of ischemic events, resulting in superior net clinical benefit (death, MI, stroke, major bleeding: 8.2% vs. 10.4%; HR, 0.78; $p = 0.004$) [96]. Catheter thrombosis was more common in patients receiving fondaparinux (0.9%) than enoxaparin alone (0.4%), but was largely prevented by using UFH at the time of PCI, without any increase in bleeding. Fondaparinux should not be used as the sole anticoagulant to support PCI because of the risk of catheter thrombosis. An additional anticoagulant with anti-factor IIa activity should be administered [7].

COMPLICATIONS OF PCI

PCI is an invasive procedure and is associated with a number of complications, including death (Table 16.1). Periprocedural bleeding is the most common complication and merits discussion due to an associated increase in morbidity, mortality, length of stay, and cost [97–100]. An analysis of greater than 10,000 PCIs reported a 5.4% incidence of major bleeding (hemorrhagic stroke or hematocrit drop >15 or 10–15 if clinical bleeding) [101]. Periprocedural bleeding was reported in 2.4% of patients undergoing PCI in data from the CathPCI registry [102]. Independent predictors of bleeding are advanced age, cardiogenic shock, use of intra-aortic balloon pump, renal failure, female sex, ST-elevation MI, and emergent/salvage PCI [101,102]. Retroperitoneal hematoma is rare (0.57%) but is associated with high mortality [98]. Patients with high femoral arterial puncture, female sex, use of Gp IIb/IIIa inhibitors, acute MI, and angioseal closure device are more likely to have retroperitoneal hematoma. Fluoroscopy or ultrasound guided femoral access, ideally using a micro-puncture needle, in elderly or obese patients may mitigate major access site related bleeding complications. Of course, radial artery access, when possible, would also be

TABLE 16.1 Complications of Coronary Angiography and Percutaneous Coronary Intervention

Vascular	Acute vessel occlusion
	Arterio-venous fistula
	Compartment syndrome (radial access)
	Dissection
	Hematoma
	Pseudo aneurysm
	Retroperitoneal hematoma
	Thrombosis and embolism
	Vasospasm (radial access)
Systemic	Bleeding
	Cholesterol embolization syndrome
	Contrast induced anaphylactoid reaction
	Contrast induced nephropathy
	Death
	Heparin induced thrombocytopenia infections
Cardiac and cerebrovascular	Acute vessel closure
	Air embolism
	Arrhythmias
	Cardiac tamponade
	Coronary spasm
	Death
	Dissection and perforation of coronary and aortic vasculature
	Guidewire fracture
	Myocardial infarction
	Retained devices
	Stroke

associated with lower rates of vascular complications and bleeding.

Patient selection for PCI is important as it is associated with vascular complications, potential for life-threatening major bleeding secondary to DAPT, contrast-induced nephropathy, iatrogenic coronary artery dissection, acute vessel closure, and rarely, even death. Benefits of therapy have to be weighed against the risks of the procedure and the approach individualized for each patient. PCI generally should not be offered to patients with terminal or metastatic cancer, severe liver or pulmonary disease, severe coagulopathy, major life-threatening cerebral or gastrointestinal bleeding, patients who will be noncomplaint with medications, and those who refuse to undergo invasive coronary angiography. Chronological age cut-off alone, however, should not be the sole criterion for not considering PCI [6].

CONTROVERSIES AND FUTURE DIRECTIONS

Complete Versus Culprit-Only Revascularization During ACSs

Emergent PCI on the infarct related culprit lesion is the recommended treatment of choice for acute ST-elevation MI. Intervention on the non-infarct related artery is not recommended during the cardiac catheterization for STEMI in the current guidelines unless there is cardiogenic shock. However, approximately 50% of patients are found to have significant disease in the non-infarct related arteries at the time of emergent cardiac catheterization and this is associated with worse 30-day outcomes [103]. Recently, the dogma regarding culprit vessel only revascularization was challenged by three modestly sized prospective randomized controlled trials—PRAMI (Preventive Angioplasty in Myocardial Infarction) [104], CvLPRIT (Complete versus Lesion-Only Primary PCI trial) [105], and DANAMI3-PRIMULTI (The Third DANish Study of Optimal Acute Treatment of Patients With STEMI: PRImary PCI in MULTIvessel Disease) [106]. It was hypothesized that complete revascularization at the time of angiography for STEMI will reduce the ischemic burden and provide medium- and long-term benefits. PRAMI trial randomized 465 patients to either culprit-only revascularization (231 patients) or culprit and preventive revascularization (234 patients). The primary end point was a composite of death, nonfatal MI, or refractory angina. At a mean 23-month follow-up, primary events were higher with culprit-only revascularization (53 patients vs. 21 patients, $HR_{preventive-PCI}$ 0.35; 95% CI 0.21–0.58; $p < 0.001$) [104]. The CvLPRIT trial randomized 296 patients to in-hospital complete revascularization (150 patients) or culprit-only revascularization (146 patients). The primary outcome was death, recurrent MI, heart failure, or repeat ischemia driven revascularization within 12 months. In-hospital complete revascularization (non-culprit revascularization performed either during primary PCI or during the hospital stay) was shown to be superior to culprit-only revascularization (HR 0.45; 95% CI 0.24–0.84; $p = 0.009$) [105,107]. These trials were well conducted but had modest sample sizes (and hence were not powered to demonstrate differences in hard outcomes such as death). A larger trial (Complete versus Culprit-only Revascularization to Treat Multi-vessel Disease After Primary PCI for STEMI; COMPLETE) scheduled to be completed in 2018 will hopefully answer more questions on individual hard end points and the role of FFR in these challenging situations. Additionally, the role of complete revascularization versus culprit-only revascularization still needs to be further evaluated in non-ST-elevation ACS [108].

At this time, we would consider intervening on the non-infarct related artery if there is hemodynamic compromise, ongoing clinical symptoms that are not resolved by intervention on the presumed culprit lesion (e.g., cases with inferior infarct on electrocardiogram and involvement of both right coronary artery and left circumflex systems), or unstable lesions in the non-infarct related artery (>90% stenosis, high thrombotic burden or ruptured plaque). Hemodynamically stable patients or patients with complex lesions that do not appear to be unstable should likely undergo staged intervention at a later date, preferably during the index hospitalization, pending the results of ongoing randomized trials.

The Long and Short of DAPT After DES Implantation

Current guidelines have a class I recommendation for 1 year of uninterrupted DAPT and lifelong aspirin after DES implantation due to risk of stent thrombosis and associated mortality. However, in patients who are at high risk of bleeding it is reasonable to discontinue clopidogrel less than 12 months and continue aspirin indefinitely (class IIa) [7]. The exact minimum (to prevent stent thrombosis) and maximum duration of DAPT (to provide secondary prevention and systemic benefit beyond the culprit lesion) have been a matter of intense debate. Data from recent trials and meta-analyses using newer generations of DESs suggest that a minimum of 3–6 months of DAPT might be noninferior to the conventional 12 months of DAPT, and may be associated with a decreased risk of bleeding, at least in patients at relatively low risk of ischemic events [109–112]. The

DAPT trial demonstrated that long-term use of DAPT (30 months vs. 12 months) was associated with reductions in stent thrombosis, adverse cerebrovascular and cardiovascular events (including at non-culprit locations) with a significant increase in bleeding risk [113]. The study, however, was in patients who could tolerate long-term antiplatelet therapies and were not at a high bleeding risk after an initial 12 months of DAPT. Of concern in the DAPT trial, there was an increase in all-cause mortality associated with prolonged DAPT, essentially confined to the subgroup of non-ACS patients [114]. In the ACS patients undergoing PCI, the risk benefit of DAPT was much more favorable [111].

Balancing the minimum required duration of DAPT for prevention of stent thrombosis with providing long-term vascular protection has challenged physicians. On the one hand are the issues of identifying patients at high risk of stent thrombosis and on the other hand are the patients at risk for bleeding from prolonged use of DAPT. The approach has to be individualized based on bleeding risk and risk of adverse thrombotic events—both stent thrombosis, but also the underlying atherothrombotic risk of the patient. After implantation of a newer generation DES, a minimum of 3–6 months seems prudent. After ACS, with or without a stent, a minimum of a year seems indicated, with longer durations of DAPT likely beneficial in patients who remain at high ischemic risk and at low bleeding risk [115–117].

Hybrid Coronary Revascularization: Ready for Primetime?

Hybrid coronary revascularization has been in existence for almost two decades [118]. It involves left internal mammary artery (LIMA) graft anastomosis to a diseased left anterior descending (LAD) artery (usually by minimally invasive direct coronary artery bypass graft technique, or MIDCAB) and percutaneous stenting of the revascularizable non-LAD territory (using newer generation DESs). The goal of this procedure is to shorten the hospital stay, offer revascularization to those at a high risk for conventional CABG, promote early ambulation, and improve patient satisfaction. The LIMA to LAD graft has patency rates approaching 95% at 10 years and is relatively resistant to atherosclerotic insult, unlike SVGs that have graft patency rates of only 32% at 15 years [119]. The newer generation DESs are an excellent choice for non-LAD territories as they have extremely low target vessel revascularization rates. The procedure can be performed in a single-stage procedure (MIDCAB followed by PCI immediately) or two-stage procedure (MIDCAB first, followed by PCI or PCI first followed by MIDCAB). A single-stage procedure offers better patient comfort by offering the entire revascularization in a single sitting, however, this approach requires hybrid operating rooms and the DAPT increases the risk of bleeding. The current guidelines prefer MIDCAB followed by PCI if a two-stage approach is selected as it offers the advantage of securing the LAD territory first and avoids perioperative bleeding in the setting of DAPT [120]. Hybrid coronary revascularization is not used widely: 0.5% of all CABG (85% staged) from 2011 to 2013 according to the Adult Cardiac Surgery databases [121]. This lack of acceptance is likely secondary to the need for extensive resource and capital investment in the form of comprehensive cardiac care teams, hybrid operating rooms, skilled minimally invasive surgical operators, and also due to the lack of large randomized clinical trial data. The first, modestly sized, randomized controlled trial comparing hybrid coronary revascularization to CABG (POL-MIDES, Prospective randomized Pilot Study Evaluating the Safety and Efficacy of Hybrid Revascularization in Multivessel Coronary Artery Disease) established the safety and feasibility of the procedure as there were no differences between the two groups in terms of mortality, repeat revascularization, MI, or major bleeding at 1 year [122]. It is yet to be seen if hybrid coronary revascularization, combining the advantages of PCI and minimally invasive coronary surgery, is ready for primetime.

Drug-Coated Balloons

The US FDA recently approved drug-coated balloons (DCBs) for symptomatic femoro-popliteal peripheral artery disease [123]. DCBs offer the advantage of better local drug uptake, theoretically less vessel distortion secondary to stenting, and might offer therapeutic benefits to lesions that are not compatible with stenting (in-stent restenosis, small vessels, and complex carinal bifurcation lesions). The DCB surface is composed of an antiproliferative drug and a carrier substance. So far, data have been most promising for coronary in-stent restenosis and have shown noninferiority to paclitaxel-eluting stents and superiority over balloon angioplasty alone [124]. DCB offers the potential to prevent adverse vessel remodeling due to repetitive stent placement. Trials comparing DCB to conventional DES have been performed for small coronary vessels [125] and bifurcation lesions [126] and have not shown clinical benefit over DES. In a recent meta-analysis of 11 randomized controlled trials, target lesion revascularization was the lowest with DCB and DES when compared with plain old balloon angioplasty [127]. DCB hold promise in treating in-stent restenosis, but current data do not support their use in *de novo* lesions.

CONCLUSIONS

Coronary angiography and PCIs are the most widely performed invasive cardiac procedures worldwide. The field of interventional cardiology has witnessed immense improvement in guiding catheters, guidewires, intracoronary stents and devices, interventional techniques, antiplatelet and anticoagulant adjunct therapies. From plain old balloon angioplasty to DCBs and bioabsorbable stents, we have come a long way in treating CAD. However, there are controversies and unresolved issues in the field that further research will attempt to answer.

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CHAPTER

17

Current Topics in Bypass Surgery

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INTRODUCTION

Coronary artery bypass grafting (CABG) is an established and highly effective method of coronary revascularization. It has remained the mainstay of treatment for patients with symptomatic multivessel coronary artery disease (CAD) despite significant improvements in medical and percutaneous therapy. The purpose of this chapter is to review relevant topics in CABG including CABG versus percutaneous coronary intervention (PCI), multiarterial grafting, perioperative stroke, minimally invasive CABG, and hybrid coronary revascularization (HCR), and off- versus on-pump CABG.

CABG VERSUS PCI

CABG continues to be one of the most common surgical procedures performed in the United States, with an estimated annual volume of 300,000 cases [1]. Both CABG and PCI have proven effective at alleviating symptoms but CABG has consistently been shown to have a survival advantage compared to PCI for patients with multivessel CAD. Numerous randomized trials and registry studies have been performed comparing PCI to CABG over the last 30 years but two randomized trials have received the most attention: SYNTAX [2] (SYNergy between PCI with TAXUS and Cardiac Surgery) and FREEDOM [3] (Future REvascularization Evaluation in patients with Diabetes mellitus: Optimal Management of multivessel disease).

In SYNTAX, a multicenter, international trial, 1800 patients with multivessel and/or left main CAD were randomly assigned to multivessel CABG or PCI after being evaluated by both an interventional cardiologist and cardiac surgeon. For the primary endpoint of major adverse cardiac and cerebrovascular events (MACCE) at 1 year, PCI failed to meet the goal of noninferiority compared to CABG, because of a higher incidence of repeat revascularization with PCI [2]. At 3 years, the rates of MACCE, repeat revascularization, and myocardial infarction (MI) were significantly increased with PCI compared to CABG [4]. After 5-years' follow-up, MACCE remained significantly higher for PCI compared to CABG (37.3% vs. 26.9%; $p < 0.0001$) [5]. Both MI (3.8% in the CABG group vs. 9.7% in the PCI group; $p < 0.0001$) and repeat revascularization (13.7% vs. 25.9%; $p < 0.0001$) were higher with PCI compared to CABG at 5 years; but, all-cause mortality (11.4% in the CABG group vs. 13.9% in the PCI group; $p = 0.10$) and stroke (3.7% vs. 2.4%; $p = 0.09$) were not significantly different between groups [5]. The advantages of CABG over PCI were most apparent in those with more complex coronary anatomy (medium to high SYNTAX scores), with higher 5 year MACCE in intermediate (25.8% vs. 36.0%; $p < 0.0001$) and high (26.8% vs. 44.0%; $p < 0.0001$) SYNTAX scores for PCI compared to CABG. Numerous post hoc subgroup analyses have been performed in this trial population, including patients with left main disease. Although these comparisons can be utilized to guide real world practice decisions, the results must be taken in context. The SYNTAX trial was originally designed and powered for the original MACCE endpoint and was a noninferiority design. PCI failed to meet the noninferiority endpoint. Thus, the results from all of the post hoc analyses should be considered hypothesis generating and not definitive. Important conclusions from this trial include: (i) CABG should be considered the standard therapy for all symptomatic patients that present with multivessel CAD that warrant revascularization; (ii) the benefits from CABG are most apparent in those with complex CAD determined by the SYNTAX score; and (iii) low SYNTAX score patients, or those with less complex coronary anatomy may be considered for multivessel PCI. Although critics of this trial

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00017-6

criticize the use of first generation stents, it is important to concede that there is no definitive data, in contrast with CABG, that supports the superiority of PCI over medical therapy for mortality in patients with stable angina [6,7]. However, support for this argument comes from an individual patient-data meta-analysis comparing 4989 patients randomized either to a new-generation DES versus early-generation DES, showing lower rates of death (3.2% vs. 5.1%), cardiac death or MI (4.4% vs. 6.3%) and stent thrombosis (0.7% vs. 1.7%) after 3 years of follow-up in those treated with newer-generation DES [8].

The second large multicenter randomized control trial that warrants discussion is FREEDOM [3]. In this trial, 1900 patients with diabetes and multivessel CAD were enrolled in 140 international sites to CABG or multivessel PCI with DES. The primary outcome was a composite of all-cause mortality, nonfatal MI, and nonfatal stroke at 5 years. The primary outcome occurred more frequently in the PCI group with 5-year rates of 26.6% in the PCI group and 18.7% in the CABG group ($p = 0.005$). The benefit of CABG was driven by differences in rates of both MI ($p < 0.001$) and death from any cause ($p = 0.049$) even though this study was powered on the composite endpoint and not all-cause mortality. This definitive study confirmed that CABG should be the first-line treatment for patients with diabetes and multivessel CAD.

Recently published randomized data comparing everolimus-eluting stents versus CABG confirm the results from the other large randomized trials that there is a survival advantage for patients with multivessel disease treated with CABG [9]. In this study, patients with multivessel CAD were randomized to CABG versus PCI with everolimus-eluting stents. At a median follow-up of 4.6 years, the composite endpoint of death, MI, or repeat revascularization occurred more frequently with PCI versus CABG (17.0% vs. 11.7%; $p = 0.04$), even with the latest generation stent technology.

There are several explanations for the advantages of CABG over PCI in patients with multivessel CAD. One is the superiority of arterial grafting using the left internal mammary artery (LIMA) to bypass the LAD which largely accounts for the survival advantage with CABG [10]. This is attributable to the superior long-term patency of the LIMA compared to either stents or saphenous vein grafts [11]. Furthermore, with CABG, a vessel targeted strategy is utilized over a lesion targeted strategy. Bypass grafting typically treats the proximal 2/3 of the native coronary artery such that the vessel in addition to the lesion is treated. This addresses not only the lesion responsible for ischemia but any future disease burden that may develop. This is in contrast to a lesion treatment strategy that occurs with PCI, and this advantage of CABG will persist even with lower in-stent restenosis rates associated with newer generation stent technology, as seen with the BEST trial. Another advantage seen with CABG is that patients are more likely to undergo complete revascularization with CABG compared to PCI [12], and that incomplete revascularization is associated with a higher risk of mortality, MI, and repeat revascularization [12,13]. Finally, graft failure results in different clinical sequelae compared to stent thrombosis. Although stent thrombosis rates have improved with newer generation stent technology and medical therapy, stent thrombosis usually results in vessel occlusion and distal ischemia; whereas graft occlusion does not always result in ischemic events. Because bypass grafts are usually sewn onto the distal third of the coronary artery, clinical events related to bypass graft occlusion usually involve less myocardium. The impact of stent thrombosis compared to graft occlusion has been shown to have a negative impact on survival compared to graft occlusion in the 5-year results from SYNTAX [13,14].

Observational comparative effectiveness trials have generally supported the conclusions rendered by these two randomized trials. Weintraub and colleagues compared 86,244 patients undergoing CABG to 103,549 patients undergoing PCI (2004–2008) in patients 65 years of age or older with two- or three-vessel CAD presenting without acute MI [15]. Although there was no difference observed in all-cause mortality at 1 year, there was a significantly lower mortality with CABG compared to PCI at 4 years (16.4% vs. 20.8%; risk ratio, 0.79; 95% CI, 0.76–0.82). However, this study may not accurately reflect contemporary practice with PCI using advanced drug-eluting stent technology or current techniques with CABG including multiarterial grafting strategies. Similarly, a recent meta-analysis of randomized trials comparing CABG versus PCI found a mortality advantage with CABG across all types of patients, including those with multivessel CAD as well as diabetes [16]. Repeat revascularization declined from the prestent era to the drug-eluting stent era. Considerable attention has been given to the development of second- and third-generation stents that promise to further reduce in-stent restenosis and stent thrombosis. It has been suggested that these improvements may further narrow the gap in mortality, MI, and repeat revascularization seen in the previous randomized trials versus CABG. In an observational analysis, Bangalore and coworkers compared patients with multivessel CAD treated with CABG (9223) to patients undergoing PCI with everolimus-eluting stents (9223) [17]. Although follow-up was relatively short (2.9 years), all-cause mortality was similar in both groups (3.1% per year and 2.9% per year, respectively; hazard ratio, 1.04; 95% CI, 0.93–1.17; $p = 0.50$). However, the randomized trial comparing everolimus-eluting stents with CABG revealed that the advantages of CABG did not emerge until longer follow-up was obtained (4.6 years) [9]. Thus, the long-term benefits of CABG for patients with multivessel CAD persist despite the use of second generation drug-eluting stents.

Although historically delegated to surgical revascularization, there has been increased interest in PCI for treatment of left main CAD. In SYNTAX, subgroup analysis of left main disease suggested equivalent outcomes in the primary endpoint (MACCE) at 12 months for CABG and PCI (13.7% and 15.8%, respectively; $p = 0.44$) [2]. Although this suggests equipoise regarding the most effective treatment strategy for patients with isolated left main disease or left main disease plus two- or three-vessel CAD, the statistical design was a noninferiority assessment of all patients in the trial. Thus, the subgroup analyses are observational in nature and hypothesis generating. In a relatively small randomized controlled trial, Park and colleagues compared PCI versus CABG for patients with left main disease [18]. Although the results suggested noninferiority at 1 year between treatment strategies, the noninferiority margin was wide and follow-up short which limited the generalizability of the results. Fortunately, data from the EXCEL trial comparing PCI to CABG in patients with left main CAD will provide much needed clinical data to guide appropriate revascularization strategies for patients with left main disease.

CABG has been definitively shown to be a highly effective revascularization modality for patients with multivessel and left main CAD with a survival advantage compared to PCI. Although significant advances in PCI have enabled more patients to be treated effectively with this strategy, significant advances have also occurred with surgical revascularization, including but not limited to multiarterial grafting strategies, off-pump CABG, improved perioperative care, and advanced myocardial preservation strategies. Current guidelines by the American Heart Association and the American College of Cardiology [19], and the European Society of Cardiology and European Association for Cardio-thoracic Surgery [20] are summarized in Tables 17.1 and 17.2.

TABLE 17.1 Summary of AHA/ACC Guidelines for Revascularization to Improve Survival Compared to Medical Therapy [19]

CLASS I INDICATIONS	
CABG	*PCI*
Unprotected left main disease	Survivors of sudden cardiac death with presumed ischemia-induced VT
3-vessel CAD with or without proximal LAD disease	
2-vessel CAD with proximal LAD disease	
Recommended in preference to PCI in patients with diabetes and multivessel CAD	
Survivors of sudden cardiac death with presumed ischemia-induced VT	
CLASS IIA INDICATIONS	
CABG	*PCI*
CABG over PCI for patients with 3-vessel CAD (SYNTAX > 22) who are reasonable candidates for CABG	Unprotected LM disease for patients with SIHD with low risk of PCI procedural complications (SYNTAX ≤ 22, ostial or trunk), high likelihood of good long-term outcome, and high-risk surgical candidate (STS PROM ≥ 5%)
2-vessel CAD without proximal LAD disease with extensive ischemia	Unprotected LM disease for patients with UA/NSTEMI if not a CABG candidate
1-vessel proximal LAD disease using LIMA	Unprotected LM disease for patients with STEMI with < TIMI 3 flow when PCI can be performed more rapidly and safely than CABG
EF 35–50%	
CLASS IIB INDICATIONS	
CABG	*PCI*
EF <35% without significant LM disease	Unprotected LM disease for patients with SIHD with low-intermediate risk of PCI procedural complications (SYNTAX < 33, bifurcation LM) with increased surgical risk (STS PROM > 2%)
	3-vessel CAD with or without proximal LAD disease
	2-vessel CAD with proximal LAD disease
	2-vessel CAD without proximal LAD disease
	1-vessel proximal LAD disease

TABLE 17.2 Summary of European Society of Cardiology and European Association for Cardio-Thoracic Surgery Guidelines [20]

	CABG		PCI	
	Rec	**LOA**	**Rec**	**LOA**
1-2VD without proximal-LAD	IIb	C	I	C
Proximal-LAD	I	A	I	A
2VD with proximal-LAD	I	B	I	C
ULMD with SYNTAX ≤ 22	I	B	I	B
ULMD with SYNTAX 23–32	I	B	IIa	B
ULMD with SYNTAX > 32	I	B	III	B
3-VD with SYNTAX ≤ 22	I	A	I	B
3-VD with SYNTAX 23–32	I	A	III	B
3-VD with SYNTAX > 32	I	A	III	B
DIABETES MELLITUS				
SYNTAX ≤ 22	I	A	IIa	B
2VD with proximal-LAD	I	A	–	–
3VD	I	A	–	–

VD, vessel disease; LAD, left anterior descending coronary artery; LOA, level of evidence; Rec, recommendation; ULMD, unprotected left main disease.

MULTIARTERIAL GRAFTING

Despite the documented benefits of CABG for patients with multivessel CAD, one of the main limitations is the failure rate of saphenous vein grafts (SVGs). The improved survival and symptom relief with CABG has been largely attributed to the superior long-term patency of the LIMA-LAD graft. Short- and long-term failure of SVGs has been well described [21–23], most notably in PREVENT-IV [24]. Despite these shortcomings, most American surgeons continue to perform CABG procedures with a single IMA to the LAD and SVGs for non-LAD targets with fewer than 10% of CABG in the United States involving > 1 arterial graft [25]. The barriers to adoption are understandable. In the era of public reporting, surgeons are "graded" on 30-day outcomes, not long-term survival. For surgeons considering bilateral internal mammary artery (BIMA) grafting, this increased risk of deep sternal wound infection, especially in an increasingly obese and diabetic population, is a major concern. Mediastinitis, one of the key Society of Thoracic Surgeons quality metrics used in public reporting, is no longer reimbursed by the Centers for Medicare and Medicaid Services, and the surgeon bears full responsibility for these events, both from the hospital as well as public reporting forums. Both BIMA and radial artery harvest frequently require additional operative time and personnel and remuneration may not be commensurate with the additional skill and effort required. Furthermore, it is often unclear which patients and what coronary targets may best be suited for additional arterial grafts. Thus, significant barriers exist that may dissuade surgeons from making a relatively straightforward case more complex.

Nevertheless, there is a resurgence of interest at the national and international level for surgeons to perform more arterial grafts. Most of the data in support of this comes from observational analyses. In all of these analyses, complex statistical methods such as propensity matching have been used to balance comparisons between patients with single versus multiple arterial grafting strategies; and in almost all of these comparisons, data is harvested from large registries such as The Society of Thoracic Surgeons National Database. Frequently missing from these registries are details about coronary lesions and target anatomy (% stenosis, target quality, and size, etc.) as well as patient-related variables such as frailty. Nonetheless, the general consensus from these studies is that long-term survival may be enhanced in carefully selected patient populations receiving additional arterial grafts. Locker and coworkers showed that in propensity score-matched groups, patients undergoing multiarterial revascularization had estimated 15-year survival rates of 70% compared to 60% for patients undergoing a single IMA (SIMA) plus SVG (hazard ratio, 0.73; 95% CI, 0.59–0.90; $p = 0.003$) [26]. Dorman and colleagues utilized propensity score matching to compare median survival for patients undergoing BIMA grafting compared to SIMA for multivessel disease (follow-up 6 weeks to 30 years, median 8.9 years) [27]. The median survival for SIMA patients was 9.8 years (95% CI, 8.6–10.5) compared with 13.1 years (95% CI, 12.2–13.9) for BIMA patients ($p < 0.001$). In an updated meta-analysis of published studies comparing BIMA versus SIMA, the BIMA group demonstrated significantly better long-term survival than the LIMA group (hazard ratio, 0.78; CI, 0.72–0.84; $p < 0.0001$) [28]. Using multiple logistic regression analysis in diabetics undergoing CABG, Raza and coworkers found that BIMA versus SIMA grafting was associated with a 21% lower late mortality (68% confidence limits, 16–26%) [29]. The incidence of deep sternal wound infection was significantly higher after BIMA versus SIMA grafting (3.4% vs. 2.1%, $p = 0.01$) which persisted after multivariable adjustment ($p = 0.002$). The conclusion was that BIMA grafting and complete revascularization provided the optimal revascularization strategy to maximize long-term survival in diabetic patients, but the authors urged careful patient selection. Buxton and associates compared patients undergoing a LIMA + SVG strategy to those undergoing revascularization with only arterial conduits [30]. After statistical modeling, total arterial

revascularization yielded a superior survival advantage on Cox proportional hazard analysis with a Hazard Ratio of 0.79 (95% CI 0.70–0.90; $p < 0.001$) [30]. In 384 propensity-matched pairs, total arterial revascularization patients had improved 15-year survival compared to single IMA patients ($54 \pm 3.3\%$ vs. 41 ± 3.0, $p = 0.0004$). However, as in previously mentioned reports, selection bias probably had some impact on these results as one can question why patients at centers with a bias towards all arterial revascularization had such a large number of patients that received only one arterial graft.

Superior patency of either arterial conduit compared to SVG has also been demonstrated. In a multicenter randomized study comparing RA versus SVG patency (Radial Artery Patency Study, RAPS), the RA was randomized to be grafted to either the circumflex or the right coronary artery, with the study SVG used for the other territory [31]. The frequency of functional graft occlusion, defined as lack of Thrombolysis In Myocardial Infarction flow grade 3, was lower in radial arteries compared with SVG (28 of 234 (12.0%) vs. 46 of 234 (19.7%), $p = 0.03$). The frequency of complete graft occlusion was also significantly lower in RA compared with SVG (24 of 269 (8.9%) vs. 50 of 269 (18.6%), $p = 0.002$). Notably, the patency superiority of the RA occurred primarily in targets with at least 90% proximal stenosis, with relative equipoise between RA and SVG in targets with 75–90% stenosis [31]. In a sub-study of RAPS, Deb and colleagues examined 5-year patency of RA versus SVG in diabetic patients less than 80 years old [32]. The within-patient randomization protocol, with the RA randomized to either the circumflex or right coronary territory (both with at least 75% proximal stenosis) and SVG utilized to the remaining territory allowed patients to serve as their own control. The proportion of complete graft occlusion was significantly lower in the RA 4/83 (4.8%) versus saphenous grafts 21/83 (25.3%), $p = 0.0004$. Cumulative patency rates for RA grafts, irrespective of diabetes status, were similar for diabetics and nondiabetic whereas cumulative patency rates of SVG were worse for diabetic patients suggesting the improved durability of the RA compared to SVG in this subset of patients. Transbaugh and colleagues, in a propensity matched comparison, concluded that there was no major patency benefit with the RIMA compared to radial artery when used to bypass the circumflex, and that radial artery use was associated with few in-hospital adverse events [33]. However, not all reports have been as supportive of a multiarterial grafting strategy. The single center Radial Artery Patency and Clinical Outcomes randomized trial (RAPCO) showed no significant difference in clinical outcome or graft patency comparing the RA to SVG in patients older than 70 [34].

What is needed is a definitive large randomized trial with long-term follow-up to address the potential survival advantage of additional arterial grafts. Long-term results of RAPCO will elucidate differences between RIMA and RA grafting in patients less than 70 years old and SVG compared to RA grafting in patients greater than 70 years old [34]. The 10-year multicenter Arterial Revascularization Trial (ART) of Taggart et al. has completed enrollment with 10-year data expected in 2018. This will help clarify the benefit of BIMA grafting compared to SIMA grafting to the left coronary circulation [35]. It will also provide comparative effectiveness data of a LIMA plus radial artery strategy in a subgroup of patients. In their 1-year results, Taggart et al. reported comparable short-term outcomes in terms of survival, MI, stroke, and repeat revascularization [35]. Importantly, the incidence of sternal wound complications requiring reconstruction was once again shown to be significantly higher in the BIMA group, 1.9% versus 0.6%.

Some general conclusions and recommendations can be made regarding additional arterial grafts during CABG until more definitive trial data becomes available:

1. The benefits of additional arterial grafts appear most pronounced when used to graft the second best left-sided target. The benefit of right coronary grafting with arterial grafts is less clear.
2. Either the radial artery or an additional internal mammary artery (IMA) is an effective second arterial conduit to graft the second best left-sided target.
3. Radial artery use should be reserved for target vessels with at least 80% and preferably >90% stenosis to minimize complications associated with graft vasospasm and competitive flow.
4. The right internal mammary can be used as an in situ graft for the LAD, ramus intermedius, or high obtuse marginal if there is adequate length. Conversely, it can also be effectively used as a free graft.
5. BIMA harvesting is associated with a slightly albeit significantly higher risk of sternal wound complications, especially in diabetic patients. Other risk categories that may be associated with higher sternal complications include morbid obesity, immunosuppression, and chronic lung disease.
6. Additional arterial grafts should be reserved for patients with an anticipated life expectancy of 10 years.
7. In appropriately selected patients, the use of a second arterial graft to an additional left-sided coronary target will likely result in a long-term (>10 years) survival advantage, lower incidence of repeat revascularization, and freedom from recurrent symptoms due to a lower incidence of graft failure with arterial grafts.

OFF-PUMP VERSUS ON-PUMP CABG

Despite an abundance of literature comparing on-pump and off-pump CABG (OPCAB) surgery, the optimal surgical strategy remains controversial. OPCAB is a more technically challenging procedure and has been criticized for concern over completeness of revascularization and graft patency, however, the technique has been associated with lower requirements for blood transfusion and fewer neurologic and renal complications. It is widely accepted that OPCAB is beneficial in certain clinical scenarios, such as severe aortic atherosclerosis which increases the risk of atheroembolism with aortic clamping. In the current era dominated by the development of minimally invasive procedures, off-pump CABG plays a key role in facilitating new technologies in coronary revascularization.

ROOBY was the first large-scale multicenter RCT comparing OPCAB versus on-pump CABG in Veteran Affairs centers [36]. ROOBY compared OPCAB versus on-pump CABG in 2203 patients at Veteran Affairs Centers. There was no difference in the short-term primary composite endpoint of death or major complications before discharge or within 30 days between OPCAB and on-pump CABG (7.0% vs. 5.6%, $p = 0.19$) [36]. However, OPCAB was associated with higher composite endpoint of mortality, MI, and repeat revascularization at 1 year (9.9% vs. 7.4%, $p = 0.04$). Criticisms of insufficient surgeon experience with OPCAB [37] and the low-risk patient population prompted two large RCTs, the CABG Off- or On-Pump Revascularization Study [38] (CORONARY) and the German Off-Pump CABG in Elderly (GOPCABE) Trial [39]. The CORONARY study randomized 4752 high-risk patients to OPCAB versus on-pump CABG [38]. The co-primary endpoints were death, stroke, MI or dialysis at 30 days and 1 year, and death, stroke, MI, dialysis, repeat revascularization at 5 years. Surgeons were required to have >2 years' experience after residency training and >100 cases of OPCAB, trainees could not be the primary surgeon, and the majority of patients had additive EuroSCORE 3–5, thus being high risk. This trial also did not find differences in primary endpoints of mortality, MI, and stroke at 30 days [38] (9.8% vs. 10.3%, $p = 0.59$) and at 1 year [40] (12.1 vs. 13.3, $p = 0.24$). However, there was a trend towards benefit with OPCAB for patients in higher EuroSCORE. Similarly, GOPCABE was a multicenter randomized trial of 2539 high-risk patients (mean logistic EuroSCORE 8.3) ≥ 75 years of age comparing OPCAB versus on-pump CABG [39]. Importantly, OPCAB was "routinely performed at all participating centers" and surgeons had an average 514 cases and median 322 cases with OPCAB. There was no difference in the primary endpoint, a composite risk of death, stroke, MI, repeat revascularization, or new renal replacement therapy at 30 days (7.8% vs. 8.2%, $p = 0.74$) and at 1 year (13.1% vs. 14.0%, $p = 0.48$) [39].

Several registry studies powered by their large sample sizes were able to detect significant differences in adverse outcomes among a broad population of patients. In a study by Hannan et al. [41], 49,830 patients from the New York state registry underwent risk-adjusted analysis (Cox proportional hazard models and propensity analysis) comparing outcomes after OPCAB versus on-pump CABG. In this study, OPCAB patients had significantly lower 30-day mortality (OR 0.81; 95% CI 0.68–0.97; $p = 0.0022$) as well as a lower incidence of postoperative stroke (OR 0.70; 95% CI 0.57–0.86; $p = 0.0006$). However, the mechanisms responsible for the observed reduction in postoperative stroke were not defined. In a large registry study of California CABG outcomes, Li and colleagues also demonstrated a significant reduction in propensity-adjusted operative mortality with OPCAB compared with on-pump CABG (2.59% 95% CI 2.52–2.67% vs. 3.22%, 95% CI 3.17–3.27%) [42]. An intention-to-treat retrospective analysis of 42,477 patients from the Society of Thoracic Surgeons National Database showed a reduction in risk-adjusted operative mortality (adjusted OR 0.83, $p = 0.03$) as well as numerous morbidity outcomes favoring patients undergoing OPCAB [43].

With the lack of randomized trial data demonstrating the superiority of one approach over another, there does not appear to be a short- or long-term mortality advantage with OPCAB. However, most surgeons would agree that in certain high-risk subgroups of patients, an off-pump approach may lower the risk of specific complications associated with extracorporeal circulation and aortic manipulation, specifically postoperative stroke.

POSTOPERATIVE STROKE

Patients undergoing CABG with ascending aortic atheromatous disease are known to carry increased risk of death, stroke, and major adverse cardiac events [44–46]. Published reports indicate that up to 75% of all strokes following CABG are embolic and early (≤24 h postop) in nature [47]; therefore, it becomes critical to make every effort to reduce the risk of intraoperative cerebral embolic events. Emboli can arise from intraoperative manipulation of the aorta during (i) aortic cannulation, (ii) institution and maintenance of cardiopulmonary bypass, or (iii) application and removal of the aortic cross-clamp for cardioplegic arrest (Figure 17.1), or with partial aortic clamping (Figure 17.2) for proximal anastomoses. During on-pump CABG, most surgeons in the United States prefer to use a cross-clamp for cardioplegic arrest, followed by removal of the cross-clamp and application of a partial-occluding clamp to perform proximal anastomoses.

Long-term survival of post-CABG stroke patients is negatively impacted with reductions in 1- and 5-year

survival to 66% and 44% compared to 94% and 81% without stroke [48]. The incremental cost of a postoperative stroke has been reported to be an additional $19,000 to the health system when there are no other associated complications and greater than $58,000 when combined with two or more other complications [49]. The effect of stroke on ultimate patient recovery and quality of life is immeasurable since these patients frequently require prolonged supportive care at long-term rehabilitation facilities.

Although SYNTAX and FREEDOM demonstrated significant advantages in favor of CABG, both trials showed significantly higher stroke rates for CABG compared to PCI. In SYNTAX at 1 year, stroke occurred significantly more often after CABG than after PCI (2.2% vs. 0.6%, $p = 0.003$) [2]. In FREEDOM, the incidence of stroke at 5 years was 5.4% in the CABG group compared to 2.4% in PCI ($p = 0.03$). These trials, although favorable for surgical revascularization, charged the surgical community to reduce the incidence of this devastating complication. In BEST, there was no significant difference in the incidence of postoperative stroke between CABG and PCI (2.9% vs. 2.5%, $p = 0.72$) at long-term follow-up. The authors hypothesized that the greater use of off-pump techniques and multiarterial grafts with less aortic manipulation may have played a role in these findings.

Despite convincing data from the previously mentioned randomized trials that failed to show a reduction in stroke with OPCAB compared to on-pump CABG, these trials remained underpowered to show a statistical difference in postoperative stroke. Furthermore, more evidence is emerging that the majority of postoperative strokes are embolic and related to aortic manipulation. Importantly, the randomized trials comparing on-pump CABG to OPCAB did not specify the technique for construction of proximal anastomoses; thus patients in the OPCAB groups may have undergone partial clamping, which is routinely performed during OPCAB to construct aortocoronary proximal anastomoses. In a study by Moss and associates [50], the authors aimed to determine whether minimizing aortic manipulation resulted in a clinically relevant reduction in postoperative stroke. The overall incidence of postoperative stroke was 1.4%, with an unadjusted incidence of 0.6% in the no-touch group, 1.2% in the clampless facilitating device group (Figure 17.3), and 1.5% in the clamp group ($p < 0.01$ for no-touch vs. clamp) [50]. The ratio of observed to expected stroke rate increased as the degree of aortic manipulation increased, from 0.48 in the no-touch group, to 0.61 in the clampless facilitating device group, and to 0.95 in the clamp group. Aortic clamping was independently associated with an increase in postoperative stroke compared with a no-touch technique (adjusted odds ratio, 2.50; $p < 0.01$). Both the off-pump partial clamp and the

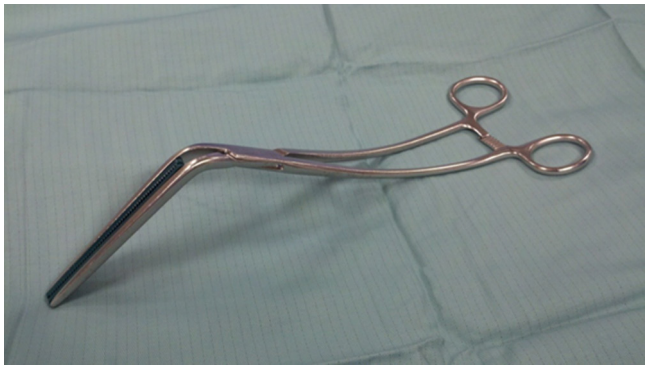

FIGURE 17.1 Aortic cross-clamp used to isolate the heart from systemic perfusion and deliver cardioplegic solution to achieve cardiac arrest.

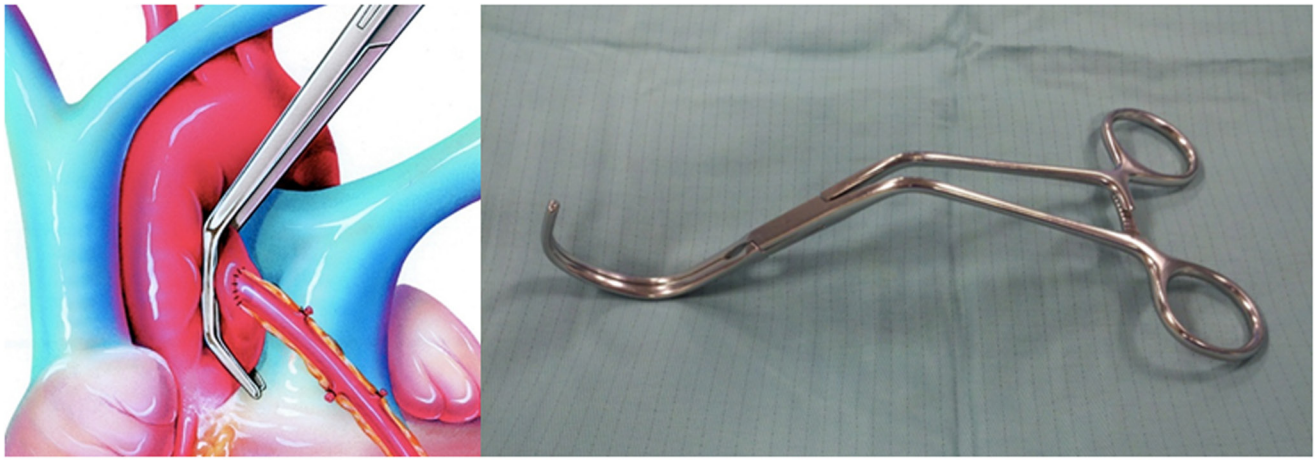

FIGURE 17.2 Partial-occluding clamp used to isolate a portion of the ascending aorta to allow for construction of aortocoronary proximal anastomoses without completely occluding the aorta. The partial clamp can be used during on- or off-pump coronary artery bypass.

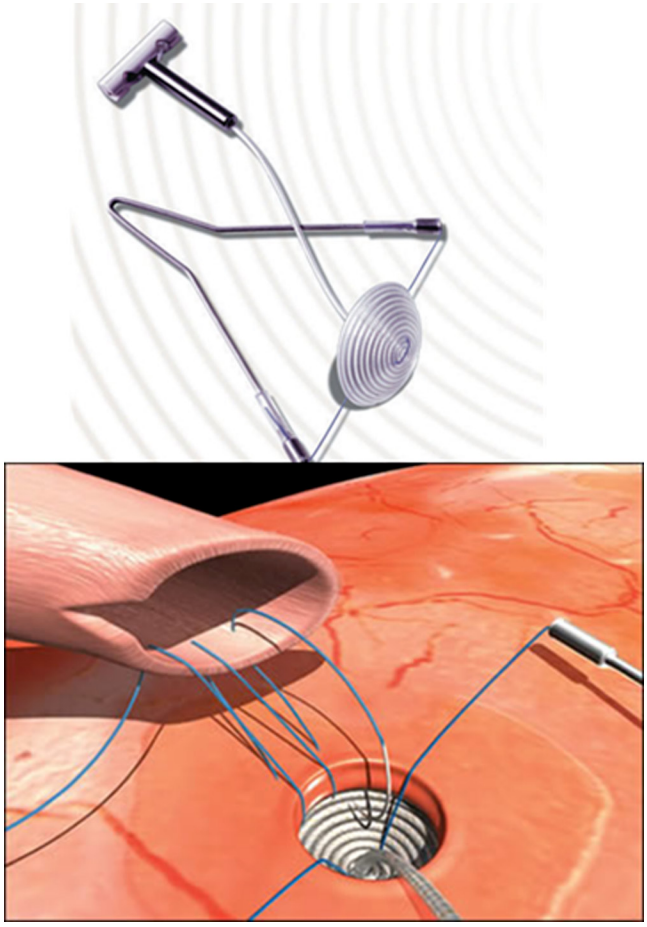

FIGURE 17.3 A clampless facilitating device, such as the Heartstring proximal anastomosis device (Maquet Cardiovascular, Rastatt, Germany) enables construction of aortocoronary proximal anastomoses without clamping the aorta.

on-pump cross-clamp techniques increased the risk of postoperative stroke compared with no-touch (adjusted odds ratio, 2.52, $p < 0.01$; and adjusted odds ratio, 4.25, $p < 0.001$, respectively). Kim and colleagues reported a lower incidence of postoperative stroke in patients undergoing OPCAB without any manipulation of the aorta compared with patients undergoing OPCAB with partial clamping and patients undergoing on-pump CABG with aortic clamping [51]. This theory is in line with data using transcranial Doppler signals to identify cerebral emboli [52–54]. Emmert and colleagues reported on a group of 4314 patients who underwent on-pump CABG or OPCAB [55]. Within the OPCAB group they compared aortic partial clamping to clampless facilitating devices (devices which do not require clamping to construct proximal anastomoses) (Figure 17.3). In this series, the off-pump group had a significantly lower incidence of stroke than the on-pump group (1.1% vs. 2.4%, $p < 0.005$). Within the off-pump group, the CFD patients had a significantly lower incidence of stroke than the partial clamp group (0.7% vs. 2.3%, $p = 0.4$), and was similar to the no-touch group.

Each maneuver to minimize aortic manipulation comes at the expense of the relative simplicity of performing coronary surgery with cardiopulmonary bypass on an arrested heart with a cross-clamped aorta. Even with OPCAB, it is relatively easier to place a partial clamp to perform proximal aortocoronary anastomoses than to use clampless facilitating devices or use *in situ* arterial grafts as inflow for other bypass grafts. Thus, surgeons have been reluctant to broadly adopt these different revascularization techniques without clear trial data to support them due to the greater technical challenges involved. These concerns have been reinforced by the randomized trials comparing off- versus on-pump CABG which have demonstrated a slightly but statistically higher risk of repeat revascularization.

MINIMALLY INVASIVE CABG AND HCR

As sternal-sparing approaches for CABG have evolved, different techniques have been adopted for minimally invasive CABG (Table 17.3). This includes minimally invasive direct coronary artery bypass (MIDCAB), endoscopic atraumatic coronary artery bypass (ENDOACAB), robotic-assisted CABG (Figure 17.4), and robotic totally endoscopic coronary artery bypass (TECAB). Although the majority of cases involve single-vessel grafting utilizing the LIMA to the left anterior descending coronary artery (LAD), multivessel grafting is also well described and performed in experienced centers.

The primary goal of minimally invasive CABG procedures is to capitalize on the major benefit of CABG, the LIMA-LAD graft, and to minimize the morbidity associated with traditional sternotomy CABG including sternal complications, postoperative stroke, prolonged recovery, and bleeding. With all of the different techniques described above, patients receive a LIMA-LAD graft as either sole therapy for single vessel disease, undergo multivessel grafting, or undergo minimally invasive LIMA-LAD grafting as part of a HCR procedure. Patients' desire to avoid a sternotomy (Figure 17.5) but maintain the survival advantage and durability of LIMA-LAD grafting have fueled these approaches which have been enabled by significant technological advances. We have previously reported equivalent 30-day and midterm results in a propensity-matched group of 597 patients undergoing nonsternotomy versus sternotomy CABG [56]. Holzhey and colleagues reported a series of 1768 patients who underwent MIDCAB with low operative mortality (0.8%), low stroke rate (0.4%), and

TABLE 17.3 Summary of Varying Approaches for Minimally Invasive Coronary Artery Bypass Grafting

Approach	Incisions	LIMA harvest	Exposure for anastomosis	Construction of anastomosis	Complexity	Advantages	Limitations
MIDCAB	Single left anterolateral thoracotomy incision (5–8 cm)	Direct visualization facilitated with specially designed retractor system to elevate anterior chest wall	Via thoracotomy incision	Manual	Low	Inexpensive, short OR time, larger incision allows for access to multivessel grafting and possible exposure to aorta	Postthoracotomy pain from chest wall retraction, possible incomplete LIMA harvest
ENDOACAB	Three left-sided port incisions, separate 3–4 cm microthoracotomy incision for anastomosis	Thoracoscopic	Through 3–4 cm microthoracotomy incision	Manual	Medium	Inexpensive, rib-sparing	LIMA harvest difficult thoracoscopically because of 2-D instruments, access adequate for LAD and/or diagonal grafting only
Robotic-assisted CABG	Three left-sided port incisions, separate 3–4 cm microthoracotomy incision for anastomosis	Robotic	Through 3–4 cm microthoracotomy incision	Manual	Medium	3-D visualization and instrumentation during LIMA harvest, rib-sparing	Expensive, access adequate for LAD and/or diagonal grafting only
TECAB	Three left-sided port incisions and one subcostal port incision for endostabilizer	Robotic	Totally endoscopic	Robotic	High	Rib-sparing, allows exposure and access to entire LAD and option for multivessel grafting	High complexity, expensive, relies on peripheral cannulation for CPB support

CABG, coronary artery bypass grafting; ENDOACAB, endoscopic atraumatic coronary artery bypass; LAD, left anterior descending coronary artery; LIMA, left internal mammary artery; OR, operating room; TECAB, totally endoscopic coronary artery bypass.

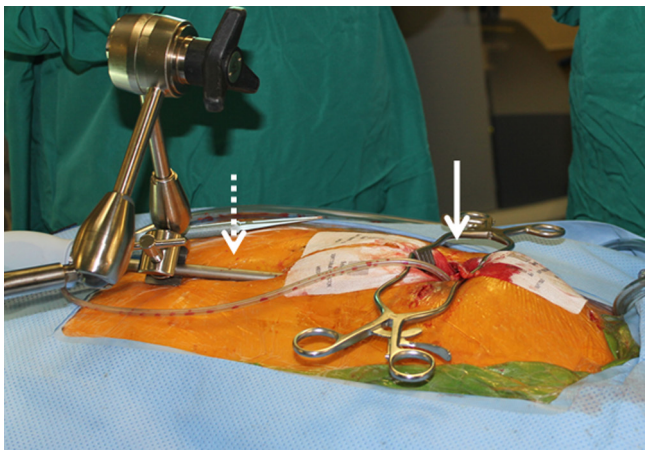

FIGURE 17.4 Example of operative setup commonly used for a robotic-assisted CABG procedure or minimally invasive direct coronary artery bypass procedure. Dashed arrow shows stabilizer arm inserted through separate incision to stabilize left anterior descending coronary artery. Solid arrow shows mini-thoracotomy incision through which the anastomosis is performed.

excellent long-term results (85.3% and 70; 9% 5- and 10-year freedom from MACCE) [57,58]. Compared to PCI, MIDCAB has consistently shown decreased rates of target vessel revascularization and no difference in other major cardiac events at 1-year follow-up [11].

We reported excellent results in a series of 307 patients who underwent robotic-assisted CABG, consisting of robotic LIMA harvest and LIMA-LAD anastomosis via a 3–4 cm anterior minithoracotomy. Operative mortality was 1.3%, LIMA patency 97%, and MACCE occurred in 3.3% of patients [59]. Robotic-assisted CAB has also shown a benefit in hospital length of stay, return to full activity, and pain scores when compared to conventional CABG [60]. Data from centers that have adopted TECAB has been favorable as well. In 2006, a multicenter trial of 98 patients reported a low rate of conversion to sternotomy (6%), a low MACCE rate, and freedom from reintervention or angiographic failure of 91% [61]. Since that time, high volume TECAB centers have consistently shown good clinical results [62].

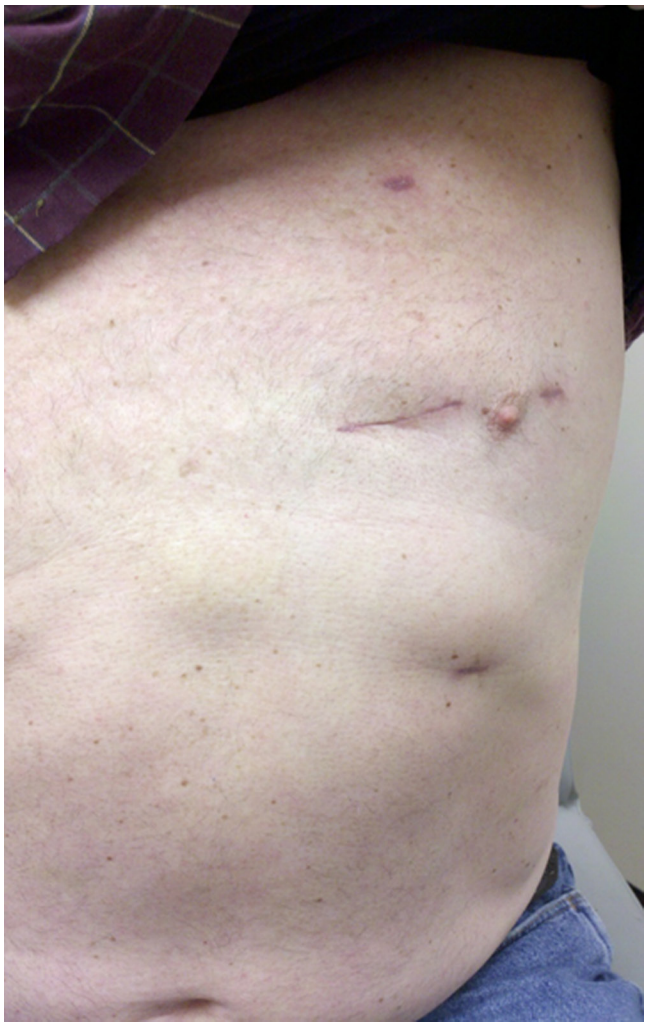

FIGURE 17.5 Postoperative appearance of incisions for robotic-assisted CABG.

HCR is defined as the planned application of both surgical and interventional techniques to treat patients with multivessel coronary disease. A surgical procedure including an internal mammary artery (IMA) graft to the left anterior descending coronary artery (LAD), typically with a minimally invasive approach, is combined with PCI to non-LAD targets (Figure 17.6). This approach capitalizes on the major strengths associated with each procedure and also eliminates the major drawbacks; for CABG, the failure rate of SVGs and for PCI, the higher repeat revascularization rates associated with proximal LAD intervention. A failure rate of approximately 20–50% associated with SVGs to non-LAD targets calls into question the best method to treat non-LAD disease [24,63]. The main limitation with PCI is the higher rates of target vessel revascularization when applied to the proximal LAD [64,65]. Therefore, the clinical utility of a hybrid approach for the treatment of coronary disease must be considered in the context of what this new approach can offer compared to currently available traditional therapy. With HCR, the LAD is treated with the best available therapy (LIMA-LAD), most commonly with a sternal-sparing approach, which makes the durability and efficacy of LIMA-LAD grafting an attractive alternative to either LAD PCI or traditional CABG via sternotomy. The circumflex and right coronary territories are treated percutaneously which may be as effective as SVGs in appropriate selected patients, when multiarterial grafting is not an option.

For HCR procedures to be adopted by cardiologists and surgeons, the following goals will need to be achieved: (i) low in-hospital morbidity and mortality which compares to or even surpasses traditional CABG; (ii) excellent short- and long-term LIMA-LAD patency rates that can be achieved with sternal sparing, minimally invasive approaches; (iii) reduced hospital resource utilization compared to traditional CABG which can be achieved by lower ventilation times, reduced intensive care requirements, lower transfusion rates, more frequent discharge to home versus subacute chronic care, and shorter overall hospitalization times despite increased procedural costs associated with the combination of two procedures; (iv) shorter postdischarge recovery times which allow patients to return to work and resume normal activity sooner than possible with sternotomy; (v) a lower incidence of repeat revascularization compared to multivessel PCI; (vi) higher patient satisfaction with HCR compared to either multivessel PCI or traditional CABG; and (vii) short- and long-term cost-effectiveness comparable to CABG or PCI. While the nuances of HCR, which include staged versus simultaneous procedures, antiplatelet regimens, utilization of hybrid operating rooms, and the use of drug-eluting or bare metal stents, are important, whether HCR proves to be a durable, safe, and ubiquitous treatment for coronary disease will largely depend on the outcomes of the minimally invasive surgical techniques. Demonstrating these results will ultimately require well-designed comparative effectiveness trials. In the absence of these data, HCR should be considered an alternative treatment strategy that should be tailored to the individual patient based on coronary anatomy and patient-related variables.

There have been several single-institution series recently describing larger experiences with modern minimally invasive hybrid revascularization [66–73]. They have documented excellent early LIMA patency rates, low mortality rates, and a low incidence of target vessel repeat revascularization. Comparisons of HCR with CABG have also shown favorable clinical outcomes in different subsets of patients [74–76]. The main limitations with most of the currently available HCR series to date are their retrospective design, relatively small sample size, and single institutional experience. Nonetheless,

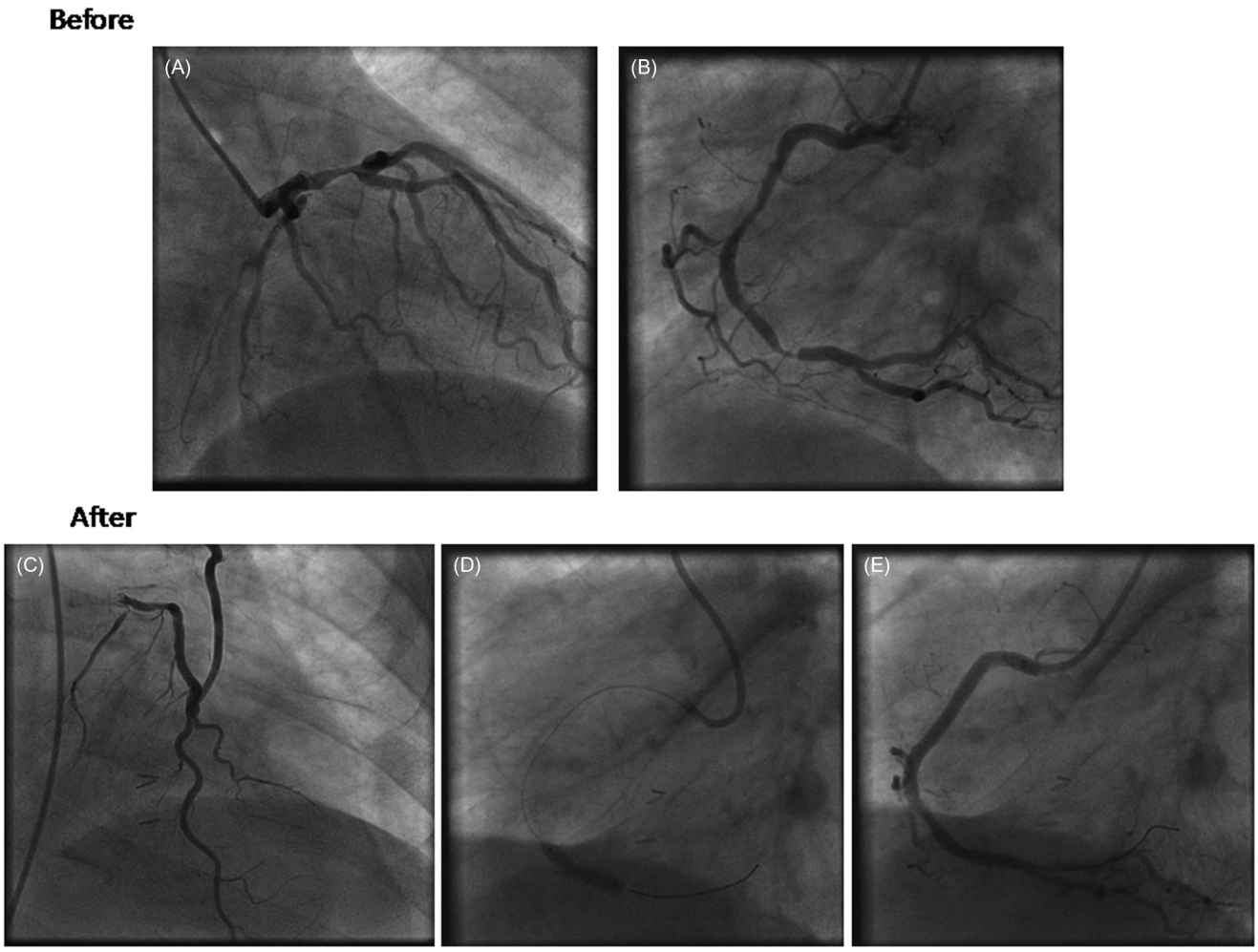

FIGURE 17.6 Example of coronary anatomy suitable for HCR. This patient underwent staged HCR with surgical session first and PCI on first postoperative day. (A) Diagnostic angiography revealing significant proximal LAD stenosis; (B) Diagnostic angiography revealing significant RCA stenosis; (C) LIMA angiography after robotic-assisted LIMA-LAD grafting demonstrating LIMA-LAD patency before PCI of RCA; (D) PCI of RCA with deployment of DES; (E) Completion angiogram of RCA revealing no residual stenosis in RCA.

HCR appears to be a safe and effective therapy as well as a valuable alternative to either traditional CABG or multivessel PCI in carefully selected patients.

DISCLOSURES

Consultant for Intuitive Surgical, Maquet Cardiovascular, Medtronic.

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CHAPTER

18

Peripheral Veno-arterial Extracorporeal Membrane Oxygenation for Treatment of Ischemic Shock

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INTRODUCTION

Extracorporeal membrane oxygenation (ECMO), often referred to as extracorporeal life support (ECLS), provides temporary support to critically ill patients who cannot maintain their respiratory and/or circulatory function. A basic circuit composed of cannula, tubing, a pump, oxygenator, and heat exchanger; there are two approaches: veno-venous (VV) ECMO for solely respiratory support, and veno-arterial (VA) ECMO for cardiac support, cardio-respiratory support, or undifferentiated etiology support. Since first use in 1971 [1], ECMO circuits have improved in design and function [2–4]. This review focuses on contemporary insertion techniques and management considerations, which have improved clinical outcomes in VA ECMO, specifically, peripherally inserted VA ECMO for cardiogenic shock secondary to ischemic presentation.

EVOLUTION TO CONTEMPORARY ECMO

Gibbon's development of the heart-lung machine facilitated the first open heart surgery in 1953 [5]. Initial use of heart-lung machine was restricted to the operating room because of damage to blood due to direct exposure to oxygen. The advent of spiral coil type membrane oxygenators, circumvented this concern, and lead to the first experiences with maiden ECMO circuits in the early 1970s [1]. As heart-lung machine technology continued to develop as cardiac surgery developed, this improved technology became applied to future iteration ECMO circuits with favorable results. Centrifugal pumps have replaced early rotor pumps in contemporary ECMO circuits [2], reducing hemolysis and improving flow dynamics. New biocompatible surfaces [6] such as heparin coated circuits allow reductions in systemic anticoagulation, potentially reducing incidence of bleeding events and systemic inflammatory response syndrome (SIRS). Innovations leading to the development of solid hollow fiber membranes [3,4] resulted in reduced incidence of air embolism and blood trauma due to oxygen exposure. Smaller and portable configurations facilitated VA ECMO as a tool to initiate management of patients in less intensive settings and enable ease of transport [7] to advanced care centers (e.g., Cardiohelp™ device, Maquet). Newer duel lumen cannula [8,9] (e.g., Avalon™, Maquet; and TandemHeart™ Right Ventricular Assist Device cannula with oxygenator) have made it possible to provide VV ECMO respiratory support with single cannula, peripheral inserted in the internal jugular vein via Seldinger technique [10]. Currently, ECMO is utilized for not only pulmonary and circulatory failure, but for

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00018-8

transport, retrieval of organs, and extracorporeal CPR. Most recent data from the Extracorporeal Life Support Organization (ELSO) [11] illustrated more than 14,000 patients have utilized short- or medium-term ECMO support, with reasonable overall survival to discharge, ~60% in respiratory and ~45% in cardiac failure.

INDICATIONS

The use of ECMO is often considered in critically ill patients, and often indicated when the pre-ECMO mortality exceeds 80% [12]. Contraindications include cerebral hemorrhage due to the need for anticoagulation, severe immunosuppression due to SIRS, or terminal diagnosis.

Veno-Arterial ECMO

VA ECMO is one of many options available for circulatory support. Other options being various ventricular assist devices both surgically implanted and percutaneously placed. The advantages of VA ECMO over the above two options include ease of emergent insertion, potential for biventricular support, and ability to provide respiratory support [13]. These features are especially suited to acute coronary syndrome presentation. It may be used as a bridge to myocardial recovery, myocardial revascularization, ventricular assist device implantation, or heart transplantation.

Indications for VA ECMO include spectrum of both isolated cardiac failure and combined cardiorespiratory failure with evidence of poor tissue perfusion despite optimal intervention. VA ECMO is most commonly employed in the setting of cardiogenic shock due to variety of etiologies, such as postmyocardial infarction, fulminant myocarditis, peripartum cardiomyopathy, septic shock causing cardiac depression, decompensated heart failure, and postcardiotomy shock (failure to wean off cardiopulmonary bypass). Recent novel yet less common indications for VA ECMO support include: extracorporeal cardiopulmonary resuscitation (eCPR), resuscitation in cases of severe hypothermia, and extracorporeal interval support for organ retrieval (EISOR) [14].

VA ECMO in the setting of ischemic shock is emerging to have a notable role. Despite early revascularization, cardiogenic shock complicating acute myocardial infarction carries a high mortality. Intra-aortic balloon pump (IABP) support was recently found to confer no mortality benefit over medical therapy in the IABP-SHOCK II trial [15].

TECHNICAL CONSIDERATIONS

Veno-Arterial ECMO

VA ECMO support can be initiated via intrathoracic or peripheral cannulation. Intrathoracic cannulation is usually performed after open heart surgery (postcardiotomy shock) or to solve peripheral ECMO complications. The venous cannula is placed in the right atrium. This siphons blood volume from body and acts as the inflow to the ECMO circuit, which subsequently contains the oxygenator and heat exchanger in series. The outflow from the ECMO goes into an arterial cannula, which is placed in the ascending aorta. Both cannulas can be tunneled through the skin to allow a definite chest closure and potential patient extubation.

For peripheral cannulation in the acute presentation of ischemic shock, the femoral vein is the preferred venous line. The venous cannula is placed in the right atrium through the femoral vein, also using Seldinger technique [16]. The arterial line can be placed in the femoral artery, axillary artery or even in the carotid artery in the pediatric population. In all cases, cannula insertion can be totally percutaneous, in which case, a short arterial cannula is positioned distally to prevent distal ischemia [17]. The completely percutaneous access is the desired one in emergency cases as it allows support to be initiated in minutes. However, a surgical cut down and sewing a 6–8 mm graft onto the vessel and connecting to the ECMO circuit prevents distal arterial ischemia [18,19]. Occasionally, the use of a graft may cause hyperperfusion of the corresponding limb, more frequently in the axillary artery and can be managed by doing a distal banding of the artery or external wrapping of the affected upper extremity.

MANAGEMENT CONSIDERATIONS

Once ECMO support has been initiated, the goal is to preserve all organs and recover those injured. It is important to obtain a baseline arterial blood gas (ABG) and visceral labs. Daily metabolic panel verifies proper perfusion and oxygenation. Arterial gases and coagulation panels must be obtained hourly, especially in the first hours of support.

Protective ventilation mode should be maintained to allow the cardiorespiratory recovery if possible. Oxygenator settings (FiO_2 and "sweep" (air flow rate)) will be adjusted according to the ABG results. It is important to avoid overcorrection and/or fast correction of pCO_2 levels, especially in chronic hypercarbic patient due to the risk of cerebral damage.

Inotropic support and ventricular assist devices (e.g., Impella™, Abiomed; IABP; TandemHeart™ transseptal cannula) should be maintained to facilitate the left-sided chamber unloading and coronary perfusion, if cardiac recovery is a possibility. This is critical in patients with acute coronary syndrome.

ECMO flows should be adjusted according to the patient needs. On one hand, flows should be enough

to keep a good systemic perfusion measured by urine output, lactic acid levels, and mixed venous saturation. On the other hand, ECMO flows should not be high enough to prevent lung circulation. To facilitate lung recovery and avoid development of pulmonary thrombi, at least 0.5 L/min of flows through the lung circulation should be permitted. To enable it, full-flow support should not be maintained for a long period of time.

Harlequin Syndrome

Harlequin syndrome describes the situation where the upper body is hypoxemic ("blue") whereas the lower body is fully oxygenated ("pink"). This situation occurs under peripheral femoral VA ECMO support, and it is the result of partially preserved heart function with poor lung function. Because of the poor lung function, the left-side heart chambers receive nonoxygenated blood. This nonoxygenated blood is ejected as the heart function is partially preserved. The principal recipient of the nonoxygenated blood would be the coronary arteries and the cerebral vessels. The visceral organ would receive oxygenated blood through the femoral cannula. The level of the mixture happens at different levels, depending on how preserved is the heart function [20].

In order to detect that problem that leads to a continuous myocardial and/or cerebral perfusion with nonoxygenated blood, it is important to obtain all the arterial gases from the right upper extremity as it is the closest arterial site to the aortic root, or cerebral oximetry placed on the forehead or upper extremity. This problem can be resolved by switching the arterial cannula to axillary artery or central cannulation.

Left Ventricular Distention

Left ventricular (LV) distension is the result of the bronchial circulation, certain degree of aortic regurgitation, and complete ECMO support. It is a significant complication that requires a prompt solution. LV distention increases myocardial wall tension leading to reduced coronary perfusion and chance of myocardial recovery. LV distension also leads to increased pulmonary capillary wedge pressure and pulmonary edema. Lastly, it may result in flow stasis and development of LV thrombus with risk of embolization and stroke.

Contemporary advances in ECMO approaches avoid LV distension by unloading the LV. LV unloading in partially recovered heart function can be achieved by reducing the ECMO support/flow and maintaining the patient's pulsatility. In some cases, IABP insertion increases coronary perfusion to improve contractility, and reduces the afterload, to facilitate the previous pulsatility. If these strategies are insufficient, an active LV drain is needed. In the case of central ECMO, a vent can be inserted in the left ventricle by opening the previous incision via the right superior pulmonary vein or pulmonary artery. In peripheral ECMO support, LV drainage can be obtained by placing a cannula in the left atrium using a transeptal approach [21]. In cases where the transeptal puncture is not feasible, a small left thoracotomy may be necessary for direct insertion of the LV vent. In all circumstances, the LV vent is connected to the venous line. Other devices such as the Impella™ (Abiomed) can be used concurrently to decompress the LV [22].

Anticoagulation

Improvements in biocompatible materials for the ECMO circuit have reduced the difficulty in the anticoagulation of patients on ECMO support. There is no clear consensus regarding anticoagulation protocols; most centers have developed their own, using unfractionated heparin IV infusion. In special situations, alternatives such as bivalirudin [23] or argatroban [24] can be used. The anticoagulant effect is monitored using activated clotting time (ACT) or PTT. In certain occasions, other measures as antifactor Xa levels or thromboelastogram (TEG) may be used [25].

Every phase of the ECMO support requires a different anticoagulation range. Our protocol recommends—cannulation and initiation support start: 50–100 U/kg of heparin to achieve an ACT 200–250 s; stable ECMO support: ACT between 180 and 225 s, PTT 60–80; weaning period, once flows are <2.5 L/min: PTT higher than 80 s and ACT round 250–300 are recommended. ACT or PTT should be checked every hour for the first 4 days of support or until a stable therapeutic level is achieved [25]. For an early detection of thrombotic complications all ECMO lines and the oxygenator should be inspected twice a day to assess for the presence of clots.

Weaning Off ECMO

Weaning ECMO support is normally a gradual and closely monitored process. For VA ECMO support, the weaning process is supported by increased arterial pulsatility, stable Swan Ganz parameters, and daily assessment of heart function using echocardiography. Once the arterial pulsatility and contractility have improved, ECMO flows can be reduced after optimizing inotropic support and ventilator settings. The flow is reduced to 50% of the cardiac output supported by the ECMO. If the contractility and the hemodynamic parameters remain stable for 15–30 min, the ECMO flows can be safely reduced another 50% until the complete wean. In VA ECMO weaning, process longer than 4 h should be avoided.

CLINICAL OUTCOMES

ECMO techniques and management carry inherently high rates of complications, some with devastating outcomes. Complications include patient-related adverse events and/or adverse events related to the ECMO circuit. Patient adverse events primarily include neurological, renal, vascular, cardiac, and respiratory. Circuit adverse events include problems related to oxygenator, heat exchanger, lines, pump itself (mainly thrombosis), and/or air embolization.

ECMO outcomes have been poor historically; this is especially true for adult patients undergoing ECMO. ELSO clearly recommends against ECMO consideration if the predicted mortality is <50%. Early studies for ECMO described very high mortality (>90%); [1] leading to decreased interest, especially in adult patients. Following technical advances, clinicians started using ECMO in highly selective otherwise healthy patient group with very high mortality risk [26]. These stringent selection criteria were gradually extended to other patients with less severe condition following increased expertise and improved technology.

ELSO statistics suggests that overall adult survival for cardiac failure patients receiving ECMO is 40%. Myocarditis (67%) and ischemic shock (49%) represents better survival compared to congenital cardiac defect (33%) [27,28].

Table 18.1 displays recent studies for VA ECMO for cardiac failure. Early (30 days) survival ranges from 24% to 65%, with survival to discharge ranges from 14% to 59%. Different complications are not mentioned in all studies, but common complications include infection, bleeding, acute renal insufficiency, neurological events, and limb ischemia. Early studies showed higher rates of complications. Infection rates as high as 58% has been observed [4]. Highest rates for acute renal insufficiency were reported by Bakhtiary and colleagues of almost 87% [5]. Neurological complication ranged from 9% to 33%. Incidence of limb ischemia ranged from 7% to 36% [3].

ETHICS

ECMO has inherent ethical challenges. As traditional definitions of death usually include cessation of cardiorespiratory function, the role of ECMO itself poses challenges to the ethos of end-of-life discussions with the patient's family. It is imperative that physicians provide family members with detailed knowledge of the implications on continued ECMO care versus discontinuation, in patients who are not being bridged to recovery, or bridge to treatment—such as revascularization, permanent ventricular assist device, or transplantation. Due to emergent nature of the procedure it may not be feasible to have these discussions beforehand, but should be initiated early in the course of treatment to avoid potential disagreements [40]. Surrogates such as the Sequential Organ Failure Assessment (SOFA) and APACHE score have been used to aid in discussions of probability of recovery with patients' families [41].

VA ECMO is further perplexing in that VA ECMO itself provides both cardiac as well as respiratory support superior to cardiopulmonary resuscitation. Do not resuscitate (DNR) order or comfort measures are therefore incompatible with this strategy [42].

TABLE 18.1 Summary of Recent Studies with Survival to Discharge Results after VA ECMO

Study	Indication	Study period	Number of patients	Survival to discharge (%)	30-day survival (%)
Bakhtiary [29]	Cardiogenic shock	2003–2006	45	28.9	47
Belle [30]	Cardiogenic shock; Cardiac arrest	2006–2010	51	27.4	NA
Chamogeorgakis [31]	Cardiogenic shock	2006–2011	61	14.8	36.1
Formica [32]	Cardiogenic shock	2002–2009	42	38.1	52.4
Kim [33]	Cardiogenic shock	2006–2010	27	59.3	63
Lamarche [34]	Cardiogenic shock	2000–2008	32	44	43.8
Lin [35]	Cardiac arrest	2004–2006	55	29.1	34.5
Liu [36]	Cardiac arrest	2007–2010	10	40	40
Smedira [37]	Cardiogenic shock	1992–1999	202	38	24
Doll [38]	Cardiogenic shock	1997–2002	219	24	24
Beurtheret [39]	Cardiogenic shock; Cardiac arrest	2005–2009	87	36.8	NA

ECONOMICS

Despite the heterogenous nature of ECMO, there have been some economic analyses that are enlightening. The most recent CESAR trial collaboration reviewed 180 patients in a randomized fashion to ECMO center referral versus optimal medical management for ARDS and found the cost to approximate £19,000 ($30,000US) per quality adjusted life year (QALY) [43]. With hemodialysis treatment used as a benchmark, where the cost is $50,000–70,000US/QALY and threshold to initiate dialysis is low, ECMO may be considered cost-effective when used in selective patients with ARDS.

Cost analysis for VA ECMO is less clear. Maxwell et al. [44] analyzed almost 9000 hospital admissions using the Nationwide Inpatient Sample, between 1998 and 2009. Average daily and total hospital costs were approximately $40,000/day and $344,000 (total) respectively. When analyzed, the postcardiotomy shock cohort had most favorable outcomes and lowest resource use/cost. From 1998 to 2009 the total annual cost of ECMO in this cohort studied increased from $109 million/year to $765 million/year. Analyses showed this was not solely driven by increased ECMO volume. Charges per patient and lengths of stay increased significantly. However, patterns showed an increased proportion of VA ECMO were from non-post-cardiotomy cohorts, resulting in worse outcomes and cost-effectiveness.

CONCLUSIONS

ECMO has evolved in design, technology, patient selection, insertion techniques, adjunct devices, and management in the past 45 years since it began. Outcomes have improved and indications have expanded. It remains an expeditious, cost-effective tool for rapid resuscitation of patients with cardiorespiratory failure, whose outcomes without ECMO intervention are predominantly fatal. However, results are still guarded and the ethical aspects of ongoing care needs to be at the forefront of daily family discussions, in those where a bridge to transplant or definitive device are not possible.

Peripherally inserted VA ECMO for resuscitation and as a bridge to definitive treatment in the cohort of patients presenting with ischemic shock, is an expeditious approach with acceptable success. Adjuncts to prevent LV distension, maintain coronary perfusion, and alternative cannulation strategies to provide optimal support to the brain and peripheral organs, have improved outcomes and led to this having a prominent role in the current algorithm for managing of acute coronary syndromes.

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CHAPTER

19

Biostatistics Used for Clinical Investigation of Coronary Artery Disease

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INTRODUCTION

Clinical research to assess alternative medical or surgical treatments in coronary artery diseases can be classified as observational and experimental research based on the assignment of exposures (e.g., treatments). In this chapter, the terms *exposure* and *treatment* will be used interchangeably since treatment can be viewed as a part of exposure. If an investigator assigns treatments to the study, the study is an experimental study. Otherwise, the study is an observational study [1].

Observational studies can be divided to an analytical study and a descriptive study based on the presence or absence of comparison group. If there is no comparison group, the study is a descriptive study, which includes a case-report study and a case-series study. If there is a comparison group, the study is an analytical study. An analytical study can be further divided to a case–control study, a cross-sectional study, and a cohort study based on the temporal direction of the study. Experimental studies can be classified as randomized clinical trials and nonrandomized clinical trials depending on the allocation of subjects to treatment groups.

American College of Cardiology Foundation and American Heart Association (ACC/AHA) provided the criterion for hierarchical rankings on the basis of research design for different types of studies in evidence-based medicine [2]. ACC/AHA provided the highest level of evidence (Level A) for treatment recommendations to studies with "data derived from multiple randomized clinical trials or meta-analyses" and the second-highest level (Level B) to studies with "data derived from a single randomized trial, or nonrandomized studies". The lowest level of evidence (Level C) was assigned to studies with "Consensus opinion of experts, case studies, or standard of care".

The advantages of randomized clinical trials (RCTs) are simplicity and universal acceptance. The disadvantages are time and effort involved in their effective implementation, dealing with the resistance of patients and clinicians, and large sample size for comparison, especially with low-incidence outcomes. RCTs may not be feasible for outcomes that are rare or have long lag times.

OBSERVATIONAL STUDY

An observational study is conducted when an RCT is not feasible. Suitable design and statistical analysis methods need to be carefully chosen for an observational study. There are a number of available designs for observational studies with each developed for specific situations in coronary artery disease research.

Case-Report/Case-Series Study

A case-report is a descriptive study of a single patient, which does not have a comparison (control) group. A case-report usually describes an unusual or novel occurrence. A case-series is a descriptive study of a small group in which the possibility of an association between an observed effect and a specific exposure is based on detailed clinical evaluations and histories of the patients. Case-report and case-series designs are useful when the disease is uncommon and when it is caused exclusively or almost exclusively by a single kind of exposure. Use of five Ws and one H (who, what, why, when, where,

Translational Research in Coronary Artery Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-802385-3.00019-X

and how) is recommended for good descriptive reporting of case-report and case-series studies. For example, who has the disease? What is the disease being studied? Why did the disease arise? When did the disease occur? Where did the disease arise? How to design a future study based on the results of the study?

Case-report and case-series are considered the lowest level of evidence. However, they provide the first line of evidence since they are where new issues and ideas emerge. Case-series are used to generate the hypothesis about the cause of the disease. They do not allow assessment of causal association.

Example: Boyer et al. [3] conducted a case-series study to present an extensive review of the existing literature and associated clinical guidelines, and proposed a management algorithm for patients with coronary artery aneurysm (CAA). CAA is an abnormal dilatation of part of the coronary artery, an uncommon clinical finding with an incidence rate of 1.5–4.9% in adults.

Cross-Sectional Study

Exposure and disease are determined at one specific point in time in a given population in a cross-sectional study. A cross-sectional study is conducted to estimate the prevalence of disease and an exposure at a particular time. A cross-sectional study is relatively inexpensive and takes up little time to conduct.

The temporal relationship between exposure and disease cannot be determined since both outcome and exposure are ascertained at the same time. Since a cross-sectional study only takes a snapshot, the study may provide differing results if another time-frame had been chosen. A cross-sectional study yields prevalence-incidence bias (also called Neyman bias). Any risk factor that results in death will be under-represented among those with the especially longer-lasting diseases.

Example: Stack and Bloembergen [4] conducted a cross-sectional study to investigate the prevalence and clinical associations of coronary artery disease in a national random sample of new end stage renal disease in the United States in 1996–1997.

Case–Control Study

A case–control study is always retrospective because it starts with an outcome such as disease and then looks backward in time for exposures that might have caused the outcome. Here, a case means a subject with a disease or outcome of interest where a control means a subject without a disease or outcome of interest. A case–control study aims to retrospectively determine the exposure to the risk factor of interest from cases and controls. The investigators ascertain the prevalence of exposure to a risk factor in both groups of cases and controls through chart reviews or other means. If cases have significantly higher prevalence rate of the exposure than controls, then the exposure is significantly associated with an increased risk of the outcome.

Case–control studies are especially useful for outcomes that are rare or that take a long time to develop, such as cardiovascular disease and cancer. These studies often require less time, effort, and money than cohort studies. Therefore, a case–control study may be the only feasible method for very rare disorders or those with long lag between exposure and outcome. A case–control study can examine many risk factors at once. Disadvantage of a case–control study includes reliance on recall or records to determine exposure status, difficulty in establishing cause and effect due to temporal backwards relationship, and potential recall and selection bias. Incidence-prevalence bias (also called Neyman bias) also occurs in a case–control study. Suppose that cases are interviewed 1 month after the coronary attack in a study that investigates association between tobacco smoking and acute myocardial infarction (AMI). If death occurs more frequently in smokers with AMI, the remaining cases will show lower frequency of smoking than the dead AMI patients, which will decrease the association between smoking and AMI. This bias occurs only if the risk factor influences mortality from the disease being studied [5].

Matched case–control study designs are commonly implemented to eliminate confounding in clinical studies. The main potential benefit of matching in case–control studies is a gain in efficiency [6].

Example: Pierre-Louis et al. [7] used a case–control design to investigate the severity of coronary artery disease by coronary angiography in age-matched and sex-matched patients with diabetes mellitus with atrial fibrillation versus sinus rhythm.

Cohort Study

Cohort studies follow groups of individuals over time to investigate the causes of disease, establishing links between risk factors and outcomes. Cohort studies prospectively proceed from exposure to outcome. Investigators identify groups with and without an exposure of interest, and then follow the exposed and unexposed groups over time to determine outcomes. As the study is conducted, outcome from subjects in each cohort is measured and relationships with specific characteristics determined. If the exposed group has a higher incidence of the outcome than the unexposed, then the exposure is associated with an increased risk of the outcome.

The cohort study has important strengths. In a cohort study standardization of criteria/outcome is possible. A cohort study limits the influence of confounding variables since subjects in cohorts can be matched. The

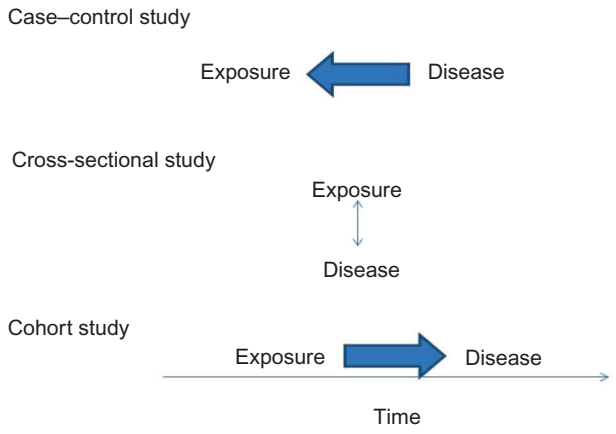

FIGURE 19.1 Temporal relationships between exposure and disease.

cohort study has less recall bias than the case–control study. However, the cohort study can be slow to yield results and thus prohibitively expensive for the study of rare diseases or diseases that take years to develop.

Example: Thanassoulis et al. [8] showed that a genetic risk score composed of 13 single nucleotide polymorphisms (SNPs) associated with coronary disease is an independent predictor of cardiovascular events and of high coronary artery calcium with a Framingham Heart cohort study.

Figure 19.1 shows temporal relationship between exposure and disease in a case–control study, a cross-sectional study, and a cohort study.

Case-Cohort Study

The case-cohort study was originally designed to allow efficient analysis of studies where the population size was too large to collect detailed data on all the study subjects. The case-cohort study randomly selects a subcohort from the original sample at entry and then only analyzes data on members of the randomly selected subcohort and the remaining cases. Randomly selected subcohort includes both cases and noncases that are identified after a certain follow-up time. For example, blood samples would be collected over time for all study participants. Then, the biochemical analysis would only be performed on participants in the randomly selected subcohort or subjects that developed the disease of interest. Then, the case cohort is comprised of subcohort and the remaining cases from other than subcohort. Sharp et al. [9] reviewed recent practice in reporting case-cohort studies, and developed recommendations about reporting of the study design, subcohort definition, descriptive information, and statistical methods.

Example: Weighted Cox proportional hazard regression analysis [10] was used to investigate if there was a significant interaction between soluble thrombomodulin (sTM) and soluble intercellular adhesion molecule-1 (sICAM-1) in predicting risk of CHD event using the case-cohort data from ARIC (Atherosclerosis Risk In Communities) cohort [11].

Statistical Analyses

In this section, statistical methods for data analysis are briefly described. Statistical methods described here have been widely used for the analysis of observational and experimental studies.

Analysis of Continuous Response Variables

Continuous response variables are analyzed using *t*-tests, analysis of variance (ANOVA), analysis of covariance (ANCOVA), or mixed models, to test the null hypothesis of equal means in different groups with and without adjusting by covariates. For all models, the data is tested to ensure that the underlying assumptions (i.e., normality and homoscedasticity) are met. If not, standard transformations (e.g., log, inverse, square root, and Box-Cox) are taken on the data in order to meet these assumptions. If data transformation is inadequate to meet the analysis assumptions, then rank transformation of the data is performed and one-way ANOVA on the rank-transformed response variables are analyzed and reported. Nonparametric alternatives such as the Wilcoxon signed-rank test, the Wilcoxon rank-sum test, the Kruskal–Wallis test, or permutation tests, are used as appropriate. When covariates could affect a response variable in an ANOVA context, analysis of covariance (ANCOVA) is used to adjust for treatment effects. The underlying assumptions of the ANCOVA model (e.g., homogeneity of slopes across treatment groups) are tested. Standard regression criteria are used to assess the appropriateness of including particular covariates. When more than one covariate is being included in the model, the possibility of multicollinearity will be reduced through the careful initial assessment of correlations among all study covariates. Multicollinearity is a phenomenon in which two or more predictor variables in a multiple regression model are highly correlated, meaning that one can be linearly predicted from the others.

Analysis of Categorical Response Variables

Where response variables are categorical, Pearson's chi-square test or Fisher's exact test is used to test for differences among treatment groups. Cochran–Mantel–Haenszel test is used when we must stratify on additional variables. Logistic regression is used to model the relationship between a binary outcome variable and covariates. Logistic regression diagnostics is employed to ensure that the logistic model is appropriate.

Polychotomous logistic regression can be applied for ordinal categorical variables under the proportional odds assumption. When the outcome is truly multinomial, generalized logit models can be applied. Poisson regression is used if the outcome is a count of events. Construction of composite measures can be formed, if necessary, to combine information among highly correlated covariates [12,13].

Analysis of Survival Data

The method of Kaplan and Meier is used to estimate the distributions of time-to-event outcomes, and these distributions among treatment groups are tested using the log-rank test. Multivariable proportional hazards models are used to test for treatment or prognostic effects in the presence of covariates. The proportional hazards assumption can be evaluated graphically and analytically, and regression diagnostics (e.g., martingale and Schoenfeld residuals) are examined to ensure that the models are appropriate [14]. Violations of the proportional hazards assumption can be addressed in one of the following ways: (i) Stratify by the levels of a categorical variable for which the proportionality assumption fails. (ii) Fit separate Cox models to different time intervals. (iii) Use the extended Cox model instead of the ordinary Cox model. The extended Cox model permits time-dependent covariates [15].

Analysis of Longitudinal Data

Some observations will be measured repeatedly over time, and thus the ordinary independence assumption of observations no longer holds. In situations where one has prior knowledge about the measurement correlation structure, one can use linear mixed models for Gaussian outcomes and generalized linear mixed models (or nonlinear mixed models) for categorical outcomes [16]. In situations where measurement correlation structure is not plausible to predict, one can apply the generalized estimating equations (GEE) for either continuous or categorical outcomes [17,18]. This population average model allows potential misspecification of the measurement correlation structure, yet maintains the consistency of a treatment effect estimate. Missing data arise in almost all serious longitudinal data analyses. Missing data can be handled using the generalized-EM algorithm [19,20] and multiple imputation techniques [21].

Multiple Comparisons

Multiple comparison problems arise when investigators assess the statistical significance in more than one test in a study. When more than one comparison is made, the chance of falsely detecting a nonexistent effect increases. Therefore, statistical adjustment needs to be made for multiple comparisons to account for this. One of the most basic and popular fixes to the multiple comparison problem is the Bonferroni correction. The Bonferroni correction adjusts the p-value based on the total number of comparisons being performed. Bonferroni-adjusted p-value is calculated by dividing the original p-value by the number of tests being performed. For example, Bonferroni-adjusted p-value is $0.05/5 = 0.01$ if the number of tests being performed is 5. Although Bonferroni correction reduces the number of false rejections, it also increases the number of cases that the null hypothesis is not rejected when it should have been rejected. That is, the Bonferroni correction severely reduces the power to detect an important effect. To overcome the shortcomings of the Bonferroni correction, investigators have proposed more sophisticated procedures that reduce the familywise error rate (the probability of having at least one false positive) without sacrificing power. A variety of such corrections exist that rely upon bootstrapping methods or permutation tests [22,23].

Sample Size and Power Calculations

Commercially available software such as nQuery Advisor and PASS can be used to compute the sample size and power for standard statistical problems. For the Cox proportional hazards model, simulations in SAS, or R can be used to compute the power given specified effect parameters and sample size. Sample size for repeated measurement data [24] can be estimated using the methods of GEE [25–27] and linear mixed models [28,29].

Statistical Tools for Observational Studies

Estimation of the causal effect of an exposure on an outcome may be biased due to confounding in observational studies. Proper estimation of causal effects must account for confounding [30]. Here, we describe statistical tools commonly used for the analysis of observational data.

Propensity Score

Investigators have used the regression adjustment to account for differences in measured baseline characteristics between treated and untreated subjects. Recently, there has been increasing interest in methods based on the propensity score (PS) to reduce or eliminate the effects of confounding when using observational data. The PS is the probability of treatment assignment conditional on observed baseline characteristics. The PS is called the balancing score, which allows one to design and analyze an observational study so that it mimics some of the particular characteristics of a randomized controlled trial. That is, conditional on the PS, the distribution of observed baseline covariates will be similar between treatment and control subjects. The PS can be used for matching, stratification, and covariate adjustment [31].

Example: Banach et al. [32] estimated PS for SBP ≤120 mm Hg for each of the 7785 patients in the Digitalis Investigation Group trial using a multivariable logistic regression model and then assembled a cohort of 1869 pairs (n = 3838) of propensity-matched patients with SBP ≤120 and >120 mm Hg score to reduce or eliminate the confounding effects from observational data. They investigated the association between baseline SBP and outcomes in a propensity-matched cohort of mild to moderate chronic systolic and diastolic HF patients.

Instrumental Variable

An Instrumental Variable (IV) is used to control for confounding and measurement error in observational studies so that causal inferences can be made. Suppose X and Y are the exposure and outcome of interest, and we can observe their relation to a third variable Z. Let Z be associated with X but not associated with Y except through its association with X. Here, Z is called an IV or instrument [33]. That is, an IV is a factor that is associated with the exposure but not with the outcome. For example, the price of beer can affect the likelihood of drinking beer in expectant mothers, but there is no reason to believe that it directly affects the child's birthweight.

Example: When surgeons show strong preference for one of the two antifibrinolytic agents, surgeon's choice does not depend on characteristics of the patient. Then, it is possible to use the surgeon's preferred agent as a substitute for the actual exposure (i.e., as an IV). Schneeweiss et al. [34] conducted an IV analysis to investigate the association between the use of aprotinin and death.

Marginal Structural Model

In observational studies, statistical inferences are often subject to confounding caused by both observed and unobserved confounding variables. Conventionally, statistical techniques such as stratification and multivariable regression analysis methods have been used to control the confounding effects. However, such statistical techniques may still lead to biased estimates in the presence of time-dependent confounders. Marginal Structural Models (MSMs) are powerful tools that adjust for time-dependent confounding in observational studies of time-varying treatments [35]. MSMs assess causality in complicated longitudinal data sets. MSMs generate a pseudo-population via inverse treatment probability weighting to separate confounding control from the estimation of the parameters of interest allowing the investigator to obtain unbiased estimates.

Example: Gerhard et al. [36] used a marginal structural Cox model (MSCM) to investigate if aggressive treatment was associated with a lower risk for serious cardiovascular outcomes compared to conventional treatment.

EXPERIMENTAL STUDY

If subjects are randomly assigned to treatment groups in an experimental study, the trial is a RCT. Otherwise, the trial is a nonrandomized clinical trial. A nonrandomized clinical trial provides less scientific rigor than a randomized clinical trial. In this section, we concentrate on the RCT. RCTs are considered the gold standard for the estimation of the treatment effects, interventions, and exposures (hereafter referred to as treatments) on outcomes since random assignment removes the chance of confounding due to extraneous variables that create differences before the experiment. There are a number of available RCT designs with each developed for specific situations in coronary artery disease. Here, we present a few RCT designs commonly used in coronary artery disease research. More detailed information on the types of RCT designs can be found in Ahn and Ahn [37].

Run-In Design

A run-in period is a period prior to randomization during which potential participants who meet all eligibility criteria for an RCT are assigned to take the study medication. A major advantage of a run-in design is the increase in trial efficiency through screening out ineligible or potentially noncompliant participants using clinical data during a run-in period, which has a direct effect on the power of the trial. In addition, a run-in period can serve as the training period for investigators, staffs, and participants. However, a run-in period leads to increased cost, potential reduction of the enthusiasm of the participants, and the increased length to complete a clinical trial.

Example: Davidson et al. [38] proposed a randomized, multicenter, prospective, double-blind, placebo-controlled, phase III study that evaluates the effect of once-daily fenofibric acid or placebo on carotid intima-media thickness progression in patients with controlled low-density lipoprotein cholesterol (LDL-C) levels achieved through atorvastatin treatment, but with high triglycerides and low HDL-C levels. The planned duration of the study is 118 weeks, composed of a 2- to 10-week diet and atorvastatin run-in period, a 104-week treatment period, and a 30-day safety follow-up period. Six-hundred eighty-two patients were randomized to receive once-daily delayed-release capsules of choline fenofibrate 135 mg or placebo plus atorvastatin treatment after a run-in period.

Superiority, Noninferiority, and Equivalence Design

Most RCTs are superiority trials that aim to determine whether a new treatment is superior to the standard

treatment. The null hypothesis is that the two treatments are not different with respect to the mean response while the alternative hypothesis is that the two treatments are different with respect to the mean response.

An equivalence trial aims to confirm the absence of meaningful difference (Δ) between treatment groups, where Δ is the prespecified margin of treatment effect. That is, an equivalence design aims to show that the mean responses to two treatments differ by amount that is clinically unimportant. The null hypothesis is that the two treatments are different with respect to the mean response while the alternative hypothesis is that the two treatments are not different with respect to the mean response. That is, equivalence trials aim to investigate if a new treatment is therapeutically similar to an existing treatment with the treatment effect being between $-\Delta$ and Δ.

A noninferiority trial aims to show that a new treatment is not less effective than a standard treatment by more than the margin of noninferiority (Δ). The null hypothesis is that a new treatment is inferior to a standard treatment with respect to the mean response while the alternative hypothesis is that a new treatment is noninferior to a standard treatment with respect to the mean response.

The question of interest in an equivalence trial is "Can I say that the response rate lies within 5% of each other for these two treatments with 95% certainty?" The question of interest in a noninferiority trial is "Can I say that the new treatment has response rate no worse than 5% than the standard treatment with 95% certainty?"

Example of Superiority Design: Aronow et al. [39] conducted a prospective randomized clinical trial and showed that propranolol reduces mortality, decreases mortality plus nonfatal myocardial infarction, improves left ventricular (LV) ejection fraction, and reduces LV mass in older patients with congestive heart failure (CHF), prior myocardial infarction, and a LV ejection fraction ≥40% treated with diuretics plus angiotensin-converting enzyme (ACE) inhibitors.

Example of Noninferiority Design: Pilgrim et al. [40] conducted a randomized, single-blind, noninferiority trial to compare the safety and efficacy of a novel, ultra-thin strut cobalt-chromium stent releasing sirolimus from a biodegradable polymer with a thin strut durable polymer everolimus-eluting stent. A noninferiority margin of 3.5% was used for the biodegradable polymer sirolimus-eluting stent compared with the durable polymer everolimus-eluting stent. In this trial, the primary endpoint was a composite of cardiac death, target vessel myocardial infarction, and clinically-indicated target lesion revascularization at 12 months.

Example of Equivalence Design: Through an equivalence trial using a predetermined equivalence threshold <10% for relative difference in coronary flow reserve (CFR), Murthy et al. [41] showed that coronary microvascular dysfunction (CMD) is highly prevalent both in men and women among at risk individuals and is associated with adverse outcomes regardless of sex.

Data from experimental studies can be analyzed using the statistical methods described in statistical analysis section.

DISCUSSION

Observational studies and randomized clinical trials are two primary types of studies that test new drugs or medical devices or procedures or compare competing drugs, medical devices or procedures. Even though RCTs are considered as important means of advancing our knowledge in coronary artery disease and gold standard for estimating treatment effects due to elimination of selection bias with random assignment, RCTs have some limitations such as time and effort involved in their effective implementation, dealing with the resistance of patients and clinicians, and large sample size for comparison especially with low-incidence outcomes.

There are many aspects to be considered for the design of observational studies and RCTs due to many possible limitations in both studies. These limitations must be carefully assessed in the design and analysis of the studies. To promote high-quality studies, clinicians should know variations in the type of observational studies and RCTs, and use appropriate designs and statistical analyses.

Meta-analysis is a statistical technique that combines the findings from independent studies. The validity of the meta-analysis depends on the quality of the systematic review on which it is based. Meta-analysis has been widely used for coronary artery disease studies. For example, D'Ascenzo et al. [42] conducted meta-analysis to demonstrate that severe coronary disease is more common in patients with acute coronary syndrome or stable coronary disease than generally perceived, and that simple tools may help in the selection of the most appropriate therapeutic approach. Meta-analyses need to complete coverage of all relevant studies examining the presence of heterogeneity and exploring the robustness of the main findings with sensitivity analysis.

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