Heparin-Induced Thrombocytopenia
Third Edition, Revised and Expanded

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32. Intravascular Ultrasound Imaging in Coronary Artery Disease, edited by Robert J. Siegel
33. Saphenous Vein Bypass Graft Disease, edited by Eric R. Bates and David R. Holmes, Jr.
35. Cardiovascular Drug Development: Protocol Design and Methodology, edited by Jeffrey S. Borer and John C. Somberg
37. Clinical Neurocardiology, Louis R. Caplan, J. Willis Hurst, and Mark I. Chimowitz
39. Heparin-Induced Thrombocytopenia, edited by Theodore E. Warkentin and Andreas Greinacher
40. Silent Myocardial Ischemia and Infarction: Fourth Edition, by Peter F. Cohn
41. Foundations of Cardiac Arrhythmias: Basic Concepts and Clinical Approaches, edited by Peter M. Spooner and Michael R. Rosen
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47. Heparin-Induced Thrombocytopenia: Third Edition, Revised and Expanded, edited by Theodore E. Warkentin and Andreas Greinacher

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

T.E.W.

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

A.G.
Series Introduction

Although I used to think heparin-induced thrombocytopenia (HIT) was an extraordinarily rare and elusive disease, nothing could be further from the truth. If a high level of suspicion is maintained, HIT will be diagnosed frequently. Surprisingly, it causes deep venous thrombosis and pulmonary embolism, which tend to be massive and life-threatening, far more often than it causes arterial thrombosis. These blood clots cannot be treated with standard anticoagulation, and routine management may yield catastrophic results. Therefore, it is imperative that clinical cardiologists, internists, hematologists, and vascular medicine and surgery specialists familiarize themselves with HIT. To achieve this objective, it is a pleasure to present this updated landmark book, *Heparin-Induced Thrombocytopenia: Third Edition, Revised and Expanded*, edited by two of the most renowned clinical investigators in the field.

I will keep my personal copy on my closest bookshelf to help me as I consult on these perplexing patients. Nowhere else is the research from this emerging field pulled together so comprehensively and lucidly as in this scholarly and practical book.

*Samuel Z. Goldhaber*
Preface to the Third Edition

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

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

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

In 2002, danaparoid was withdrawn from the United States, leaving the direct thrombin inhibitors, lepirudin and argatroban, as the two main agents in that market for managing the difficult clinical situation of HIT complicated by thrombosis. (As of October 2003, danaparoid remained available in Canada and the European Union.) Another recent clinical development is the recognition that lepirudin bolus infusion can (rarely) trigger life-
threatening anaphylaxis, perhaps because of antibodies that have been formed against this foreign protein (although lepirudin is manufactured using recombinant biotechnology, its blueprint is drawn from the medicinal leech).

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

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

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

Theodore E. Warkentin
Andreas Greinacher
Preface to the Second Edition

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

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

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

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

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

More and more, it seems, HIT is an issue in cases of alleged medical malpractice. This is because HIT often occurs in patients who received heparin for prophylaxis of thrombosis. So it can seem evident even to a nonmedical person that something fundamentally went wrong if the patient ended up with severe pulmonary embolism or even limb loss.

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

*Theodore E. Warkentin*

*Andreas Greinacher*
Preface to the First Edition

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

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

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

Our focus on HIT as a clinicopathologic syndrome has implications for the terminology we have used in this book. Because the causative role of heparin can generally be established—in the appropriate clinical context—by the demonstration of pathogenic, *heparin-induced thrombocytopenia* to describe this syndrome (i.e., heparin can be shown convincingly to have “induced” the platelet count fall in a particular patient). In contrast, we use the term *nonimmune heparin-associated thrombocytopenia* (nonimmune HAT) to describe patients who have developed thrombocytopenia during heparin treatment and in whom a pathogenic role for HIT antibodies cannot be shown. In our view, this term unambiguously denotes that HIT antibodies are not responsible for the thrombocytopenia, while leaving open the possibility that heparin may have played a role in the causation of the platelet count fall by nonimmune mechanisms (although coinciding thrombocytopenia from another cause is probably the most frequent explanation for this event). We have also introduced the term *pseudo-HIT* to indicate those patients with nonimmune HAT that, by virtue of associated thrombosis or the timing of onset of thrombocytopenia, closely mimics HIT (see Chap. 12).

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

Marked thrombin generation in HIT also helps explain its association with *coumarin-induced venous limb gangrene*, an unusual syndrome now recognized as a potential complication of coumarin treatment of HIT-associated deep venous thrombosis (see Chap. 3). This iatrogenic disorder
represents perhaps the most striking of all the HIT treatment paradoxes (see Chap. 13): two antithrombotic agents with distinct adverse event profiles that interact to produce a profound disturbance in procoagulant-anticoagulant balance, i.e., increased thrombin generation (secondary to HIT) together with acquired, severe protein C deficiency (secondary to coumarin treatment). Finally, the concept of HIT as a hypercoagulable state with in vivo thrombin generation provides a rationale for understanding the efficacy of new therapies that either reduce thrombin generation by inhibition of factor Xa (e.g., danaparoid) or directly inactive thrombin (e.g., lepirudin).

A third theme of this book is the peculiarly inconstant nature of HIT, in particular, its variable frequency and clinical presentation among different patient populations treated with heparin. Figure 1 depicts HIT as an iceberg within which a variety of clinical and laboratory factors interact to influence antibody formation, development of thrombocytopenia, and, finally, resulting clinical complications, such as thrombosis. A recent, novel concept is that the size and buoyancy of the HIT icebergs can vary among different patient populations who receive heparin. This concept of multiple icebergs of HIT is shown in Chapter 4, Fig. 3. Unraveling the clinical and laboratory determinants for these differences in the icebergs among patient populations is a major challenge of current and future investigation.

Figure 1  The iceberg model of heparin-induced thrombocytopenia as an index for this book.
Why should HIT be the subject of a book? First and foremost, HIT is common, and nonimmune HAT is very common. According to the Council for International Organization of Medical Sciences (CIOMS III), adverse drug reactions can be classified as common if they occur in 1–10% of patients, and very common if they occur in 10% or more of patients. There is convincing evidence that HIT occurs in as many as 5% of certain patient populations, such as postoperative orthopedic patients receiving unfractionated heparin. The clinical influence of HIT is substantial: about half of these patients develop HIT-associated thrombosis. Nonimmune HAT occurs in as many as 30% of certain patient populations. Thus, physicians need to be able to reliably distinguish among the various thrombocytopenic disorders that occur during heparin treatment. This will minimize the risk of inappropriate treatments, such as failing to stop heparin administration in a patient with probable HIT, or deciding to stop heparin in a patient with nonimmune HAT or pseudo-HIT. Because HIT is a life- and limb-threatening iatrogenic illness with many diagnostic and treatment pitfalls, medicolegal consequences of caregiver’s action or inaction can be significant (see Chap. 18).

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

Heparin has been, and will continue to be, one of the most important agents for the prophylaxis and treatment of venous and arterial thromboembolism. Consequently, HIT will continue to be an important management problem for some time to come. Both of us have spent a decade of our scientific careers providing, in the context of other investigators’ work, a rational management approach aimed at minimizing morbidity and mortality among the many patients who develop the most common immune-mediated adverse drug reaction in clinical medicine. The importance of controlling thrombin generation in HIT is now widely appreciated. The book should help guide clinicians through the often paradoxical clinical and management problems posed by patients with HIT (see Chaps. 13–17).

The book is a tribute to our scientific mentors, John G. Kelton and Christian Mueller-Eckhardt, and the close cooperation of many of our scientific colleagues and personal friends whose efforts made this project possible.
We would also like to acknowledge the help of many individuals in this project. In North America, we thank Jo-Ann Sheppard for technical support over the years, and also for preparing many of the figures in this book; Aurelio Santos, Jr., for preparing the key Fig. 13.1; James W. Smith, for help with vexing computer problems; Katherine Bean and Maria Adamek, for able secretarial assistance; and Erica Warkentin, for checking references and manuscripts. In Germany, we thank Uta Alpen for excellent secretarial assistance and Petra Eichler, Norbert Lubenow, Lena Carlsson, and Oliver Ranze for valuable discussion and review of manuscripts.

Theodore E. Warkentin  
Andreas Greinacher

REFERENCES


Contents

Series Introduction (Samuel Z. Goldhaber) v
Preface to the Third Edition vii
Preface to the Second Edition ix
Preface to the First Edition xi
Contributors xxi

1. History of Heparin-Induced Thrombocytopenia
   Theodore E. Warkentin 1

2. Differential Diagnosis of Acute Thrombocytopenia
   Volker Kiefel 25

3. Clinical Picture of Heparin-Induced Thrombocytopenia
   Theodore E. Warkentin 53

4. Frequency of Heparin-Induced Thrombocytopenia
   David H. Lee and Theodore E. Warkentin 107

5. Nonimmune Heparin–Platelet Interactions: Implications
   for the Pathogenesis of Heparin-Induced Thrombocytopenia
   McDonald K. Horne III 149

6. Heparin-Dependent Antigens in Heparin-Induced
   Thrombocytopenia
   Jean Amiral and Dominique Meyer 165

7. Molecular Immunopathogenesis of Heparin-Induced
   Thrombocytopenia
   Gian Paolo Visentin, Chao Yan Liu, and Richard H. Aster 179

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<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Role of Sulfated Polysaccharides in the Pathogenesis of Heparin-Induced Thrombocytopenia</td>
<td>Susanne Alban and Andreas Greinacher</td>
<td>197</td>
</tr>
<tr>
<td>9</td>
<td>The Platelet Fc Receptor in Heparin-Induced Thrombocytopenia</td>
<td>Gregory A. Denomme</td>
<td>223</td>
</tr>
<tr>
<td>10</td>
<td>Immune Vascular Injury in Heparin-Induced Thrombocytopenia</td>
<td>Gowthami M. Arepally, Mortimer Poncz, and Douglas B. Cines</td>
<td>251</td>
</tr>
<tr>
<td>11</td>
<td>Laboratory Testing for Heparin-Induced Thrombocytopenia</td>
<td>Theodore E. Warkentin and Andreas Greinacher</td>
<td>271</td>
</tr>
<tr>
<td>12</td>
<td>Pseudo–Heparin-Induced Thrombocytopenia</td>
<td>Theodore E. Warkentin</td>
<td>313</td>
</tr>
<tr>
<td>13</td>
<td>Treatment of Heparin-Induced Thrombocytopenia: An Overview</td>
<td>Andreas Greinacher and Theodore E. Warkentin</td>
<td>335</td>
</tr>
<tr>
<td>14</td>
<td>Danaparoid for the Treatment of Heparin-Induced Thrombocytopenia</td>
<td>Beng Hock Chong and Harry N. Magnani</td>
<td>371</td>
</tr>
<tr>
<td>15</td>
<td>Lepirudin for the Treatment of Heparin-Induced Thrombocytopenia</td>
<td>Andreas Greinacher</td>
<td>397</td>
</tr>
<tr>
<td>16</td>
<td>Argatroban Therapy in Heparin-Induced Thrombocytopenia</td>
<td>Bruce E. Lewis and Marcie J. Hursting</td>
<td>437</td>
</tr>
<tr>
<td>17</td>
<td>Bivalirudin for the Treatment of Heparin-Induced Thrombocytopenia</td>
<td>John R. Bartholomew</td>
<td>475</td>
</tr>
</tbody>
</table>
### Contents

18. Hemodialysis in Heparin-Induced Thrombocytopenia 509  
   Karl-Georg Fischer

19. Management of Cardiopulmonary Bypass Anticoagulation in Patients with Heparin-Induced Thrombocytopenia 531  
   Bernd Poetzsch and Katharina Madlener

20. Heparin-Induced Thrombocytopenia in Children 553  
   Anne F. Klenner and Andreas Greinacher

21. Legal Aspects of Heparin-Induced Thrombocytopenia: U.S. Perspectives 573  
   Kevin M. McIntyre and Theodore E. Warkentin

22. Legal Aspects of Heparin-Induced Thrombocytopenia: European Perspectives 587  
   Klaus Ulsenheimer

### Appendixes

1. Ten Clinical “Rules” for Diagnosing HIT 597
2. Estimating the Pretest Probability of HIT: The Four T’s 599
3. Platelet Count Monitoring for HIT 600
4. Treatment Recommendations 602
5. Danaparoid Dosing Schedules in HIT Patients 606
6. Dosing Schedules for Lepirudin Treatment of Patients with HIT 608
7. Dosing Schedule for Lepirudin in Patients with HIT and Renal Impairment 610
8. Dosing Schedules for Argatroban Treatment of Patients with HIT (Approved Indications) 611
9. Timelines of an Episode of HIT 612

Index 613
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1
History of Heparin-Induced Thrombocytopenia

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I. THE DISCOVERY OF HEPARIN AND ITS FIRST CLINICAL USE

The following account of the discovery and first clinical development of heparin was recorded by the physiologist Charles H. Best (1959), a codiscov- erer of insulin as well as a pioneer in studies of heparin. Incidentally, in 1916, while working at Johns Hopkins University to characterize procoagulant substances, Dr. Jay McLean (1916) identified a natural anticoagulant sub- stance. Further studies of this material were performed by his supervisor, Dr. Howell, who coined the term, “heparin” to indicate its first extraction from animal hepatic tissues (Gr. Ἐπαρ [hepar], liver) (Howell and Holt, 1918).

Despite its in vitro anticoagulant action, the inability of heparin to prevent platelet-mediated thrombosis (Shionoya, 1927) made it uncertain whether it had antithrombotic potential. However, animal (Mason, 1924) and human studies (Crafoord, 1937) showed that heparin could prevent thrombosis. By the 1950s, heparin was established as an important therapeutic agent in the treatment of venous and arterial thrombosis.

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

A. Weismann and Tobin

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

This 62-year-old white woman was admitted to the Hitchcock Hospital Feb 8, 1955, with left retinal detachment, complicating longstanding myopia. . . . Left scleral buckling was carried out on Feb. 10, and strict bed rest was required during the ensuing three weeks. On her beginning ambulation, on March 6, signs and symptoms of left iliofemoral thrombophlebitis were noted, for which systemic heparinization was begun ( . . . heparin sodium in divided subcutaneous doses, totaling 150–300 mg per day . . . ). On March 16 . . . . , after 10 days of anticoagulation therapy, sudden signs of right common femoral arterial occlusion led to the diagnosis of common femoral arterial embolism. Successful femoral embolectomy was carried out. She was kept on adequate heparinization and made a satisfactory initial recovery until March 19, . . . when signs of sudden occlusion of the distal aorta appeared.
... [P]rompt transperitoneal distal aortic and bilateral iliac embolectomies were performed. In the ensuing 24 hours, because unsatisfactory distal circulation persisted, the patient underwent left femoral exploration, with negative findings, and right popliteal exploration, revealing an embolus. She subsequently pursued a favorable course, ... never showing more serious ischemic changes than a small area of superficial gangrene of the right great toe and several small areas of skin infarction of the right leg (Weismann and Tobin, 1958).

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

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

The authors noted that further embolization stopped when the heparin was discontinued, leading to their recommendation that “heparin should be promptly reduced in dosage, and, if possible, discontinued if the presence of fibrin-platelet thrombi adherent to the intima of the aorta is suspected.” Aggressive surgical management of emboli was also recommended, as some limbs were salvageable in this way. The authors summarized well the clinical dilemma: “In each instance there was a feeling of futility in the management of the problem, due to anticipation of further emboli from the same or similar sources. Heparin was badly needed to retard distal thrombosis; yet the agent was probably seriously altering the integrity and attachment of the thrombotic source” (Weismann and Tobin, 1958).

B. Roberts and Colleagues

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

To witness a series of apparently paradoxical events is disconcerting as well as challenging. When such paradoxes involve totally unexpected results following the use of a major therapeutic agent, it is at first difficult to know whether the relationship is causal or merely coincidental. When, however, the same series of events has been seen repeatedly it is difficult to escape the conclusion that there is some causal relationship, even though the mechanism by which it is accomplished may be unknown. . . . During the last 9 years at the Hospital of the University of Pennsylvania we have seen a group of 11 patients who suffered unexplained arterial embolization for the first time while being treated with heparin for some condition that could not of itself reasonably be expected to cause arterial emboli. . . . All patients had been receiving heparin for 10 days or more when the initial embolus occurred. . . . All emboli removed were of a light color, seemingly made up primarily of fibrin and platelets, and microscopically appeared to be relatively free of red cells. . . . All patients in this group had multiple emboli. . . . Of the 4 deaths, 3 were attributed to cerebral vascular accidents presumably embolic in origin and 1 was thought to have resulted from a perforation of the small bowel 2 weeks after the removal of a mesenteric embolus (Roberts et al., 1964).

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

C. An Immune Basis for Heparin-Induced Thrombosis?

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

III. HEPARIN-INDUCED THROMBOCYTOPENIA AND PARADOXICAL THROMBOSIS

A. Heparin-Induced Thrombocytopenia

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

B. Rhodes, Dixon, and Silver: “Heparin-Induced Thrombocytopenia with Thrombotic and Hemorrhagic Manifestations”

Laboratory evidence implicating an immune basis for heparin-induced thrombocytopenia (HIT) was first provided by studies performed by a vascular surgeon (Donald Silver; Fig. 2), in collaboration with two residents (Glen R. Rhodes and R. H. Dixon). The first two patients described by Silver’s group (Rhodes et al., 1973) developed severe thrombocytopenia (platelet count
nadirs, 8 and 10 × 10^9/L), myocardial infarction, petechiae, and heparin resistance, with complete platelet count recovery on discontinuing heparin treatment. Both patients developed rapid recurrence of thrombocytopenia when heparin rechallenges were given within 1 week of platelet count recovery.

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

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

C. Platelet-Activating Antibodies in the Pathogenesis of HIT

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

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

A consistent theme was evident from these various reports. Patients developed arterial or venous thrombotic complications, in association with
thrombocytopenia that generally began after 5 or more days of heparin treatment. A platelet-activating antibody that aggregated platelets suspended in citrated plasma was usually detected. The platelet count nadirs seen in some of the larger series (e.g., 33 and 48 × 10⁹/L, respectively) observed by Cimo et al. (1979) and Hussey et al. (1979), were higher than in previous reports, indicating that as recognition of the syndrome grew, less severely thrombocytopenic patients were recognized.

D. The “White Clot Syndrome”

Jonathan Towne, a vascular surgeon in Milwaukee, reported with his colleagues (1979) that the pale thrombi characteristic of this syndrome consisted of fibrin–platelet aggregates (electron microscopy). These workers coined the term “white clot syndrome” to describe the characteristic appearance of these arterial thromboemboli. Ironically, their report is also the first to note the occurrence of phlegmasia cerulea dolens (severe venous limb ischemia) that progressed to venous limb gangrene in two of their patients (i.e., a syndrome of limb loss due to extensive venous thrombosis without arterial white clots). Nonetheless, the designation of white clot syndrome has become virtually synonymous with HIT in both North America and Europe (Benhamou et al., 1985; Stanton et al., 1988), despite the lack of specificity of these thrombi for HIT (see Chap. 12).

IV. NONIMMUNE HEPARIN-ASSOCIATED THROMBOCYTOPENIA

A. Nonimmune Mechanisms in Heparin-Associated Thrombocytopenia

Klein and Bell (1974) reported on two patients who developed severe thrombocytopenia, thrombotic complications, and DIC, with hypofibrinogenemia and microangiopathic red cell abnormalities; i.e., these patients likely had severe HIT. This experience prompted Bell to perform the first prospective study investigating the frequency of thrombocytopenia complicating therapeutic-dose UFH (Bell et al., 1976). Sixteen of 52 patients (31%) developed a platelet count fall to less than 100 × 10⁹/L, and some of these patients developed hypofibrinogenemia and elevated fibrinogen degradation products. The authors speculated that a “thromboplastic contaminant” extracted along with heparin from beef lung could explain the thrombocytopenia. A subsequent randomized controlled trial by Bell and Royall (1980) found the frequency of thrombocytopenia to be higher in patients who received bovine heparin (26%) compared with heparin of porcine intestinal origin (8%).
These investigators found no platelet-activating antibodies in plasma from the patients who developed thrombocytopenia (Alving et al., 1977), leading Bell (1988) to challenge the view that an immune pathogenesis explained HIT. However, as the Johns Hopkins group did not report thrombotic complications in any of their 37 patients who developed thrombocytopenia in their prospective studies, and given the apparent early onset of thrombocytopenia in many of their patients, it is likely that most of their patients did not have immune-mediated HIT.

B. Nonimmune (Type I) Versus Immune (Type II) Heparin-Induced Thrombocytopenia

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

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

V. LABORATORY TESTING TO CHARACTERIZE THE HIT SYNDROME

A. A Sensitive and Specific Platelet Activation Assay for HIT

Many clinical laboratories began to use platelet aggregation assays (Fratantoni et al., 1975; Babcock et al., 1976) to diagnose HIT. Problems with this type of assay, however, included low sensitivity (Kelton et al., 1984) as well as technical limitations in simultaneous evaluation of multiple patient and control samples. In 1983–1984, while working as a research fellow in the
McMaster University laboratory of John Kelton, Dave Sheridan overcame problems of low test sensitivity by showing that washed platelets, resuspended in a buffer containing physiological concentrations of divalent cations, were very sensitive to platelet activation by HIT sera (Sheridan et al., 1986). The assay, known as the “platelet serotonin release assay (SRA),” was adapted from a method of platelet washing developed at McMaster University by the laboratory of Dr. Fraser Mustard. In particular, the emphasis on using physiological calcium concentrations was based on observations that “artifacts” of agonist-induced platelet activation were caused by use of citrate anticoagulation resulting in low plasma calcium concentrations. One example of an artifact induced by citrate is that of two-phase aggregation triggered by ADP. At physiological calcium concentrations, only weak single-phase aggregation without thromboxane generation is triggered by ADP (Kinlough-Rathbone et al., 1983). Fortuitously, the washed platelet technique previously developed at McMaster University by Mustard and colleagues that Sheridan evaluated for its HIT serum-sparing properties rendered platelets far more sensitive to the platelet-activating properties of HIT antibodies than assays based on citrated platelet-rich plasma. Modified washed platelet assays have subsequently been developed by other investigators (see Chap. 11).

Sheridan and colleagues also made the observation that heparin concentrations strongly influenced platelet activation by HIT sera: therapeutic (0.05–1 U/mL), but not high (10–100 U/mL), heparin concentrations resulted in platelet activation, i.e., the characteristic “two-point” serotonin release activation profile of HIT. Later, Greinacher and colleagues (1994) showed that high heparin concentrations in solution release platelet factor 4 (PF4) from PF4–heparin complexes bound covalently to a solid phase, with a corresponding decrease in binding of HIT antibodies to the surface. Thus, the inhibition of platelet activation by high heparin concentrations probably results from a similar disruption of the multimolecular antigen complex on the platelet surface.

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

B. Prospective Studies of Serologically Defined HIT

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

In a prospective study of HIT that performed systematic testing for HIT antibodies (Warkentin et al., 1995), the authors showed the dramatic clinical effects of HIT. Of 665 patients participating in a clinical trial of UFH versus low molecular weight heparin (LMWH) after orthopedic surgery, 9 patients developed “late” thrombocytopenia serologically confirmed to represent HIT. These patients had a thrombotic event rate far greater than controls. Moreover, the spectrum of thrombosis in HIT patients included venous thromboembolism, rather than only the classic problem of arterial thrombosis. This study also showed that early postoperative thrombocytopenia occurred frequently, but was not explained by HIT antibodies (see Chap. 4).

However, even this study did not initially capture the complete clinical profile of HIT. This is because it defined the platelet count fall indicating possible HIT using the “standard” definition of thrombocytopenia, i.e., a platelet count fall to less than 150 × 10^9/L (Warkentin et al., 1995). Subsequent review of the database, together with correlative analysis of the results of systematic serological testing for HIT antibodies (performed in most study subjects), showed that this standard definition underestimated the number of patients who had HIT (Warkentin et al., 2003a). Rather, a proportional fall in platelet count (50% or greater)—in relation to the postoperative peak platelet count—provided a more accurate definition of thrombocytopenia (applicable at least to this postoperative patient population). This improved definition identified twice as many patients as having had HIT in this clinical trial, without compromising diagnostic specificity. Indeed, the study suggested that the risk of immune HIT is about 5% (16/332 = 4.8%) in postoperative orthopedic surgery patients receiving UFH for a week or more (see Chap. 4).

VI. THE TARGET ANTIGEN OF HIT: PLATELET FACTOR 4–HEPARIN

In 1992, Jean Amiral, working in the laboratory of Dominique Meyer, reported that the antigen recognized by HIT antibodies was a complex between heparin and platelet factor 4, an endogenous platelet α-granule protein (Ami-
ral et al., 1992). This important discovery led to an explosion of basic studies in numerous laboratories that led to further characterization of the basic pathogenesis of HIT (see Chaps. 6–8). Amiral’s discovery also led to the development of new assays for HIT antibodies based on enzyme immunoassay techniques (see Chap. 11).

The antigen site(s) recognized by HIT antibodies were identified as being on PF4, rather than on heparin itself or a compound antigen (Li et al., 2002) (see Chaps. 6–8). This observation highlights intriguing parallels between HIT and the antiphospholipid syndrome. This latter disorder is also characterized by pathogenic antibodies directed against one or more proteins that express neoepitopes when bound to certain negatively charged phospholipid surfaces (see Chap. 12). The presence of neoepitopes on the “self” protein, PF4, suggests the HIT can be conceptualized as a transient, drug-induced, platelet- and coagulation-activating autoimmune disorder. Indeed, high-titer HIT antibodies that are able to activate platelets in vitro even in the absence of pharmacologic heparin have been associated with the onset of thrombocytopenia and thrombosis beginning several days after heparin has been discontinued, so-called delayed-onset HIT (Warkentin and Kelton, 2001) (see Chap. 3).

VII. TREATMENT OF THROMBOSIS COMPLICATING HIT

The treatment of HIT is discussed in Chapters 13–17. Here we will discuss only a few vignettes relating to the initial use of selected treatments for HIT.

A. Danaparoid Sodium

In 1982, a 48-year-old vacationing American developed deep venous thrombosis and pulmonary embolism following a transatlantic flight to Germany. Heparin treatment was complicated by thrombocytopenia and progression of venous thrombosis. Professor Job Harenberg of Heidelberg University, who had performed phase I evaluations of the experimental glycosaminoglycan anticoagulant danaparoid, requested this agent from the manufacturer (NV Organon, The Netherlands). The platelet count recovered and the venous thrombosis resolved (Harenberg et al., 1983, 1997). Over the next 6 years, this patient developed recurrent thromboembolic events, each time successfully treated with danaparoid. This favorable experience led to a named-patient, compassionate-release program ending in March 1997, during which time somewhat over 750 patients were treated with this agent. Additionally, Chong and colleagues (2001) performed the first randomized, controlled clinical trial evaluating danaparoid (see Chap. 14).
B. Recombinant Hirudin (Lepirudin)

The medicinal leech, *Hirudo medicinalis*, has been used for medical purposes for many centuries. Given the observation that the medicinal leech can prevent clotting of blood it has ingested, crude preparations derived from this animal were given experimentally at the beginning of the twentieth century. However, because this treatment’s daily cost (75 Reichsmark) in 1908 was equivalent to the monthly salary of a factory worker, it was judged to be infeasible. After World War I, Haas, at Justus-Liebig University in Giessen, began his experiments using crude extracts of leech heads for hemodialysis. The major complication in these animal experiments was severe bleeding. The first human hemodialysis patients were treated by him with hirudin during dialysis when a more purified, but still crude protein extract of leech heads became available (Haas, 1925).

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

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

C. Warfarin-Induced Venous Limb Gangrene

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

In December 1992, in Hamilton, Canada, while receiving ancrod and warfarin treatment for deep vein thrombosis complicating HIT, a 35-year-old woman developed progressive venous ischemia, culminating in venous limb gangrene. This occurred despite a supratherapeutic international normalized ratio (INR). The following day, Kelton observed an area of skin necrosis on the abdomen of this patient, suggesting the diagnosis of warfarin-induced skin
necrosis. The author questioned whether the warfarin had also contributed to the pathogenesis of the venous limb gangrene. This hypothesis was directly tested just 2 months later when a second young woman developed severe phlegmasia cerulea dolens of an upper limb during treatment of deep vein thrombosis complicating HIT with ancrod and warfarin. Treatment with vitamin K and plasma given by pheresis reversed the phlegmasia. Further laboratory studies supported this hypothesis of a disturbance in procoagulant-anticoagulant balance during treatment of HIT with warfarin (Warkentin et al., 1997) (see Chaps. 3, 12, and 13).

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

VIII. TREATMENT OF ISOLATED HIT

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

A. Natural History of Isolated HIT

During the mid-1990s, new data indicated a high risk for venous thrombosis in postoperative orthopedic patients who developed HIT, particularly for pulmonary embolism (Warkentin et al., 1995) (see Chap. 3). Thus, HIT came to be viewed as a dramatic, albeit transient, prothrombotic state, even when the original indication for heparin was routine antithrombotic prophylaxis.

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

To address this problem, the author performed a study of the natural history of HIT (Warkentin and Kelton, 1996). From a database of patients with serologically proven HIT, a 62-patient cohort with isolated HIT was identified: the cumulative 30-day thrombotic event rate was 52.8% (see Fig. 2 in Chap. 4). The rate of thrombosis was similarly high whether heparin was simply stopped or substituted with warfarin.

Similar findings were reported later by Wallis and colleagues (1999) from Loyola University. These investigators also found a high frequency of subsequent thrombosis (43 of 113, or 38%) in patients with isolated HIT managed by discontinuation of heparin. Surprisingly, a trend was observed for the highest risk of thrombosis in those patients in whom heparin was stopped most promptly (see Table 5 in Chap. 4).

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

B. Argatroban

A synthetic small-molecule thrombin inhibitor derived from L-arginine, now known as argatroban, was used in Japan during the 1980s as a treatment for chronic arterial occlusion (Tanabe, 1986). During this time, argatroban also underwent investigation as treatment for HIT in Japan, particularly in the setting of hemodialysis (Matsuo et al., 1988). In 1993, exclusive rights to the compound for the United States and Canada were acquired from Mitsubishi-Tokyo Pharmaceuticals, Inc. by Texas Biotechnology Corporation (TBC) of Houston. In 1995, clinical evaluation of this agent for HIT began in the United States, using a prospective, multicenter, open-label design with historical controls, the ARG-911 study (Lewis et al., 2001) (see Chap. 16). Two groups of patients were studied: HIT without thrombosis (i.e., isolated HIT) and HIT complicated by thrombosis (heparin-induced thrombocytopenia/thrombosis syndrome, or HITTS). Eligibility was based on clinical suspicion of HIT, and serological confirmation of the diagnosis, therefore, was not required. Both patient groups received the identical therapeutic-dose regimen of argatroban (initially, 2 µg/kg/min, then adjusted by activated partial thromboplastin time [aPTT]). The favorable results of the ARG-911
and subsequent studies (ARG-915, ARG-915X) led to the approval of argatroban on June 30, 2000, by the U.S. Food and Drug Administration (FDA) as “anticoagulant for prophylaxis or treatment of thrombosis in patients with heparin-induced thrombocytopenia.” Thus, for the first time in the United States, a drug was approved for the novel indication of prevention of thrombosis in isolated HIT. A marketing partnership between TBC and SmithKline Beecham (now, GlaxoSmithKline) commenced in August 1997. Marketing of argatroban began on November 13, 2000.

C. Therapeutic-Dose Anticoagulation for Isolated HIT

The approval by the FDA of identical therapeutic-dose regimens of argatroban for both prophylaxis and treatment of HIT highlighted the emerging view that HIT is a high-risk prothrombotic state. This contrasted with the earlier concept that HIT was generally benign, provided that thrombocytopenia was promptly recognized and heparin discontinued. Further support for the new view included studies showing HIT to be a profound hypercoagulable state (markedly elevated molecular markers of in vivo thrombin generation) (Warkentin et al., 1997; Greinacher et al., 2000) and recognition that many patients already have subclinical deep vein thrombosis at the time that isolated HIT is first recognized (Tardy et al., 1999).

Indeed, therapeutic doses of an alternative anticoagulant might be generally applicable for treatment of most patients with isolated HIT (Farner et al., 2001) (see also Chaps. 13–16). For example, although the prophylactic-dose regimen of lepirudin for HIT is initially lower than the therapeutic-dose regimen (0.10 mg/kg/h, rather than 0.15 mg/kg/h, and without an initial lepirudin bolus), subsequent dose adjustments are made using the aPTT; thus, the eventual infusion rate approaches the one given using the therapeutic regimen. A high success rate (91.4%) was observed using such “prophylactic” doses of lepirudin for isolated HIT (Farner et al., 2001).

In contrast, the prophylactic-dose regimen using danaparoid (750 U bid or tid) may be somewhat less effective than therapeutic-dose danaparoid (usually, 150–200 U/h) for preventing new thromboembolic complications in acute HIT: 81.4% versus 91.6% (Farner et al., 2001) (see Chap. 14). If this difference is real, it could be explained by greater efficacy of the therapeutic-dose regimen, in which at least twice as much danaparoid is usually given (3600–4800 vs. 1500–2250 U/24 h). The implication of Farner’s study is that the approved prophylactic-dose regimen of danaparoid may not be optimal, either when used for its approved indication in Europe (i.e., prevention of HIT-associated thrombosis) or for the corresponding “off-label” use for HIT elsewhere (Warkentin, 2001) (see Chap. 13).
IX. REDUCING THE RISK OF HIT

A. Low Molecular Weight Heparin

For over 50 years, UFH has been used in numerous clinical situations. However, UFH has several limitations, and efforts to develop potentially superior LMWH preparations began during the 1980s. Advantages of LMWH included better pharmacokinetics (e.g., improved bioavailability, predictable and stable dose response obviating the need for monitoring, lower risk of resistance to anticoagulation, longer plasma half-life) and favorable benefit-risk ratios in experimental animals (Hirsh, 1994; Hirsh et al., 2001). Advantages of UFH include its low cost, widely available laboratory monitoring, and potential for neutralization using protamine. But the question remained: Was the risk of HIT lower with LMWH? This was an important and relevant question, particularly as differences in risk of HIT exist even among UFH preparations derived from different animal sources (see Chap. 4). As discussed earlier (Sec. V.B), there is indeed evidence that LMWH has both a lower risk of HIT antibody formation and (more importantly) a lower risk of HIT and HIT-associated thrombosis. Table 1 provides a historical timeline of the introduction of the LMWH enoxaparin in the United States in various clinical situations.

B. Fondaparinux

Fondaparinux (Arixtra) is a synthetic pentasaccharide modeled after the antithrombin-binding site of heparin. It selectively binds to antithrombin, causing rapid and specific inhibition of factor Xa. Fondaparinux does not bind to PF4, and as a corollary, HIT antibodies fail to recognize PF4 mixed with fondaparinux, both in platelet activation and PF4-dependent antigen assays.

Preliminary evidence suggests that although HIT antibody formation occasionally occurs in association with fondaparinux use, such antibodies fail to react in HIT assays in which fondaparinux replaces UFH or LMWH in vitro (Warkentin et al., 2003b). Thus, this pentasaccharide anticoagulant seems unlikely to cause an adverse effect resembling HIT. Although no patients developed HIT with either LMWH (enoxaparin) or fondaparinux in the two orthopedic surgery trials reported, the duration of anticoagulant therapy may have been too brief to reveal a true difference in risk of immune thrombocytopenia between LMWH (frequency 0.1–1.0%) and fondaparinux (anticipated negligible frequency). Fondaparinux is approved in the United States, Canada, and the European Union for antithrombotic prophylaxis in orthopedic surgery situations (Table 1).
<table>
<thead>
<tr>
<th>Indication</th>
<th>Date of U.S. approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis after hip replacement surgery</td>
<td>March 29, 1993</td>
</tr>
<tr>
<td>Prophylaxis after knee replacement surgery</td>
<td>March 9, 1995</td>
</tr>
<tr>
<td>Prophylaxis after general (abdominal) surgery</td>
<td>May 6, 1997</td>
</tr>
<tr>
<td>Extended prophylaxis after hip replacement surgery</td>
<td>January 30, 1998</td>
</tr>
<tr>
<td>Prophylaxis for unstable angina and non-q wave myocardial infarction (given together with aspirin)</td>
<td>March 27, 1998</td>
</tr>
<tr>
<td>Acute deep-vein thrombosis, with or without pulmonary embolism, together with warfarin</td>
<td>December 31, 1998</td>
</tr>
<tr>
<td>Prophylaxis in medical patients at risk for deep-vein thrombosis or pulmonary embolism</td>
<td>November 17, 2000</td>
</tr>
<tr>
<td>Use as an anticoagulant in patients with unstable angina undergoing percutaneous transluminal coronary angioplasty (PTCA)</td>
<td>Bivalirudin</td>
</tr>
<tr>
<td>Anticoagulation in patients with or at risk for HIT undergoing percutaneous coronary interventions (PCI)</td>
<td>Argatroban</td>
</tr>
</tbody>
</table>

a Other LMWH preparations have been approved (at later times) for various antithrombotic indications (not shown).

b Wording of fondaparinux approval in U.S.: “ARIXTRA is indicated for the prophylaxis of deep vein thrombosis, which may lead to pulmonary embolism:
- in patients undergoing hip fracture surgery
- in patients undergoing hip replacement surgery
- in patients undergoing knee replacement surgery.”
C. Bivalirudin

The 20-amino-acid hirudin analogue bivalirudin (Angiomax, formerly, Hirulog) was used 10 years ago in the United States on a compassionate use basis for the treatment of four patients with HIT (Nand, 1993; Reid and Alving, 1994; Chamberlin et al., 1995). Currently, this agent is approved in the United States and Canada for anticoagulation of patients with unstable angina undergoing percutaneous transluminal coronary angioplasty (Table 1) (see Chap. 16). It is now seeing some off-label use for the treatment of HIT (Francis et al., 2003). This agent is also being evaluated as an anticoagulant to permit “on-pump” and “off-pump” cardiac surgery, in patients with or without HIT (Warkentin and Greinacher, 2003). It is possible that increasing use of bivalirudin or other nonheparin anticoagulants, e.g., argatroban (Table 1) or ximelagatran (oral thrombin inhibitor currently under investigation), in diverse clinical situations will result in reduced numbers of patients developing HIT.

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History of HIT


Differential Diagnosis of Acute Thrombocytopenia

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I. INTRODUCTION

Platelet count is affected by the rate of platelet production, the platelet life span, and the distribution of platelets among different compartments (Wintrobe et al., 1981). These three aspects of platelet kinetics have been extensively studied with platelets radiolabeled with $^51$Cr (Aster and Jandl, 1964) or $^{111}$In (Heaton et al., 1979).

Platelet production is equivalent to platelet turnover in a “steady state” and can be estimated by determining platelet mean life span and count: approximately $44 \times 10^9/L$ per day of platelets are produced by normal persons (Branehög et al., 1974). Platelet turnover is decreased in certain marrow disorders (e.g., aplastic anemia and hereditary thrombocytopenia) and often as a result of cytotoxic drugs used for the therapy of malignant disease. Platelet distribution is mainly influenced by spleen size. Normally, approximately 30% of platelets are sequestered in the spleen. In patients with splenomegaly, this fraction can increase to 90%, and mild to moderate thrombocytopenia can result. Conversely, in splenectomized subjects, more than 90% of the total platelet mass is circulating.

This chapter will focus on the differential diagnosis of thrombocytopenic states that are characterized by a shortened platelet survival that is mediated by immune mechanisms. Pathological conditions of enhanced platelet destruction caused by nonimmune mechanisms will also be briefly discussed. Autoantibodies responsible for autoimmune thrombocytopenic purpura (AITP), acquired platelet dysfunction, cyclic thrombocytopenia, and
some forms of drug-induced immune thrombocytopenia (e.g., gold-induced thrombocytopenia), react with monomorphic epitopes on platelet glycoproteins present on platelets of all healthy individuals. Alloantibodies recognize polymorphic, genetically determined epitopes on platelet glycoproteins. They are a specific finding in sera of patients with post-transfusion purpura. Other alloimmune thrombocytopenic disorders will not be discussed here. Drug-dependent antibodies can mediate platelet destruction by recognizing monomorphic determinants on platelet glycoproteins in the presence of the causative drug. Table 1 gives an overview of the platelet glycoproteins known to carry antigenic determinants.

II. AUTOIMMUNE THROMBOCYTOPENIC PURPURA

A. Pathogenesis

Thrombocytopenia in AITP results from rapid clearance of platelets sensitized with autoantibodies, usually of the IgG class, reacting with glycoproteins on the platelet surface (reviewed in Kiefel et al., 1992). Van Leeuwen and

Table 1 Antigens on Platelet Glycoproteins, Determined Under Reducing Conditions

<table>
<thead>
<tr>
<th>Glycoproteins</th>
<th>Number of subunits</th>
<th>Molecular weight</th>
<th>Antigenic determinants for&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP Ib/IIa, CD41/CD61</td>
<td>3</td>
<td>Iib</td>
<td>allo, iso, auto, drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ii</td>
<td>22,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IIa</td>
<td>105,000</td>
</tr>
<tr>
<td>GP Ib/IX/V, CD42</td>
<td>4</td>
<td>Ibα</td>
<td>allo, auto, drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ibβ</td>
<td>22,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IX</td>
<td>17,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>85,000</td>
</tr>
<tr>
<td>GP Ia/IIa, VLA-2, CD49b/CD29</td>
<td>2</td>
<td>Ia</td>
<td>allo, auto</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IIa</td>
<td>130,000</td>
</tr>
<tr>
<td>GP IV, GP IIIb, CD36</td>
<td>1</td>
<td></td>
<td>iso, auto</td>
</tr>
<tr>
<td>CD109</td>
<td>1</td>
<td></td>
<td>allo</td>
</tr>
<tr>
<td>HLA Class I</td>
<td>2</td>
<td>heavy chain</td>
<td>allo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β₂M</td>
<td>12,000</td>
</tr>
</tbody>
</table>

<sup>a</sup> allo, alloantibody; auto, autoantibody; drug, drug-dependent antibody; iso, isoantibody; VLA, very late activation antigen; β₂M, β₂-microglobulin.
coworkers (1982a) were the first to identify the glycoprotein (GP) IIb/IIIa complex as the major target antigen recognized by platelet autoantibodies in AITP, a finding confirmed by others. Epitopes of some antibodies have been assigned to GP IIIa (Beardsley et al., 1984) or GP IIb (Tomiyama et al., 1987). The other major target antigen is the platelet GP Ib/IX complex (Woods et al., 1984; Kiefel et al., 1991). Other “rare” autoantibodies have been shown to react with GP Ia/IIa (Castaldi et al., 1989), GP V (Mayer and Beardsley, 1996), GMP-140 (CD62), and the thrombopoietin receptor (Malloy et al., 1995).

B. Clinical Manifestations

In its various manifestations AITP is a relatively common disorder. Different forms are usually distinguished by clinical criteria.

Acute Postinfectious Autoimmune Thrombocytopenia

Acute AITP usually affects children younger than 10 years of age, boys and girls equally. The thrombocytopenia typically begins suddenly 10 days to 3 weeks after an acute viral infection (Waters, 1992). The incidence of AITP in childhood is estimated at 1:25,000 children per year. Bleeding symptoms may be severe in patients with platelet counts of fewer than 10 × 10^9/L. However, most patients recover within 3 months, even if they have not been treated with corticosteroids or intravenous IgG (ivIgG). By convention, AITP is referred to as “chronic” if thrombocytopenia persists for more than 6 months. However, about 90% of children with AITP develop the acute self-limited form.

Chronic AITP

Chronic AITP is the most common form of immune thrombocytopenia in adults, with women more frequently affected (3:1). The annual incidence has been estimated at approximately 1:31,000 in adults (Frederiksen and Schmidt, 1999). It may occur as “idiopathic” immune thrombocytopenia or as “secondary AITP”; that is, observed together with other immunological diseases, such as systemic lupus erythematosus (Waters, 1992), rheumatoid arthritis (Hegde et al., 1983), Crohn’s disease (Kosmo et al., 1986), primary biliary cirrhosis (Panzer et al., 1990); malignant diseases, such as lymphoproliferative disorders (Hegde et al., 1983) and solid tumors (Mueller-Eckhardt et al., 1983a); infectious diseases including viral hepatitis (Pawlotsky et al., 1995; Ibarra et al., 1986), human immunodeficiency virus (HIV) infection (Van der Lelie et al., 1987; Walsh et al., 1985); after bone marrow transplantation (Benda et al., 1989); and in diseases of unknown origin, such as sarcoidosis.
AITP with concomitant warm-type autoimmune hemolytic anemia is known as Evans’s syndrome (Waters, 1992). Onset of hemorrhagic diathesis is often insidious in chronic AITP. Most commonly, patients present with hemorrhagic manifestations of the skin, and in more severe forms, mucosal bleeding (“wet purpura”) with bloody blisters in the mouth, oozing from gums, epistaxis, melena, and menorrhagia (Crosby, 1975). Concerns about life-threatening intracranial hemorrhage are an important reason for therapy in AITP. The clinical course in AITP is difficult to predict, and it is estimated that in 10% of patients in childhood with acute AITP it will become chronic. The likelihood of chronic thrombocytopenia is much higher in adults. Many patients experience remissions and relapses that occur spontaneously or following infections. Chronic AITP may be an early manifestation of systemic lupus erythematosus.

C. Diagnosis

Clinical diagnosis of idiopathic AITP is based on its typical clinical picture and the exclusion of other causes for thrombocytopenia: isolated thrombocytopenia without evidence of impaired thrombopoiesis (normal numbers of megakaryocytes in the bone marrow). Moreover, spleen size is normal and no other conditions known to enhance platelet clearance are found, such as disseminated intravascular coagulation (DIC), thrombotic thrombocytopenia purpura (TTP), or large hemangiomas characteristic of the Kasabach-Merritt syndrome. Familial thrombocytopenia implies a nonimmune pathogenesis (Greinacher and Mueller-Eckhardt, 1994), for most patients with hereditary thrombocytopenia do not have enhanced platelet destruction (Najean and Lecompte, 1990).

It may be difficult to diagnose AITP on clinical grounds in patients with malignancy, because immune thrombocytopenia may coexist with splenomegaly or neoplastic marrow infiltration. Moreover, not all “platelet antibody tests” are diagnostically helpful (George et al., 1996). In particular, “platelet-associated immunoglobulin G” (PAIgG) assays developed as “platelet Coombs tests” focused on technical considerations, rather than on clinical usefulness (Dixon et al., 1975; Mueller-Eckhardt et al., 1978; Hegde et al., 1985; Follea et al., 1982; Morse et al., 1981; Kunicki et al., 1982; Leporrier et al., 1979; McMillan et al., 1979; Court and LoBuglio, 1986; Kiefel et al., 1987a). However, it appears that platelet-bound IgG in these assays bears little or no direct relation to immune-mediated platelet destruction (Mueller-Eckhardt et al., 1982; Kiefel et al., 1986). Rather, PAIgG reflects IgG stored in the platelet α-granules (George, 1990). However, with the advent of platelet glycoprotein-specific assays (McMillan et al., 1987; Kiefel et
D. Therapeutic Considerations

Therapy should be based on the degree of hemorrhagic diathesis observed. Thus, patients with “wet purpura” are generally treated more aggressively because they are considered to be at greatest risk for bleeding. In children with severe acute AITP, it is important to reduce physical activity. Drugs that inhibit platelet function, such as acetylsalicylic acid, should be avoided. If therapy is necessary, prednisone at an initial dose of 1 mg/kg body weight should be given for a limited period. The ivIgG preparations are very effective in childhood AITP (Imbach et al., 1981, 1985), with doses of 2 g/kg body weight (0.4 g/kg daily for 5 days or 1 g/kg for 2 days) usually effective for a limited time in adult patients. Alternatively, blockade of immune phagocytosis with sensitized autologous red blood cells has been proposed (Salama et al., 1983; Becker et al., 1986): IgG anti-D (Rh0) may be given intravenously to rhesus (D)-positive patients with AITP. Two doses of approximately 20 µg/kg body weight result in an increase in platelets in most patients. Although the therapeutic effect of anti-D appears less rapidly than with high-dose ivIgG, it is often more sustained. Immunosuppressive therapy with azathioprine (Quiquandon et al., 1990) alone or together with corticosteroids may be attempted in patients refractory to other forms of treatment. Refractory patients with dangerous bleeding complications have been successfully treated with cyclophosphamide (Reiner et al., 1995). One of the most effective therapeutic measures in AITP is splenectomy. It should not be performed in children younger than 6 years of age and not in the first 6 months of initially diagnosed acute AITP. It results in partial or complete remissions in 50–80% of patients (Shulman and Jordan, 1987). Possibly, patients with predominantly splenic sequestration of platelets have a higher chance of remission after splenectomy (Najean et al., 1997). A good response to ivIgG may indicate a higher remission rate after splenectomy (Law et al., 1997). Therapy of AITP has been reviewed (Berchtold and McMillan, 1989; Waters, 1992; Eden and Lilleyman, 1992; George et al., 1996).

E. Other Manifestations of Autoimmunity Against Platelets

Acquired Antibody-Mediated Platelet Dysfunction

In “typical” AITP, platelet autoantibodies induce thrombocytopenia with relatively moderate bleeding tendency that often is less pronounced than that
observed with similar platelet counts caused by impaired thrombocytopoiesis (Waters, 1992). Therefore, it has been concluded that platelet autoantibodies normally do not, or only slightly, affect platelet function. In 1986, the first case of a patient with normal platelet counts, but an IgG1 autoantibody-induced platelet dysfunction resembling Glanzmann’s thrombasthenia, was described (Niessner et al., 1986). Interestingly, patients with antibody-mediated platelet dysfunction may develop immune thrombocytopenia (Kubota et al., 1989) and vice versa (Meyer et al., 1991).

Cyclic Thrombocytopenia

Cyclic thrombocytopenia, which predominantly occurs in women, is characterized by rhythmic fluctuations of platelet counts. These fluctuations are in phase with the menstrual cycle, lowest platelet counts being observed during menses. Normal to high platelet counts are observed at midcycle (Tomer et al., 1989). In many patients with this condition, thrombocytopenia is the result of accelerated platelet clearance, as determined with $^{111}$In-labeled platelets. In two of the three patients described by Tomer, autoantibodies with GP Ib/IX specificity were identified during both the thrombocytopenic period and the period with normal platelet counts. These authors correlated platelet counts with changing densities of the Fcγ-receptor on the patients’ autologous monocytes. In one patient studied by Menitove, IgG anti-GP IIb/IIIa was found (Menitove et al., 1989). In another case, an IgM anti-GP IIb/IIIa has been reported (Kosugi et al., 1994). Data from another group suggest that the pathophysiology underlying the clinical picture of cyclic thrombocytopenia may be heterogeneous: an autoimmune form with cyclic changes in platelet destruction and a distinct condition with cyclic changes in thrombocytopoiesis (Nagasawa et al., 1995).

Onyalai

An exceptionally severe variant of AITP, onyalai, is observed in some black populations in southern Africa. Whites living in the same regions do not appear to suffer from this disease. In a series of 103 patients (Hesseling, 1987), all patients presented with hemorrhagic bullae of the mucous membranes of the oropharynx. Six died, four of cerebral hemorrhage and two of hemorrhagic shock. Clinical diagnosis is based on the criteria of AITP and, in addition, to the presence of hemorrhagic bullae (Hesseling, 1992). Antibodies against GP IIb/IIIa—often of the IgM class—have been implicated. The bone marrow contains normal counts of megakaryocytes, but patients may be anemic at presentation owing to blood loss. Therapeutic options are discussed elsewhere (Hesseling, 1992).
III. POSTTRANSFUSION PURPURA

A. Pathogenesis

Posttransfusion purpura (PTP) is a rare, but severe, transfusion reaction. Typically, 6–8 days after transfusion of whole blood, packed red blood cells, or platelet concentrates, patients experience an abrupt platelet count drop. The patient’s serum almost invariably contains a high-titered platelet-specific alloantibody, typically reacting with a determinant on the platelet GP IIb/IIIa complex. In most cases, it reacts with HPA-1a, but other specificities have been observed (Table 2). The duration of this immune-mediated thrombocytopenia is normally limited to 5–60 days. Although the patient’s autologous platelets do not carry the corresponding alloantigen, nevertheless, they undergo enhanced destruction. The alloimmune response is anamnestic, because nearly all patients with PTP have a documented prior exposure to the platelet alloantigen during previous pregnancy or transfusion.

Although PTP was first described in 1961, its pathogenesis remains debated even today. It has been suggested that circulating HPA-1a antigen from the platelets of the immunizing blood product persists, and thus antigen–antibody complexes are adsorbed onto the autologous platelets (Shulman et al., 1961). Others have proposed that during the secondary, anamnestic immunization precipitating PTP, a second autoreactive antibody arises, causing

<table>
<thead>
<tr>
<th>Alloantigen</th>
<th>Original designation</th>
<th>Percent positive&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Localization</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-1a</td>
<td>Pl&lt;sup&gt;A1&lt;/sup&gt;, Zw&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.5</td>
<td>GP IIIa</td>
<td>Shulman et al., 1961</td>
</tr>
<tr>
<td>HPA-1b</td>
<td>Pl&lt;sup&gt;A2&lt;/sup&gt;, Zw&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.8</td>
<td>GP IIIa</td>
<td>Taaning et al., 1985; Chapman et al., 1987</td>
</tr>
<tr>
<td>HPA-2b</td>
<td>Ko&lt;sup&gt;a&lt;/sup&gt;, Sib&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.8</td>
<td>GP Ib</td>
<td>Lucas et al., 1998</td>
</tr>
<tr>
<td>HPA-3a</td>
<td>Bak&lt;sup&gt;a&lt;/sup&gt;, Lek&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.1</td>
<td>GP IIb</td>
<td>Boizard and Wautier, 1984; Keimowitz et al., 1986</td>
</tr>
<tr>
<td>HPA-3b</td>
<td>Bak&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.9</td>
<td>GP IIb</td>
<td>Kieckler et al., 1988; Kiefel et al., 1989</td>
</tr>
<tr>
<td>HPA-4a</td>
<td>Yuk&lt;sup&gt;b&lt;/sup&gt;, Pen&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;99.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>GP IIIa</td>
<td>Simon et al., 1988</td>
</tr>
<tr>
<td>HPA-5b</td>
<td>Br&lt;sup&gt;a&lt;/sup&gt;, Zav&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.6</td>
<td>GP Ia</td>
<td>Christie et al., 1991</td>
</tr>
<tr>
<td>HPA-15b</td>
<td>Gov&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.2</td>
<td>CD109</td>
<td>Kelton et al., 1990; Berry et al., 2000</td>
</tr>
</tbody>
</table>

<sup>a</sup> Alloantigen frequency observed in Germany (Kiefel et al., 1993).

<sup>b</sup> Alloantigen frequency observed in Japan.
the patient’s severe thrombocytopenia. It is our experience that alloantibodies observed in the early anamnestic response in PTP are always high-titered. Moreover, they can be eluted from the autologous (alloantigen-negative) platelets in most cases studied (Kroll et al., 1993). Therefore, it can also be hypothesized that this pseudospecific alloantibody for a limited time cross-reacts with a structurally related epitope on the patient’s autologous platelets.

B. Clinical Picture

The clinical course of PTP has been summarized by the European PTP study group (Mueller-Eckhardt et al., 1991). Women were predominantly affected (99 out of 104). Mean age was 58.4 years. In 28 of 51 patients, marked febrile transfusion reactions were observed following the transfusion that precipitated PTP. The interval between transfusion and onset of severe thrombocytopenia was generally 6–10 days. In 68 out of 84 patients, the initial platelet count was fewer than 10 × 10⁹/L. Bleeding persisted for 3–37 days (mean, 10.2 days). Most patients required treatment. Fatal bleeding was not rare, occurring in 7 of 75 patients (Shulman and Jordan, 1987) and in 2 of 38 patients (Kroll et al., 1993) in two studies.

C. Diagnosis

The diagnosis of PTP should be considered in all patients with a sudden drop in platelet count to fewer than 10 × 10⁹/L. Thus, PTP and HIT may occur in similar clinical situations, (i.e., about 1 week after surgery). In PTP, however, thrombocytopenia is more pronounced and associated with bleeding (Lubenow et al., 2000), in contrast to the absence of petechiae and presence of thromboembolic complications characteristic of HIT (see Chap. 3).

Moreover, PTP only occurs following recent transfusion. PTP has occurred in association with delayed hemolytic transfusion reaction (Chapman et al., 1987; Maslanka and Zupanska, 1993). In a single case, PTP was observed concurrently with drug-dependent immune hemolytic anemia (Mueller-Eckhardt et al., 1987). Diagnosis is confirmed by detection of a platelet-specific alloantibody against an epitope on platelet GP IIb/IIIa, usually anti-HPA-1a. Platelets of the patient are always negative for the corresponding antigen. Eluates prepared from patients’ autologous platelets usually contain anti-HPA-1a (Kroll et al., 1993).

D. Therapeutic Considerations

The efficacy of corticosteroids is uncertain. In contrast, high-dose ivIgG (Mueller-Eckhardt et al., 1983b) is clearly effective in most (Berney et al.,
1985; Chong et al., 1986; Mueller-Eckhardt and Kiefel, 1988) but not all cases (Kroll et al., 1993). Thus, ivIgG is the treatment of choice for PTP. Platelet transfusions are ineffective, even if platelets from HPA-1a-negative donors are given (Gerstner et al., 1979). As the bleeding tendency is often pronounced, immediate therapy following clinical diagnosis is mandatory.

IV. PASSIVE IMMUNE THROMBOCYTOPENIA

Acute thrombocytopenia in humans resulting from experimental transfer of platelet autoantibodies has been observed in studies on the pathogenesis of AITP (Harrington et al., 1951). However, inadvertent transfer of platelet autoantibodies has not been recognized as a problem in clinical transfusion practice. In contrast, platelet alloantibodies with HPA-1a (Moilan et al., 1985; Ballem et al., 1987; Scott et al., 1988; Brunner-Bollinger et al., 1997) and HPA-5b (Warkentin et al., 1992) specificities can cause abrupt-onset immune thrombocytopenia. In three cases the antibody was transfused with plasma, in one case by whole blood, and once by a red blood cell concentrate. The condition may be accompanied by a febrile transfusion reaction. It is important to investigate these cases to identify and exclude donors with “harmful” platelet alloantibodies.

V. DRUG-INDUCED IMMUNE THROMBOCYTOPENIA

A. Pathogenesis

Drugs can induce various immune-mediated cytopenias, such as immune hemolytic anemia, neutropenia, and drug-induced immune thrombocytopenia (DIT) (Salama and Mueller-Eckhardt, 1992). Different mechanisms are involved in causing DIT, with drug-dependent antibodies being most extensively investigated. Drug-dependent antibodies typically react with monomorphic epitopes on virtually the same platelet glycoproteins recognized by platelet-specific autoantibodies: GP Ib/IX (Kunicki et al., 1978; van Leeuwen et al., 1982b; Berndt et al., 1985), GP V (Stricker and Shuman, 1986) or GP IIb/IIIa (Christie et al., 1987, 1993; Pfueller et al., 1990; Visentin et al., 1991). They bind to the platelet target antigen only in the presence of the causative drug: thus, if the drug is removed from the buffer during in vitro studies, the antibody detaches (see Fig. 2). The antibody specifically recognizes the antigen on platelet glycoprotein by the Fab fragment (Christie et al., 1985).

The precise role of the drug in antigen–antibody binding is unclear. Experimental studies suggest that in some individuals, the drug induces conforma-
tional change either in the antibody or in the platelet glycoprotein to induce complementarity required for antibody binding (Shulman and Jordan, 1987).

The drugs most commonly implicated in DIT are quinidine and quinine. Many substances have been suspected to induce this condition, but only a few have been documented by appropriate laboratory analysis (Aster and George, 1990; George et al., 1998). Table 3 lists drugs implicated in the author’s laboratory. Sometimes a metabolite, rather than the drug itself, mediates drug-dependent antibody binding to the platelet surface (Eisner and Shahidi, 1972; Eisner and Kasper, 1972; Kiefel et al., 1987c; Meyer et al., 1993). Similar observations have been made for drug induced immune hemolysis (Salama and Mueller-Eckhardt, 1985).

B. Clinical Picture and Therapy

Thrombocytopenia begins at least 7 days after the first exposure to the drug (Shulman and Jordan, 1987). Drug-dependent antibodies can persist; thus, reexposure to the drug can cause a sudden drop in platelet count, and is not recommended as a diagnostic maneuver. DIT often causes severe thrombocytopenia and bleeding.

The most important measure is to discontinue the offending drug. This is normally followed by a rise of platelet count. If bleeding symptoms are life-threatening, transfusion of large doses of platelets together with ivIgG should be considered.

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinidine</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Quinine</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Quinidine + quinine</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim–sulfamethoxazole</td>
<td>6</td>
<td>1 metabolite—specific ddAb (sulfamethoxazole)</td>
</tr>
<tr>
<td>Rifampicin (rifampin)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Nomifensine</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Paracetamol (acetaminophen)</td>
<td>1</td>
<td>1 metabolite—specific ddAb</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>3</td>
<td>1 metabolite—specific ddAb</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Source:* Institute for Clinical Immunology and Transfusion Medicine, University of Giessen.
C. Immune Thrombocytopenia Induced by GP IIb/IIIa Inhibitors

Specific inhibitors of GP IIb/IIIa are used with increasing frequency during cardiovascular interventions. Acute thrombocytopenia is a well-documented side effect of abciximab, a chimeric antibody inhibiting GP IIb/IIIa receptor function. In most cases, antibodies reacting with GP IIb/IIIa-abciximab complexes seem to be involved, but many issues of the pathogenesis remain unresolved. Typically, abciximab-induced thrombocytopenia occurs within the first 24 hours, most frequently at 4 hours. About 0.7% of patients are affected after first exposure to the drug (Jubelirer et al., 1999) and 4.6% of patients after repeated administration of abciximab (Tcheng et al., 2001). The rapid onset of thrombocytopenia even in those patients receiving the drug for the first time suggests that preformed antibodies are involved. A complicating issue is that pseudothrombocytopenia also can develop after abciximab administration; this was reported by Sane et al. (2000) to occur in 2.1% of patients (0.6% of placebo controls), whereas Schell et al. (2002) found pseudothrombocytopenia in 27% of patients treated with abciximab. Immuno-logical tests for antibodies reacting with abciximab-coated platelets are of little predictive value, as such antibodies are found in sera of more than 70% of healthy normal individuals (Curtis et al., 2002; Kiefel, unpublished observations). Whether these antibodies react with the abciximab molecule itself or against structural changes of GP IIb/IIIa complex after binding of the drug is not clear (Curtis et al., 2002).

Acute immune-mediated thrombocytopenia has also been observed after treatment with the GP IIb/IIIa inhibitors tirofiban and eptifibatide (Bougie et al., 2002). Sera from these patients contain naturally occurring antibodies that react against GP IIb/IIIa in the presence of the drug. However, they showed no reactivity with abciximab-treated platelets. Similar antibodies have been identified in patients who developed thrombocytopenia during treatment with the oral GP IIb/IIIa-receptor antagonists xemilofiban and orbofiban (Brassard et al., 2002). However, in these patients the median time of treatment before onset of thrombocytopenia was 11 days.

Therapy of thrombocytopenia induced by GP IIb/IIIa inhibitors is best established for abciximab. In contrast with other forms of immune thrombocytopenia, abciximab-induced immune thrombocytopenia is most effectively treated with platelet transfusions (Kereiakes et al., 1996). However, only in few cases with severe thrombocytopenia does bleeding occur and require therapy. In every suspected case, pseudothrombocytopenia should be ruled out first by review of the blood smear to avoid inappropriate discontinuation of the GP IIb/IIIa inhibitor or giving an unnecessary platelet transfusion.
D. Drug-Induced Autoimmune Thrombocytopenia

Some drugs cause autoimmune cytopenias by inducing autoantibodies indistinguishable from those encountered in “idiopathic” autoimmune cytopenia. A well-known example is α-methyldopa, which in 10–36% of patients induces formation of red blood cell autoantibodies (Petz and Garratty, 1980). However, only 1% of patients develop clinical hemolysis. Similarly, autoimmune thrombocytopenia has been observed during the course of gold therapy (von dem Borne et al., 1986). The antibodies found in these patients do not require the presence of the drug for binding to platelets in vitro (i.e., they resemble autoantibodies).

VI. CONSUMPTIVE THROMBOHEMORRHAGIC DISORDERS

A. Pathogenesis

A heterogeneous group of events, including sepsis, malignancies, trauma, obstetric complications, snake venoms, and hemolytic transfusion reactions, can be complicated by a systemic syndrome characterized by dysregulated thrombin formation, leading to activation and consumption of coagulation factors and resulting in the formation of intravascular fibrin thrombi. Secondary plasmin generation helps to lyse the fibrin formed. Additionally, the vessel wall and platelets are usually involved in this pathological process of “disseminated intravascular coagulation” (DIC). Indeed, thrombocytopenia is a common clinical manifestation of DIC (Mammen, 1998). Both bleeding and widespread thrombotic microvascular occlusion, leading to organ failure, can result from DIC.

B. Clinical Disorders

Septicemia

Disseminated intravascular coagulation can complicate infections, especially with gram-negative bacteria (Marder et al., 1994; Mammen, 1998). It has been suggested that thrombocytopenia in septic patients is the consequence of immune-mediated platelet damage, based on the observation of elevated PAIgG levels (Kelton et al., 1979). However, this does not prove an autoimmune basis for the thrombocytopenia (Shulman and Reis, 1994), for PAIgG is often also elevated in thrombocytopenia of nonimmune origin.

The pathogenesis of DIC in sepsis is multifactorial and includes direct endothelial damage and platelet activation by endotoxins, resulting in exposure of procoagulant material. In addition, the cytokines interleukin-1 and tumor necrosis factor increase tissue factor activity, thereby shifting the bal-
ance toward a prothrombotic tendency (Mammen, 1998). Plasminogen activator inhibitor-1 (PAI-1) blocks plasmin generation during the course of sepsis, thereby contributing to fibrin deposition in the microcirculation (Müller-Berghaus, 1987).

Malignant Disease

About 9–15% of patients with cancer have DIC at some point during their disease (Pasquini et al., 1995). Overt bleeding is uncommon; rather, recurrent thromboembolism is characteristic, an entity known as Trousseau’s syndrome. An exception: acute promyelocytic leukemia is often accompanied by a severe DIC and bleeding, often induced or worsened by chemotherapy (Marder et al., 1994).

Other Conditions

Disseminated intravascular coagulation occurs in obstetric situations characterized by release of thrombogenic material [e.g., the retained dead fetus syndrome (Marder et al., 1994; Baglin, 1996), amniotic fluid embolism, or placental separation]. Bites of certain snakes may cause hypofibrinogenemia induced by enzymes that clot fibrinogen or directly activate platelets. Severe hemolytic transfusion reactions can cause DIC, especially in association with red cell antibodies, causing intravascular complement-mediated hemolysis (e.g., ABO-incompatible transfusion). DIC seems to be aggravated by complement-mediated damage of endothelial cells. Whether red cell lysis alone (not mediated by complement) is able to induce DIC in humans remains unclear (Mollison et al., 1993; Baglin, 1996). Other conditions associated with DIC are trauma and localized processes in which activation of coagulation occurs within giant hemangiomas (Kasabach-Merritt syndrome) or aortic aneurysms.

C. Diagnosis

“Global” coagulation tests, such as prothrombin and activated partial thromboplastin times, are usually prolonged; fibrinogen concentrations are often reduced. However, these parameters can be normal in DIC. Fibrin degradation products mirror the action of plasmin on fibrin clots, and therefore are elevated in most patients with DIC. The D-dimer test readily detects cross-linked fibrin degradation products. Elevated prothrombin fragment F1 + 2 levels reflect thrombin activation as one of the central mechanisms underlying DIC. Examination of a blood smear sometimes will show red cell fragmentation in DIC. On the other hand, a high percentage of red cell frag-
Thrombotic thrombocytopenic purpura (TTP) is a severe disease characterized by intravascular platelet aggregation, nonimmune hemolytic anemia, neurological symptoms and signs, and renal failure. Red cell fragmentation, hemolysis with a negative direct antiglobulin test, and platelet-rich thrombi occluding small blood vessels are characteristic.

Different hypotheses have been proposed to explain the peculiar phenomenon of platelet deposition within the precapillary arterioles (Moake and Eisenstaedt, 1994). Plasma of patients with TTP and the hemolytic-uremic syndrome (HUS) contains unusually large von Willebrand factor (vWF) forms (Moake et al., 1982) that may mediate platelet aggregation. These can be explained by hereditary deficiency of vWF-cleaving protease (ADAMTS13) (Bianchi et al., 2002) in patients with chronic, relapsing TTP. An IgG inhibitor of the vWF-cleaving protease is responsible for the more common acute, self-limited form of TTP (Furlan et al., 1998). Various disorders can be associated with a TTP-like illness, including treatment with immunosuppressive drugs, metastatic cancer or its therapy (Gordon and Kwaan, 1997), and infections.

Vascular damage in HUS is usually confined to the kidneys, and neurological sequelae are less pronounced than in TTP (Moake and Eisenstaedt, 1994). HUS developing after bloody diarrhea is most commonly related to infections with verocytotoxin-producing bacteria [e.g., Escherichia coli serotype O157 (Taylor and Monnens, 1998)]. Transfer of verocytotoxin to the target organs and damage of endothelial cells has been implicated in the pathogenesis of HUS following infectious diarrhea and enterocolitis. Other forms of HUS may be caused by drugs (cyclosporine, tacrolimus, quinine) or complement abnormalities, e.g., factor H (Ohali et al., 1998; Noris et al., 1999). Moreover, HUS can be found associated with SLE, during pregnancy, and following bone marrow transplantation.

The cornerstone of therapy for TTP is transfusion of homologous plasma, usually given by plasma exchange. Corticosteroids and immunosuppressive drugs may be effective in cases of auto-anti-vWF-cleaving protease. Details of clinical features and therapeutic options are discussed elsewhere (Remuzzi, 1987; George and Aster, 1990; Moake and Eisenstaedt, 1994; Alford et al., 2003).
Laboratory testing for immune-mediated thrombocytopenia requires specific knowledge of the underlying clinical problem. For example, an accurate drug history is needed to evaluate DIT in vitro.

A. Analysis of Platelet Autoantibodies and Alloantibodies in Serum Samples

Often, only serum samples from a thrombocytopenic patient are available for study. Depending on the clinical problem, it may be useful to screen for platelet-reactive serum antibodies. A reliable, standardized immunoglobulin-binding assay for platelet antibodies is the platelet suspension immunofluorescence test (von dem Borne et al., 1978). A simplified alternative is more convenient for large-scale screening (Schneider and Schnaidt, 1981), but may be less sensitive. Usually a “panel” of platelets with different alloantigens is employed: platelet suspensions are incubated with the serum to be studied, and immunoglobulin binding to platelets is determined by platelet immunofluorescence. Reactivity with all platelets from the panel is often observed with platelet autoantibodies, but may also occur with antibodies against “high-frequency” antigens (e.g., HPA-4a [Yuk b]; Nak c), or mixtures of alloantibodies, if the panel does not include antigen-negative platelet suspensions. The most compelling way to exclude that a broadly reactive alloantibody is an autoantibody is to test the serum against autologous platelets. With the rare exception of PTP, alloantibodies do not react with autologous platelets.

Whenever possible, the platelet glycoprotein target of the antibodies should be identified. This is important because many sera of patients who have previously been exposed to allogeneic blood cells via transfusion or pregnancy contain “contaminating” alloantibodies reacting with HLA class I antigens present in high density on platelets.

Laboratory diagnosis of AITP, PTP, DIT, and certain thrombocytopenic states in newborns is based on the characterization of platelet-specific antibodies. This may be accomplished with assays that include electrophoretic determination of molecular weight of glycoproteins, including immunoblot (Herman et al., 1986; Huisman, 1986) or (radio-) immunoprecipitation (Mulder et al., 1984; Santoso et al., 1989; Smith et al., 1993). These techniques are cumbersome and time-consuming. Therefore, assays that allow identification of target antigens with well-characterized monoclonal antibodies are now preferred. These include the monoclonal antibody immobilization of platelet antigens (MAIPA) assay (Kiefel et al., 1987b; Kiefel, 1992) and the
immunobead assay (McMillan et al., 1987). These monoclonal antibody-based immunoassays are suitable to detect, and discriminate among, antibodies against GP IIb/IIIa, GP Ia/IIa, GP Ib/IX, GP V, HLA class I antigen, and other structures of the platelet membrane.

Virtually all antibodies reacting with the HLA class I antigens, and most antibodies against GP Ia/IIa, are alloantibodies. In contrast, GP IIb/IIIa and GP Ib/IX/V carry determinants recognized by autoantibodies, drug-dependent antibodies, and alloantibodies (see Tables 1 and 2). The serological diagnosis of PTP is based on detection of platelet alloantibodies, mainly against GP IIb/IIIa.

B. Characterization of Platelet-Bound Antibodies

Determination of specific autoantibodies against GPs IIb/IIIa and Ib/IX on a patient’s autologous platelets performed with “direct” glycoprotein-specific immunoassays (McMillan et al., 1987) is much more specific than quantitation of PAIgG. As an alternative, testing of eluates prepared at pH 2.8 from autologous patient platelets in an antibody-binding assay (platelet immunofluorescence) is also specific for AITP (Kiefel et al., 1996). Direct immuno-

![Figure 1](summary_of_drug-dependent_antibody_detection_in_immunoglobulin-binding_assays)
precipitation detected anti-HPA-5b on the patient’s platelets in a case of passive alloimmune thrombocytopenia (Warkentin et al., 1992).

C. Determination of Drug-Dependent Antibodies

Drug-dependent antibodies against platelets can be characterized using various techniques. If antiglobulin-binding assays are used, they should include the following steps (Fig. 1). Test platelets should be incubated in buffer containing the drug, and the serum sample to be tested is added. Platelets are washed with buffer containing the same concentration of the drug. Following incubation in buffer with the conjugated antihuman IgG (also containing the same drug concentration as the incubation mixture), platelets are again washed, and binding of IgG is detected by enzyme-linked immunosorbent assay (ELISA) or by radioimmunoassay. With each experiment, several controls should be included (Table 4). Figure 2 shows a typical experiment.

**Table 4  Interpretation of Immunoglobulin-Binding Assays for Detection of Drug-Dependent Antibodies**

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Drug or metabolite</th>
<th>Serum</th>
<th>Reaction</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>Patient</td>
<td>+(^a)</td>
<td>Drug-dependent antibody</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Patient</td>
<td>–(^b)</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Normal donor serum</td>
<td>–(^b)</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Normal donor serum</td>
<td>–(^b)</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Patient</td>
<td>+</td>
<td>Autoantibody (alloantibody)</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Patient</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Normal donor serum</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Normal donor serum</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Patient</td>
<td>+</td>
<td>(Nonspecific) adsorption of immunoglobulins to platelets induced by the drug</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Patient</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Normal donor serum</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Normal donor serum</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Patient</td>
<td>–</td>
<td>Negative result</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Patient</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Normal donor serum</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Normal donor serum</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The experiment for ddAb detection, as depicted in Figure 2.  
\(^b\) Control experiments.
with two quinidine-dependent platelet antibodies: evidently the drug-dependent antibodies do not remain fixed to the platelet membrane if the drug is not included in the washing buffer. As already discussed, drug-dependent antibodies can be caused by a drug metabolite, rather than by the drug itself. In this case, positive results are obtained only with metabolites. If metabolites are renally excreted, urine from a person ingesting the drug may be sufficient as a crude “metabolite preparation” (Kiefel et al., 1987c). This approach has also been explored for the detection of drug-dependent antibodies against red blood cells (Salama and Mueller-Eckhardt, 1985).

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Clinical Picture of Heparin-Induced Thrombocytopenia

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I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a distinct clinicopathologic syndrome caused by platelet-activating antibodies that recognize complexes of platelet factor 4–heparin (PF4/H). Its strong association with venous and arterial thrombosis represents a striking paradox. However, thrombocytopenia itself is common in clinical medicine. Furthermore, heparin is usually given to patients who either have thrombosis, or who are judged to be at high risk for thrombosis. Thus, thrombocytopenia with or without thrombosis during heparin treatment does not necessarily indicate a diagnosis of HIT. Indeed, several disorders can closely resemble HIT (see Chap. 12).

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

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

A. Timing

The characteristic delay of 5 or more days between initiation of heparin and onset of thrombocytopenia was the major clue that led early investigators to

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**Table 1** Thrombotic and Other Sequelae of HIT

<table>
<thead>
<tr>
<th>Venous thrombosis</th>
<th>Arterial thrombosis</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep vein thrombosis (DVT) (50%): new, progressive, recurrent; lower limb (often bilateral); upper limb (at site of venous catheter); phlegmasia cerulea dolens</td>
<td>Aortic or iliofemoral thrombosis resulting in acute limb ischemia/infarction (5–10%) or spinal cord infarction (rare)</td>
<td>Heparin-induced skin lesions at heparin injection sites (10–20%); Erythematous plaques Skin necrosis</td>
</tr>
<tr>
<td>Coumarin-induced venous limb gangrene (~5–10% of DVT treated with warfarin)</td>
<td>Acute thrombotic stroke (3–5%)</td>
<td>Coumarin-induced skin necrosis complicating HIT involving “central” sites (breast, abdomen, thigh, leg, etc.) (rare)</td>
</tr>
<tr>
<td>Pulmonary embolism (25%): with or without right-sided cardiac intra-atrial or intraventricular thrombus</td>
<td>Myocardial infarction (3–5%)</td>
<td>Acute systemic reactions postintravenous heparin bolus (~25% of sensitized patients who receive an intravenous heparin bolus): Inflammatory: e.g., fever, chills, flushing Cardiorespiratory: e.g., tachycardia, hypertension, dyspnea; cardiopulmonary arrest (rare)</td>
</tr>
<tr>
<td>Cerebral dural sinus thrombosis (rare)</td>
<td>Cardiac intraventricular or intra-atrial thrombosis, in situ or via embolization of DVT (rare)</td>
<td>Gastrointestinal: nausea, vomiting, diarrhea Neurological: transient global amnesia, headache</td>
</tr>
<tr>
<td>Adrenal hemorrhagic infarction (rare): bilateral (acute or chronic adrenal failure) or unilateral</td>
<td>Thrombosis involving miscellaneous arteries (rare); upper limb, renal, mesenteric, spinal, and other arteries</td>
<td></td>
</tr>
</tbody>
</table>
| Disseminated intravascular coagulation (DIC), with hypofi

---

Estimated frequencies of the various complications of HIT are taken from reports with serological confirmation of the diagnosis (Warkentin et al., 1995; Warkentin and Kelton, 1996; Warkentin et al., 1997). “Rare” indicates an estimated frequency <3% of HIT patients.
recognize the immune pathogenesis of HIT (Roberts et al., 1964; Rhodes et al., 1973). King and Kelton (1984) noted that thrombocytopenia occurred between days 6 and 15 for more than 90% of patients in whom HIT occurred during their first exposure to heparin. In contrast, for patients who developed HIT during a repeat course of heparin, the onset of thrombocytopenia was often more rapid, occurring within 2 days. These data have been interpreted as indicating an “anamnestic” (Gr., memory) or “secondary” immune response in HIT, i.e., the immune system produces HIT antibodies more quickly on reencountering an antigen “remembered” within its memory cell repertoire. Recent data, however, suggest another explanation for these two temporal profiles of HIT, typical and rapid (discussed subsequently).

**Typical Onset of HIT**

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

**Rule 1**

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

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

A recent study (Warkentin and Kelton, 2001a) that analyzed temporal aspects of the platelet count fall in 243 patients with serological confirmed HIT in relation to heparin use (both past and present) also found that the onset of the platelet count fall typically occurs between days 5 and 10 (Fig. 2). Interestingly, among these patients with typical onset of HIT, there was no significant difference in the time to onset of HIT, irrespective of whether or not the patients had been exposed to heparin in the past. For most patients with typical onset of HIT, previous heparin exposure had occurred in the “remote” past, arbitrarily defined as more than 100 days previous (Fig. 2).

Gruel and colleagues (2003) have reported that the onset of the platelet count fall may occur on average several days later in patients who develop
Figure 1  HIT in a clinical trial of postoperative orthopedic patients. (a) Serial platelet counts of nine patients with HIT (platelet count nadir $<150 \times 10^9/L$). The bold line and shaded area indicate the mean ($\pm 2$ SD) platelet count in the reference population (367 patients who tested negative for HIT antibodies). The reference population indicates the occurrence of postoperative thrombocytopenia (days 1–3), followed by postoperative thrombocytosis (maximal, days 11–14). Nine patients developed serologically confirmed HIT, with a platelet count fall to $<150 \times 10^9/L$; eight of the nine patients developed HIT-associated thrombosis (see insert for description of the types of thrombi observed; all thrombi were venous, except for a mesenteric artery thrombosis). (b) Serial platelet counts of nine patients with HIT (platelet nadir $>150 \times 10^9/L$, but platelet count fall $>50\%$). Five patients developed DVT (*). HIT developed in seven patients receiving unfractionated heparin (UFH) and two receiving low molecular weight heparin (LMWH) (\textit{y}). The platelet count fell abruptly on postoperative day 13 when 5000 U of intravenous UFH was given. (c) Day of onset of HIT for 18 patients observed in a clinical trial. HIT began between days 5 and 10, inclusive, in all 18 patients. Length of heparin treatment was variable; thus, the remaining number of patients at risk for HIT for each day of follow-up is shown (\textit{n}). * For one of the patients, the platelet count began to fall on day 5 after UFH “flushes” were received through an intra-arterial catheter placed at the time of surgery. \textit{l} The platelet count fell abruptly on day 12 (postoperative day 13), together with symptoms and signs of an acute systemic reaction, following administration of a 5000 U intravenous UFH bolus (see Fig. 1a, Chap. 4). However, the first clinical manifestation of HIT was on day 9 (erythematous skin lesions at heparin injection sites), and HIT antibodies were first detected on day 5. \textit{l} The platelet count fell abruptly on day 10 after administration of a 5000 U intravenous UFH bolus, followed by therapeutic-dose UFH infusion. However, positive HIT antibodies were first detected by PF4/H-EIA on day 6 of treatment with subcutaneous UFH, 7500 U twice daily. (a, c, Warkentin et al., 1995; Warkentin, 2000; b, Warkentin et al., 1995, 2003.)
Clinical Picture of HIT

Figure 1  Continued.

(b) Platelet count (× 10^9/L) vs. Postoperative day

- UFH, n=7
- LMWH, n=2
- * Thrombosis (DVT), n=5
- † UFH bolus in LMWH patient

Normal postoperative platelet count range
(mean ± 2 SD)

150 x 10^9/L (standard definition of thrombocytopenia)

(c) Onset of HIT (days after starting heparin)

- >50% Platelet count fall
  but nadir>150 x 10^9/L
- >50% Platelet count fall
  and nadir<150 x 10^9/L
Figure 2  Temporal patterns of HIT in 243 patients in relation to previous treatment with heparin. (A) Data are shown for the patients in whom the day of onset of HIT could be determined to within a 3-day period. Among 170 patients with typical onset of HIT, there was no significant difference in onset of HIT (median day), irrespective of whether previous heparin exposure had been definite (6.5, \( n = 47 \)), possible (7.0, \( n = 49 \)), or unlikely (6.0, \( n = 74 \)) (\( p = 0.88 \), definite vs. unlikely). Among 120 patients who had definite previous exposure to heparin, 73 had rapid onset of HIT. (B) For the subgroup of patients with definite previous exposure to heparin, the 73 patients with rapid onset of HIT invariably had been exposed to heparin within the past 100 days (i.e., no patients shown at the asterisk [*]); in contrast, only 16/47 patients with typical onset of HIT had been exposed to heparin within the past 100 days (\( p < 0.001 \)). (From Warkentin and Kelton, 2001a).
HIT during low molecular weight heparin (LMWH) therapy. More time may be required to generate clinically important levels of HIT-IgG so as to activate platelets in the presence of PF4/LMWH, rather than PF4/H, complexes.

**Diminishing Risk of HIT After Day 10**

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

**Rapid Onset of HIT**

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

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

This explanation is supported by other observations. First, for patients with typical onset of HIT, there was no difference in its median day of onset, irrespective of whether or not patients had previously been exposed to heparin. Second, patients did not generally develop thrombocytopenia that began between days 2 and 4. Had there truly been an anamnestic immune response more rapid than the usual 5- to 10-day period, one might have expected to identify such a group of patients. Third, patients reexposed to heparin following disappearance of HIT antibodies do not necessarily form HIT anti-
bodies again; those who do appear to form antibodies after day 5 (Gruel et al., 1990; Warkentin and Kelton, 2001a). Indeed, several patients with well-documented previous HIT have received full treatment courses of heparin several months or years later without incident (Warkentin and Kelton, 2001a; Lindhoff-Last et al., 2002).

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

**HIT Antibodies Are Transient**

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

Rule 2

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

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

Figure 4  Proportion of patients with HIT antibodies after an episode of HIT. The time (in days) to a negative test by the activation assay (n = 144) or the antigen assay (n = 93) is shown. The antigen test tended to become negative more slowly than did the activation assay (p = 0.007). (From Warkentin and Kelton, 2001a.)
A Hypothesis to Explain the Timing of HIT

There is a possible explanation for these unusual temporal features of HIT: because the HIT antigen(s) is a “cryptic” autoantigen (or neoantigen) comprised of two autologous substances (PF4 and heparin), HIT can be regarded as an autoimmune disorder. Indeed, the target of the immune response appears to be one of at least three dominant conformation-dependent neoepitopes formed on PF4 when it binds to heparin (see Chaps. 6–8). There is evidence that transient IgG-mediated autoimmune responses can occur, particularly when the responsible antibodies have relatively low affinity for the neoepitope (thus having avoided prior clonal deletion as occurs with lymphocytes that have high-affinity binding to autoantigens). In this situation, the antibodies are generated only as long as the autoantigen is present, thus explaining why there is a rapid fall in anti-PF4/H antibodies soon after discontinuation of heparin. The affinity of the HIT antibodies may be substantially enhanced when both Fab “arms” of the IgG molecule can bind to linked epitopes, i.e., two PF4 molecules bound to a single heparin molecule (Newman and Chong, 1999).

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

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

The (1) transient nature of the HIT antibody, the (2) apparent minimum of 5 days to regenerate clinically significant HIT antibodies even in a patient who once had HIT, and (3) the observation that HIT antibodies do not necessarily recur, despite heparin rechallenge in a patient with definite prior HIT, all suggest that it may be safe to readminister heparin to such patients. Fortunately, this potentially risky situation is not frequently necessary, as there are
several alternative anticoagulants that can be substituted for heparin (see Chaps. 13–19).

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

For planning a brief reexposure to heparin in a patient who had HIT in the past few weeks or months, a dilemma would arise if the follow-up patient serum now tested negative using a sensitive activation assay (e.g., platelet serotonin release assay), but positive by antigen assay. There is evidence that activation assays are better at detecting clinically significant levels of HIT antibodies (Warkentin et al., 2000) (see Chap. 11). Thus, use of heparin in this situation might be a reasonable option, provided that one had confidence in the activation assay performed, the antigen assay result was “weak” (e.g., 0.400–0.750 OD units), there was a strong indication for surgery requiring heparin, and there was limited experience with an alternative anticoagulant. Continued watchful waiting is another option, given the transience of HIT antibodies.

Sensitization by Incidental Heparin Exposure

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

Delayed Onset of HIT

Rarely, HIT begins several days after discontinuing heparin therapy or persists for several weeks even though heparin administration has been stopped (Castaman et al., 1992; Tahata et al., 1992; Warkentin and Kelton, 2001b; Rice et al., 2002; Warkentin and Bernstein, 2003; Shah and Spencer, 2003) (see Fig. 5). A dramatic case encountered by the author was a female outpatient who presented with transient global amnesia and a platelet count of $40 \times 10^9/L$ 7 days after receiving two doses of UFH; despite the diagnosis and serologic confirmation of HIT and avoidance of all heparin, this patient’s

![Figure 5](image)

**Figure 5** Delayed onset of HIT: a 68-year-old woman who received UFH for heart surgery was noted to have a platelet count of $40 \times 10^9/L$ on postoperative day 19 and a “rash” of her lower extremities. She presented on day 38 with symptomatic DVT and developed rapid-onset recurrent thrombocytopenia after receiving iv UFH. The patient was successfully treated with danaparoid sodium (D.S.) and warfarin. In retrospect, the thrombocytopenia first observed on postoperative day 19 almost certainly was caused by delayed onset of HIT.
platelet count fell over the next 4 days to $14 \times 10^9/L$, along with laboratory evidence for DIC (low fibrinogen and elevated fibrin D-dimer levels). This patient’s platelet counts gradually recovered to normal over several months, during which time recurrent thrombotic events were managed successfully with an alternative anticoagulant.

The unusual clinical course of these patients could be related to very high titers of platelet-activating IgG antibodies (Warkentin and Kelton, 2001b). Moreover, substantial platelet activation in vitro can be caused by some of these patients’ sera even in the absence of added heparin. This finding of substantial heparin-independent platelet activation resembles that described in other patients with drug-induced immune thrombocytopenia, in which prolonged thrombocytopenia has been reported in association with drug-independent binding of IgG to platelets (Kelton et al., 1981). Given the apparent rarity of these cases, it is perhaps surprising that this syndrome does not occur more frequently, given that HIT—once initiated—resembles somewhat an autoimmune disorder, with IgG recognizing an autologous protein, PF4. On the other hand, earlier discharge from the hospital and a higher index of suspicion for this syndrome might mean that delayed onset of HIT will become a relatively more common presentation of HIT in the future.

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

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

Figure 6 shows the platelet count nadirs of 142 patients with laboratory-proved HIT in one medical community: the median platelet count nadir was approximately $60 \times 10^9/L$ (Warkentin, 1998a). This contrasts with “typical” drug-induced immune thrombocytopenic purpura (DITP; e.g., caused by quinine/quinidine, sulfa antibiotics, or rifampin [see Chap. 2]), for which the median platelet count nadir is $15 \times 10^9/L$ or less, and patients usually develop bleeding (Pedersen-Bjergaard et al., 1997). The platelet count is $15 \times 10^9/L$ or

![Platelet count nadirs in 142 patients with serologically confirmed HIT](image)

**Figure 6** Platelet count nadirs in 142 patients with serologically confirmed HIT: the data are taken from a study of 127 patients with serologically confirmed HIT that used a definition of $<150 \times 10^9/L$ (Warkentin and Kelton, 1996), together with a group of 15 patients diagnosed with serologically confirmed HIT over the same time period whose platelet count nadir was $>150 \times 10^9/L$. There is a lognormal distribution of the platelet count nadirs, with a median platelet count of $59 \times 10^9/L$. HIT is only occasionally complicated by very severe thrombocytopenia. HIT-associated thrombosis occurred in most patients irrespective of the severity of the platelet count nadir. For comparison, schematic platelet count nadir distributions are shown for typical drug-induced immune thrombocytopenic purpura (DITP) and atypical DITP caused by abciximab. (Modified from Warkentin, 1998a.)
fewer in only about 5% of patients with HIT (Warkentin, 2003). But even in this minority of HIT patients with very severe thrombocytopenia, thrombosis, rather than bleeding, predominates. Patients with atypical drug-induced thrombocytopenic purpura caused by anti-GPIIb/IIIa-blocking drug (e.g., abciximab [ReoPro]) appear to develop severity of thrombocytopenia intermediate between that of typical DITP and HIT (Fig. 6).

Definition of Thrombocytopenia

Figure 6 illustrates that HIT is associated with thrombosis even when the platelet count nadir is more than 150 × 10^9/L. This suggests that the standard definition of thrombocytopenia (<150 × 10^9/L) may be inadequate for many patients with HIT. Particularly in postoperative patients, a major fall in the platelet count can occur without the nadir falling to less than 150 × 10^9/L (see Figs. 1b and 3). Indeed, studies indicate that a 50% or greater fall in the platelet count from the postoperative peak is strongly associated with HIT antibodies, even when the platelet count nadir remains higher than 150 × 10^9/L (Ganzer et al., 1997; Warkentin et al., 2003). Moreover, this patient subgroup is at increased risk for thrombosis.

Rule 3

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

It is possible that a greater than 50% platelet count fall definition is also appropriate for medical patients (Girolami et al., 2003). Regardless of the patient population, a clinician should have a high index of suspicion when unexpected large-percentage declines in the platelet count occur during heparin treatment, irrespective of whether an arbitrary absolute threshold for “thrombocytopenia” is crossed.

Platelet Count Monitoring in Patients Receiving Heparin

In postoperative patients, the onset of HIT coincides with rising platelet counts (postoperative thrombocytosis); thus, the platelet count profile of HIT resembles an “inverted V” (Δ; see Fig. 1a, b). The postoperative peak platelet count preceding HIT is often higher than the preoperative platelet count. Therefore, the postoperative peak platelet count is the appropriate baseline for calculating the magnitude of a subsequent platelet count fall (Table 2).
Table 2 Determining the Day of Onset of Thrombocytopenia: A 35-Year-Old Woman Who Developed HIT After Heart Surgery

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

* Pulmonary embolism (PE) occurred on postoperative day 8, in association with a platelet count fall of 41%, from 378 (postoperative peak) to 224 × 10^9/L. The platelet count began to fall on day 7. The case illustrates why it is wrong to use the preoperative platelet count value as the “baseline,” as the fall in platelet count from 227 (preoperative) to 224 (day 7) would be considered trivial, even though HIT-associated pulmonary embolism occurred. The preoperative and first three postoperative days are in shaded boxes to indicate that these data should be censored in the interpretation of platelet counts in HIT. In this patient, the abrupt fall in platelet count from 227 to 98 (day 0) is expected (heart surgery). This patient was treated successfully with danaparoid sodium (D.S.), with longer-term anticoagulation with warfarin. The patient’s clinical course is also shown in Fig. 4B in Chap. 12.
Figure 1  Pathogenesis of HIT: a central role for thrombin generation. (See Fig. 13.1, p. 340 for full legend.)

Figure 2  Model of the interaction between argatroban and thrombin. (See Fig. 16.2, p. 440.)
Figure 3  (a) Model of the human platelet factor 4 tetramer. (b) AC dimer view of the amino acids (“ring of charge”) crucial for heparin binding. [C-terminal α-helix residues encompassing lysines 61–62 and 65–66 (cyan), arginines 20, 22, and 49 (green), and histidine 23, threonine 25, lysine 46 (yellow).] (See Fig. 7.2, p. 185, for full legend. From a, Zhang et al., 1994; b, Loscalzo et al., 1985; Mayo et al., 1995a.)

Figure 4  (a) Heparin-induced erythematous plaques. (b) Heparin-induced skin necrosis. (See Fig. 3.13a,b, p. 86, for full legend.)
Figure 5  Primary and secondary structure of platelet factor 4 (PF4) in relation to HIT neoepitopes. (Top) 3-D representation of the PF4 tetramer, indicating two neoepitope sites (per monomer). The “ring of positive charge” is formed by lysine residues in the C-terminus (light blue) and other lysine and arginine residues (dark blue). (Bottom) The linear sequence of the 70-amino acid polypeptide of a single PF4 molecule is shown. (From Li et al., 2002.) (See Fig. 7.4, p. 187.)
Figure 6 Ischemic limb syndromes in HIT. (a) Warfarin-induced venous limb gangrene complicating HIT-associated deep vein thrombosis. (b) Warfarin-induced digital necrosis complicating HIT and Raynaud’s phenomenon (from Warkentin et al., 2004). (c) Livedo reticularis (thigh) and (d) patchy necrosis (foot) complication HIT with hypofibrinogenemic DIC (no warfarin, no DVT). (See Figs. 3.9, p. 77, Fig. 3.11, p. 80, and Fig. 3.12a,b, p. 82, for full legends.)
Anecdotal reports indicate that HIT-associated thrombosis can occur in the absence of thrombocytopenia, as conventionally defined (Phelan, 1983; Hach-Wunderle et al., 1994; Warkentin, 1996a, 1997; Houston, 2000). However, most of these patients do have an associated fall in the platelet count, although the nadir remains higher than $150 \times 10^9/L$. Perhaps the most dramatic example of this phenomenon was a patient with essential thrombocytopenia who developed serologically confirmed HIT: the platelet count fell by 49% from 1235 to 633, i.e., concomitant “thrombocytopenia” and thrombocytosis (Risch et al., 2000).

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

\[\text{HIT antibody formation} \rightarrow \text{Thrombocytopenia} \rightarrow \text{Thrombosis} \]

**Figure 7** “Iceberg” model of HIT: Model A indicates that thrombosis occurs in patients who develop HIT antibody formation and thrombocytopenia. This model is supported by clinical data. In contrast, model B indicates the possibility of HIT antibody formation contributing to thrombosis without the intermediary process of thrombocytopenia. Although anecdotal experience suggests occasional patients consistent with model B, controlled studies indicate that HIT antibody formation without thrombocytopenia does not have an increased frequency of thrombosis, compared with controls (Warkentin et al., 1995, 2003). Note that thrombocytopenia is broadly defined and includes patients with large relative falls in the platelet count, even if the platelet nadir is $>150 \times 10^9/L$. (From Warkentin, 1999.)
vidence suggesting that in vivo platelet activation by HIT antibodies probably contributes to the pathogenesis of HIT-associated thrombosis.

Platelet Count Recovery Following Discontinuation of Heparin

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

III. THROMBOSIS

A. The HIT Paradox: Thrombosis but Not Hemorrhage

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

Rule 4

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

The explanation for this clinical feature is unknown, but could be related to unique pathophysiological aspects of HIT, such as in vivo platelet activation, generation of procoagulant, platelet-derived microparticles, and procoagulant alterations of endothelium and monocytes (see Chaps. 9 and 10).

B. HIT Is a Hypercoagulable State

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

Thrombosis occurs in association with HIT in at least four ways. Only the last three situations are conventionally considered as HIT-associated thrombosis. First, thrombosis can precede heparin treatment, for which it usually represents the initial indication for heparin therapy. Second, HIT can be the presenting clinical manifestation of HIT, often occurring early during the platelet count fall. Indeed, new thrombosis is the initial clinical manifestation in about half of all HIT patients (Warkentin and Kelton, 1996; Greinacher et al., 1999) (see Fig. 1a).

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

Natural History of “Isolated HIT”

There is a high probability of subsequent thrombosis even when heparin administration is stopped because of thrombocytopenia caused by HIT. A retrospective cohort study (Warkentin and Kelton, 1996) identified 62 patients with serologically confirmed HIT in whom the diagnosis was clin-
ically suspected because of thrombocytopenia alone, and not because of signs and symptoms indicative of possible new thrombosis. Thus, this cohort was identified without an apparent recognition bias caused by symptomatic thrombosis. Nevertheless, the 30-day thrombosis event rate was about 50% (see Fig. 2 in Chap. 4). This high frequency of thrombosis occurred whether the heparin administration was simply stopped or substituted by warfarin.

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

Meta-analysis of two prospective cohort studies also found a high initial thrombotic event rate (6.1% per day after stopping heparin therapy and before beginning alternative anticoagulant therapy with lepirudin (Greinacher et al., 1999, 2000) (see Fig. 4 in Chap. 15). Taken together, these large retrospective and prospective cohort studies suggest the following rule:

**Rule 5**

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

About 5% of patients (3 of 62) in the largest study died suddenly, two with proved or probable pulmonary embolism (Warkentin and Kelton, 1996). This experience supports the recommendation that further anticoagulation be considered for patients in whom isolated HIT has been diagnosed (Hirsh et al., 1998, 2001; Warkentin et al., 1998) (see Chaps. 1 and 13–16).

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

Clinical factors help determine the location of thrombosis in HIT. For example, Makhoul and colleagues (1986) observed prior vessel injury (e.g.,
Clinical Picture of HIT

recent angiography) in 19 of 25 patients with lower limb HIT-associated thrombosis. Similarly, central venous catheters are crucial for the occurrence of an upper limb DVT in patients with HIT (Hong et al., 2003).

Prospective studies of HIT in medical patients show that venous and arterial thrombotic events occur in approximately equal numbers; in contrast, there is a marked predominance of venous thrombosis when HIT occurs in surgical patients (see Table 4 in Chap. 4). In a retrospective study, Boshkov and colleagues (1993) found that HIT patients with cardiovascular disease were more likely to develop arterial thrombosis, whereas venous thrombosis was strongly associated with the postoperative state.

Rule 6

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

E. Venous Thrombosis

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

Rule 7

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

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

Lower Limb DVT

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

<table>
<thead>
<tr>
<th>Patient population (Ref.)</th>
<th>Thrombosis</th>
<th>Thrombosis rate in:</th>
<th>Odds ratio (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HIT</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Post-orthopedic surgery&lt;sup&gt;a&lt;/sup&gt; (Warkentin et al., 1995, 2003)</td>
<td>Proximal DVT</td>
<td>8/18 (44.4%)</td>
<td>26/647 (4.0%)</td>
<td>19.1 (5.9–58.3)</td>
</tr>
<tr>
<td></td>
<td>Bilateral proximal DVT</td>
<td>2/18 (11.1%)</td>
<td>4/647 (0.6%)</td>
<td>20.1 (1.7–150)</td>
</tr>
<tr>
<td></td>
<td>Pulmonary embolism</td>
<td>2/18 (11.1%)</td>
<td>2/647 (0.3%)</td>
<td>40.3 (2.7–572)</td>
</tr>
<tr>
<td></td>
<td>Any thrombosis</td>
<td>13/18 (72.2%)</td>
<td>112/647 (17.3%)</td>
<td>12.4 (4.0–45.2)</td>
</tr>
<tr>
<td>Patients with central line&lt;sup&gt;b&lt;/sup&gt; (Hong et al., 2003)</td>
<td>Upper-limb DVT</td>
<td>14/145 (9.7%)</td>
<td>3/484 (0.6%)</td>
<td>17.1 (4.9–60.5)</td>
</tr>
<tr>
<td>Medical&lt;sup&gt;b&lt;/sup&gt; (Girolami et al., 2003)</td>
<td>Any thrombosis</td>
<td>3/5 (60%)</td>
<td>21/593 (3.5%)</td>
<td>40.8 (5.2–163)</td>
</tr>
</tbody>
</table>

<sup>a</sup> HIT defined as >50% platelet count fall.

<sup>b</sup> HIT defined as any abnormal platelet count fall with positive HIT serology (platelet fall was >50% in 93% of study patients).
Sometimes the DVT is sufficiently severe on clinical grounds as to merit use of the term “phlegmasia cerulea dolens” (i.e., an inflamed, blue, painful limb). However, progression of phlegmasia to venous limb gangrene is rare in the absence of coumarin anticoagulation (discussed subsequently).

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

Upper Limb DVT

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

Recurrence of Venous Thromboembolism

Gallus and colleagues (1987) identified HIT as a significant risk factor for recurrence of venous thromboembolism in a prospective treatment study: 3 of the 9 patients with HIT developed recurrent venous thromboembolism, compared with 12 of the 223 patients in whom HIT was not diagnosed (odds ratio, 8.8; p < 0.01).

Warfarin-Induced Venous Limb Gangrene

Venous limb gangrene is one of two clinical syndromes associated with HIT in which coumarin anticoagulation paradoxically plays an important pathogenic role (Fig. 8). Venous limb gangrene is defined as acral (extremity) necrosis that occurs in a limb affected by DVT. Additional features include (1) absence of large artery occlusion (i.e., there are palpable or doppler-
identifiable pulses); (2) extensive thrombotic occlusion of large and small veins, as well as venules; and (3) the characteristic hallmark of a supra-therapeutic international normalized ratio (INR), generally > 4.0. (Fig. 9).

Anticoagulation with warfarin, phenprocoumon, or other coumarins is a crucial factor to explain the progression of DVT to venous limb gangrene (Warkentin, 1996b; Warkentin et al., 1997). A case–control study of 8 patients with HIT-associated venous limb gangrene found a higher median INR, compared with 58 control HIT patients treated with warfarin for DVT who did not develop venous gangrene (5.8 vs. 3.1; \( p < 0.001 \)). Laboratory studies

Figure 8  Coumarin-induced skin necrosis (CISN): HIT is associated with two forms of CISN: (1) venous limb gangrene, affecting extremities with active deep vein thrombosis, and (2) “classic” CISN, which involves central (nonacral) tissues, such as breast, abdomen, thigh, flank, and leg, among other tissue sites. CISN complicating HIT typically manifests as venous limb gangrene (~90%) (Warkentin et al., 1997, 1999), whereas CISN in other settings most commonly affects central tissues (~90%) (Cole et al., 1988). (From Warkentin, 1996b.)
showed a characteristic hemostatic profile for patients with venous gangrene: persisting in vivo thrombin generation (elevated thrombin–antithrombin complex levels), together with reduced protein C activity (Fig. 10). The high INR is a surrogate marker for severely reduced protein C (through parallel coumarin-induced reduction in factor VII). Thus, venous limb gangrene appears to result from a profound disturbance in procoagulant-anticoagulant balance.

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

Patients have also developed venous limb gangrene during combined treatment with both ancrod and warfarin (Warkentin et al., 1997; Gupta et al., 1998); because thrombin generation increases during treatment of HIT with ancrod (Warkentin, 1998b; see Fig. 2 in Chap. 13), ancrod could predispose to a greater risk for venous gangrene during warfarin treatment.

Recently, several patients have been reported who developed venous limb gangrene during the transition to coumarin from parenteral anticoagu-
lation with a direct thrombin inhibitor (lepirudin or argatroban) (Smythe et al., 2002; Srinivasan et al., 2003). Typically, patients had symptomatic DVT in the affected limb and had their direct thrombin inhibitor started and stopped while they remained thrombocytopenic. Additionally, the INR was supratherapeutic at the time that limb ischemia or gangrene occurred after stopping the direct thrombin inhibitor. This experience indicates that the transition from parenteral anticoagulation to coumarin therapy should proceed cautiously, as suggested by the following “rule” (see also Chap. 13):

**Rule 8**

Venous limb gangrene is characterized by (1) in vivo thrombin generation associated with acute HIT; (2) active DVT in the limb(s) affected by venous gangrene; and (3) a supratherapeutic INR during coumarin

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**Figure 10** Thrombin-antithrombin (TAT) complexes compared with protein C activity in patients with HIT: Each data point represents TAT complexes and protein C activity per single treatment day per patient. In both panels the open symbols represent three patients with warfarin-induced venous limb gangrene and one patient with phlegmasia cerulea dolens (open squares). The diagonal line represents an arbitrary ratio of TAT complex to protein C of 400. (Left) Results when HIT was first diagnosed and before warfarin therapy. Control samples included 8 patients (closed circles) who subsequently received warfarin for DVT without developing venous limb gangrene and 14 patients without DVT who did not later receive warfarin. (Right) Results in 16 patients who were receiving warfarin for HIT, including 4 patients (open symbols) who developed venous limb gangrene/phlegmasia and 12 patients (closed circles) who received warfarin without developing venous limb gangrene. The data suggest that patients who develop venous limb gangrene or phlegmasia have a higher ratio of TAT to protein C, consistent with a disturbance in procoagulant-anticoagulant balance during warfarin treatment of HIT. (From Warkentin et al., 1997.)
anticoagulation. This syndrome can be prevented by (1) delaying initiation of coumarin anticoagulation during acute HIT until there has been substantial recovery of the platelet count (to at least 100–150 × 10⁹/L) while receiving an alternative parenteral anticoagulant (e.g., lepirudin, argatroban, danaparoid), and only if the thrombosis has clinically improved; (2) initiating coumarin in low, maintenance doses (e.g., 2–5 mg warfarin); (3) ensuring that both parenteral and oral anticoagulant overlap for at least 5 days, with at least the last 2 days in the target therapeutic range; and (4) if applicable, physicians should reverse coumarin anticoagulation with vitamin K in a patient recognized with acute HIT after coumarin therapy has been commenced.

The frequency of venous limb gangrene in HIT patients with DVT who receive warfarin is unknown. This complication happened in 8 of 66 (12.1%; 95% CI 5.4–22.5%) patients with HIT-associated DVT treated with warfarin (with or without ancrod) in Hamilton; venous limb gangrene was a more frequent cause of limb loss in HIT patients than was arterial occlusion in this medical community. Venous gangrene also occurred in 1 of 21 (4.8%; 95% CI 0.12–23.8%) patients treated with phenprocoumon in Germany (Greinacher et al., 2000). In contrast, this complication was not observed by Wallis and colleagues in any of 51 patients who received warfarin with a diagnosis of HIT, although only 16 patients received warfarin to manage HIT-associated thrombosis (95% CI for 0/16 0–20.6%). Besides cotherapy with ancrod, factors that could influence the risk for venous gangrene include the dosing of coumarin, the rate of coagulation factor turnover/consumption related to DIC, and vitamin K deficiency.

Rarely, coumarin therapy contributes to microvascular thrombosis and acral limb ischemia in the absence of DVT. Figure 11 (also see color insert, Fig. 6b) shows multiple digital necrosis of the right hand complicating the initiation of warfarin therapy (maximal INR = 4.3) in a patient with Raynaud’s phenomenon who developed HIT following aortic valve replacement for adenocarcinoma-associated noninfective thrombotic endocarditis (Warkentin et al., 2004). Although digital necrosis occurred in all four limbs in this patient, only the right foot (which exhibited the greatest amount of ischemic necrosis) was found to have DVT by duplex ultrasonography. It was hypothesized that microcirculatory disturbances secondary to paraneoplastic Raynaud’s phenomenon interacted with altered procoagulant–anticoagulant balance (secondary to HIT and warfarin therapy) to cause this dramatic clinical syndrome.

Cerebral Venous (Dural Sinus) Thrombosis

Thrombosis of the dural venous sinuses is an unusual cause of stroke in HIT patients that was first reported by Stevenson (1976). Often, there is a
second hypercoagulable state, such as pregnancy (Van der Weyden et al., 1983; Calhoun and Hesser, 1987) or myeloproliferative disease (Kyritsis et al., 1990), that may have interacted with HIT to cause this complication. Platelet-rich “white clots” were identified in the superior sagittal venous sinus in one necropsy study (Meyer-Linderberg et al., 1997). Clinicians should have a high index of suspicion for dural sinus thrombosis when a patient develops progressive focal neurological signs, decreased level of consciousness, seizures, or headache during or soon after stopping heparin treatment (Beland et al., 1997; Pohl et al., 1999, 2000; Warkentin and Bernstein, 2003). Treatment includes immediate discontinuation of heparin, use of an alternative anticoagulant, and possibly, intravenous gammaglobulin (see Chap. 13).

Adrenal Hemorrhagic Infarction

Clinicians should suspect bilateral adrenal hemorrhagic infarction when thrombocytopenic patients develop abdominal pain and hypotension in association with heparin treatment (Arthur et al., 1985; Dahlberg et al., 1990; Ernest and Fisher, 1991; Delhumeau and Granry, 1992; Bleasel et al., 1992; Kovačević et al., 2001). Fever and hyponatremia occur in some patients. These patients require corticosteroid replacement to prevent death from acute or chronic adrenal failure (Rowland et al., 1999). Unilateral adrenal hemor-
rhagic infarction typically presents with ipsilateral flank pain without signs of adrenal failure (Warkentin, 1996a). HIT explained at least 5% of patients with adrenal hemorrhage at one institution (Vella et al., 2001).

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

**DIC and Acquired Anticoagulant Deficiency**

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

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

Other patients with HIT-associated DIC evince clinical signs of microvascular thrombosis. For example, Figure 12 shows livedo reticularis and patchy foot necrosis (despite palpable foot pulses) in a postoperative cardiac surgery patient with HIT (platelet count nadir, 39 × 10^9/L) complicated by hypofibrinogenemic DIC. Evidence for acquired natural anticoagulant failure included mildly reduced antithrombin levels (0.76 U/mL; normal, 0.77–1.30 U/mL) and moderately reduced protein C activity (0.50 U/mL; normal, 0.70–1.80 U/mL) that subsequently resolved. Free protein S levels were normal (1.12 U/mL; normal, 0.62–1.38 U/mL). Evidence for DIC included a fibrinogen of 1.2 g/L (normal, 1.5–4.0 g/L) that rose to 4.7 g/L one week later during therapeutic-dose danaparoid therapy, a strongly positive protamine sulfate paracoagulation assay (4+ reactivity at 15 min; normal, no reactivity), a fibrin D-dimer level that was greater than 2000 µg/L (normal, <500 µg/L), and the presence of red cell
Figure 12  Clinical manifestations of DIC. (a) Livedo reticularis. (b) Patchy ischemic necrosis of right foot. This 70-year-old woman developed HIT-associated DIC with hypofibrinogenemia, elevated INR, and reduced antithrombin and protein C activity levels 9 days after emergency cardiac surgery for cardiac catheterization-associated dissection of the left main coronary artery (see text for additional clinical information). (See color insert, Figs. 6c and 6d.)
fragments. Additionally, the INR was elevated at 1.6 (normal, 0.9–1.2), even though coagulation factors VII, V, X, and II all measured between 0.73 to 0.83 U/mL (normal, 0.50–1.50 U/mL). The anticoagulant treatment was successful in avoiding limb amputation. In my experience, limb ischemia and necrosis associated with DIC that occurs in the absence of large artery thrombotic occlusion or warfarin therapy is the least common explanation for limb loss in HIT.

Livedo reticularis is also discussed on pp. 88–89.

Congenital Hypercoagulability and HIT-Associated Thrombosis

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

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

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

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

F. Arterial Thrombosis

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

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

Angiographic Appearance

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

G. Graft, Prosthetic Device, and Extracorporeal Circuit Thrombosis

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

IV. MISCELLANEOUS COMPLICATIONS OF HIT

A. Heparin-Induced Skin Lesions at Subcutaneous Injection Sites

Clinical Picture

Skin lesions that occur at the site(s) of subcutaneous heparin injection are a manifestation of the HIT syndrome. For unknown reasons, only 10–20% of patients who form HIT antibodies during subcutaneous UFH or LMWH treatment develop these lesions. Furthermore, about 75% of patients who develop heparin-induced skin lesions do not develop thrombocytopenia, even though heparin-dependent, platelet-activating HIT antibodies are readily detectable (Warkentin, 1996a, 1997).

The skin abnormalities range in appearance from indurated, erythematous nodules or plaques (Fig. 13a) to frank necrotizing lesions (Fig. 13b) (see color insert, Fig. 4) that start 5 or more days (median, day 8) after beginning heparin injections (Hasegawa 1984; MacLean et al., 1990; Wüschert et al., 1999). The lesions can occur earlier if there was recent treatment with heparin given by another route that resulted in formation of HIT antibodies. Some erythematous plaques have an eczematous appearance. Necrotic lesions typically consist of a central black eschar surrounded by a cuff of induration and erythema (Fig. 13b). Complex skin lesions can result—for example, several discrete areas of necrosis (each lesion corresponding to a different heparin injection site), each with a surrounding violaceous halo, with all circumscribed by a diffuse erythema. Even the least severe forms of heparin-induced skin lesions usually cause pain or pruritus.

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

Histopathology

Lymphocyte infiltration of the upper and middermis that can extend into the epidermis characterizes the erythematous plaque (Bircher et al., 1990). Dermal and epidermal edema (spongiosis) is observed in lesions that appear eczematous. The T lymphocytes of helper–suppressor (CD4+) phenotype
Figure 13  Heparin-induced skin lesions. (a) Heparin-induced erythematous plaques: UFH injections into the lower abdomen resulted in painful erythematous plaques beginning on day 7 of sc UFH treatment; at this time, the platelet count fell only by 9% from 340 to $311 \times 10^9/L$. HIT antibody seroconversion from a negative baseline was shown using the serotonin release assay (from 0% to 84% serotonin release). (From Warkentin, 1996a.) (b) Heparin-induced skin necrosis: UFH injections into the right anterior thigh led to skin necrosis: a large black eschar with irregular borders is surrounded by a narrow band of erythema. The platelet count fell to $32 \times 10^9/L$; despite stopping heparin, the patient developed symptomatic proximal DVT 10 days later. (See color insert, Fig. 4.)
predominate, together with CD1+/DR+ dendritic (Langerhans) cells, consistent with a type IV delayed hypersensitivity immune response. Cytokine synthesis by activated CD4 cells could explain the peripheral blood eosinophilia that has been reported in a few patients (Bircher et al., 1994). In contrast, histopathology of lesions associated with cutaneous necrosis usually shows intravascular thrombosis of dermal vessels, with or without perivascular inflammation and red cell extravasation of variable degree (Hall et al., 1980; Kearsley et al., 1982; Cohen et al., 1988; MacLean et al., 1990; Balestra et al., 1994).

Management

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

Rule 9

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

B. Classic Coumarin-Induced Skin Necrosis

Classic coumarin-induced skin necrosis (CISN) is a very rare complication of oral anticoagulant therapy (Cole et al., 1988). In its classic form, it is characterized by dermal necrosis, usually in a central (nonacral) location, such as breast, abdomen, thigh, or leg, that begins 3–6 days after starting therapy with warfarin or other coumarin anticoagulants (see Fig. 8). Initially, there is localized pain, induration, and erythema that progresses over hours to central purplish-black skin discoloration and blistering, ultimately evolving to well-demarcated, full-thickness necrosis involving skin and subdermal tissues. Some patients require surgical debridement. Case reports suggest that
congenital deficiency of natural anticoagulant proteins, especially protein C,
is sometimes a pathogenic factor (Broekmans et al., 1983; Comp, 1993).

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

C. Other Heparin-Associated Skin Lesions

Skin Necrosis in the Absence of Coumarin Therapy

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

Other Skin Lesions Associated with Heparin Treatment

**Livedo Reticularis.** The bluish, reticulated (network-like), mottled appearance of livedo reticularis was reported in a patient with HIT complicating intravenous UFH given for atrial fibrillation after heart surgery (Gross et al., 1993). This patient also had DIC, microangiopathic peripheral blood abnormalities, and fibrin thrombi noted within small dermal vessels. The livedo appearance results from microvascular thrombosis, with slowing of blood flow and dilation of the horizontally oriented dermal venous drainage channels (Copeman, 1975). Figure 12a (see p. 82) shows livedo reticularis associated with HIT and DIC.
Urticaria and Other Miscellaneous Lesions. Other dermatological consequences of heparin treatment do not appear to be related to HIT. These range from common lesions (ecchymosis) to rare effects of intravenous heparin, such as vasculitis (Jones and Epstein, 1987) and cutaneous necrosis with hemorrhagic bullae (Kelly et al., 1981). Some patients have developed widespread urticarial lesions, sometimes accompanied by angioedema, during treatment with subcutaneous or intravenous heparin (Odeh and Oliven, 1992; Patriarca et al., 1994). In one patient skin testing suggested a generalized reaction against the preservative chlorbutol (Dux et al., 1981). Although LMWH injections were claimed to have caused distal extremity dermal lesions in a patient with HIT (Payne and Kovacs, 2003), it is possible these were related to concomitant warfarin therapy.

D. Acute Systemic Reactions Following an Intravenous Heparin Bolus

Acute systemic reaction (ASR) refers to a variety of symptoms and signs that characteristically begin 5–30 min after an intravenous heparin bolus is given to a patient with circulating HIT antibodies (Nelson et al., 1978; Warkentin et al., 1992, 1994; Popov et al., 1997; Ling and Warkentin, 1998; Warkentin, 2002) (Table 5; see Fig. 3). Only about one quarter of at-risk patients who receive a heparin bolus develop such a reaction. The most common signs and symptoms are fever and chills, hypertension, and tachycardia. Less common are flushing, headache, chest pain, dyspnea, tachyypnea, and large-volume diarrhea. In some patients, severe dyspnea is the predominant sign, termed “pseudo-pulmonary embolism” by Popov and colleagues (1997); multiple

| Timing: onset 5–30 min after intravenous heparin bolus |
| Clinical context: recent use of heparin (past 5–100 days) |
| Laboratory features: abrupt, reversible fall in the platelet count |
| Signs and symptoms: |
| Inflammatory: chills, rigors, fever, flushing |
| Cardiorespiratory: tachycardia, hypertension, tachypnea, dyspnea, chest pain or tightness, cardiopulmonary arrest (rare) |
| Gastrointestinal: nausea, vomiting, diarrhea |
| Neurological: headache, transient global amnesia (rare) |
small perfusion defects on radionuclide lung scans can be shown (Nelson et al., 1978; Ling and Warkentin, 1998). Fatal cardiac and respiratory arrest has been reported (Ansell et al., 1986; Platell and Tan, 1986; Hewitt et al., 1998).

An abrupt fall in the platelet count invariably accompanies these reactions. However, the platelet count drop is often transient (see Fig. 1A in Chap. 4). Thus, physicians should determine the platelet count immediately on suspecting the diagnosis and test for HIT antibodies. Heparin must be discontinued, as further use can lead to fatal complications (Ling and Warkentin, 1998).

**Rule 10**

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

The clinical features of postheparin bolus ASR are not typical of IgE-mediated anaphylaxis (i.e., urticaria, angioedema, and hypotension are not seen). Rather, the syndrome resembles febrile transfusion reactions commonly observed after platelet transfusions, suggesting a common pathogenesis of proinflammatory cytokines associated with cellular activation (Heddle et al., 1994). Moreover, there are similarities between ASR and the administration of ADP in humans, including acute dyspnea, tachycardia, and transient thrombocytopenia (Davey and Lander, 1964).

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

### E. Heparin Resistance

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

A. Cardiac and Neurological Complications of HIT

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

B. HIT in Pregnancy

Heparin-induced thrombocytopenia has complicated UFH treatment given for venous thromboembolism complicating pregnancy (Van der Weyden et al., 1983; Meytes et al., 1986; Copplestone and Oscier, 1987; Greinacher et al., 1992) or the postpartum period (Calhoun and Hesser, 1987). HIT seems to be rare in this patient population; no pregnant patients have been diagnosed with HIT over a 20-year period in Hamilton. Plasma glycosaminoglycans are increased during pregnancy (Andrew et al., 1992), which could contribute to lower frequency or pathogenicity of HIT antibodies. HIT antibodies cross the placenta (Greinacher et al., 1993), so it is at least theoretically possible that a heparin-treated newborn delivered from a mother with acute HIT could develop this drug reaction.

Pregnant patients with HIT have developed unusual events, such as cerebral dural sinus thrombosis (Van der Weyden et al., 1983; Calhoun and Hesser, 1987). Treatment options for pregnant patients with life-threatening thrombosis include danaparoid or fondaparinux as these drugs do not cross the placenta (see Chaps. 13 and 14). The more benign syndrome of heparin-induced skin lesions without thrombocytopenia has also been reported in pregnant patients (Drouet et al., 1992). Danaparoid was reported to be effective in a patient who developed LMWH-induced skin lesions (de Saint-Blanquat et al., 2000).

C. HIT in Children and Neonates

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

The frequency and clinical import of HIT in neonates receiving heparin in intensive care settings is controversial. Spadone and colleagues (1992) investigated 34 newborn infants (average gestational age, 29 weeks) who de-
oped thrombocytopenia or thrombosis, beginning an average of 22 days after starting heparin therapy. Platelet aggregation studies suggested the presence of HIT antibodies in 41% of these neonates. Aortic thrombosis complicating umbilical artery catheter use was the most common complication. Another group (Butler et al., 1997), also using platelet aggregation studies, reported a neonate who may have developed fatal HIT shortly after birth. More specific activation or antigen assays were not performed in either study, however. A

Table 6  Cardiological and Neurological Complications of HIT

<table>
<thead>
<tr>
<th>Cardiological complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction (Rhodes et al., 1973; Van der Weyden et al., 1983)</td>
</tr>
<tr>
<td>Occlusion of saphenous vein grafts post–coronary artery bypass surgery&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intra-atrial thrombus (left and right&lt;sup&gt;b&lt;/sup&gt; heart chambers) (Scheffold et al., 1995; Olbrich et al., 1998)</td>
</tr>
<tr>
<td>Intraventricular thrombus (left and right&lt;sup&gt;b&lt;/sup&gt; heart chambers) (Commeau et al., 1986; Dion et al., 1989; Vignon et al., 1996)</td>
</tr>
<tr>
<td>Prosthetic valve thrombosis (Bernasconi et al., 1988; Vazquez-Jimenez et al., 1999)</td>
</tr>
<tr>
<td>Right heart failure secondary to massive pulmonary embolism</td>
</tr>
<tr>
<td>Cardiac arrest postintravenous heparin bolus (Ansell et al., 1986; Platell and Tan, 1986; Hewitt et al., 1998)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neurological complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke syndrome</td>
</tr>
<tr>
<td>In situ thrombosis</td>
</tr>
<tr>
<td>Progressive stroke in patients receiving heparin for treatment of stroke (Ramirez-Lassepas et al., 1984)</td>
</tr>
<tr>
<td>Cardiac embolization (Scheffold et al., 1995)</td>
</tr>
<tr>
<td>Cerebral vein (dural venous sinus) thrombosis (Van der Weyden et al., 1983; Kyritsis et al., 1990; Meyer-Lindenberg et al., 1997; Warkentin and Bernstein, 2003); complicating pregnancy (Calhoun and Hesser, 1987)</td>
</tr>
<tr>
<td>Amaurosis fugax (Theuerkauf et al., 2000)</td>
</tr>
<tr>
<td>Ischemic lumbosacral plexopathy (Jain, 1986)</td>
</tr>
<tr>
<td>Paraplegia, transient (Maurin et al., 1991) or permanent (Feng et al., 1993), associated with distal aortic thrombosis</td>
</tr>
<tr>
<td>Transient global amnesia (Warkentin et al., 1994)</td>
</tr>
<tr>
<td>Headache&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Thrombosis preferentially affects saphenous vein grafts rather than internal mammary artery grafts (Liu et al., 2002; Ayala et al., 2002).

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

<sup>c</sup> Headache as a feature of HIT is suggested by (1) its occurrence in patients with acute systemic reactions post–heparin bolus (see Fig. 1a, Chap. 4) and (2) its concurrence with onset of thrombocytopenia in several patients who developed HIT in a clinical trial (unpublished observations of the author).
recent study of 108 neonates who received UFH flushes found no HIT antibodies using a sensitive antigen assay (Klenner et al., 2003).

D. HIT in Bone Marrow Transplantation

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

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

VI. ESTIMATING THE PRETEST PROBABILITY OF HIT

A. Scoring Systems for HIT

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

B. The “Four Ts”

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

<table>
<thead>
<tr>
<th></th>
<th>2</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombocytopenia (acute)</strong></td>
<td>Nadir, 20–100 (at least 30% fall); or any &gt;50% fall (nadir ≥20)</td>
<td>Nadir, 10–19 × 10^9/L or any 30–50% fall (or &gt;50% fall associated with heart surgery)</td>
<td>Nadir, &lt;10 × 10^9/L or any &lt;30% fall</td>
</tr>
<tr>
<td><strong>Timing</strong>^b of platelet count fall, thrombosis, or other sequelae (first day of heparin course = day zero)</td>
<td>Clear onset between days 5–10 or ≤1 day (if heparin exposure within past 30 days)</td>
<td>Consistent with day 5–10 fall, but not clear (e.g., missing platelet counts) or ≤1 day (heparin exposure within past 31–100 days) or platelet fall after day 10</td>
<td>Platelet count fall ≤4 days without recent heparin exposure</td>
</tr>
<tr>
<td><strong>Thrombosis or other sequelae (e.g., skin lesions, ASR)</strong></td>
<td>New thrombosis; skin necrosis; ASR after iv heparin bolus</td>
<td>Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis (not yet proven); asymptomatic upper-limb DVT</td>
<td>None</td>
</tr>
<tr>
<td><strong>Other cause of thrombocytopenia not evident</strong></td>
<td>No explanation (besides HIT) for platelet count fall is evident</td>
<td>Possible other cause is evident</td>
<td>Definite other cause is present</td>
</tr>
</tbody>
</table>

*Abbreviations:* ASR, acute systemic reaction (see Table 5); DVT, deep venous thrombosis.

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

^b First day of immunizing heparin exposure considered day zero; the day the platelet count begins to fall is considered the day of onset of thrombocytopenia (it generally takes 1–3 more days until an arbitrary threshold that defines thrombocytopenia is passed). The scoring system shown here has undergone minor modifications from previously published scoring systems (Warkentin, 2003; Warkentin and Heddle, 2003).
Estimated pretest probabilities of HIT thereby range from low (0–3) to high (6–8), with an intermediate score (4–5) indicating moderate risk.

Maximal scores for each category are given when the clinical features are highly consistent with HIT. Thus, a patient will score 8 if there is a substantial fall in the platelet count that begins 5–10 days after commencing heparin, together with thrombosis, and where no other plausible cause is apparent during clinical assessment. Even a patient with no clinical evidence of thrombosis can be assigned a high pretest probability (score 6 of 8) if the clinical features are otherwise consistent with HIT. Another feature of this system is that very low platelet count values (i.e., 10–19 and <10 \times 10^9/L) score only 1 and 0 points, respectively, thus reducing the chance that a patient with posttransfusion purpura (PTP) or DITP will be misclassified as HIT and inappropriately given anticoagulant therapy.

C. Clinical Use of a Scoring System

A practical use of the scoring system is to help make initial clinical decisions regarding therapy. Based on preliminary evaluation, we believe it is likely that a low score (0–3) is associated with a very low risk (<5%) of clinically significant HIT antibodies (defined arbitrarily as >50% serotonin release in a washed platelet activation assay) (see Chap. 11). In contrast, a high score (6–8) appears to be associated with a high risk (>80%) of such strong HIT antibodies. Further, 50–75% of patients evaluated for clinical HIT will have low or high scores. This leaves a smaller number of patients in whom the clinical suspicion of HIT is more uncertain (score 4–5) and in whom the results of diagnostic testing will be especially important for supporting (or refuting) the diagnosis of HIT.

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Van der Weyden MB, Hunt H, McGrath K, Fawcett T, Fitzmaurice A, Sawers RJ,
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Warkentin TE. Hemostasis and atherosclerosis. Can J Cardiol 1995; 11(suppl C):29C–34C.


Frequency of Heparin-Induced Thrombocytopenia

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I. INTRODUCTION

Thrombocytopenia is a common problem encountered in hospitalized patients. For patients receiving heparin, there are three general explanations for thrombocytopenia: (1) heparin-induced thrombocytopenia (HIT), (2) non-idiosyncratic heparin-induced platelet activation (see Chap. 5), and—perhaps most often—(3) an unrelated clinical problem, either common (e.g., hemodilution, septicemia) or rare (e.g., posttransfusion purpura, drug-induced immune thrombocytopenic purpura) (see Chaps. 2 and 12). The availability of sensitive and specific laboratory assays (e.g., enzyme immunoassay [EIA] and serotonin release assay [SRA]) for pathogenic HIT antibodies means that patients with HIT can usually be readily distinguished from the other conditions (see Chap. 11). However, the role of heparin in causing thrombocytopenia because of nonimmune platelet activation cannot readily be separated from other common medical problems encountered in hospitalized patients, either on clinical or laboratory criteria. Furthermore, these two conditions can coexist (Chong and Castaldi, 1986). Thus, the term “nonimmune heparin-associated thrombocytopenia” (nonimmune HAT) has been recommended to describe patients who develop thrombocytopenia during
heparin treatment in which a role for HIT antibodies cannot be implicated (Warkentin et al., 1998a).

Unfortunately, many early studies of HIT frequency either did not perform laboratory testing or used relatively insensitive or nonspecific assays to diagnose HIT. In contrast, more recent studies have used one, or even two, sensitive and complementary assays. Perhaps for this reason, the understanding of the frequency and clinical import of HIT has shifted over the years. Formerly, the range of views on immune-mediated HIT were divergent: it was considered both nonexistent (Bell, 1988) and common (Kelton, 1986). Nevertheless, both viewpoints acknowledged that thrombosis resulting from HIT was very uncommon. Today’s perspective on HIT is very different. The frequency of HIT is now shown to be variable, partly depending on patient population and type of heparin used. For example, the frequency ranges from less than 1% (cardiac medical patients) to 5% (orthopedic surgical patients) receiving unfractionated heparin (UFH); HIT antibody formation following UFH use ranges from 2% (cardiac medical patients) to 15% (orthopedic surgical patients) to 50% (cardiac surgical patients). Most importantly, however, it is now becoming clear that the risk for thrombosis in patients who develop HIT is at least 33–50%, a frequency that is far greater than in control patients who do not develop HIT (see Chap. 3).

The biological basis for this variability in frequency of HIT and HIT antibody formation is now apparent. The HIT antigen is a cryptic autoantigen, or neoantigen, on platelet factor 4 (PF4) that is formed when PF4 binds to heparin (see Chaps. 6–8). Only stoichiometric concentrations of heparin and PF4 will form the antigen. Thus, it can be hypothesized that the frequency of HIT antibody formation will be influenced not only by heparin dose and composition, but also by circulating PF4 levels. Conditions associated with fluctuating, but at times high, circulating PF4 and heparin levels (e.g., cardiac surgery) might be ideal for immunization to the HIT antigen. Thus, real differences in HIT frequency observed among prospective studies can be understood in a biologically plausible context.

The key role of the pathogenic HIT antibodies and the availability of sensitive and specific assays for their detection suggest that HIT should be considered a clinicopathologic syndrome. Consequently, this chapter will focus on studies that have used in vitro testing to evaluate HIT antibodies. However, other features known to be useful to diagnose HIT, such as the timing of the onset of thrombocytopenia in typical HIT, and the rapid platelet count fall on heparin rechallenge, will also be used (see Chap. 3). The importance of confirmatory laboratory testing should not be underestimated: prospective (Greinacher et al., 1994; Lee et al., 1996) and retrospective (Look et al., 1997) studies suggest that only 25–55% of sera referred for evaluation
Figure 1  Initially unrecognized HIT during prospective studies. (a) A 57-year-old man developed skin lesions at the sites of LMWH (enoxaparin) injections on day 9. An acute systemic reaction (ASR; see Chap. 3) developed after a 5000 U intravenous UFH bolus. This patient was recognized as having had the HIT syndrome only following systematic testing for HIT antibodies performed later (Warkentin et al., 1995, 2003a). (b) A 73-year-old woman developed bowel infarction necessitating resection while receiving UFH prophylaxis after hip replacement surgery. The thrombocytopenia was initially attributed to “sepsis.” However, the patient was later recognized as having the HIT syndrome following systematic testing for HIT antibodies performed later (Warkentin et al., 1995, 2003a).
test positive for HIT antibodies. Furthermore, systematic analysis of a large clinical trial of heparin treatment (Warkentin et al., 1995, 2003a) revealed several patients in whom unusual clinical events subsequently linked to HIT were initially attributed to other problems (Fig. 1).

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

II. EARLY- VERSUS LATE-ONSET THROMBOCYTOPENIA

The distinction between thrombocytopenia that begins early (within 4 days) or late (5 or more days after beginning heparin treatment) is a simple clinical
feature that is useful to distinguish nonimmune HAT, which begins early, from (immune) HIT, which begins late. For this assessment, the first day of heparin use is considered day 0 (see Table 2 in Chap. 3). There is an important exception to this rule of timing for HIT: a rapid fall in platelet count on starting heparin therapy can represent acute HIT, but only if a patient already has circulating HIT antibodies, usually the result of a recent heparin exposure. HIT antibodies are transient, which could explain why the risk for rapid-onset HIT is restricted to a period of about 100 days following exposure to heparin (Warkentin and Kelton, 2001) (see Chap. 3).

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

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

III. FREQUENCY OF IMMUNE HIT

Tables 2 and 3 list prospective studies of the frequency of HIT that employed in vitro testing for HIT antibodies or were studies in which the likelihood of HIT could be judged based on information provided, especially the timing of the onset of thrombocytopenia. Relevant variables
Table 2  The Frequency of HIT: Prospective Studies of HIT in Medical Patients Using In Vitro Testing of Patient Serum/Plasma for HIT Antibodies or Indicating a High Likelihood of HIT Based on Timing of Platelet Count Fall

<table>
<thead>
<tr>
<th>Study</th>
<th>Major indication for heparin</th>
<th>In vitro test</th>
<th>Route, dose</th>
<th>Frequency of (immune) HIT (%)</th>
<th>Timing of platelet fall reported?</th>
<th>Definition of thrombocytopenia (&lt;10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bovine UFH</td>
<td>Porcine UFH</td>
<td>LMWH</td>
</tr>
<tr>
<td>Comparisons between bovine UFH and porcine UFH [studies and data in bold are randomized, controlled trials (RCTs)]:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ansell et al., 1980</td>
<td>VTE</td>
<td>PRP(SR)</td>
<td>iv ther</td>
<td>4/21 (19.0)</td>
<td>0/22 (0)</td>
<td>Yes</td>
</tr>
<tr>
<td>Green et al., 1984 (RCT)</td>
<td>VTE</td>
<td>HIPA</td>
<td>iv ther</td>
<td>2/45 (4.4)</td>
<td>0/44 (0)</td>
<td>Yes</td>
</tr>
<tr>
<td>Powers et al., 1984 (RCT)</td>
<td>VTE</td>
<td>SRA&quot;</td>
<td>iv ther</td>
<td>2/65* (3.1)</td>
<td>0/66 (0)</td>
<td>Yes</td>
</tr>
<tr>
<td>Bailey et al., 1986 (RCT)</td>
<td>VTE, ATE</td>
<td>No test</td>
<td>iv ther</td>
<td>1/21 (4.8)</td>
<td>0/22 (0)</td>
<td>Yes</td>
</tr>
<tr>
<td>Cipolle et al., 1983; Ramirez-Lassepas et al., 1984 [stroke]</td>
<td>VTE, ATE</td>
<td>PRP</td>
<td>iv ther</td>
<td>6/100b (6.0)</td>
<td>1/111 (0.9)</td>
<td>Yes</td>
</tr>
<tr>
<td>Bell et al., 1976</td>
<td>VTE, ATE</td>
<td>PRP/SRAc</td>
<td>iv ther</td>
<td>3/52c (5.8)</td>
<td>2/120d (1.7)</td>
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<tr>
<td>Alving et al., 1977</td>
<td>VTE</td>
<td>No test</td>
<td>iv ther</td>
<td>3/166c (1.8)</td>
<td>0/5 (0)</td>
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<tr>
<td>Monreal et al., 1989</td>
<td>VTE</td>
<td>No test</td>
<td>iv ther</td>
<td>2/89 (2.2)</td>
<td>0/43 (0)</td>
<td>Low</td>
</tr>
</tbody>
</table>

Predominant treatment for venous (VTE) or arterial (ATE) thromboembolism

Lee and Warkentin
<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Result</th>
<th>Clotting and Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kakasseril et al., 1985</td>
<td>VTE, ATE PRP</td>
<td>iv ther</td>
<td>No</td>
<td>1/412² (2.8)</td>
<td>&lt;100</td>
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<tr>
<td>Malcolm et al., 1979</td>
<td>Multiple PRP</td>
<td>iv ther</td>
<td>Yes</td>
<td>1/66³ (1.5)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Rao et al., 1989</td>
<td>Multiple PRP(SR)</td>
<td>sc proph</td>
<td>NA</td>
<td>0/39 (0)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Girolami et al., 2003</td>
<td>VTE, ATE HIPA, EIA</td>
<td>sc proph</td>
<td>Yes</td>
<td>5/360 (1.4)</td>
<td>&gt;50% fall</td>
</tr>
<tr>
<td>Lindhoff-Last et al., 2002</td>
<td>VTE EIA sc proph</td>
<td>1/356 (0.3)³</td>
<td>Yes</td>
<td>&lt;100 or &gt;50% fall</td>
<td></td>
</tr>
</tbody>
</table>

Predominant treatment for myocardial infarction or acute coronary syndromes (MI/ACS)

<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Result</th>
<th>Clotting and Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kappers-Klune et al., 1997</td>
<td>MI/ACS HIPA, EIA</td>
<td>iv ther</td>
<td>Yes</td>
<td>1/358 (0.3)</td>
<td>&gt;60 and &gt;50% fall (&lt;120)</td>
</tr>
<tr>
<td>Romeril et al., 1982</td>
<td>MI/ACS PRP sc proph</td>
<td>2/358 (0.6)</td>
<td>NA</td>
<td>&lt;150</td>
<td></td>
</tr>
<tr>
<td>Weitberg et al., 1982</td>
<td>MI/ACS No test sc proph</td>
<td>0/720 (0)</td>
<td>NA</td>
<td>&lt;150</td>
<td></td>
</tr>
<tr>
<td>Johnson et al., 1984</td>
<td>MI/ACS No test sc proph</td>
<td>0/66 (0)</td>
<td>Yes</td>
<td>&lt;150</td>
<td></td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>MI/ACS HIPA, EIA</td>
<td>iv ther</td>
<td>Yes</td>
<td>1/267 (0.4)</td>
<td>&gt;33% fall</td>
</tr>
<tr>
<td>Verma et al., 2003</td>
<td>Multiple EIA, SRA Multiple 1/267 (0.4)</td>
<td>Yes</td>
<td>&gt;33% fall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>New-onset hemodialysis EIA, PRP</td>
<td>6/154 (3.9)</td>
<td>Yes</td>
<td>Clotting and &gt;20% fall (&gt;50% fall)</td>
<td></td>
</tr>
<tr>
<td>Yamamoto et al., 1996</td>
<td>— PRP sc proph</td>
<td>3/154 (1.9)</td>
<td>NA</td>
<td>&lt;150</td>
<td></td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>— PRP sc proph</td>
<td>0/25 (0)</td>
<td>0/14 (0)</td>
<td>NA</td>
<td>&lt;150</td>
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</tbody>
</table>

Frequency of HIT

*Note: VTE = venous thromboembolism, ATE = arterial thromboembolism, PRP = platelet rich plasma, EIA = enzyme immunoassay, HIPA = hemipatia, SRA = serological reactivity assay.*
HIT was excluded if the platelet count rose during continued heparin after an early fall (e.g., Johnson et al., 1984). Also, where uncertainty existed as to the number of patients with probable HIT, the lower number was indicated in the table, to avoid overestimating the number of patients with HIT (contrast the analysis shown in Table 4). Some data relating to Cipolle et al. (1983) were obtained by personal communication, as reported (Warkentin and Kelton, 1991). The study by Gallus et al. (1980) was excluded because the source of heparin was not specified. Some reports (e.g., Nelson et al., 1978) were excluded because timing of thrombocytopenia was not reported.

Abbreviations: ATE, arterial thromboembolism; EIA, PF4-heparin enzyme-immunoassay; HIPA, heparin-induced platelet activation test; iv ther, intravenous therapeutic-dose heparin; LMWH, low molecular weight heparin; MI/ACS, myocardial infarction/acute coronary syndromes; PRP, HIT assay using citrated platelet-rich plasma (PRP/SR, with serotonin release); sc proph, subcutaneous prophylactic-dose heparin; sc ther, subcutaneous therapeutic; RCT, randomized controlled trial; SRA, serotonin release assay using washed platelets; UFH, unfractionated heparin; VTE, venous thromboembolism.

Table 2
Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Major indication for heparin</th>
<th>In vitro test</th>
<th>Route, dose</th>
<th>Bovine UFH</th>
<th>Porcine UFH</th>
<th>LMWH</th>
<th>Timing of platelet fall reported?</th>
<th>Definition of thrombocytopenia (% EIA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schwartz et al., 1985</td>
<td>—</td>
<td>No test</td>
<td>Bolus iv ther</td>
<td>3/20 (15.0)</td>
<td>0/10 (0)</td>
<td>Yes</td>
<td>&lt;150</td>
<td></td>
</tr>
</tbody>
</table>
Table 3  The Frequency of HIT: Prospective Studies of Surgical Patients Using Confirmatory In Vitro Laboratory Testing of Patient Serum or Plasma or Indicating a High Likelihood of HIT Based on Timing of Platelet Count Fall

<table>
<thead>
<tr>
<th>Study</th>
<th>Major indication for heparin</th>
<th>In vitro test</th>
<th>Route, dose</th>
<th>Frequency of (immune) HIT</th>
<th>Timing of platelet fall reported?</th>
<th>Definition of thrombocytopenia (×10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparisons between porcine UFH and LMWH</td>
<td>Bovine UFH</td>
<td>2/204 (1.0)</td>
<td>Yes</td>
<td>&lt;100, &gt;40% fall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leyvraz et al., 1991</td>
<td>Porcine UFH</td>
<td>0/205 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warkentin et al., 1995, 2003</td>
<td>LMWH</td>
<td>9/332 (2.7)</td>
<td>Yes</td>
<td>&lt;150</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16/332 (4.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/333 (0.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other studies</td>
<td>Bovine UFH</td>
<td>2/246 (0.8)</td>
<td>Yes</td>
<td>&lt;150 or &gt;50% fall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Louridas, 1991</td>
<td>Porcine UFH</td>
<td>5/114b (4.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LMWH</td>
<td>15/307 (4.9)</td>
<td>Yes</td>
<td>&gt;50% fall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warkentin et al., 1998b</td>
<td>PRP</td>
<td>0/51 (0)</td>
<td>Yes</td>
<td>Not stated</td>
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<tr>
<td>Ganzer et al., 1997</td>
<td>PRP/EIA</td>
<td>1/100 (1.0)</td>
<td>Yes</td>
<td>&gt;50% fall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trossaert et al., 1998</td>
<td>PRP/EIA</td>
<td>9/263 (3.4)</td>
<td>No</td>
<td>&lt;100 or &gt;40% fall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warkentin et al., 2000</td>
<td>SRA</td>
<td>1/370 (0.3)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pouplard et al., 1999, 2002</td>
<td>SRA, EIA</td>
<td>1/370 (0.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: EIA, PF4-heparin enzyme-linked immunosorbent assay; HIPA, heparin-induced platelet activation test (aggregation of washed platelets); LMWH, low molecular weight heparin; PRP, HIT assay using citrated platelet-rich plasma; sc proph, subcutaneous prophylactic-dose heparin; SRA, serotonin release assay using washed platelets; UFH, unfractionated heparin.

a Ineffective testing was used (platelet aggregation without UFH).

b Of seven patients with thrombocytopenia reported, two were excluded because of early onset of thrombocytopenia.
influencing the frequency of HIT include the type of heparin used and the patient population.

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

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

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

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

A higher frequency of immune HIT with bovine lung heparin is biologically plausible. Bovine heparin has a higher sulfate:disaccharide ratio than does porcine heparin (Casu et al., 1983), and it is better able to activate platelets in vitro (Barradas et al., 1987). These properties could lead to greater platelet activation in vivo and, consequently, greater potential for PF4 release. Moreover, the bovine heparin chains would be expected to better form the large multimolecular complexes that compose the target antigen for HIT antibodies.
Lot-to-lot variability within heparin of a particular animal source could also contribute to variable frequency of HIT. Stead and coworkers (1984) reported a striking cluster of six patients with pulmonary embolism complicating HIT identified within a few weeks at one institution. A particular lot of bovine lung heparin in use in the operating room was linked to these events: patient serum-induced platelet aggregation occurred in the presence of this particular lot of heparin, but not when other lots of bovine lung heparin from the same manufacturer were used.

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

Table 2 also lists the frequency of HIT observed in several prospective studies that have evaluated medical patients receiving intravenous, therapeutic-dose porcine UFH, usually for venous thromboembolism (VTE). Excluding a study of hemodialysis, an overall frequency of HIT of slightly less than 1% is suggested (0.7% [11/1604]; 95% CI 0.3–1.2%). This is a relatively low number, particularly when one considers that, paradoxically, the frequency appears to be much higher in postoperative surgical patients who received lower (prophylactic) doses of porcine heparin (discussed subsequently).

In contrast, HIT seemed to occur more often in a prospective study of acute hemodialysis patients receiving porcine UFH (Yamamoto et al., 1996). Whether this is a real difference that reflects increased platelet activation (and PF4 release) during hemodialysis or reflects a more sensitive definition of thrombocytopenia (any platelet count fall associated with line clotting) requires further investigation.

There is an anecdotal report of a patient developing HIT when she received UFH to permit extracorporeal exposure of her leukocytes to ultraviolet irradiation (photopheresis) for treatment of lupus (Dittberner et al., 2002).

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

Two large prospective studies suggest that HIT is an important problem in orthopedic patients receiving UFH (Warkentin et al., 1995, 2003a; Ganzer et al., 1997). When using a definition of a 50% fall in platelet count that began on or after day 5 of heparin treatment, and that was confirmed by serologic testing for HIT antibodies, both studies observed a frequency of HIT of about 5% (see Table 3). Each study used porcine mucosal heparin, derived from a different manufacturer, that was given by the subcutaneous route at a dosage of 15,000 U/day. Thrombocytopenia that was likely attributable to HIT has
also been observed in several other clinical trials of patients receiving UFH following orthopedic surgery (see Warkentin et al., 1995, for discussion), but only one study (Leyvraz et al., 1991) reported confirmatory in vitro testing. There is little prospective information of the frequency of HIT in other postoperative surgical populations treated with UFH (see Table 3). Three studies have been performed on postoperative cardiac surgical patients who also received postoperative UFH in addition to high doses of heparin during preceding cardiopulmonary bypass (Trossaert et al., 1998; Warkentin et al., 2000; Pouplard et al., 1999) (see Table 3). Pooling the three studies, about 2.4% of the patients developed serologically confirmed HIT. Interestingly the frequency of HIT in this population appears to be lower than in orthopedic patients receiving UFH, even though the cardiac surgical patients appear to have a higher frequency of formation of HIT antibodies (Warkentin et al., 2000).

Isolated limb perfusion (ILP) with melphalan employs extracorporeal circulation (and thus high-dose UFH) to treat melanoma or unresectable sarcoma limited to an extremity. In one study, HIT occurred in 3 of 108 patients (2.8%), who also received subcutaneous UFH prophylaxis following ILP (Masucci et al., 1999). The occurrence of arterial thrombosis and partial limb amputation in two of these patients with HIT led the investigators to discontinue routine UFH prophylaxis post-ILP. The hypothesis that ILP is a high-risk situation for HIT was supported by a prospective study showing HIT antibody seroconversion in nine of nine patients who underwent this procedure (despite not receiving postoperative UFH prophylaxis), with eight patients having HIT antibodies “strong” enough to cause serotonin release.

D. HIT is Less Frequent in Orthopedic Patients Receiving LMWH Compared with UFH Prophylaxis

Anecdotal reports indicate that HIT can occur during treatment with LMWH (Ball et al., 1989; Tardy et al., 1990; de Raucourt et al., 1996; Plath et al., 1997; Elalamy et al., 1996; Warkentin, 1998; Gruel et al., 2003; Ng and Lee, 2003). Clinical trial data suggest that the frequency is low, however. Using a sensitive definition for HIT (>50% fall on or after day 5 and confirmed by positive HIT antibodies), two studies in Hamilton found an overall frequency of only 4/439 (0.9%; 95% CI 0.25–2.32%) for HIT complicating use of LMWH given for postoperative orthopedic patients (Warkentin et al., 1995, 1998b, 2003a). In contrast, using the same definition of HIT, patients who received UFH had a much higher frequency of HIT, 16/332 (4.8%; 95% CI 2.78–7.71%) (Warkentin et al., 1995, 2003a). A similar high frequency of UFH-induced HIT was observed in a German study, 15/307 (4.9%; 95% CI, 2.76–7.93) (Ganzer et al., 1997).
The strongest evidence that LMWH is indeed associated with a lower frequency of HIT was provided by a randomized trial that directly compared the frequency of HIT between the two types of heparin (Warkentin et al., 1995, 2003a). The frequency of HIT in patients treated with the LMWH preparation, enoxaparin (itself derived from porcine mucosal heparin), was lower than that seen in patients treated with porcine UFH, irrespective of whether a standard definition (platelet fall to $<150 \times 10^9/L$ on or after day 5 of heparin treatment) or a more sensitive definition ($>50\%$ platelet count fall from the postoperative peak) of thrombocytopenia was used. The frequency of HIT antibody formation also differed between the two patient groups, using either the serotonin release assay (Warkentin et al., 1995) or the PF4-heparin EIA (Warkentin et al., 2000). Thrombocytopenia also appeared to be infrequent in other trials of LMWH (Leyvraz et al., 1991; Simonneau et al., 1997; ENOXACAN Study Group, 1997).

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

Gruel and colleagues (2003) performed a systematic study that identified 11 patients with HIT (3 with HIT-associated thrombosis) that had been exclusively treated with a LMWH preparation (dalteparin, nadroparin, or enoxaparin). Clinical and serologic features were similar to patients with HIT developing during UFH, except that there was evidence that thrombocytopenia may begin somewhat later during LMWH therapy. Based upon estimated relative use of UFH and LMWH in France, the authors estimated the frequency of HIT to be 40-fold less with LMWH, compared with UFH.

E. Frequency of HIT During Pregnancy

HIT appears to be uncommon during pregnancy even with UFH treatment. Fausett and colleagues (2001) reported that none of 244 pregnant women developed HIT during UFH use, although HIT occurred in 10 of 244 (4%) nonpregnant patients who received UFH ($p = 0.0014$). In a literature review, Sanson and coworkers (1999) identified no cases of HIT among 486 women who received LMWH during pregnancy. Ellison et al. (2000) studied 57 pregnancies in 50 patients and also found no episodes of HIT in pregnant women who received enoxaparin. More recently, Lepercq and colleagues...
(2001) found no cases of HIT in 624 pregnancies among 604 women treated with LMWH.

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

There are two ways that incidental exposure to heparin by “flushing” of intravascular catheters can affect the frequency or clinical effect of HIT. First, such minor heparin exposures can trigger formation of HIT antibodies (Ling and Warkentin, 1998; Warkentin et al., 1998b). And second, in patients who have already formed potent HIT antibodies for any reason, any ongoing or recurrent heparin exposure—including small-dose exposure—could lead to recurrence or exacerbation of thrombocytopenia or thrombosis (Rice and Jackson, 1981). Indeed, several patients have been reported in whom severe HIT occurred while only small amounts of heparin were being given as flushes to maintain the patency of intravascular catheters (Doty et al., 1986; Heeger and Backstrom, 1986; Kappa et al., 1987; Rama et al., 1991; Brushwood, 1992; Parney and Steinke, 2000).

In most of the reports of patients developing HIT during LMWH treatment, recent prior exposure to UFH was not excluded. Indeed, incidental exposure to UFH by intraoperative invasive catheters could lead to formation of HIT antibodies that are inappropriately attributed to later postoperative LMWH prophylaxis (Shumate, 1995). However, if true, it would suggest that the apparent difference in immunogenicity between UFH and LMWH could be even greater than initially reported.

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

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

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

G. HIT and Heparin-Coated Devices

Heparin can be bonded to artificial surfaces (Larsson et al., 1987), either through ionic attachment, as used for pulmonary artery catheters (Eldhi and Jacobsson, 1974), or by end-linked covalent bonding (e.g., Carmeda BioActive Surface, or CBAS) (Larm et al., 1983). CBAS has been used for cardiopulmonary bypass circuits and filters (Borowiec et al., 1992a,b, 1993), extracorporeal membrane oxygenation (ECMO) devices (Koul et al., 1992), and coronary stents (Serruys et al., 1996). During use in patients, ionically attached heparin is displaced by albumin from the catheter surface, where it could contribute to HIT (discussed subsequently). End-linked heparin is an effective and longer-lasting anticoagulant, as the immobilized, but flexible, heparin chains are able to interact with fluid-phase antithrombin and thrombin (Elgue et al., 1993). Nevertheless, the end-linked, but relatively unconstrained, heparin is capable of interacting with PF4 (Suh et al., 1998). Therefore, it is theoretically possible that covalent heparin-bonded devices could result in formation of HIT antibodies or could cause HIT in a patient who has formed antibodies. Alternatively, even covalently bonded heparin might “leach” into blood by proteolytic mechanisms, thereby contributing in a more conventional way to the pathogenesis of HIT (Almeida et al., 1998a).

Use of heparin-coated pulmonary catheters in contributing to HIT has been implicated by Laster and Silver (1988). These workers reported 10 patients with HIT whose platelet counts did not rise until the removal of their heparin-coated pulmonary catheters, despite discontinuing all other sources of heparin. Incubation of the heparin-coated catheters with platelets in the
presence of patient sera resulted in “catheter-induced” platelet aggregation. Based on the identification of four such cases, during which time 1112 heparin-coated catheters had been used, they estimated the frequency of catheter-associated HIT to be 0.4%.

H. HIT Caused by Other Sulfated Polysaccharides

The cryptic HIT autoantigen comprises conformationally altered PF4 when it forms a multimolecular complex with heparin. Other negatively charged polysaccharides can interact with PF4 to produce the HIT antigen (Wolf et al., 1983; Greinacher et al., 1992a,b,c; Anderson 1992) (see Chap. 8). These considerations explain why a number of high-sulfated polysaccharides, 10 or more subunits in length, have been reported to cause a syndrome of thrombocytopenia and thrombosis that essentially mimics HIT. These drugs include the semisynthetic 5-carbon subunit–based “heparinoid” pentosan polysulfate (Gouault-Heilman et al., 1985; Vitoux et al., 1985; Follea et al., 1986; Goad et al., 1994; Tardy-Poncet et al., 1994; Rice et al., 1998), as well as polysulfated chondroitin sulfate (Bouvier, 1980; Wolf et al., 1983; Greinacher et al., 1992a). The frequency of immune-mediated thrombocytopenia, with or without thrombosis, after exposure to these compounds is unknown, but may be high.

I. Variable Duration of Heparin Treatment

As HIT typically begins 5–10 days after starting therapy with heparin, it follows that the length of heparin treatment can influence the risk for HIT (e.g., a 10-day course is far more likely to produce clinical HIT than a 3-day treatment period). On the other hand, there is evidence that the risk of HIT begins to decrease after 10 days of uninterrupted heparin use (see Fig. 1C, Chap. 3). In a large study of postoperative orthopedic surgical patients receiving postoperative heparin prophylaxis, no patient developed HIT antibodies after day 10, even though many patients received heparin for up to 14 days (Warkentin et al., 1995). These data are consistent with a “point exposure” model for risk of HIT in this patient population, such as a brief time shortly after surgery, when high circulating PF4 levels coincide with the first few subcutaneous heparin injections. However, even if HIT antibody formation occurs during the day 5–10 window period, thrombocytopenia itself can occur somewhat later, particularly if a larger dose of heparin is given, or UFH is substituted for LMWH (see Fig. 1a). The characteristic timing of HIT should assist clinicians in focusing their platelet count monitoring for HIT during the critical time period, so that the early diagnosis of HIT is improved (see Sec. VI).
J. Heparin Dose-Dependence in HIT

Analysis of individual patients with HIT often shows dose-dependence; that is, mild thrombocytopenia during subcutaneous heparin prophylaxis is followed by a marked drop in platelet count if the patient then receives therapeutic-dose heparin (Fig. 1a).

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

IV. FREQUENCY OF THROMBOSIS COMPPLICATING HIT

Ironically, although thrombosis was the first manifestation of the HIT syndrome, first recognized over 40 years ago (Weismann and Tobin, 1958), widespread recognition that thrombosis was a common complication of HIT did not occur until recently. Indeed, until 1995 no study of HIT had compared the frequency of thrombosis with a matched control population (Warkentin et al., 1995). This study quantitated the strength of the association between HIT and thrombosis and noted that the more unusual the thrombotic event (e.g., bilateral deep venous thrombosis, pulmonary embolism), the stronger the association with HIT (see Chap. 3).

Table 4 summarizes the thrombotic events that have been observed during prospective studies of HIT. The major observation is that thrombosis is relatively common in HIT patients, occurring in approximately one third of medical patients and about one half of postoperative surgical patients. The data also support findings from a prior retrospective study (Boshkov et al., 1993) that found the type of thrombotic event complicating HIT was influenced by the patient population. Table 4 suggests that the ratio of arterial to venous thrombosis is about 1:1 in medical patients, many of whom might have had arterial disease as their basis for hospitalization. Additionally, the therapeutic-dose heparin used in many of these studies may have partially protected against venous thromboembolic complications, although it may not have prevented platelet-mediated arterial occlusion. In contrast, there appears to be a strong predisposition to venous thromboembolism in post-
<table>
<thead>
<tr>
<th>Study</th>
<th>Major indication for heparin</th>
<th>In vitro test</th>
<th>Number treated</th>
<th>Definition of thrombocytopenia ($\times 10^9/L$)</th>
<th>Patients with HIT (using more sensitive definition of HIT)</th>
<th>Thrombotic complication of HIT</th>
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<tbody>
<tr>
<td>Medical patients (only intravenous therapeutic dose or hemodialysis included in table)</td>
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<td></td>
</tr>
<tr>
<td>Ansell et al., 1980</td>
<td>VTE</td>
<td>PRP (SR)</td>
<td>43</td>
<td>$&lt;150$</td>
<td>4</td>
<td>0</td>
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<tr>
<td>Green et al., 1984; Green, 1986</td>
<td>VTE</td>
<td>HIPA</td>
<td>89</td>
<td>$&lt;150$</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Powers et al., 1984</td>
<td>VTE</td>
<td>SRA</td>
<td>65</td>
<td>$&lt;150$</td>
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<td>1</td>
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<tr>
<td>Bailey et al., 1986</td>
<td>VTE, ATE</td>
<td>No test</td>
<td>43</td>
<td>$&lt;100$</td>
<td>1</td>
<td>0</td>
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<td>Cipolle et al., 1983; Ramirez-Lassepas et al., 1984</td>
<td>Cerebrovascular ischemia</td>
<td>PRP</td>
<td>137$^a$</td>
<td>$&lt;100$</td>
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<td>Gallus et al., 1980</td>
<td>VTE</td>
<td>No test</td>
<td>120</td>
<td>$&lt;150$</td>
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<td>Monreal et al., 1989</td>
<td>VTE</td>
<td>PRP</td>
<td>166</td>
<td>$&lt;100$</td>
<td>5</td>
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<tr>
<td>Kakkaresseril et al., 1985</td>
<td>VTE, ATE</td>
<td>No test</td>
<td>89</td>
<td>$&lt;100$</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Makoluk et al., 1979</td>
<td>VTE</td>
<td>PRP</td>
<td>142</td>
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<td>Kappers-Kunne et al., 1997</td>
<td>Acute coronary syndromes</td>
<td>HIPA, EIA</td>
<td>358</td>
<td>$&lt;120$ or $&gt;30%$ fall</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>($&lt;60$ or $&gt;50%$ fall)</td>
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<tr>
<td>Study</td>
<td>Type</td>
<td>PRP, EIA</td>
<td>Clotting, platelet fall</td>
<td>Total medical: Venous/arterial thrombosis ratio = 9/7 - 1.3</td>
<td>Orthopedic surgical patients (total joint arthroplasty)</td>
<td>Total orthopedic: Venous/arterial thrombosis ratio = 14/1 - 14.0</td>
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<td>----------------------------------------------------------</td>
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<td>---------------------------------------------------------------</td>
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<tr>
<td>Yamamoto et al., 1996</td>
<td>Hemodialysis</td>
<td>PRP, EIA</td>
<td>154</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total medical: Venous/arterial thrombosis ratio = 9/7 - 1.3</td>
<td>1472</td>
<td>41</td>
<td>9</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthopedic surgical patients (total joint arthroplasty)</td>
<td>Warkentin et al., 1995, 2003a</td>
<td>Hip SRA</td>
<td>332 (&lt;50% fall)</td>
<td>9</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Warkentin et al., 1998b</td>
<td>Hip, knee SRA</td>
<td>246 &lt;150</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Warkentin et al., 1999</td>
<td>Hip, knee HIPA</td>
<td>307 &gt;50% fall</td>
<td>15</td>
<td>5*</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Leyvraz et al., 1991</td>
<td>Hip PRP</td>
<td>175 &lt;100, &gt;40% fall</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Surgical, other</td>
<td>Louridas, 1991 Vascular PRP b</td>
<td>114 &lt;100</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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

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

b Ineffective testing was used (platelet aggregation without heparin).

c Another five patients developed venous thrombosis in association with a positive HIPA assay, but the platelet count did not fall by >50%.
operative orthopedic patients who have developed HIT (venous:arterial ratio at least 14:1) (see Table 4).

The retrospective identification of patients with serologically confirmed HIT permits analysis of large groups of HIT patients (Table 5). This provides an alternative assessment of the spectrum of thrombotic complications in HIT. Three large studies (Warkentin and Kelton, 1996; Nand et al. 1997; Wallis et al., 1999) showed a predominance of venous thrombosis complicating HIT. Indeed, pulmonary embolism was even more frequent than all types of arterial thromboses combined.

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

Another pattern that emerges from the Missouri series is a progressively decreasing frequency of reported thrombotic or hemorrhagic complications, from 61% in 1983, to 23% in 1987, then to 7.4% in 1998. The authors believe this to be the result of earlier recognition of HIT. However, an alternative explanation could be greater awareness of HIT over time, and thus a higher likelihood of identifying patients with less severe HIT. Indeed, a study by Wallis and colleagues (1999) suggests that earlier recognition of HIT may not reduce the risk of thrombosis (see Table 5).

A progressive reduction in HIT-associated mortality over time was also observed by the Missouri group (see Table 5). However, early discontinuation of heparin was not associated with significantly lower mortality in another study (Wallis et al., 1999). This issue is complicated by the observation that deaths apparently unrelated to thrombosis are relatively common in patients with HIT (Warkentin and Kelton, 1996; Greinacher et al., 1999).

It is possible that nonthrombotic mortality may be higher than expected by chance in patients with HIT. This speculation is based on the observation that only a minority of patients who form HIT antibodies develop HIT (discussed subsequently); a corollary to this statement is that comorbid factors that tend to result in increased pathogenicity of HIT antibodies may also independently contribute to increased patient morbidity and mortality (i.e., patients with septicemia or multisystem organ failure may be more likely to have platelet activation in the presence of HIT antibodies than “well” patients).
Table 5: Frequency of Thrombosis Complicating HIT in Retrospective Cohort Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients with HIT</th>
<th>Mean platelet count nadir (\times 10^{9}/L)</th>
<th>Patients with thrombosis (%)</th>
<th>Ratio of venous/arterial thrombosis</th>
<th>Number of deaths (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warkentin and Kelton, 1996</td>
<td>127</td>
<td>59(^{a})</td>
<td>97 (76%)</td>
<td>4.3</td>
<td>26 (20%)</td>
</tr>
<tr>
<td>Subgroup with “isolated” thrombocytopenia</td>
<td>62</td>
<td>57</td>
<td>32 (52%)(^{b})</td>
<td>4</td>
<td>13 (21%)</td>
</tr>
<tr>
<td>Nand et al., 1997</td>
<td>108</td>
<td>58</td>
<td>32 (29%)</td>
<td>2.5</td>
<td>5 (5%)(^{c})</td>
</tr>
<tr>
<td>Wallis et al., 1999</td>
<td>113</td>
<td>54</td>
<td>43 (38%)</td>
<td>1.4</td>
<td>31 (27%)</td>
</tr>
<tr>
<td>Subgroup with heparin cessation &lt;48 h</td>
<td>40</td>
<td>56</td>
<td>18 (45%)</td>
<td>1.4</td>
<td>10 (25%)</td>
</tr>
<tr>
<td>Subgroup with heparin cessation &gt;48 h</td>
<td>73</td>
<td>54</td>
<td>25 (34%)</td>
<td>1.4</td>
<td>21 (29%)</td>
</tr>
<tr>
<td>Silver et al., 1983</td>
<td>62</td>
<td>Range: 5–83</td>
<td>38 (61%)</td>
<td>0.6</td>
<td>20 (32%)(^{d})</td>
</tr>
<tr>
<td>Laster et al., 1987</td>
<td>169</td>
<td>57</td>
<td>30 (18%)</td>
<td>0.5</td>
<td>20 (12%)</td>
</tr>
<tr>
<td>Almeida et al., 1998b</td>
<td>94(^{e})</td>
<td>&gt;108</td>
<td>7 (7%)</td>
<td>0.6(^{f})</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

\(^{a}\) The mean platelet count nadir for 127 patients with HIT and platelet count \(<150 \times 10^{9}/L\), and the median platelet count nadir for all 142 patients diagnosed with HIT (including those whose platelet count nadir was \(\geq 150 \times 10^{9}/L\), were both \(59 \times 10^{9}/L\) (Warkentin, 1998a) (see Fig. 6 in Chap. 3).

\(^{b}\) The cumulative 30-day frequency of new thrombosis in patients with isolated thrombocytopenia following recognition of HIT was 52.8% by Kaplan-Meier analysis.

\(^{c}\) Only deaths in patients who developed thrombosis were reported. Total number of deaths in the HIT cohort was not reported.

\(^{d}\) Fourteen of the 20 deaths were judged to be caused by HIT-associated thrombosis.

\(^{e}\) Of 100 consecutive patients with positive in vitro testing, 6 were previously known to have heparin-dependent antibodies and were not subsequently reexposed to heparin.

\(^{f}\) Two thromboses of arteriovenous grafts were excluded from classification into arterial or venous thrombosis.
A. Natural History of Isolated HIT

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

Figure 2  Cumulative frequency of thrombosis in HIT patients presenting with isolated thrombocytopenia. Approximately 50% of HIT patients initially recognized with isolated thrombocytopenia developed objective evidence for thrombosis during the subsequent 30-day period. The 1- and 2-day thrombotic event rates were approximately 10 and 18%, respectively. (From Warkentin and Kelton, 1996.)
The study identified 62 patients who met the definition of isolated thrombocytopenia. The 30-day cumulative risk for thrombosis in this study was 52.8% (see Fig. 2). This risk did not differ whether the heparin had been discontinued, or whether warfarin had been substituted for the heparin. Similar findings were reported from a much smaller earlier study performed in Europe (Boon et al., 1994). This high risk for thrombosis in HIT is also supported by a prospective study (Warkentin et al., 1995), in which five of six HIT patients developed thrombosis either on the first day that their platelet count fell below $150 \times 10^9/L$ or within the next few days despite the discontinuation of heparin.

Subsequent to the Hamilton study, a report by Wallis and colleagues (1999) from Loyola University confirmed the high risk for thrombosis among patients in whom HIT is identified by platelet count monitoring, even with discontinuation of heparin (see Table 5). Overall, the 30-day thrombotic event rate was 43/113 (38%), with a ratio of venous to arterial thrombosis of just 1.4. The relatively low predominance of venous thrombosis could be explained by the large number of patients (59%) in this study who developed HIT following cardiac surgery (i.e., a patient population had relatively high risk for arterial thrombosis).

An intriguing finding of the Wallis report is that early cessation of heparin did not appear to improve clinical outcomes. For 40 of the 113 patients with HIT (35%), heparin was discontinued within 48 h of onset of thrombocytopenia (defined as a platelet count fall to less than $100 \times 10^9/L$, or a greater than 50% fall from the peak platelet count after initiating heparin). Indeed, there was a trend to a higher rate of thrombosis in the patients with early heparin cessation, compared with the remaining 65% of patients in whom heparin was stopped later (45 vs. 34%; $p = 0.26$) (see Table 5).

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

Taken together, these studies of the natural history of isolated HIT provide the basis for the recent recommendation that prophylactic anticoagulant therapy is appropriate for most patients with isolated HIT (Hirsh et al., 2001) (see Chaps. 1 and 13–16). Other data to support this concept include the high probability of detecting subclinical deep vein thrombosis by duplex ultrasonography in patients with isolated HIT (Tardy et al., 1999), as well as the persistence of marked in vivo thrombin generation for several days in
Table 6  Studies Describing Systematic Screening for HIT Antibodies Using Sensitive Assays in Patients Receiving Heparin

<table>
<thead>
<tr>
<th>Study</th>
<th>Trial design</th>
<th>Heparin (porcine UFH used unless otherwise indicated)</th>
<th>HIT assay used</th>
<th>Number of patients</th>
<th>Patients with HIT antibodies (%)</th>
<th>Patients with HIT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amiral et al., 1996</td>
<td>Retrospective</td>
<td>iv ther UFH</td>
<td>EIA-IgM/A/G</td>
<td>109</td>
<td>19 (17.4)</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EIA-IgG</td>
<td></td>
<td>3 (2.8)</td>
<td></td>
</tr>
<tr>
<td>Kappers-Klunne et al., 1997</td>
<td>Prospective</td>
<td>iv ther UFH</td>
<td>EIA-IgG</td>
<td>358</td>
<td>9 (2.5)</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HIPA</td>
<td></td>
<td>30 (8.4)</td>
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</tr>
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<td>Hemodialysis patients</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Greinacher et al., 1996</td>
<td>Prevalence</td>
<td>iv ther UFH</td>
<td>HIPA</td>
<td>165</td>
<td>7 (4.2%)</td>
<td>0 (0)</td>
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<tr>
<td>de Sancho et al., 1996</td>
<td>Prevalence</td>
<td>iv ther UFH</td>
<td>EIA-IgM/A/G</td>
<td>45</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<td>0 (0)</td>
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<td>EIA-IgG</td>
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<td>3 (2.3)</td>
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</tr>
<tr>
<td>Boon et al., 1996</td>
<td>Prevalence</td>
<td>iv ther UFH</td>
<td>EIA-IgM/G</td>
<td>133</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
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<td>EIA-IgG</td>
<td></td>
<td>1 (0.8)</td>
<td>0 (0)</td>
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<td>Prevalence</td>
<td>iv ther UFH</td>
<td>EIA-IgG</td>
<td>50</td>
<td>6 (12.0)</td>
<td>0 (0)</td>
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<td>Orthopedic postoperative surgical patients</td>
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<tr>
<td>Warkentin et al., 1995, 2000</td>
<td>Substudy of RCT</td>
<td>sc proph UFH</td>
<td>EIA-IgG</td>
<td>205</td>
<td>29 (14.1)</td>
<td>10 (4.9)</td>
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<td>19 (9.5)</td>
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<td>Warkentin et al., 2000</td>
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<td>sc proph LMWH</td>
<td>EIA-IgG</td>
<td>182</td>
<td>22 (12.1)</td>
<td>2 (0.8)</td>
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<td>SRA</td>
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<td>9 (3.5)</td>
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<tr>
<td>Amiral et al., 1996</td>
<td>Retrospective</td>
<td>sc proph LMWH</td>
<td>EIA-IgM/A/G</td>
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<td>8 (3.5)</td>
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<td>EIA-IgG</td>
<td></td>
<td>2 (0.8)</td>
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<tr>
<td>Cardiac postoperative surgical patients (all received porcine UFH at cardiopulmonary bypass except where otherwise stated)</td>
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<td></td>
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<tr>
<td>Visentin et al., 1996</td>
<td>Retrospective</td>
<td>CPB: UFH (NPH)</td>
<td>EIA-IgM/G</td>
<td>44</td>
<td>27 (61.4)</td>
<td>0 (0)</td>
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<td>EIA-IgG</td>
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<td>23 (52.3)</td>
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<tr>
<td>Bauer et al., 1997</td>
<td>Prospective</td>
<td>CPB: bovine UFH (NPH)</td>
<td>EIA-IgM/A/G</td>
<td>111</td>
<td>57 (51.4)</td>
<td>0 (0)</td>
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<tr>
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<td>23 (52.3)</td>
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<tr>
<td>Trossaert et al., 1998</td>
<td>Retrospective</td>
<td>CPB: UFH sc proph UFH</td>
<td>EIA-IgM/A/G</td>
<td>51</td>
<td>14 (27.5)</td>
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<td>9 (17.6)</td>
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<td>PRP</td>
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<td>2 (3.9)</td>
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</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Prophylaxis</td>
<td>Patient Population</td>
<td>EIA-IgM/A/G</td>
<td>EIA-IgG</td>
<td>SRA</td>
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<td>Pouplard et al., 1999</td>
<td>Prospective</td>
<td>sc proph UFH</td>
<td>EIA-IgM/A/G 157</td>
<td>46 (29.3)</td>
<td>24 (15.3)</td>
<td>6 (3.8)</td>
</tr>
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<td>sc proph LMWH</td>
<td>EIA-IgM/A/G 171</td>
<td>37 (21.6)</td>
<td>24 (14.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Warkentin et al., 2000</td>
<td>Prospective</td>
<td>CPB: UFH</td>
<td>EIA-IgM/A/G 100</td>
<td>50 (50.0)</td>
<td>20 (20.0)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Francis et al., 2003</td>
<td>Prospective RCT</td>
<td>CPB: bovine UFH</td>
<td>EIA-IgM/A/G 99</td>
<td>44 (44.4)</td>
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<tr>
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<td>CPB: porcine UFH</td>
<td>EIA-IgM/A/G 108</td>
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<td>Vascular surgical patients</td>
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<td>Jackson et al., 1998</td>
<td>Prospective</td>
<td>iv ther UFH</td>
<td>EIA-IgM/A/G 50</td>
<td>17 (34.0)</td>
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<tr>
<td>Lindhoff-Last et al., 2000</td>
<td>Prospective</td>
<td>iv ther UFH</td>
<td>EIA-IgM/A/G 48</td>
<td>6 (12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>× 7 days, then sc proph LMWH</td>
<td>EIA-IgM/A/G 48</td>
<td>3 (6.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

- *Thrombocytopenia defined as platelet count fall >50% from baseline.
- **Two patients with HIT antibodies had mild thrombocytopenia, but a causal relation to heparin was not stated.
- †20% serotonin release used in Warkentin et al. (2000) rather than 50% serotonin release cutoff used in Warkentin et al. (1995).
- ‡Ten patients had HIT-IgG preoperatively; incidence of new seroconversion was 17/44 (38.6%).
- §Incidence of new seroconversion was 43% for EIA and 9% for SRA.
- ¶Two patients had HIT-IgG preoperatively; incidence of new seroconversion was 12/51 (23.5%) for EIA-IgM/A/G and 7/51 (13.7%) for EIA-IgG. None had a positive aggregation assay preoperatively.
- ‖80.2% underwent CPB, remainder “off-pump” surgery; 18.8% received postoperative UFH, LMWH, or both.
- ‡Excludes patients testing positive for HIT antibodies at baseline.
- †Includes only HIPA patients who also tested positive in EIA.
- †‡Two patients had HIT-IgG preoperatively; incidence of new seroconversion was 1/54 (1.9%).
patients with acute HIT even following discontinuation of heparin (Warkentin et al., 1997; Greinacher et al., 2000).

B. Summary of Observations from Prospective and Retrospective Studies

Observations emerging from these studies include the following:

1. The risk of thrombosis in patients with HIT is higher than previously recognized (up to 50%), and remains high despite the discontinuation of heparin. Mortality in patients with HIT is significant, although it remains uncertain what proportion is related to HIT-associated thrombosis, and whether these can be prevented by effective treatment.

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

V. POPULATION-BASED STUDIES OF HIT ANTIBODY SEROCONVERSION

Usually, serological investigation for HIT antibodies is performed on patients who develop thrombocytopenia during heparin treatment. Since 1995, however, many studies have performed systematic studies of HIT antibody seroconversion using sensitive assays (EIA, SRA, or both), irrespective of whether or not thrombocytopenia occurred. Some interesting insights into the pathogenesis of HIT have emerged from these reports.

As shown in Table 6, three general types of patient population have been investigated: medical patients receiving therapeutic-dose UFH; orthopedic patients receiving UFH or LMWH; and cardiac surgical patients receiving UFH or LMWH. There appear to be distinct frequencies of HIT antibody formation, as well as varying risks of “breakthrough” of HIT, among these different populations (Fig. 3). Several observations emerge from these studies:

1. The prevalence of seroconversion depends on the diagnostic assay used. PF4–heparin EIA is more sensitive than the SRA for the detection of HIT antibodies (Bauer et al., 1997; Pouplard et al., 1999; Warkentin et al., 2000); however, this increase in sensitivity does not necessarily translate into greater predictive value for clinical HIT (see Chap. 11).
2. With use of PF4–heparin EIA, the frequency of seroconversion following cardiac surgery approaches 50% (Visentin et al., 1996; Bauer et al., 1997; Warkentin et al., 2000). A high frequency of seroconversion (13–20%) was also observed using the SRA. Despite the highest frequency of HIT seroconversion reported in this patient population, the likelihood of developing HIT appears to be less than in other orthopedic patients also treated with postoperative UFH.

3. Seroconversion occurs frequently without thrombocytopenia or thrombosis. Indeed, most patients who form HIT antibodies do not develop HIT. The proportion who develop HIT, however, is highest among the patients who have a positive SRA. This suggests that HIT antibodies “strong” enough to activate platelets are
more likely to be clinically significant. Patient-dependent factors also must be important, however, because the probability of a positive SRA indicating clinical HIT ranges from about $<10\%$ (cardiac surgery) to approximately $50\%$ (orthopedic surgery).

4. Regardless of which diagnostic assay is used, new seroconversion occurs more frequently after exposure to UFH than LMWH (Warkentin et al., 1995, 2000, 2003a; Amiral et al., 1996; Lindhoff-Last et al., 2002; Ahmad et al., 2003a).

**A. HIT in Patients Undergoing Cardiac Surgery**

Three prospective studies have evaluated the frequency of HIT in postoperative cardiac surgical patients who also have received postoperative antithrombotic prophylaxis with UFH (Trossaert et al., 1998; Pouplard et al., 1999, 2002; Warkentin et al., 2000). Pooling the data, the frequency of HIT appears to be about $2\%$. This frequency is consistent with a number of retrospective studies (Glock et al., 1988; Walls et al., 1992a,b; Singer et al., 1993) that reported a frequency of HIT of up to $5\%$, but overall, also noted a frequency of about $2\%$ (Table 7). Furthermore, HIT was associated with a

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**Table 7** Frequency of HIT and Thrombosis in Retrospective Studies of HIT in Cardiovascular Surgery Patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients at risk, n</th>
<th>Patients with HIT, n (%)</th>
<th>Patients with HIT and thrombosis, n (%)</th>
<th>Ratio of venous: arterial thrombosis</th>
<th>Total deaths in patients with HIT, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walls et al., 1992a</td>
<td>4261</td>
<td>82 (1.9)</td>
<td>31 (38)</td>
<td>0.3:1</td>
<td>23 (28)</td>
</tr>
<tr>
<td>Walls et al., 1992b</td>
<td>764</td>
<td>35 (4.5)</td>
<td>17 (49)</td>
<td>0.3:1</td>
<td>15 (43)</td>
</tr>
<tr>
<td>Visentin et al., 1996</td>
<td>51</td>
<td>0 (0)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glock et al., 1988</td>
<td>—</td>
<td>21</td>
<td>17 (81)</td>
<td>0.7:1</td>
<td>8 (38)</td>
</tr>
<tr>
<td>Singer et al., 1993</td>
<td>1500</td>
<td>11 (0.75)</td>
<td>7 (64)$^a$</td>
<td>0.3:1$^b$</td>
<td>2 (18)</td>
</tr>
</tbody>
</table>

$^a$ In seven patients, 17 thrombotic events occurred.

$^b$ Precise number of arterial and venous events is unclear from the published data. For this analysis, of six limb amputations associated with intravascular catheters or devices, five were assumed to be arterial and one venous, based on the type of intravascular catheter or device associated with the amputated limb.
risk of thrombosis of 38–81%, and with an overall mortality of 18–43% in these studies. In contrast to the orthopedic patient population, the predominant thrombotic event appears to be arterial.

Only one study has examined the influence of postoperative antithrombotic prophylaxis with UFH or LMWH on the frequency of HIT antibody formation and HIT following heart surgery (Pouplard et al., 1999, 2002). In this prospective, but nonrandomized comparative trial, one group of patients \((n = 263)\) judged to be at highest risk for thrombosis received postoperative therapeutic-dose UFH (200 U/kg once daily by subcutaneous injection, adjusted by activated partial thromboplastin time, for at least 10 days). The second group of patients \((n = 370)\) received LMWH.

Overall, formation of HIT antibodies was similar in the two patient groups, using a commercial EIA that detects IgM, IgA, and IgG antibodies. However, among 19 patients who tested positive in the platelet activation assay (serotonin release), 10 evinced a platelet count fall consistent with HIT. Further, there appeared to be an association with HIT and postoperative treatment with UFH \((9/263 = 3.4\%)\) compared with LMWH \((1/370 = 0.3\%)\) (Pouplard et al., 2002). Thus,

1. The frequency of HIT antibody formation following heart surgery is influenced primarily by UFH given at cardiopulmonary bypass, rather than the type of heparin preparation given postoperatively.
2. Among patients who form HIT antibodies following heart surgery, the risk of HIT may be higher in those receiving UFH (however, this cannot be definitively concluded because the study was non-randomized and UFH and LMWH dosing differed).
3. The study indicates a greater clinical usefulness of the platelet serotonin release assay (SRA), compared with the PF4–heparin EIA (see Chap. 11).

The potential to reduce risk of “breakthrough” of HIT among postcardiac surgery patients who form HIT antibodies is a major reason why antithrombotic agents with low (danaparoid) or possibly absent (antithrombin-binding pentasaccharide, fondaparinux) cross-reactivity against PF4–heparin might be ideal anticoagulants for this clinical situation (Warkentin et al., 2003) (see Chap. 8).

### B. Anti-Xa–Inhibiting Pentasaccharide

Fondaparinux (Arixtra) is a novel antithrombin-binding pentasaccharide that inhibits factor Xa without inhibiting thrombin (factor IIa). It has been shown to be safe and effective for antithrombotic prophylaxis following
orthopedic surgery. Fondaparinux does not bind to PF4, and therefore its use might avoid HIT. In systematic studies of HIT antibody formation associated with fondaparinux or enoxaparin prophylaxis after elective hip or knee replacement therapy, low frequencies of HIT antibody formation were seen with both anticoagulants (Warkentin et al., 2003b). However, whereas HIT antibodies invariably “cross-reacted” with enoxaparin, they failed to recognize PF4 mixed with fondaparinux (by fluid-phase EIA) (see Chap. 11). Thus, fondaparinux offers the possibility of absent or negligible risk of causing HIT.

C. Implications of Subclinical HIT Antibody Seroconversion

Two retrospective studies found that patients with acute coronary syndrome who either had HIT antibodies at presentation (Williams et al., 2003) or developed HIT antibodies following heparin treatment (Mattioli et al., 2000) had a higher frequency of vascular events during long-term follow-up ranging from 1 month to 1 year, despite absence of clinical HIT in any patient. Unrecognized confounders could explain these findings; e.g., a short interval from recent hospitalization/heparin exposure (former study) or a longer duration time of hospitalization (latter study) could be associated independently with adverse outcomes and greater likelihood of forming HIT antibodies.

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

Until recently, studies of HIT frequency have yielded seemingly confusing and inconsistent results. However, as argued in this chapter, by taking into consideration (1) type of heparin used, (2) patient population treated, and (3) laboratory and clinical evidence to distinguish (immune) HIT from nonimmune HAT, distinct profiles for HIT antibody seroconversion, HIT itself, and HIT-associated thrombosis can be discerned (see Fig. 3). New research questions will be generated in the search for the biological basis for these intriguing differences in HIT risk. But perhaps the most important insight to emerge from these collective studies is the simple and clinically relevant observation that new, progressive, or recurrent thrombosis occurs in at least 33–50% of patients who develop proven HIT. This underscores the need for prompt recognition and urgent therapy in all patients suspected of having this adverse drug reaction.

Practically, these findings suggest strategies for platelet count monitoring in patients receiving heparin. Some physicians are hesitant to institute
Table 8  Recommendations for Platelet Count Monitoring for Heparin-Induced Thrombocytopenia During Heparin Treatment

1. Monitoring for typical-onset HIT: stratifying the intensity of platelet count monitoring for HIT based upon its risk
   A. Patients at highest risk for HIT (1–5%) (e.g., postoperative patients receiving prophylactic-dose UFH after major surgery): monitoring during heparin therapy, at least every second day from day 4 to day 14
   Patients receiving therapeutic-dose UFH: platelet count monitoring once daily from day 4 to day 14
   B. Patients at intermediate risk for HIT (0.1–1%) (e.g., medical/obstetrical patients receiving prophylactic-dose UFH, or postoperative patients receiving prophylactic-dose LMWH, or postoperative patients receiving intravascular catheter “flushes” with UFH): monitoring during heparin therapy, at least every 2 or 3 days from day 4 to day 14, when practical
   C. Patients at low risk for HIT (<0.1%) (e.g., medical/obstetrical patients receiving prophylactic- or therapeutic-dose LMWH, or medical patients receiving only intravascular catheter “flushes” with UFH): routine platelet count monitoring is not recommended

2. Monitoring for rapid-onset HIT: for a patient recently exposed to heparin (within the past 100 days), a repeat platelet count within 24 hours following reinitiation of heparin

3. When to suspect HIT:
   A relative (proportional) platelet count fall of 50% or greater that is otherwise clinically unexplained should be considered suspicious for HIT, even if the platelet count nadir remains above 150 x 10^9/L.
   For any patient who develops thrombosis during or within several days after heparin therapy, or who develops an unusual clinical event in association with heparin therapy (e.g., inflammatory or necrotic skin lesions at heparin injection sites, acute systemic reaction post-intravenous heparin therapy), a repeat platelet count should be measured promptly and compared with recent values.

These are draft recommendation (Seventh American College of Chest Physicians Consensus Conference on Antithrombotic Therapy, September 2003). Readers should consult the publication (Warkentin and Greinacher, 2004) to obtain the final recommendations.

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

b Once-daily platelet count monitoring recommended as daily blood draws required for aPTT monitoring.

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

d Monitoring as per “intermediate” risk is appropriate if UFH was given before initiating LMWH.
regular platelet count monitoring for HIT. One explanation is the almost ubiquitous use of heparin in hospitalized patients. Thus, a requirement that regular, perhaps even daily, platelet count monitoring be performed seems excessive. Additionally, there is no convincing evidence that regular platelet count monitoring can prevent the thrombotic complications of HIT if the physician response is merely to stop the heparin (Wallis et al., 1999). However, a worthy consideration is that instituting alternative, parenteral anticoagulation may prevent thrombosis in patients recognized as having isolated HIT.

These comments notwithstanding, marked differences in risk for HIT are apparent among different patient populations. Thus, it seems prudent to recommend that patients at the highest risk of HIT, and for HIT-associated thrombosis (e.g., postoperative patients receiving UFH, or any patient receiving bovine lung UFH) should have platelet counts monitored regularly, perhaps at least every other day. For patients whose risk for HIT appears to be 0.1–1% (e.g., medical patients receiving UFH, surgical patients receiving LMWH), less frequent monitoring may be appropriate. Since HIT is unlikely to occur before day 5, or after day 14, the monitoring could be performed 2 or 3 times per week from days 4 to 14. Most patients have frequent complete blood counts performed during the first few days of hospitalization, so comparative platelet count results for days 0–3 are usually available.

Two recent consensus conferences have examined the issue of platelet count monitoring for HIT (Warkentin, 2002; Warkentin and Greinacher, 2004). Although the recommendations were not identical, they had in common the concept of stratifying the intensity of platelet count monitoring based upon the risk of developing HIT and focusing the monitoring during the time when HIT usually occurs. Table 8 summarizes draft recommendations (Warkentin and Greinacher, 2004).

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

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I. INTRODUCTION

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

II. HEPARIN BINDING TO PLATELETS

Appreciation of the functional effects of heparin on platelets led to studies of heparin binding to these cells, which is specific and saturable (Sobel and Adelman, 1988; Horne, 1988; Horne and Chao, 1989). The negative charge density of heparin is largely responsible for its binding specificity (Horne,
1988). Polysaccharides with various primary structures can displace heparin from platelets if the molecules are sufficiently charged (Horne, 1988; Greinacher et al., 1993) (Table 1). The identity of the platelet binding site(s), which provides a complementary positive charge, is uncertain. One report indicates that glycoprotein IIb/IIIa (integrin $\alpha_{\text{IIb}}\beta_{3}$) contains a heparin-binding site (Sobel et al., 2001), but this is inconsistent with other studies (Horne, 1988, 1991).

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

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

<table>
<thead>
<tr>
<th>Heparin $M_r$ range (Da)</th>
<th>Sulfate/carboxylate (mol/mol)</th>
<th>Dissociation constant (mg/L)</th>
<th>$K_d$ (nM)</th>
<th>Binding capacity (mg/10$^{15}$ cells)</th>
<th>(molecules/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14,000–16,000</td>
<td>2.0 ± 0.29$^{a}$</td>
<td>4.6 ± 1.1</td>
<td>310 ± 73</td>
<td>66 ± 2.5</td>
<td>2600 ± 100</td>
</tr>
<tr>
<td>9,500–10,500</td>
<td>1.8 ± 0.26</td>
<td>3.9 ± 2.1</td>
<td>390 ± 210</td>
<td>56 ± 8.4</td>
<td>3400 ± 500</td>
</tr>
<tr>
<td>4,500–5,500</td>
<td>1.9 ± 0.15</td>
<td>3.2 ± 1.0</td>
<td>640 ± 200</td>
<td>23 ± 5.7</td>
<td>2800 ± 680</td>
</tr>
<tr>
<td>2,700–3,300</td>
<td>1.7 ± 0.25</td>
<td>4.0 ± 2.0</td>
<td>1300 ± 650</td>
<td>10 ± 5.4</td>
<td>2000 ± 1100</td>
</tr>
</tbody>
</table>

$^{a}$ Values are means ±1 standard deviation.

Figure 1 Schematic binding of heparin to platelets comparing heparin of $M_r$ 5000 with heparin of $M_r$ 15,000. Each “platelet-binding domain” of heparin is hypothesized to have $M_r > 3000$, whereas heparin-binding sites on the platelets (indicated as $+++$) can bind 7000 Da heparin. Therefore, each binding site is not quite filled with $M_r$ 5000 heparin, but is too occupied to allow binding of a second heparin molecule. In contrast, $M_r$ 15,000 heparin has adequate length to occupy two binding sites, but physical constraints, such as limited heparin flexibility and spacial distribution of binding sites, allow it to occupy only one site at a time. The scheme is consistent with the binding parameters shown in Table 1.
Because of its high charge density, as well as the high linear flexibility conferred by its constituent L-iduronic acid residues, heparin binds readily to a variety of basic plasma proteins, which theoretically could compete with platelets for heparin (Casu et al., 1988; Young et al., 1994). However, heparin binding to only two plasma proteins, antithrombin and fibronectin, interferes with heparin-induced platelet activation (Salzman et al., 1980; Chong and Ismail, 1989) or with binding of heparin to platelets (Horne and Chao, 1990).

III. NONIDIOSYNCRATIC HEPARIN-INDUCED PLATELET ACTIVATION

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

The platelet proaggregatory effect of heparin does not appear to be an artifact of low ionized calcium concentration in the presence of citrate anticoagulant: Chen and colleagues (1992) observed that heparin enhanced collagen-induced platelet aggregation in a dose-dependent fashion even in whole blood anticoagulated with hirudin (i.e., physiological calcium concentrations). On the other hand, the responsiveness of washed platelets to agonists, when resuspended in buffers containing physiological calcium, has been reported to be both increased and decreased by heparin (Saba et al., 1984; Westwick et al., 1986). Although the data are not always consistent, this much seems clear: direct heparin-induced platelet aggregation requires metabolic energy and is mediated by fibrinogen; therefore, it depends on platelet fibrinogen receptors (platelet glycoprotein IIb/IIIa complexes) and divalent cations (Chong and Ismail, 1989). There is also evidence that heparin can antagonize platelet inhibition by prostacyclin (Saba et al., 1979; Eldor and Weksler, 1979; Fortini et al., 1985; Berglund and Wallentin, 1991).
The properties of heparin that influence its platelet binding also influence its stimulatory effects on platelets: heparin of a high molecular weight is more active than low molecular weight heparin, and heparin with low affinity for antithrombin and fibronectin is more active (because it is more available) than heparin with high affinity for these plasma proteins (Salzman et al., 1980; Holmer et al., 1980; Westwick et al., 1986; Chong and Ismail, 1989; Brace and Farred, 1990; Xiao and Théroux, 1998; Aggarwal et al., 2002; Klein et al., 2002). The latter observation implies that the anticoagulant (antithrombin-dependent) activities of heparin are distinct from its platelet stimulatory effects. Furthermore, nonheparin polysaccharides can mimic the proaggregatory effects of heparin on platelets if they are sufficiently large and charged (Tiffany and Penner, 1981). In contrast, heparan sulfate (the predominant anticoagulant glycosaminoglycan found in danaparoid) has negligible platelet-activating properties, as it has a relatively low degree of sulfation, despite sharing a carbohydrate backbone similar to heparin (Lindahl and Kjellén, 1991; Burgess and Chong, 1997).

IV. PLATELET-RELATED PROHEMORRHAGIC EFFECTS OF HEPARIN

Paradoxically, despite the in vitro evidence that heparin stimulates platelets, there is evidence that heparin causes bleeding partly because of its effects on platelet function (Hirsh, 1984; John et al., 1993). Heparin, for example, causes prolongation of the skin-bleeding time unrelated to any effects on platelet counts (Hjort et al., 1960; Heiden et al., 1977; Kelton, 1986; Ljungberg et al., 1988). Also, the structural characteristics of heparin associated with platelet stimulation in vitro (e.g., increased heparin size and sulfation; decreased affinity for antithrombin) are associated with enhanced bleeding in animal models (Hjort et al., 1960; Carter et al., 1982; Ockelford et al., 1982; Fernandez et al., 1986; Borowska et al., 1988; Van Ryn-McKenna et al., 1989).

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

V. NONIMMUNE HEPARIN-ASSOCIATED THROMBOCYTOPENIA (NONIMMUNE HAT)

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

Nonimmune HAT is typically mild, transient, and clinically inconsequential (Gollub and Ulin, 1962; Johnson et al., 1984; Chong, 1988; Warkentin and Kelton, 1994). There is debate whether this represents a real in vivo phenomenon, or whether the apparent thrombocytopenia is instead related to ex vivo platelet aggregation (Davey and Lander, 1968). Indeed, some investigators were unable to show this event at all (Heinrich et al., 1988; Xiao and Théroux, 1998). Sometimes, however, apparent nonimmune HAT is a dramatic clinical syndrome that can be confused with HIT (Chong et al., 1982) (see Chap. 12).

Balduini et al. (1993) observed that an early fall in platelet count was more frequent and of greater magnitude in patients receiving heparin following streptokinase therapy for acute myocardial infarction, compared with control patients who received streptokinase alone. The heparin-treated patients also showed greater ex vivo spontaneous platelet aggregation, suggesting that heparin may have had a direct proaggregatory effect.

VI. HEPARIN–PLATELET INTERACTIONS IN THE PATHOGENESIS OF HIT

Heparin also binds to several proteins that are secreted from stimulated platelets. One of these, platelet factor 4 (PF4), binds heparin with unusually high affinity (Capitanio et al., 1985; Loscalzo et al., 1985; Horne, 1993). Unlike antithrombin or fibronectin, however, PF4 does not prevent heparin...
Nonimmune Heparin–Platelet Interactions

from binding to platelets. In fact, it appears that heparin can bind to platelets in complexes with PF4 (Horne and Hutchison, 1998).

Heparin-bound PF4 has been identified as the primary target of the antibodies characteristic of HIT (Amiral et al., 1992; Visentin et al., 1994; Kelton et al., 1994). Because platelets have specific-binding sites for PF4, this protein was originally assumed to mediate the attachment of HIT immune complexes to the platelet surface (Kelton et al., 1994). However, it was subsequently demonstrated that heparin, rather than PF4, serves this purpose: that is, the binding to platelets of heparin-PF4 complexes occurs at heparin-binding sites, rather than PF4-binding sites (Greinacher et al., 1993; Horne and Hutchison, 1998).

The sequence of interactions leading to platelet activation has recently been described (Newman and Chong, 2000). It begins with the nonimmune binding of heparin to the cells, which stimulates them to secrete a relatively small amount of PF4. The released PF4 binds to heparin in solution to reveal the target for HIT IgG antibodies (HIT-IgG). Immune complexes of heparin, PF4, and HIT-IgG then attach to the platelets at heparin-binding sites. This facilitates contact between the Fc termini of the IgG molecules and the platelet Fc receptors, thereby activating the cells further and causing more PF4 secretion. As this sequence accelerates, platelet aggregation occurs.

Because immune complexes comprised of heparin, PF4, and IgG from HIT patients bind to platelets at heparin-binding sites, they must compete with free heparin for binding to the platelet surface (Horne and Alkins, 1996; Horne and Hutchison, 1998). When free heparin is in molar excess, heparin–PF4–IgG complexes are displaced from the platelet surface (Fig. 2). When PF4 is in excess, there is no free heparin, and binding of the complexes is the only option.

Variable heparin–PF4 stoichiometry may explain why some patients develop HIT-IgG without developing thrombocytopenia, and why HIT is more common in certain clinical settings than in others, such as following surgery (Boshkov et al., 1993; Amiral et al., 1995, 1996; Warkentin et al., 1995, 2000; Visentin et al., 1996; Kappers-Klunne et al., 1997; Bauer et al., 1997). When a patient is given heparin, the plasma concentration of PF4 rises because PF4 is displaced from the endothelial surface, where it is normally bound to heparan sulfate (Dawes et al., 1982; Rao et al., 1983; O’Brien et al., 1985). PF4 neoantigens (or cryptic antigens) are formed, leading to the HIT immune response (Chong and Newman, 1997). However, complexes of heparin, PF4, and IgG are harmless unless they become bound to platelets, and this cannot happen as long as there is sufficient free heparin to compete effectively for the limited number of platelet-binding sites (Horne and Hutchison, 1998). Therefore, as long as heparin remains in molar excess over
PF4, heparin–PF4–IgG binding to platelets is minimized, and platelet activation and thrombocytopenia do not develop. The importance of heparin–PF4 stoichiometry in HIT was demonstrated in a serotonin-release assay using platelets from a patient with the gray platelet syndrome, which lack PF4 (Horne and Alkins, 1995). Platelet release was stimulated only as PF4 was added and approached or exceeded molar excess. In the absence of PF4 or with 5 nM PF4 (heparin:PF4 ~10:1), 0.1 unit/mL heparin (~50 nM) and HIT antibody caused 4–5% release. However, 25 nM PF4 (heparin:PF4 ~2:1) caused 20% release, 50 nM PF4 (heparin:PF4 ~1:1) caused 34% release, and 100 nM PF4 (heparin:PF4 ~1:2) caused 60% release.

In most clinical settings, free heparin is in considerable molar excess over PF4. For example, therapeutic concentrations of heparin (0.2–0.4 U/mL) correspond to about 100–200 nmol/L of heparin. When heparin is given...
to normal individuals, plasma concentrations of PF4 from endothelial reservoirs reach only about 8 nM (Dawes et al., 1982). For PF4 concentrations to approach 100–200 nmol/L, marked activation of circulating platelets is necessary. Complete activation of platelets in a concentration of $250 \times 10^9/L$ will generate a plasma PF4 concentration of about 200 nM (Horne, 1993). Therefore, a molar excess of PF4 over therapeutic concentrations of heparin would be highly unlikely outside extreme clinical circumstances. On the other hand, prophylactic doses of heparin (e.g., 5000 U every 8–12 h by subcutaneous injection) administered in a setting associated with some platelet activation (e.g., postoperative orthopedic patients) might well produce molar ratios of heparin and PF4 that would favor platelet binding of heparin–PF4 complexes, and—if an immune response had occurred—platelet binding of heparin–PF4–IgG complexes. Indeed, such scenarios are the ones in which HIT is reported most frequently (Warkentin et al., 1995, 2000; Ganzer et al., 1997).

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

Heparin’s molecular size influences its platelet-binding affinity and capacity and its stimulating effect on platelets. Similarly, heparin’s affinity and capacity for PF4 are related to its size: large heparin molecules have higher affinity for PF4 and can bind several PF4 molecules, whereas smaller heparin molecules have less affinity for PF4 and can bind fewer PF4 molecules. (Bock et al., 1980; Lane et al., 1984; Marshall et al., 1984). Therefore, it should be no surprise that low molecular weight heparin is associated with a lower frequency of HIT than standard heparin (Warkentin et al., 1995; Lindhoff-Last et al., 2002) and that in some instances low molecular weight heparin has been given to patients with HIT without adverse consequences (Slocum et al., 1996). Smaller heparins complex less efficiently with PF4, thereby reducing immunogenicity, and bind less avidly to platelets, thereby reducing immune complex binding to the cells. Indeed, the very smallest heparin, the antithrombin-binding pentasaccharide ($M_r$ 1714), theoretically is an ideal anticoagulant agent for patients with HIT (see Chap. 8). The pentasaccharide binds to neither platelets nor PF4 and therefore cannot support platelet activation by HIT antibodies (Elalamy et al., 1995; Walenga et al., 1997; Ahmad et al., 1999).

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

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Nonimmune Heparin–Platelet Interactions 161


Mikhailidis DP, Barradas MA, Jeremy JY, Gracey L, Wakeling A, Dandona P.


Heparin-Dependent Antigens in Heparin-Induced Thrombocytopenia

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I. INTRODUCTION

About 10 years ago, platelet factor 4 (PF4) complexed to heparin (PF4-H) was identified as the major target antigen for heparin-dependent antibodies involved in the pathogenesis of immune heparin-induced thrombocytopenia (HIT) (Amiral et al., 1992, 1995; Gruel et al., 1993; Greinacher et al., 1994, 1995; Kelton et al., 1994; Visentin et al., 1994). Occasionally, other antigens can be involved, such as interleukin-8 (IL-8) or neutrophil-activating peptide-2 (NAP-2), two CXC chemokines of the PF4 superfamily (Amiral et al., 1996a). There is now increasing evidence that the risk of HIT depends on the type of heparin used, its sulfation grade, the therapy duration, and the patient’s clinical context (Kelton, 1992; Warkentin and Kelton, 1996) (see Chap. 4). However, many questions remain unresolved: How are antibodies generated? Why are they observed in only a subgroup of patients receiving heparin? How do they become pathogenic in only a few of the latter? Indeed, antibodies to PF4-H develop surprisingly often in many heparin-treated patients, especially in the context of platelet activation (e.g., heart surgery using cardiopulmonary bypass) (Amiral et al., 1996b; Visentin et al., 1996). Clinical complications of HIT are especially associated with high-titer PF4-H antibodies of the IgG isotype, usually in patients with comorbid disease who are receiving unfractionated heparin (UFH). The frequency of
HIT is lower with the use of low molecular weight heparin (LMWH) (Warkentin et al., 1995). However, recent studies suggest that this complication can also develop in the absence of IgG isotypes (Amiral et al., 1996c). In some patients with apparent HIT, only IgA or IgM isotypes are present, usually in high concentrations.

In this chapter, the current understanding of PF4–H antibody generation and its contribution to the complications of HIT will be discussed. Formation of the PF4–H antigen complexes and their binding to blood and endothelial cells, thus targeting the immune response onto these cells (Cines et al., 1987; Visentin et al., 1994; Visentin and Aster, 1995; Horne and Hutchison, 1998), will be analyzed. Finally, the possibility that HIT can be caused in the absence of detectable antibodies to PF4–H will be discussed, including the hypothesis that preexisting antibodies to other chemokines could become pathogenic during heparin treatment.

II. ANTIGENICITY OF PF4 IN THE PRESENCE OF HEPARIN

Antibodies to self-antigens, including certain autologous plasma proteins, can develop as result of immune dysfunction, resulting in chronic autoimmune disease. Sometimes, however, formation of complexes between an autologous protein and a foreign substance leads to new antigens, potentially even on the self-protein. These can be described as cryptic autoantigens, or neoantigens. The immune stimulation resulting from such an altered self-epitope quickly abates when the inducing foreign substance is no longer present. Such a model appears relevant to explain some of the clinical complications observed in HIT (see Chap. 3). In HIT, PF4 constitutes the self-antigen, with one or more cryptic auto-epitopes formed when complexes are formed under optimal conditions with the foreign substance, heparin (discussed subsequently). Thus, the antibodies to PF4–H complexes effectively behave as autoantibodies in the presence of heparin (Shoenfeld, 1997).

PF4 is a positively charged tetrameric glycoprotein member of the CXC chemokine family (Brandt and Flad, 1992). The tetramer forms by sequential noncovalent association of PF4 monomers: two dimers are formed that self-associate into the fundamental tetrameric structure. As found within platelet α-granules, PF4 is released into blood only after platelet activation, such as seen with trauma, surgery, atherosclerosis (Dunlop et al., 1987), diabetes, cardiopulmonary bypass, inflammation, cancer, infections, and so on. In vivo, PF4 has many different biological functions, including immunoregulation, inhibition of megakaryocytosis and angiogenesis, and mediation of cell response. When released from platelets, PF4 is in a complex of eight tetramers linked to a chondroitin-containing proteoglycan dimer: the entire
complex has a molecular weight (MW) of 350 kDa. The PF4 complexes can also bind to endothelial cell proteoglycans (heparan sulfate). Heparin, when present, having a greater affinity for PF4, displaces PF4 from the endothelial cell glycosaminoglycans, thereby forming PF4–H complexes that are released into the circulation.

The interaction between heparin and PF4 has been intensively studied (Bock et al., 1980; Cowan et al., 1986; Stuckey et al., 1992; Maccarana and Lindahl, 1993). In the presence of a stoichiometric concentration of heparin and PF4 (which corresponds to 27 international units [IU] of heparin per milligram of PF4), multimolecular PF4–H complexes (Greinacher et al., 1994; Amiral et al., 1995) are generated. With stoichiometric concentrations, heparin wraps around the PF4 molecule, altering its structure and rendering it antigenic. Figure 1 shows the different complexes that can be formed between heparin and PF4, depending on the respective concentrations of both substances. Only multimolecular complexes are believed to be antigenic in heparin-treated patients. Thus, the immunogenicity of complexes is strictly dependent on the respective concentrations of heparin and PF4. If we consider the usual therapeutic range for heparin (0.1–1 IU/mL), the amount of PF4 required for the generation of multimolecular PF4–H complexes is from 3 to 40 μg/mL. In patients undergoing cardiopulmonary bypass who receive higher heparin concentrations (up to 3 IU/mL), the corresponding

![Figure 1](image-url)

**Figure 1** Formation of heparin and PF4 complexes at different concentrations of heparin and PF4: In the presence of stoichiometric concentrations of both substances, multimolecular complexes are formed. Heparin then wraps around the PF4 tetramer, altering its structure and rendering it antigenic.
higher PF4 concentrations required for the formation of the immunogenic PF4–H complexes may result from intense platelet activation, resulting from exposure of blood to the extracorporeal circuit. In general, the existence of favorable conditions allowing the formation of multimolecular PF4–H complexes may depend as much on the underlying disease that is promoting platelet activation as on the dose of heparin given (see Chap. 4).

The intensity of the heparin-dependent immune response thus depends on the presence and, presumably, persistence of the multimolecular PF4–H complexes. In particular, high concentrations of PF4–H complexes may be important in triggering an immune response. However, heparin concentrations vary considerably in treated patients, and the concentrations allowing PF4–H complex formation may occur frequently. But, if low PF4 concentrations are present, formation of immunogenic complexes can occur only at corresponding very low levels of heparin (e.g., 0.027 IU/mL of heparin for 100 ng/mL of PF4, which is the approximate PF4 concentration in normal subjects receiving heparin). The chances of developing a significant immune response in this setting would be low. The potentially important role of individual responsiveness to a given PF4 antigenic stimulus is unknown.

Although antibodies to PF4–H complexes are present in most patients who develop HIT, they are absent in some patients with apparent HIT, including patients with positive activation assays for HIT antibodies. Antibodies to IL-8 or to NAP-2 have been observed in some of these patients (Amiral et al., 1996a; Regnault et al., 2003), but in others, no specific heparin-dependent antibodies have been identified. As discussed later, antibodies to IL-8 or to NAP-2 are generated by mechanisms different from those involved in PF4–H antibody formation, and may be true autoantibodies (Bendtzen et al., 1995).

III. PATHOGENICITY OF HEPARIN-DEPENDENT ANTIBODIES

Anti-PF4–H antibodies of the IgG isotype are present in at least 80% of patients with clinical HIT. In the remaining cases, only IgA, IgM, or both, isotypes, in high concentrations, are observed. These intriguing observations require explanation for how these antibodies trigger thrombocytopenia, with or without thrombosis.

Antibodies to PF4–H are believed to become pathogenic when they interact with platelets or other blood and endothelial cells. This can occur only if the PF4–H complexes bind to the cell surfaces, predominantly through their heparin-binding sites (Van Rijn et al., 1987; Horne and Alkins, 1996; Horne and Hutchison, 1998), but possibly also through PF4-binding sites.
Heparin-Dependent Antigens in HIT

(Capitanio et al., 1985; Rybak et al., 1989). Although the HIT antibodies recognize PF4–H complexes in the fluid phase (Newman et al., 1998), it is uncertain whether this typically occurs in vivo before interaction of PF4–H–IgG complexes with the platelet surface, or whether HIT antibodies only bind after PF4–H complexes are attached to the platelet surface.

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

Another factor determining HIT antibody formation is the type of heparin used for heparin binding to PF4, which depends on its oligosaccharide composition, polysaccharide length, and grade of sulfation (Lindahl et al., 1994; Greinacher et al., 1995). Formation of PF4–H complexes requires a heparin molecule with at least 12–14 oligosaccharide units and a high sulfation grade (more than three sulfate groups per disaccharide) (Amiral et al., 1995). Furthermore, binding of heparin to blood and endothelial cells also increases with heparin molecule length and sulfation grade (Sobel and Adelman, 1988; Horne and Chao, 1990; Harenberg et al., 1994). Heparin structure thus has a dual effect in HIT: it is required to form PF4–H complexes and also to target these complexes onto cells. These factors could explain the higher frequency of PF4–H antibody development and of HIT in patients receiving UFH, compared with LMWH. With UFH, PF4–H complexes are more easily formed and require a lower heparin concentration than with LMWH. For the latter drug, only the subset of molecules containing at least 12–14 oligosaccharide units (MW > 3600 Da) can generate immunoreactive PF4–H complexes. Thus, because LMWH has a lower propensity to form PF4–H complexes and binds less readily to platelets and endothelial cells, LMWH therapy may be less likely to result in thrombocytopenia even in the presence of pathogenic HIT antibodies.

PF4–H–reactive antibodies targeted to platelets induce platelet activation, resulting in thrombocytopenia and, often, thrombosis. Occasionally, heparin-induced thrombosis occurs in the absence of thrombocytopenia (Hach-Wunderle et al., 1994; Bux-Geweir et al., 1996). Platelet activation by the IgG isotype antibodies is mediated by interaction with the platelet FcγRIIA receptors (Kelton et al., 1994; Denomme et al., 1997). Some studies suggest an important role for FcγRIIA polymorphism (Brandt et al., 1995;
Burgess et al., 1995). However, the role of the FcγRIIA receptor polymorphism is controversial (Arepally et al., 1997; Denomme et al., 1997; Suh et al., 1997; Bachelot-Loza et al., 1998) (Chap. 9).

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

Various characteristics of PF4–H antibodies are another key factor for induction of HIT. Platelet activation induced by PF4–H antibodies is usually weak and is only pathogenic when amplified. This is demonstrated by the variable lag phase observed in platelet aggregation studies with different plasmas or sera from HIT patients. Antibody concentration is an important factor for determining the extent of platelet activation. Antibody affinity is also very important: the higher the affinity, the lower the concentration of antibodies required for activating platelets. Recently, a subset of antibodies to PF4–H complexes that had platelet-activating properties was isolated in three patients with HIT. These antibodies had the highest avidity for PF4–H. In contrast, the bulk of antibodies to PF4–H in these patients had no effect on platelet activation (Amiral et al., 2000). When IgM or IgA isotypes are present, affinity for PF4–H complexes is usually lower than that of IgG isotypes and, consequently, high concentrations are necessary for pathogenicity. Lastly, HIT antibodies do not all bind to the same epitope on PF4–H complexes, and this specificity could be an important factor in their action (Horsewood et al., 1996; Pouplard et al., 1997; Suh et al., 1998). At least two neoepitopes have
been identified on PF4 that are distinct from the “region of positive charge” to which heparin binds (Ziporen et al., 1998; Li et al., 2002) (see also color insert and Chap. 7). Thus, anti-PF4–H antibodies are not equivalent, and those with the strongest affinity are most pathogenic.

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

**Figure 2** Occurrence of cell–cell interactions at the neighborhood of blood activation or inflammation sites. Presence of heparin-dependent antibodies increases the amount of cells available at these sites, amplifies cell–cell interactions and cellular activation, and can lead to blood clotting or release of circulating cell aggregates. PF4-H, heparin–platelet factor 4; IL-8, interleukin-8; NAP-2, neutrophil-activating peptide 2; βTG, β-thromboglobulin.
IV. PREEXISTING ANTICHEMOKINE ANTIBODIES

Preexisting antibodies to chemokines, such as IL-8 or NAP-2, or possibly to PF4 itself, may be present in some patients even before heparin therapy (Sylvester et al., 1992; Bendtzen et al., 1995). These antibodies may occur naturally and have a regulatory role in inflammation (Reitamo et al., 1993). In some disease states, they are present at high concentration. Antibodies to IL-8 are most common (Reitamo et al., 1993). However, in some patients true autoantibodies to PF4 alone can also be observed. In the absence of heparin, these antibodies do not demonstrate clear pathogenicity. During heparin therapy, PF4 and other chemokines are released into the circulation from their storage pools. Heparin localizes these chemokines to blood and endothelial cells. Thus, naturally occurring heparin-dependent antibodies could then be targeted to these cells, initiating immune injury. The amount of chemokine–heparin complexes bound to blood and endothelial cells depends on different factors: the amount of releasable chemokines (i.e., the patient’s clinical state); the type and dose of heparin used; and the presence of activated cells with an increased capacity to bind heparin-like antibodies against PF4–H complexes. As with antibodies against PF4–H complexes, these natural antichemokine antibodies could initiate cell activation and cell–cell interactions, as well as generate circulating cell aggregates that could lead to vessel occlusion.

V. CONCLUSIONS

The conditions that permit formation of the molecular PF4–H target antigen for HIT antibodies involve the properties of the heparin used, dose and duration of therapy, and the clinical context of the treated patient. Immuno-reactive complexes between PF4 and heparin are formed only under certain conditions. Their formation in high concentrations is facilitated if underlying disease favors platelet activation and release. Similar conditions enhance the pathogenicity of the HIT-generated antibodies. These considerations help unravel the apparent random generation of HIT antibodies in heparin-treated patients, as well as the seemingly random occurrence of thrombotic events.

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I. INTRODUCTION

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

Today, there is an emerging consensus that this immune-mediated syndrome, designated heparin-induced thrombocytopenia (HIT), is an important life- and limb-threatening complication of heparin therapy. HIT is more common in patients receiving certain types of heparin, such as unfractionated heparin (UFH) of beef lung versus porcine mucosal origin, or unfractionated versus low molecular weight forms of porcine-derived heparin.
HIT can be triggered by standard therapeutic-dose heparin, low-dose (prophylactic) treatment (Hrushesky, 1978), low molecular weight heparin (LMWH) (Lecompte, 1991; Tardy, 1991), and even by minute quantities given to “flush” intravascular catheters (Heeger and Backstrom, 1986; Ling and Warkentin, 1998; Kelton and Warkentin, 1998). Various aspects of the pathogenesis of this disorder are also summarized in Chapters 5, 6, 8, 9, and 10.

Early investigations showed that IgG antibodies associated with HIT could induce platelet activation in the presence of pharmacological (0.2–1 U/mL) or even lower doses of heparin. By taking advantage of this property, two “activation” diagnostic tests were developed for HIT: the platelet aggregation test (PAT) and the serotonin release assay (SRA) (see Chap. 11). Because activation of platelets by IgG from patients with HIT in the presence of heparin could be inhibited by a monoclonal antibody that blocks the platelet FcγRIIA receptor (Kelton et al., 1988), it was assumed that the antibodies react with heparin to form immune complexes, which, in turn, activate platelets. However, early studies failed to demonstrate binding of heparin-induced antibodies to platelets in the presence of heparin, in contrast to the behavior of platelet-reactive antibodies induced by other drugs, such as quinidine and quinine. Moreover, the putative heparin–IgG complexes could not be identified in most studies (Green et al., 1978; Warkentin and Kelton, 1991; Greinacher et al., 1992). Thus, it remained unclear how heparin induces platelet activation and thrombocytopenia in patients with HIT.

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

II. HEPARIN AND PLATELET FACTOR 4

Heparin and heparan sulfate constitute a distinct class of glycosaminoglycans (GAG). GAGs are long, linear polymers composed of repeating di-
saccharide subunits. Heparin and heparan sulfate belong to a family of polysaccharide species, the chains of which are made up of alternating 1-4-linked and variously sulfated residues of hexuronic (D-glucuronic or L-iduronic) acid and D-glucosamine. The two substances differ in their hexuronic acid composition and pattern of substitution, with heparin having a higher content of sulfates and, consequently, a greater linear charge density. Commercially available heparin preparations are heterogeneous and polydisperse, consisting of polysaccharide fragments ranging in length from 3,000 to 30,000 Da (10–100 saccharide residues) (see Chap. 8).

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

PF4, a heparin-binding protein normally found in platelet α-granules, is secreted when platelets are activated by various stimuli. Human PF4 is a member of a large family of homologous proteins, encoded by genes located on chromosomes 4 and 17, which have been designated “chemokines,” and are involved in chemotaxis, coagulation, inflammation, and cell growth (Oppenheim et al., 1991; Rollins, 1997; Luster, 1998). This family has been separated into four branches, designated CX3C, CXC, CC, and C, based on the relative position of the first two conserved cysteines. PF4 belongs to the CXC family, which includes, among others, interleukin-8 (IL-8), interferon-γ-inducible protein (IP-10), platelet basic protein (PBP), and two proteins derived from PBP by proteolytic cleavage: β-thromboglobulin (β-TG) and neutrophil-activating protein-2 (NAP-2). Human PF4 is a symmetrical, tetrameric molecule made up of identical subunits, each containing 70 amino acid residues of known sequence (Poncz et al., 1987), including two disulfide bonds, a single tyrosine, but no tryptophan. The molecule is positively charged at physiological pH (Handin and Cohen, 1976). The crystal structure of human PF4 has been resolved (Zhang et al., 1994). Lysine residues on the exterior faces of α-helices at the COOH-terminus of each monomer are critical for heparin binding (Loscalzo et al., 1985). However, residues located elsewhere on the tetramer are probably also important for this interaction (Maoccara and Lindahl, 1993; Mayo et al., 1995b).
III. NATURE OF THE EPITOPE RECOGNIZED BY HIT ANTIBODIES

A. The Role of Polyanion

The HIT antibodies fail to recognize PF4 or heparin alone, but bind avidly to the PF4-heparin complex (Visentin et al., 1994). Antibody epitopes, therefore, could be composed of either combinatorial epitopes, consisting partly of heparin and partly of PF4, or conformational epitopes on the PF4 molecule induced by heparin binding. Alternatively, a conformational change elsewhere on the PF4 molecule, created when the complex forms, could be targeted.

Heparin is a linear polyanion, and Maccarana and Lindahl (1993) have suggested that it binds to positively charged PF4 by nonspecific, electrostatic interactions, rather than by specific oligosaccharide sequence recognition. However, Stringer and Gallagher (1997) have described a sequence on heparan sulfate consisting of a 9 kDa fragment, with sulfated domains at each end separated by a central, N-acetylated region, that may confer some specificity for PF4 binding. Regardless of whether PF4-heparin interaction is to some extent specific, non-GAG molecules can be substituted for heparin in detecting HIT antibodies. Kelton et al. (1994) found that highly sulfated polysaccharides, including heparan sulfate, pentosan polysulfate, and dextran sulfate, could be used, provided that they contained 1.0–1.5 sulfate groups per saccharide residue. Chondroitin sulfates A, B, and C, containing an average of only 0.5 sulfates per saccharide residue, were inactive. Highly sulfated but low molecular weight substances such as glucose-1,3,6-trisulfate, 1,2-cyclohexanediol disulfate, and heparin disaccharide, were likewise inactive. Greinacher and colleagues (1992, 1995) also characterized the structural requirements of polysaccharides active in generating HIT antibody epitopes. They showed that the β1,4-linkage between disaccharides, characteristic of heparin and other GAGs, was not essential, that heparin fractions containing fewer than 10 residues were unable to promote platelet serotonin release by HIT antibodies, and that branched glucan sulfates were more effective than linear glucan sulfates of the same molecular weight. Similarly, Amiral and coworkers (1995) found that the extent of polysaccharide sulfation is positively correlated with the ability to interact with PF4 in facilitating the binding of HIT antibodies.

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

To determine whether or not a polysaccharide structure is necessary for the formation of HIT antibody epitopes, we evaluated a series of linear, nonsaccharide, polyanionic compounds and, unexpectedly, polyvinyl sulfate, polyvinyl sulfonate, polyvinyl sulfonate, polyvinyl phosphate, polyvinyl phosphonate, and polyvinyl sulfonate all react with PF4 to form complexes recognized by HIT-associated antibodies (Visentin et al., 2001) (Fig. 1). Thus, neither a saccharide chain nor sulfate side groups is essential for a polyanion to react with PF4 and to create sites for antibody binding, arguing strongly against the possibility that HIT antibodies are specific for “compound epitopes” consisting partially of GAG and partially of peptide sequence at sites where the molecules making up the complex come into close contact. This observation, together with the finding that HIT antibodies fail to recognize heparin complexed with protamine (unpublished observation), excludes the possibility that they recognize a configuration of the sulfated saccharide that is stabilized on binding to a small, positively charged, spherical protein.

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

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

Crystallographic studies have shown that both bovine (St. Charles et al., 1989) and human (Zhang et al., 1994) PF4 contain a ring of positively charged lysine, arginine, and histidine residues that encircle the tetramer along a line perpendicular to the α-helices and are available for interaction with solvent. Modeling studies (Stuckey et al., 1992) support the possibility that a negatively charged heparin molecule, containing 18 saccharide residues (MW ~ 5.4 kDa), interacts with these positively charged residues spanning about half the tetramer. Mayo et al. (1995a,b) created a PF4 mutant (PF4-M2) in which the NH2-terminal 11 residues were replaced by eight residues from the homologous CXC chemokine interleukin-8, to create a tetramer that binds heparin with the same avidity as native PF4, but is more nearly symmetrical around all three axes, facilitating NMR structural analysis. Their data,
contrary to PF4–heparin–binding models that center around COOH-terminal α-helix lysines, indicate that arginines 20, 22, and 49, and to a lesser extent histidine 23, threonine 25, and lysine 46, are also important for heparin binding (see Fig. 2b [see color insert, Fig. 3b] and Fig. 3). On the basis of these findings, it was speculated that heparin does not bind perpendicularly to the α-helices of the AB dimer, as had been suggested (Stuckey et al., 1992), but instead reacts with the α-helix at an angle, interacting preferentially with PF4 along the AD dimer, where it would encounter arginine and other positively charged residues. In either model, it is plausible that binding of a linear polyanion of sufficient length and linear charge density to positively charged residues on the surface of PF4 could cause the structural rearrangement throughout the entire tetramer necessary for generation of HIT antibody epitopes.

On the basis of these reports and our own observations, it is possible to propose a model of how heparin and other linear polyanions react with PF4 to produce configurational changes in the tetramer and create sites for HIT antibody binding. We suggest that linear polyanions, such as heparin, that carry appropriately spaced, strong negative charges interact with PF4 by binding to the ring of positive charges extending between the A and D or B and C subunits, or both. The minimum length for a fully active polyanion is about 50 Å, equivalent to six disaccharide subunits (12-mer), with each di-
B. The Role of Protein

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

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

Ziporen et al. (1998) studied the binding of antibodies from 50 HIT patients to different constructs of PF4, which contained a single amino acid substitution, and chimeric proteins, which contained various portions of human PF4 and NAP-2. Mutation to alanine of three (K62, K65, K66) of the four lysine residues in the COOH-terminal α-helix, had only minimal effect on the binding of HIT antibodies, and the K61 → A mutation reduced antibody binding by only about 50%, suggesting that the COOH-terminal lysines of PF4 do not constitute the major antigenic site for HIT antibodies. NH2-terminal PF4–NAP-2 chimeras exhibited only slightly reduced antibody binding. In contrast, the PF4–NAP-2 chimera, in which the portion of PF4 lying between the third and fourth cysteine residue (amino acids 37–47) was

**Figure 4** Primary and secondary structure of platelet factor 4 (PF4) in relation to HIT neoeptopes. (Top) 3-D representation of the PF4 tetramer, indicating two neoeptope sites (per monomer). The “ring of positive charge” is formed by lysine residues in the C-terminus (light blue) and other lysine and arginine residues (dark blue). (Bottom) The linear sequence of the 70-amino acid polypeptide of a single PF4 molecule is shown. (From Li et al., 2002.) (See color insert, Fig. 5.)
substituted by the corresponding NAP-2 sequence, was almost totally non-reactive.

With a different approach we found that, although human PF4 has 74% protein sequence identity to bovine and rat PF4, neither bovine nor rat PF4 complexed to heparin are recognized by HIT antibodies (Visentin, 1999). Yet, rat PF4 differs from its human counterpart at only 6 of its 47 COOH-terminal amino acids (Doit et al., 1987; Poncz et al., 1987) (see Fig. 3). To characterize the binding sites for HIT antibodies on PF4–heparin, we constructed seven PF4 mutants in which the human sequence (reactive) was converted to the corresponding residues of rat PF4 (nonreactive) and determined the effect of each change on HIT antibody binding to the construct complexed with heparin. The PF4 constructs tested were comparable with wild-type PF4 in their avidity for heparin. Each of 15 antibodies from HIT patients recognized PF4–heparin complexes containing PF4 constructs bearing mutations: E4 → S, L11 → V, and T16 → S at the NH2-terminus, or A57 → V at the COOH-terminus just as well as wild-type human PF4-heparin complexes. In contrast, complexes containing other COOH-terminal mutants: P37 → A/T38 → V/A39 → P, R49 → S, and L55 → R exhibited varying degrees of reduced binding. The HIT antibodies tested recognized PF4 mutated at

Figure 5 Spectratype analysis of the βV 6.1 family; PBMC from a patient with HIT were cultured in the presence or in the absence of the antigen (heparin–PF4). “Oligoclonal” expansion of T cells is observed only under stimulation with heparin–PF4.
positions 49 and 55 only at a higher ratio of heparin to PF4 (0.8 U/mL vs. 0.5 U/mL). None of the 15 antibodies recognized peptides comprising the 26 or 15 COOH-terminal amino acid residues of the PF4 monomer, or reduced alkylated human PF4 either in presence or absence of heparin.

These results, together with the observations by Ziporen and associates (1998), point to the region of PF4 between the third and fourth cysteine residues as the major antigenic site for HIT antibody binding (Fig. 4 [see color insert, Fig. 5]). Li et al. (2002) using a series of mouse/human PF4 chimeras identified another antigenic site, on PF4–heparin that requires both P34 and an intact N-terminus (Fig. 4 [see color insert, Fig. 5]). The latter results, together with our studies utilizing biotin-labeled affinity-purified HIT antibodies in a competitive inhibition assay (Suh et al., 1998), indicate that at least three dominant HIT antibody recognition sites can be distinguished and further support the idea that HIT antibodies recognize conformation-dependent “neoepitopes” formed on PF4 when it binds to heparin.

IV. THE CELLULAR IMMUNE RESPONSE

The finding that HIT antibodies can be of the IgM, IgG, or IgA isotype (Visentin et al., 1994; Greinacher et al., 1994; Kelton et al., 1994; Amiral et al., 1995, 1996b; Arepally et al., 1997; Suh et al., 1997) indicates that class switching, likely requiring helper T cells, takes place in patients mounting a humoral immune response to heparin–PF4. Although HIT is a drug-induced disorder, parallels for the role of T cells in HIT may be drawn from studies of autoimmune conditions, such as systemic lupus erythematosus, systemic sclerosis, and insulin autoimmune syndrome (Ito et al., 1993; Crow et al., 1994; Kuwana et al., 1995b). In both lupus and scleroderma, T-helper cells mediate antigen-specific autoantibody production by B cells (Adams et al., 1991; Mohan et al., 1993; Kuwana et al., 1995a).

We hypothesize that the heparin–PF4 complex not only is the target for antibody, but also is the stimulus for T-cell activation, and have used T-cell receptor (TCR) spectratyping (Maslanka et al., 1995), also called immunoscope (Cochet et al., 1992; Pannetier et al., 1993), and clonotyping (Maslanka et al., 1996) to characterize the T-cell response to PF4–heparin complexes in HIT. The TCRs of more than 95% of peripheral blood T cells are composed of two highly variable α- and β-chain glycoproteins, which function together in a complex with five other invariant molecules (CD3 complex) on the surface of the cell. The genes encoding the TCR β-chain subunit undergo sequential rearrangements analogous to that of the immunoglobulin superfamily of genes, during which D and J segments first, and then V segments, are combined to form various VDJ sequences (LaRoque and Robinson, 1996). Diver-
sity is further increased by the random removal and insertion of nucleotides between the V, D, and J segments. The resulting VDJ sequence encodes the so-called third complementarity region or CDR3 loop, the primary region involved in peptide recognition by the TCR (LaRoque and Robinson, 1996). Through allelic exclusion, only a single β-chain is expressed on the surface of a T cell, thus the β-chain CDR3 sequence provides a clonal marker for T-cell lineages and has been used to assess T-cell repertoires. TCR spectratyping is a polymerase chain reaction (PCR)-based technique that provides a readout of TCR β-chain diversity in a given T-cell population. TCR clonotyping is a refinement of spectratyping whereby oligonucleotide probes specific to a given CDR3 loop region from a particular TCR β-chain (thus a “clonotype”) are used to detect the presence or absence of the clonotype in a given T-cell population.

Culture of peripheral blood mononuclear cells (PBMC) from patients experiencing HIT incubated with heparin–PF4 complexes, but not heparin or PF4 alone, leads to selective expansion of T-cell subsets (Liu et al., 2000; Bacsí et al., 2001). On in vitro culturing of PBMC from two HIT patients, the PF4–heparin complexes preferentially stimulated CD4 T cells expressing TCR with β-chains of the V 5.1 family, with a shared core CDR3 region amino acid motif (PGTG) (Bacsí et al., 1999). In a study of a third HIT patient, we found heparin–PF4–specific expansion of several βV 17 TCR clonotypes with yet another shared core CDR3 region amino acid motif (TSG) (Bacsí et al., 2001). However, T-cell lines derived from this third patient and maintained in culture in the presence of heparin–PF4 demonstrated selective expansion of the βV 6.1 (Fig. 5) and 17 families sharing the conserved core GTG motif previously identified in the βV5.1 family of the first two HIT patients (Liu et al., 2001).

Our findings provide evidence for the existence of T-cell subpopulations specific for heparin–PF4 complexes in the peripheral blood of patients experiencing HIT and suggest that a common CDR3 TCR motif may be important for recognition of a peptide derived from PF4 processed by antigen-presenting cells in the presence of heparin. The observations are consistent with the possibility that only a limited number of helper T cells are used in mounting an antibody response to heparin–PF4. Further studies of the cellular immune response in HIT may lead to insights concerning drug-induced breakdown of immune tolerance to a self-protein.

It is presently unclear why patients with HIT mount a brisk humoral immune response to an autologous protein (PF4). PF4 is undoubtedly processed, under normal circumstances, by antigen-presenting cells (APC) without triggering immunity. One possible explanation for the induction of a response to PF4 after injection of heparin is that heparin may perturb the processing of PF4 by APC in a way such that peptides not ordinarily pro-
duced (cryptic peptides) are generated and presented to T cells in the context of class II MHC molecules. Studies in murine systems provide examples of "autoimmune" states triggered by exogenous agents that perturb protein processing (Hess et al., 1991; Griem et al., 1996), but this phenomenon is not well characterized in the context of human disease. Our studies support a model in which pharmacological doses of heparin cause aberrant processing of PF4 by APCs, leading to the presentation of peptides not ordinarily seen by the immune system. This hypothesis can be tested directly if T-cell clones can be developed from mononuclear cells responding to heparin–PF4 cultures.

It appears that multiple factors influence the formation of antibodies specific for heparin–PF4 complexes in patients receiving heparin. Currently, there is no evidence to support genetic predisposition as a basis for antibody formation in patients receiving heparin. Unlike the situation in alloimmune thrombocytopenia (de Waal et al., 1986; Mueller-Eckhardt et al., 1989), no connection between HIT and human leukocyte antigens (HLA) has been found (Greinacher and Mueller-Eckhardt, 1993). IgM antibodies specific for heparin–PF4 complexes are a common finding in HIT (Visentin et al., 1994, 1999), indicating a primary immune response, and it could be speculated that patients who received UFH previously may be at greater risk to produce heparin–PF4–specific antibodies and develop HIT, if rechallenged with heparin. However, Cadroy et al. (1994) described a patient with a history of HIT who mounted a brisk IgM response when challenged again with UFH 3 years later. A report by Warkentin and Kelton (2001) suggests that there is no anamnestic immune response in HIT (i.e., patients either have “typical” HIT [onset at days 5–10] or “rapid” HIT, the latter apparently caused by residual circulating HIT antibodies, rather than a secondary immune response). Furthermore, HIT did not necessarily recur in patients who were exposed to heparin a second time.

V. IMPLICATIONS

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

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I. INTRODUCTION

Unfractionated heparin (UFH) and low molecular weight heparin (LMWH) are the anticoagulants of choice when parenteral anticoagulation is required. Both can be given subcutaneously or intravenously, and both are effective in a variety of clinical settings (Hirsh et al., 2001). UFH in particular has several limitations. These include its poor bioavailability after subcutaneous injection as well as the marked variability in the anticoagulant response to UFH treatment in patients with acute thromboembolism (Hirsh, 1991; Young et al., 1992). Another problem is the risk of inducing heparin-induced thrombocytopenia (HIT). These limitations are closely linked (Greinacher, 1995): the underlying cause is the high density of negative charges of the heparin molecule, leading to nonspecific binding of heparin to plasma proteins other than antithrombin (AT). This results in inhibition of the anticoagulant effects of heparin, as well as changes in the conformational structure of the proteins following binding to heparin, with the potential for exposure of neoepitopes, or cryptic epitopes, toward which an immune response can be induced.

In this chapter, the mechanism and structural requirements for complex formation between sulfated carbohydrates, especially heparin, and proteins,
such as platelet factor 4 (PF4), are reviewed. The pathophysiological consequences of these interactions in causing HIT are summarized. From these considerations, the prospects for development of carbohydrate-based heparin alternatives that would not cause immune thrombocytopenia are discussed.

II. INTERACTIONS OF PF4 WITH SULFATED CARBOHYDRATES

A. Structure of PF4

Heparin activity is neutralized by PF4, a protein released from the α-granules of activated platelets (Sear and Poller, 1973; Klener and Kubisz, 1978; Luscher and Kaser-Glanzman, 1975; Niewiarowski, 1976; Walsh, 1976), which attaches to the endothelial surface by binding to glycosaminoglycans (GAGs) (Novotny et al., 1993). PF4 is a compact homotetrameric globular protein with a subunit molecular weight (MW) of 7780 Da (70 amino acid residues per subunit) (Kaplan and Niewiarowski, 1985; Mayo et al., 1995) containing 6.0% arginine, 3.2% histidine, and 12.3% lysine basic amino acids (Moore et al., 1975). The NH₂-terminal residues form antiparallel β-sheet-like structures that induce non-covalent associations between dimers and also contribute to the cohesion of the tetrameric unit. Furthermore, electrostatic interactions of multiply charged amino acid side chains and hydrogen-bonding interactions at the AB/CD dimer interface serve to stabilize the tetrameric structure. The COOH-terminal α-helices, which contain four lysine residues, are arranged as antiparallel pairs on the surface of each extended β-sheet (St. Charles et al., 1989). The lysine residues are predominantly on one side, resulting in a “ring of positive charge” that runs perpendicularly across the helices (Stuckey et al., 1992; Zhang et al., 1994) (see Figs. 2 and 4 [color inserts Figs. 3 and 5], Chap. 7).

B. Structure of Heparin

Heparin is a polydisperse mixture of GAGs with MWs ranging from 5 to 40 kDa, with an average MW of 13 kDa (Linhardt and Toida, 1997). It is composed of alternating D-glucosamine residues linked 1→4 to either L-iduronic acid or D-glucuronic acid (Casu, 1985). The principal repeating unit in heparin is the trisulfated disaccharide [→ 4]-O-α-L-iduronic acid-2-sulfate (1→4)-O-α-D-glucosamine-2,6-disulfate (1→) (Fig. 1), which represents 75–90% of the heparin chain (Linhardt et al., 1992). The remaining 10–25% of disaccharide units differ in their degree and positions of sulfation (Linhardt et al., 1988). Besides, there are disaccharides consisting of unsulfated glucuronic acid and/or N-acetylglucosamine. With a $\text{SO}_3^-\text{COO}^- \text{ ratio of 2.0–2.5}$, heparin is the GAG with the highest charge density. By binding to domains containing positively charged amino acids, especially arginine and lysine, it
interacts with many proteins, resulting in manifold biological activities. The most prominent example is a well-defined pentasaccharide sequence with a central α-D-glucosamine-2,3,6-trisulfate unit, which binds specifically to AT (Choay, 1989). About 30% (range 10–50%) of the heparin chains contain this pentasaccharide (Fig. 2). These molecules are called high-affinity heparin in contrast to the low-affinity heparin without this AT-binding site (Casu, 1990). AT is a natural serine protease inhibitor that controls blood coagulation by forming equimolar covalent complexes with certain coagulation enzymes. The anticoagulant action of heparin is based mainly on accelerating the slow rate of factor Xa (FXa) and thrombin (FIIa) inhibition by AT (Björk et al., 1989). Whereas the heparin pentasaccharide is sufficient for FXa inhibition, thrombin inhibition requires a minimum heparin chain length of 18 monosaccharides (5400 Da) to permit simultaneous binding of heparin to both AT and thrombin.

C. PF4-Sulfated Polysaccharide Complexes

Platelet factor 4 has the highest affinity to heparin among proteins stored within the platelet α-granules. Heparin molecules bind to PF4 by interactions with the positively charged residues on the surface of PF4 (see also Chap. 7). Stuckey and coworkers (1992) suggested that heparin is bound to PF4 by being wrapped around the tetramer along the ring of positive charge. A heparin molecule with 16–18 monosaccharides interacts with PF4 by spanning about half of the tetramer. As a consequence, only very long molecules are able to wrap around the complete tetramer. Mayo and coworkers (1995) identified a loop containing Arg-20, Arg-22, His-23, and Thr-25, as well as Lys-46 and Arg-49, which are more relevant for heparin binding than the terminal COOH-lysines. For optimal interaction with PF4, a heparin molecule should

![Figure 1](image-url)
Figure 2  Pentasaccharide sequence of the AT-binding site of heparin: sulfate groups essential for the AT-binding are encircled.
consist of at least 12 monosaccharides (Visentin, 1999; Mikhailov et al., 1999). At low concentrations (0.1–1.0 IU/mL) of heparin and high concentrations of PF4, several PF4 tetramers compete for heparin binding. This permits binding of a heparin chain to more than one PF4 tetramer. Particularly if a heparin molecule is longer than 16 monosaccharides, it is able to bind to, and thereby bridge, two PF4 tetramers. Thus, at certain concentrations of heparin and PF4, large, multimolecular PF4–heparin complexes are formed that can become dissociated in the presence of high heparin concentrations (Bock et al., 1980; Greinacher et al., 1995, 1994c) (see Fig. 2, Chap. 5).

Only heparin molecules containing 16 or more monosaccharides completely bind to immobilized PF4, resulting in total neutralization of their antifactor Xa (anti-Xa) and anti-thrombin (anti-IIa) activities, whereas progressively smaller oligosaccharides (without anti-IIa activity) become increasingly resistant to neutralization of their anti-Xa activity by PF4 (Denton et al., 1983; Lane et al., 1984). Because of their reduced sensitivity to inactivation by PF4, LMWHs are more active than UFH in platelet-rich plasma (Beguin et al., 1989), despite their lower activity in platelet-poor plasma (Samama et al., 1994). However, in contrast to their anti-Xa activity, the anti-IIa activity of LMWHs, which is mediated by molecules with a MW of more than 5400 Da, can be completely neutralized by higher PF4 concentrations (Padilla et al., 1992; Bendetowicz et al., 1994).

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

D. Interactions of PF4 with Sulfated Polysaccharides In Vivo

Intravenous injection of heparin causes an increase in plasma PF4 level, whereas subcutaneous injection does not (O’Brien et al., 1985). The maximum amount of PF4 released corresponds to only about 5% of total platelet PF4.
Platelets do not release PF4 when incubated with heparin in vitro (see Chap. 5). However, in vivo, heparin and some other GAGs are able to increase plasma PF4 levels (Cella et al., 1986). Thus, endothelial-bound, rather than platelet-stored, PF4 seems to be the predominant source of the PF4 released by heparin. Most likely, heparin and other high-sulfated polysaccharides are able to displace PF4 from endothelial heparan sulfate in relation to their affinity for PF4 (O’Brien et al., 1985).

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

#### A. Formation of Immune Complexes by HIT Antibodies

Two types of platelet count reduction associated with heparin treatment must be distinguished (Greinacher, 1995; Warkentin et al., 1995, 1998). Mild thrombocytopenia that occurs at the beginning of heparin treatment is most common, usually with high doses of heparin or under certain clinical circumstances (e.g., following thrombolytic therapy or during the perioperative period). Known as nonimmune heparin-associated thrombocytopenia, the platelet count fall is typically unaccompanied by clinically adverse events, and the platelet count recovers despite continued use of heparin. In contrast, HIT typically occurs between the 5th and 20th days after starting heparin therapy. HIT is often associated with thromboembolic complications.

Whereas nonimmune heparin-associated thrombocytopenia may be caused by direct platelet-activating effects of heparin (see Chap. 5), HIT results from an immune mechanism (Amiral et al., 1992). However, both thrombocytopenic syndromes are closely linked in their pathogenesis (Greinacher, 1995; Warkentin et al., 1995, 1998).
nacher, 1995), as the strong anionic character of heparin plays a pathogenic role for each. Heparin adheres to both endothelial-bound and platelet-derived PF4, with a potential further PF4 increase resulting from the platelet-activating effects of heparin (Horne and Hutchison, 1998; Newman and Chong, 2000). Heparin binding to PF4 exposes at least two neoepitopes, or cryptic autoantigens, on PF4 (Li et al., 2002). Some patients develop antibodies, predominantly IgG, but also IgM or IgA isotypes (Amiral, 1997) against the multimolecular PF4–heparin complexes, which thus represent the major antigen of HIT (Visentin et al., 1994; Greinacher et al., 1994c). Most HIT antibodies recognize noncontiguous conformational epitopes on the PF4 molecule that are produced when at least four to eight PF4 molecules are bound together by heparin (Horsewood et al., 1996; Newman and Chong, 1999). At PF4:heparin ratios equivalent to those prepared for use in PF4–heparin immunoassays, the PF4 protein exhibits a shift from a globular state to a more flexible, partially folded state (Mikhailov et al., 1999), thereby potentially exposing cryptic antigens. The antibodies recognize two, and probably three, distinct sites on the PF4–heparin complexes (Suh et al., 1998; Li et al., 2002). Furthermore, the heparin molecules must be in a flexible, relatively unconstrained state to react with PF4 in such a way that they create sites for HIT antibody binding.

In a few cases, PF4 alone can be recognized by the HIT antibodies as shown by the reaction of purified HIT antibodies with both PF4–heparin complexes and PF4 in the absence of heparin (Greinacher et al., 1994c; Newman and Chong, 1999; Amiral et al., 2000). Here, endogenous GAGs may take the role of heparin. However, patients have been reported with “delayed-onset HIT” in which thrombocytopenia and thrombosis began several days after heparin was stopped (Warkentin and Kelton, 2001). Sera from some of these patients can activate platelets strongly in vitro even in the absence of added heparin. One patient developed high levels of antibodies against PF4–heparin complexes, together with thrombocytopenia and multiple thromboses, beginning about one week after a single injection of only 5000 U of heparin (Warkentin and Bernstein, 2003).

In some patients with acute myocardial infarction, HIT antibodies were apparently detected at baseline, even though the patients had never previously been exposed to heparin (Suzuki et al., 1997). This might be due to platelet activation connected with release of PF4 binding to endogenous GAGs. Antibodies with cross-reactivity to PF4–heparin complexes may have been generated against such endogenous GAG-PF4 complexes, even before the first heparin treatment.

A retrospective analysis of patients with acute coronary syndrome found that the presence of anti–PF4–heparin antibodies at onset was associated with a higher risk of acute myocardial infarction and death (Williams et al., 2003). This observation requires prospective confirmation.
B. Effects of HIT Antibody-Containing Immune Complexes

Heparin-induced thrombocytopenia antibodies bind to PF4–heparin complexes by their F(ab’)2 domains (Horne and Alkins, 1996; Newman and Chong, 2000), with the predominant immunoglobulin isotype being IgG (Amiral et al., 1996b). Thus, divalent IgG binding to multimolecular PF4–heparin complexes leads to the formation of large immune complexes containing HIT–IgG on the platelet surface. The interaction of the HIT–IgG Fc with the platelet FcγIIa receptors leads to cross-linking of these receptors on the same or adjacent platelets, which triggers platelet activation (Kelton et al., 1988, 1994; Chong et al., 1989a) (see Chap. 9). The HIT antibody-mediated platelet activation can be inhibited by a monoclonal antibody specific for the FcγIIa receptor, by high concentrations of Fc fragments derived from normal IgG, and by excess heparin saturating all binding sites on PF4, and thus preventing the formation of multimolecular complexes (Greinacher et al., 1994b; Visentin et al., 1994).

Besides these effects on platelets, polyclonal HIT antibodies bind to endothelial cells (Cines et al., 1987; Visentin et al., 1994). The most convincing evidence demonstrating that these antibodies are the same ones that cause platelet activation was provided by classic adsorption–elution experiments (Greinacher et al., 1994c). Purified IgG obtained from sera of HIT patients gave positive reactions in both activation (serotonin release) and antigen (anti-PF4–heparin) assays. This IgG fraction was then adsorbed using cultured endothelial cells and, after extensive washing, the cells were eluted. The eluate again tested positive in both activation and antigen assays. Thus, these experiments showed that the antibodies recognize the same epitope on platelets, endothelial cells, and PF4–heparin complexes coated onto a microtiter plate. It appears most likely that the epitope on endothelial cells comprises surface GAGs (Cines et al., 1987; Greinacher et al., 1994c; Visentin et al., 1994). Endothelial cell activation by HIT antibodies can be inhibited by excess heparin, but not by anti-FcγIIa receptor monoclonal antibodies.

In addition to platelet and endothelial cell activation, there is concomitant activation of coagulation, as shown by marked elevations in thrombin–AT complex levels (Warkentin et al., 1997; Warkentin, 1998; Greinacher et al., 2000). The simultaneous activation of platelets, endothelium, and coagulation factors could explain the development of thrombocytopenia combined with thrombosis or disseminated intravascular coagulation in patients with HIT.

C. Importance of HIT Antibodies in Clinical HIT

HIT antibodies occur commonly in heparin-treated patients. However, as many patients develop neither thrombocytopenia nor thrombosis (Amiral
et al., 1996a; Kappers-Klunne et al., 1997; Arepally et al., 1997;), it is evident
that pathogenicity requires additional factors. Two possible factors are high
titers of HIT antibodies (Suh et al., 1997), as well as optimal (equimolar) con-
centrations of heparin and PF4 in the blood circulation, such that formation
of macromolecular PF4–heparin antigen complexes is permitted (Horne and
Alkins, 1996; Horne and Hutchison, 1998). Thus, during low-dose heparin
prophylaxis in a setting of minimal platelet activation, clinical HIT may occur
less often than in a patient receiving high heparin doses together with ac-
tivated platelets (Fondu, 1995). In accordance with this working hypothesis,
HIT antibodies are most frequently induced by UFH in patients following
cardiopulmonary bypass surgery (~50%), followed by patients undergoing
major orthopedic surgery (~15%), and least frequently in medical patients
(~3%) (see Chap. 4).

Further factors favoring the development of clinical HIT are prethrom-
botic or inflammatory situations (e.g., open heart surgery) (Visentin et al.,
1996), greater susceptibility of the platelets to activation by HIT antibodies
(Salem and van der Weyden, 1983), perhaps mediated by differences in PF4
binding to platelets (Capitanio et al., 1985), and increased expression of
FcγIIa receptors (Chong et al., 1993). Also, the polymorphism of the FcγIIa
receptor at position Arg–His131 seems to be associated with a predisposition
to HIT (Carlsson et al., 1998). Consequently, although HIT antibodies play
an important role in the pathogenesis of clinical HIT, they are not the only
factor responsible for its clinical manifestation. Thus, although monitoring
for HIT antibodies should identify patients at risk for HIT (Elalamy et al.,
1996), it appears unlikely that this would lead to improved clinical outcomes
versus simply monitoring the platelet count to make an early diagnosis of
HIT.

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

A. Interactions with Low Molecular Weight Heparins

Generation of the HIT antigen depends not only on the concentration, but
also on the chain length of heparin. LMWH preparations (Table 2) have
reduced affinity for platelets, endothelial cells, and plasma proteins, such as
PF4 (Horne, 1993; O’Brien et al., 1985; Turpie, 1996). Accordingly, LMWH is
less likely to form multimolecular complexes with PF4 (Greinacher et al.,
1993); hence, they may induce an immune response less often than UFH. This
is corroborated by a prospective study in which patients receiving LMWH
after hip replacement surgery had a lower frequency of HIT antibody
formation than patients receiving UFH (Warkentin et al., 1995).
Table 2  Characteristics of Commercial LMWHs

<table>
<thead>
<tr>
<th>INN (Brand name)</th>
<th>Degradation method</th>
<th>Mean MW (kDa)</th>
<th>Anti-Xa (U/mg)</th>
<th>Anti-Xa: Anti-IIa ratioa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ardeparin sodium (Normiflo)</td>
<td>Peroxidation at elevated temperature</td>
<td>4.0–6.0</td>
<td>120 ± 25</td>
<td>1.7–2.4</td>
</tr>
<tr>
<td>Bemiparin sodiumb (Hibor)</td>
<td>Basic degradation in a nonaqueous media and fractionation</td>
<td>3.6</td>
<td>80–90</td>
<td>8.1</td>
</tr>
<tr>
<td>Certoparin sodium (Mono-Embolex NM)</td>
<td>Hydrolysis with isoamylnitrite</td>
<td>4.2–6.2</td>
<td>80–120</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Dalteparin sodiumc (Fragmin)</td>
<td>Hydrolysis with HNO₂</td>
<td>5.6–6.4</td>
<td>110–120</td>
<td>1.9–3.2</td>
</tr>
<tr>
<td>Enoxaparin sodiumc (Clexane, Lovenox)</td>
<td>Benzylation and alkaline β-elimination</td>
<td>3.5–5.5</td>
<td>95–125</td>
<td>3.3–5.3</td>
</tr>
<tr>
<td>Nadroparin sodiumc (Fraxiparin)</td>
<td>Hydrolysis with HNO₂ and fractionation</td>
<td>3.6–5.0</td>
<td>95–135</td>
<td>2.5–4.0</td>
</tr>
<tr>
<td>Parnaparin sodiumc (Fluxum)</td>
<td>Radical-catalyzed degradation with H₂O₂ and Cu salts</td>
<td>4.0–6.0</td>
<td>75–110</td>
<td>1.5–3.0</td>
</tr>
<tr>
<td>Reviparin sodium (Clivar)</td>
<td>Hydrolysis with HNO₂</td>
<td>3.5–4.5</td>
<td>105</td>
<td>3.6–6.3</td>
</tr>
<tr>
<td>Tinzaparin sodiumc (Innohep)</td>
<td>Enzymatic (heparinylase) β-elimination</td>
<td>5.6–7.5</td>
<td>70–120</td>
<td>1.5–2.5</td>
</tr>
</tbody>
</table>

a Ratio of anti-FXa activity (U/mg) to antithrombin activity (U/mg).
b Bemiparin is the first example of the second-generation LMWHs, which are defined to have a mean MW <4.0 kDa, a proportion of fragments >6.0 kDa <15%, and an aXa: aIIa ratio >4:1.
c From Monograph in European Pharmacopoeia, 4th ed.
Despite its lower immunogenicity, LMWH exhibits nearly 100% in vitro cross-reactivity to HIT antibodies using sensitive assays (Greinacher et al., 1994a,b; Warkentin et al., 1995; Amiral et al., 1996b; Amiral, 1997). The small variations found with different LMWH preparations are probably based on their individual structural parameters, such as DS and MW (Farreed et al., 1988). Homogeneous heparin fragments containing 20, 18, 16, 14, and 12 carbohydrate residues form multimolecular complexes recognized by the antibodies; fragments containing 10 residues induce antigen formation only weakly; fragments containing 8 and 6 residues are less reactive (Amiral et al., 1995; Greinacher et al., 1995) or nonreactive (Visentin et al., 2001). Small heparin molecules may bind to PF4 (Bock et al., 1980; Denton et al., 1983; Greinacher et al., 1995), but only large molecules are able to bridge four to eight PF4 molecules (one to two PF4 tetramers), thus producing the noncontiguous conformational epitopes recognized by most HIT antibodies (Horsewood et al., 1996).

B. Interactions with Other Sulfated Carbohydrates

The formation of platelet-activating immune complexes is not limited to heparin (Greinacher et al., 1992, 1993). Various other sulfated polysaccharides, and even polyvinylsulfonate, bind PF4 to form antigen complexes recognized by HIT antibodies. This cross-reaction depends on their structure, especially on their DS and MW (Greinacher et al., 1992, 1995; Kelton et al., 1994; Amiral et al., 1995). In vitro assays demonstrate that pentosan polysulfate, dextran sulfate, as well as a highly sulfated chondroitin sulfate can substitute for heparin. In contrast, neither dextran, dermatan sulfate, de-N-sulfated heparin, sulfated glucosamine (Weimann et al., 2001), nor the AT-binding pentasaccharide react in these assays. Accordingly, pentosan polysulfate and highly sulfated chondroitin sulfate have induced thrombocytopenia and thrombosis in vivo (Greinacher et al., 1993; Tardy et al., 1994) (see Chap. 4). The corresponding antibodies can be detected by conventional PF4-heparin enzyme-linked immunosorbent assay (PF4-H ELISA), demonstrating the cross-reactivity with heparin (Gironell et al., 1996).

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

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

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

![Repeating unit of \( \beta \)-1,3-glucan sulfates: The primary OH group in position 6(\( \ast \ast \)) is preferentially sulfated. Glycosidic-branched \( \beta \)-1,3-glucan sulfates are substituted by a glucose, rhamnose, or arabinose unit, respectively, in position 6.](image-url)

**Figure 3** Repeating unit of \( \beta \)-1,3-glucan sulfates: The primary OH group in position 6(\( \ast \ast \)) is preferentially sulfated. Glycosidic-branched \( \beta \)-1,3-glucan sulfates are substituted by a glucose, rhamnose, or arabinose unit, respectively, in position 6.
concentrations are required to form multimolecular PF4–GluS complexes (Greinacher et al., 1995).

Compared with linear GluS having similar DS and MW, glycosidic-branched products generally exhibit higher anticoagulant activity than the respective linear derivatives (Alban, 1993, 1997). Glycosidic substitution changes the three-dimensional structure of the polysaccharide chain, enhancing its flexibility and improving the interaction with proteins (Kindness et al., 1980). As the side chains are more accessible to sulfation, they represent clusters of negative charges (Alban and Franz, 1994b), facilitating binding to PF4, which results in an increased cross-reactivity with HIT antibodies.

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

A. Structural Requirements of Carbohydrate-Based Heparin Alternatives

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

1. The molecule should not be branched to reduce its flexibility and to minimize charge clusters.
2. Its DS should be lower than 1.0 per monosaccharide, if its chain length exceeds 10 monosaccharides.
3. Its MW should be lower than 2.4 kDa (about seven monosaccharides), if its DS is between 1.0 and 1.8.
4. If the MW is higher than 2.4 kDa and the DS higher than 1.0, then at least the therapeutic concentration must be lower than that exhibiting cross-reactivity with HIT antibodies.

B. Danaparoid

Danaparoid sodium (Orgaran) is an alternative anticoagulant that is effective for treating patients with HIT (see Chap. 14). This heparinoid consists of a depolymerized mixture of GAGs extracted from porcine intestinal mucosa, with a mean MW of 6 kDa. Its components are approximately 80% low molecular weight heparan sulfate, 10% dermatan sulfate, 5% chondroitin sulfate, and a small proportion of heparan sulfate (4%) with high affinity for AT (Meuleman, 1992). Apart from the minor AT-binding heparan sulfate component, the constituents of danaparoid have a DS per monosaccharide between 0.5 and 0.7, as well as a low MW. Thus, the two important requirements to form multimolecular complexes with PF4 are not met. This is con-
sistent with the low cross-reactivity rate of danaparoid (about 10%) (Wilde and Markham, 1997) (see Chaps. 11 and 14). As danaparoid inhibits platelet activation by HIT antibodies even in the presence of heparin (Chong et al., 1989b), it is possible that the GAG mixture binds to PF4 without producing the antigen. Consequently, less PF4 is available for the small amount of higher-sulfated heparan sulfate molecules responsible for AT binding and, presumably, PF4 binding resulting in cross-reactivity with HIT antibodies (Greinacher et al., 1992).

C. Pentasaccharides

Within the scope of developing new carbohydrate-based antithrombotics, fondaparinux, a fully synthetic, chemically defined pentasaccharide (formerly named Org31540/SR90107A, MW = 1728 Da; DS = 1.6; 700 anti-Xa U/mg), has been developed, which corresponds to the AT-binding site of heparin (Petitou et al., 1997) (Fig. 4). By its highly specific binding to AT, fondaparinux selectively inhibits factor Xa and thus prevents thrombin generation (Bauer et al., 2002). In four phase III clinical trials evaluating the prevention of venous thromboembolism after major orthopedic surgery (>7300 patients), fondaparinux showed superiority over the LMWH enoxaparin without increasing clinically important bleeding (Turpie et al., 2001; Eriksson et al., 2001; Bauer et al., 2001, Lassen et al., 2002; Turpie et al., 2002a,b) and has recently been approved for this indication. At present, fondaparinux is under further investigation for antithrombotic prophylaxis in other clinical settings, as well as for treatment of deep vein thrombosis, pulmonary embolism, and

![Chemical structure of the synthetically produced pentasaccharide Org 31540/SR90107A (MW = 1728 kDa; DS = 1.6; 864 anti-Xa U/mg), with eight sulfate groups corresponding to the natural antithrombin-binding site.](image-url)
acute coronary syndromes (The Rembrandt Investigators, 2000; Coussenent et al., 2001; Eriksson et al., 2003).

As expected, owing to the structure–activity relations previously discussed, fondaparinux did not cross-react with HIT antibodies in any concentration tested, either in the PF4–H-ELISA or in the serotonin release assay (Amiral et al., 1997; Greinacher et al., 1995; Ahmad et al., 1999). Immune thrombocytopenia attributable to fondaparinux has not been observed in any of the clinical studies. Of interest, a few sera from patients treated with fondaparinux have tested positive in a PF4-dependent ELISA and have caused platelet activation in vitro in the presence of added heparin, although no cross-reactivity with fondaparinux itself could be shown (Warkentin et al., 2003). This paradox is not understood.

In contrast to fondaparinux, we have observed in our laboratory that a more highly sulfated pentasaccharide, Org 32701 (MW = 1991 Da; DS = 2.0) (Fig. 5) (Herbert et al., 1996), induces platelet activation in the presence of HIT antibodies. This demonstrates that certain highly sulfated oligosaccharides are indeed able to bind to PF4 and thus form the HIT neoantigen. But whether such a highly sulfated pentasaccharide itself could induce clinical HIT cannot yet be answered.

D. Specifically Designed Oligosaccharides

Pentasaccharides such as fondaparinux or the long-acting idraparinux (Herbert et al., 1998) have minimal, if any, undesirable interactions with blood and vessel components, but their anticoagulant activity is limited to AT-mediated

![Figure 5](image_url) - Chemical structure of the synthetically produced pentasaccharide, Org 32701 (MW = 1991 kDa; DS = 2; 1150 anti-Xa U/mg), with a higher degree of sulfation (ten sulfate groups) than the natural antithrombin-binding site.
FXa inhibition. Additional thrombin inhibitory properties might further improve the anticoagulant efficacy of heparin-related oligosaccharides. Unfortunately, as with heparin, lengthening the sulfated oligosaccharide chain increases nonspecific binding that could have undesirable effects, such as binding to PF4 and associated risk of HIT. Thus, Petitou and coworkers (1999) synthesized “heparin mimetics” that inhibited thrombin, but failed to bind other proteins, particularly PF4. The most promising structure is the hexadecasaccharide SR123781A, which is undergoing phase I evaluation (Herbert et al., 2001). It is obtained from glucose through a convergent synthesis and consists of an AT-binding pentasaccharide sequence linked to a thrombin-binding domain via a neutral methylated hexasaccharide “spacer.” It specifically catalyzes the AT-mediated inhibition of FXa (IC$_{50}$ = 77 ± 5 ng/mL, 297 ± 13 U/mg) and thrombin (IC$_{50}$ = 4.0 ± 0.5 ng/mL, 150 ± 30 U/mg), without effect on heparin cofactor II and without binding to PF4. Compared with UFH and LMWH in animal studies, SR123781A exhibited a highly favorable antithrombotic:bleeding ratio. This compound did not activate platelets in the presence of plasma from HIT patients, suggesting that it will not induce the HIT antigen. As the methylated spacer substitutes for the sulfated carbohydrates, the minimally required chain length of eight sulfated monosaccharides required for binding to PF4 (Maccarana and Lindahl, 1993) is not present.

E. Conclusions

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

1. Mixtures of GAGs consisting predominantly of low-sulfated carbohydrates with correspondingly limited capacity to form antigenic complexes with PF4: A prototype of such an anticoagulant is danaparoid.
2. Oligosaccharides with antithrombotic activity similar to the AT-binding pentasaccharide: One such agent appears promising: fondaparinux did not cause HIT in more than 4000 patients treated after orthopedic surgery.
3. GAGs with highly sulfated, but short, regions that are connected by nonsulfated “spacers”: Hereby, the thrombin-binding site and the AT-specific pentasaccharide can be expressed in a single molecule without reaching the critical length of a sulfated chain critical for HIT antigen formation (Petitou et al., 1999).
The increasing use of LMWH already seems to have reduced the incidence of HIT. We propose that the problem of HIT can be avoided further by using anticoagulants meeting the foregoing outlined criteria in our treatment arsenal.

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I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a unique immune-mediated disorder. HIT is common, occurring in as many as 5% of certain patient populations. Affected patients often develop the paradox of thrombosis, but not bleeding, despite having thrombocytopenia. One possible reason for this unique clinical profile is the central role of the platelet Fcγ receptor (FcγRIIa) in mediating platelet activation in HIT. Indirect evidence suggesting a crucial role for platelet activation in the pathogenesis of HIT is the observation that thrombocytopenia caused by HIT antibodies is strongly associated with thrombosis, whereas formation of HIT antibodies without thrombocytopenia is not (Warkentin et al., 1995).

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

One of the most important unanswered questions in the pathophysiology of this disorder is an explanation for why only a few patients who develop HIT antibodies become thrombocytopenic. This problem has led investigators to study the role of FcγRIIa in explaining, at least partly, the heterogeneous clinical sequela among patients with HIT. This chapter will (a) review the structure and function of the platelet FcγRIIa; (b) describe the mechanism of HIT antibody-induced platelet activation by FcγRIIa; and (c) summarize the studies that have attempted to identify a role for the FcγRIIa in modifying clinical manifestations of HIT.

II. PLATELET FcγRIIa STRUCTURE, DISTRIBUTION, AND FUNCTION

The platelet FcγRIIa is a member of a family of structurally related glycoproteins, many of which are expressed on hematopoietic cells (Table 1). Twelve different transcripts have been reported, derived from eight distinct genes and grouped into three different classes: I, II, and III (for review see van de Winkel and Capel, 1993; Rascu et al., 1997; Gessner et al., 1998). Allelic polymorphic variants add yet another level of diversity for FcγRIIa, FcγRIIIa, and FcγRIIIb. The genomic organization of the FcγR genes on chromosome 1q23 was resolved by Su and coworkers (2002). The multigenic region is approximately 1 mb in size and is in the following gene order and orientation: cen—FCGR2A (5′-3′)—FCGR3A (3′-5′)—FCGR2C (3′-5′)—FCGR3B (3′-5′)—FCGR2B (5′-3′)—tel.

The affinity for IgG varies among these isoforms and polymorphic variants. Most notably, the FcγRIIa–His131 allele has a significantly higher affinity for human IgG2 than FcγRIIa–Arg131 (Warmerdam et al., 1991). Furthermore, FcγRIIIa–Val158 and FcγRIIIb–NA1 bind nearly twice as much IgG as FcγRIIIa–Phe158 and FcγRIIIb–NA2, respectively (Salmon et al., 1990; Koene et al., 1997). One allelic variant of FcγRIIc has a nonsense codon, which results in the absence of expression of the glycoprotein if
<table>
<thead>
<tr>
<th>Genes</th>
<th>FCYRI (CD64)</th>
<th>FCYRII (CD132)</th>
<th>FCYRIII (CD16)</th>
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<tbody>
<tr>
<td>IA, IB, IC</td>
<td>IIA, IIB, IIC</td>
<td>IIA, IIB</td>
<td>IIA, IIBB</td>
</tr>
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<td>None</td>
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<tr>
<td></td>
<td></td>
<td>IIA: Arg/His&lt;sup&gt;31&lt;/sup&gt;</td>
<td>IIA: Phe/Val&lt;sup&gt;36&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>IIC: Gln/Stop codon&lt;sup&gt;13&lt;/sup&gt;</td>
<td>IIBb: NA1/NA2</td>
</tr>
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<td></td>
<td>IIBb: Ala/Asp&lt;sup&gt;60&lt;/sup&gt; (SH)</td>
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<td>RNA transcripts&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>IIB1, sIIb2, IIb3</td>
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<td>Hematopoietic cell distribution</td>
<td>CD34 progenitor cells, monocytes, macrophages, dendritic cells</td>
<td>IIA: platelets, endothelial cells, monocytes, macrophages, eosino-/basso-/neutrophils, Langerhans/dendritic cells</td>
<td>IIB: B cells, monocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IIC: NK cells</td>
<td>IIb: neutrophils</td>
</tr>
</tbody>
</table>

<sup>a</sup> Allelic polymorphisms that show differences in IgG binding; NA1/NA2 variants have multiple amino acid differences (Ory et al., 1989); SH<sup>+</sup> individuals (Bux et al., 1997) carry three copies of FCYRIIIb (Koene et al., 1998); lack of expression of IIc is due to a nonsense mutation (Metes et al., 1998).

<sup>b</sup> Multiple mRNA transcripts from FCYRIB, IIA, and IIB, are the result of alternative splicing of primary transcripts.

<sup>c</sup> Soluble forms of FCYRIIa and IIb (sIIa, sIIb) are devoid of the hydrophobic transmembrane exon; GPI, glycosylphosphatidylinositol.

<sup>d</sup> ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif.

<sup>e</sup> Numbers represent the relative order of IgG subclass binding to variants of FCYRHa (Warmerdam et al., 1990), FCYRHa (Koene et al., 1997; Wu et al., 1997), and FCYRIIIb (Salmon et al., 1990; Bredius et al., 1994b).
inherited in the homozygous state (Metes et al., 1998). When cross-linked, each isoform participates in biological activities through distinct signal transduction pathways that affect cell functions, including antigen presentation, immune complex clearance, phagocytosis and the oxidative burst, release of various cytokines and intracellular granular mediators, antibody-dependent cellular cytotoxicity (ADCC), and negative down-regulation of antibody production or phagocytosis. Only FcγRIIa is expressed on platelets (Rosenfeld et al., 1985; Kelton et al., 1987). The receptor is a single α-chain, 40-kDa glycoprotein, with an extracellular region consisting of two immunoglobulin-like, disulfide-linked domains responsible for ligand binding, a transmembrane region, and an intracellular domain that incorporates an immunoreceptor tyrosine-based activation motif (ITAM) essential for intracellular signal transduction (Qiu et al., 1990). The gene comprises seven exons. A soluble form of the receptor is produced by alternative splicing of primary RNA transcripts to exclude exon five containing the transmembrane region (Rappaport et al., 1993). The nucleotide region encoding the ITAM is unique in that the two tyrosine motifs are separated by 12 amino acids, rather than the usual 7 (Brooks et al., 1989).

At the amino acid level, the extracellular domain of FcγRIIa share 96% identity with FcγRIIb and FcγRIIc (Brooks et al., 1989). A G→A polymorphism at nucleotide position 519 of the cDNA (position 512 in Genbank Accession M90724) is responsible for the Arg/His131 allelic functional variants (Clark et al., 1989). An additional polymorphism, an A→G at nucleotide 207 of the cDNA, results in a Gln–Trp27 substitution in the mature polypeptide (Warmerdam et al., 1991). However, the Arg–His131 position is near or within the binding region for IgG Fc (Hulett et al., 1995), and it is this polymorphism that is associated with the affinity differences for human IgG2 (Warmerdam et al., 1991). More recently, another polymorphism proximal to Arg131 that affects IgG2 binding has been found in a single healthy individual (Norris et al., 1998): a lysine substitution for glutamine at position 127 demonstrated a significant increase in FcγR-mediated phagocytosis in this homozygous FcγRIIa–Arg131 individual.

FcγRIIa has low affinity for IgG (10^7 M^-1) and interacts only with multivalent antigen–antibody complexes (Warmerdam et al., 1991; Parren et al., 1992). The copy number of FcγRIIa expressed on normal resting platelets varies among healthy individuals, but is stable for a given individual (Rosenfeld et al., 1987). Studies using Scatchard analysis report roughly a threefold variation among individuals, with the number of binding sites ranging from 600 to 1500 molecules per platelet when assayed using intact murine monoclonal antibody IV.3 (McCrae et al., 1990), and 1500 to more than 4500 for the monovalent (Fab) preparation of IV.3 (Tomiyama et al., 1992; Brandt et al., 1995). There are no major differences in FcγRIIa number between
males and female, among platelets from persons of different ages, or among platelets representing the three possible genotypic classes of the FcγRIIa-Arg/His131 allelic variants (Brandt et al., 1995).

III. IMMUNOGLOBULIN G AGONISTS AND PLATELET ACTIVATION

A. FcγIIa Receptor-Mediated Platelet Activation

Murine monoclonal antibodies, usually of the IgG1 subclass, against CD9 were among the first studied for their platelet-activating properties. Subsequently, it was determined that monoclonal antibodies to glycoprotein (GP) IIb/IIIa, β2-microglobulin, GP IV (CD36), and other selected antigens can activate platelets (for review, see Rubinstein et al., 1995). In each instance, platelet activation occurs by a consistent mechanism: first, there is Fab-mediated binding to surface-expressed platelet antigens, then the Fc portion of the antibodies interacts with platelet FcγRIIa. Evidence for FcγRIIa-dependency includes the inhibition of platelet activation by a murine monoclonal anti-FcγRIIa antibody (IV.3). However, some platelet GPs (e.g., GP Ib) do not support activation by monoclonal antibodies; others support activation despite their usual sequestered location within platelets (e.g., multimerin), and still other GPs (e.g., GP IIb/IIIa) support activation by only certain monoclonal antibodies (Horsewood et al., 1991). This suggests that specific factors, such as target protein membrane mobility and localization of the target epitope, that permit formation of multimolecular GP antigen–IgG–FcγRIIa complexes, are crucial for platelet activation mediated by FcγRIIa clustering. These murine IgG Fc moieties within the platelet surface immune complexes can interact with either FcγRIIa on the same platelet (intraplatelet activation) or with FcγIIa receptors located on other platelets in close proximity (interplatelet activation) (Anderson et al., 1991; Horsewood et al., 1991).

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

HIT-IgG causes the generation of thromboxane A2 and associated platelet granule release (Chong et al., 1981). Indeed, several different “activation assays” have been developed that detect HIT antibodies by their ability
to cause resting platelets to aggregate (Greinacher et al., 1991), effect granule release (Sheridan et al., 1986), or generate platelet-derived microparticles (Warkentin et al., 1994) (see Chap. 11).

B. Procoagulant, Platelet-Derived Microparticles

Platelet activation by various agonists leads to procoagulant alterations of the platelet membrane. This includes loss of the usual membrane asymmetry (i.e., with platelet activation there is increased transbilayer movement of phosphatidylserine from the inner to the outer platelet membrane). This membrane "flip-flop" is a consequence of a calcium-dependent enzyme ("scramblase") that serves to undo the membrane asymmetry actively maintained in resting platelets by other enzymes (aminophospholipid translocase and "flopase") (Bevers et al., 1999). Additionally, platelet activation also leads to profound morphological changes that correlate with procoagulant activity, including the generation of procoagulant, platelet-derived "microparticles" (Sims et al., 1989).

Serum and purified IgG from patients with HIT, as well as other "IgG agonists" (immune complexes and murine platelet-activating monoclonal IgG), also generate platelet-derived microparticles via the platelet FcγIIa receptors; in contrast, quinine- and quinidine-dependent sera do not produce microparticles, even though they lead to far greater drug-dependent binding of IgG to platelets, compared with HIT samples (Warkentin et al., 1994). Indeed, HIT serum is superior in generating platelet-derived microparticles, and in producing platelet procoagulant activity (as shown using a phospholipid-dependent assay, the Russell’s viper venom time), than physiological agonists such as thrombin, collagen, and ADP. Only the nonphysiological agonist calcium ionophore produced greater numbers of microparticles and procoagulant activity than did HIT sera (Warkentin and Sheppard, 1999).

Flow cytometry can be used to detect platelet-derived microparticles generated by HIT antibodies or other platelet agonists (Fig. 1). This technique has been used to develop diagnostic assays for HIT. For example, relative quantitation of platelets and microparticles can be achieved using particle size ("forward scatter") and fluorescein-labeled platelet GP-specific monoclonal antibodies (Lee et al., 1996). Tomer (1997) used labeled annexin V, a protein that binds to phosphatidylserine expressed on activated platelets and microparticles, to test for HIT (see Chap. 11).

There is some uncertainty as to whether the "microparticles" detected by flow cytometry represent true microparticles, or rather platelets that have undergone considerable morphological changes during activation. Use of high-sensitivity settings for signal thresholding on orthogonal light scatter,
combined with fluorescence gating on platelet antigens, allows detection of significant increases in total particle count, suggesting that at least some true microparticles can be detected by flow cytometry (Bode and Hickerson, 2000). However, these techniques remain to be tested with HIT sera. Nevertheless, that true microparticles can be generated by HIT antibodies is suggested by a recent study that used confocal microscopy and scanning/transmission electron microscopy for their detection (Hughes et al., 2000) (Fig. 2).
C. ADP Potentiation of Platelet Activation

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

D. Fcγ Receptor–Mediated Signal Transduction

FcγRIIa activation, as a result of ligand (IgG Fc) binding and by action of phosphatidylinositol-3 kinase and phospholipase Cγ2 (PLCγ2), leads to release of diacylglycerol (DAG) and inositol triphosphate (IP3) and mobilization of internal calcium stores, culminating in platelet aggregation (Ander-
Ligand binding leads to clustering of the FcγRIIa, causing Src family protein tyrosine kinases to phosphorylate the ITAM region of FcγRIIa (Chacko et al., 1994; Huang et al., 1992). Following FcγRIIa phosphorylation, additional tyrosine kinase activity (e.g., p72\textsuperscript{syk}) increases through the noncovalent interaction of their SH2 domains with the phosphorylated FcγRIIa ITAMs (Greinacher et al., 1994b; Yanaga et al., 1995; Chacko et al., 1996). Subsequently, PLC\textgammay2 is phosphorylated by p72\textsuperscript{syk} (Blake et al., 1994); this phosphorylation is dependent on phosphatidylinositol-trisphosphate [PtdIns(3,4,5)P3] (Gratacap et al., 1998). PLC\textgammay2 activation is crucial for the generation of DAG and IP3.

More recently, Gratacap and coworkers (2000) showed that FcγRIIa activation alone does not produce sufficient levels of PtdIns(3,4,5)P3 to cause PLC\textgammay2 activation, platelet release, and aggregation. Additionally, ADP receptor activation by Gi-protein signaling leads to the generation of PtdIns (3,4,5)P3, which combined with activation of FcγRIIa generates optimal levels of PtdIns(3,4,5)P3, leading to PLC\textgammay2 phosphorylation. Activated PLC\textgammay2 then generates DAG and IP3 from PtdIns(4,5)P2, mobilizing calcium, and effecting platelet aggregation (Fig. 3). Moreover, lipid rafts appear to play an

**Figure 3** Fcγ receptor-mediated signal transduction. PF4–heparin–IgG complexes (1) bind to FcγRIIa, causing receptor clustering (2). The immunoreceptor tyrosine activation motifs (ITAMs) on FcγRIIa are phosphorylated by src tyrosine kinases (PTKsrc) (3). The phosphorylated ITAMs interact with SH domains on p72\textsuperscript{syk} to phosphorylate phospholipase C\textgammay2 (PLC\textgammay2) (4). ADP receptors, activated via ADP and Gi proteins, generate phosphatidylinositol-trisphosphate (PIP3) (5), which helps phosphorylate PLC\textgammay2. Activated PLC\textgammay2 acts on PIP2 to generate inositol triphosphate (IP3), and diacylglyceryl (DAG) from phosphatidylinositol-bisphosphate (6). IP3 mobilizes Ca\textsuperscript{++} to the intracellular space via Ca\textsuperscript{++} channels (7) and together with DAG activates downstream protein kinase C (PKC) signaling pathways (8).
important role in the organization of the FcγRIIa/ADP receptor/PLCγ2 signaling pathway (Bodin et al., 2003). These findings help to explain the previous observations that ADP scavengers (e.g., apyrase) fully inhibit platelet aggregation by HIT-IgG (Polgár et al., 1998).

IV. FcγRIIa ACTIVATION IN HIT

Although an association between heparin treatment and paradoxical thrombosis was first suspected about 40 years ago (Weismann and Tobin, 1958; Roberts et al., 1964), it was Rhodes and colleagues (1973) who first provided evidence that serum from HIT patients contained a substance, most likely IgG, that aggregated normal platelets in the presence of heparin. This observation was confirmed by Fratantoni et al. (1975), who reported a simple indirect aggregation method for detecting HIT antibodies. In 1986, Sheridan and coworkers (1986) reported a washed platelet activation assay, employing radiolabeled serotonin, as an activation endpoint that was sensitive and specific for detecting clinically significant HIT antibodies. This same group later reported that platelet activation by HIT antibodies was platelet FcγRIIa-dependent, as it could be completely abrogated by a murine monoclonal anti-FcγRIIa antibody, IV.3 (Kelton et al., 1988). Other workers confirmed the central importance of the platelet Fc receptor in mediating platelet activation in HIT (Adelman et al., 1989; Chong et al., 1989a,b). Subsequently, Amiral and colleagues (1992) reported that the major target antigen for HIT-IgG was PF4 complexed to heparin, a finding quickly confirmed by other workers (Greinacher et al., 1994a; Kelton et al., 1994; Visentin et al., 1994).

A. Dynamic Model of Platelet Activation in HIT

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

B. Platelet FcγRIIa Numbers
Variable expression of FcγRIIa numbers among individuals could affect susceptibility to immune complex diseases (Rosenfeld et al., 1987), or even to HIT. The number of platelet surface–expressed FcγRIIa molecules is increased dramatically in patients with HIT (Chong et al., 1993b). However, increased FcγRIIa expression is also seen after in vitro activation of platelets by HIT antibodies. Thus, elevated FcγRIIa numbers may be a consequence of platelet activation in HIT, rather than a proximate cause. This notion is supported by the fact that increased platelet FcγRIIa levels are seen in patients with atherothrombosis and diabetes mellitus (Calverley et al., 2002). It remains uncertain whether high baseline (pre-HIT) FcγRIIa numbers represents an important risk factor for HIT.

C. Plasma-Soluble FcγRIIa
Soluble FcγRIIa, which is released from α-granules on platelet activation by thrombin, has been demonstrated in plasma (Gachet et al., 1995). The soluble form of the receptor lacks the amino acids for the transmembrane domain, as a result of alternative splicing that removes exon 5 from primary transcripts (Rappaport et al., 1993). However, the relative amount of membrane versus soluble FcγRIIa is fixed (Keller et al., 1993). Gachet and colleagues (1995) reported that approximately 2 ng of soluble FcγRIIa is produced from 10⁹ platelets. This value equals 2 ag, or 300 molecules, per platelet compared with roughly 10 times as many molecules on the platelet surface. A much larger amount of plasma-soluble FcγRIIa would be needed to inhibit significantly PF4- H immune complexes from binding to platelet FcγRIIa. Therefore, there is likely no effect of soluble FcγRIIa on membrane FcγRIIa-dependent platelet activation, especially considering that immune complexes formed on the platelet surface would sterically hinder soluble receptor interaction. Moreover, plasma levels of soluble FcγRIIa are higher in patients with HIT than in heparin-treated or other nonthrombocytopenic controls, presumably as a marker of in vivo platelet activation in HIT (Saffroy et al., 1997).

D. Plasma IgG Concentrations
Plasma IgG levels appear to influence platelet activation and aggregation by HIT sera. With a platelet-rich plasma (PRP) aggregation test to detect HIT
antibodies, Chong et al. (1993a) showed variable platelet sensitivity to aggregation that was stable over time among different platelet donors. Chong and coworkers showed that the addition of purified human IgG to the PRP inhibited platelet aggregation by HIT sera, with complete inhibition at 40 mg/mL. It is possible that the effect of purified IgG is due to the presence of small IgG oligomers, because Karas et al. (1982) demonstrated that monomeric IgG does not bind to the platelet FcγRIIa. Furthermore, Greinacher et al. (1994b) showed that different preparations of intravenous IgG (ivIgG) for therapeutic use varied in their ability to inhibit HIT antibody-induced platelet serotonin release. Only Cohn alcohol-fractionated ivIgG preparations retained the ability to inhibit the reaction at concentrations that can be achieved in vivo (20 mg/mL). Preparations that were treated to reduce IgG oligomers did not inhibit heparin-dependent platelet serotonin release. Although the use of ivIgG to treat HIT does not appear to be common, it has some rationale in certain clinical settings (see Chap. 13).

E. FcγRIIa-Arg/His\textsuperscript{131} Polymorphism

There is an arginine-histidine (Arg/His) polymorphism at amino acid 131 of the human FcγRIIa (Clark et al., 1989; Warmerdam et al., 1990). This allelic variation affects the ability of human platelets to be activated by murine monoclonal IgG1 as well as by human IgG2 (Horsewood et al., 1991; Tomiyama et al., 1992; Parren et al., 1992; Bachelot et al., 1995). This prompted Burgess et al. (1995) to suggest that inherited FcγRIIa receptor variants could be a risk factor for developing HIT. In a small cohort of patients, they found an overrepresentation of the FcγRIIa–His\textsuperscript{131} variant. They hypothesized that IgG2 might be an important IgG subclass among HIT-IgG, as this could explain an apparent association between HIT and the FcγRIIa–His\textsuperscript{131} variant. However, subsequent reports argued against this hypothesis: IgG1 rather than IgG2 was the predominant subclass among HIT-IgG (Arepally et al., 1997; Denomme et al., 1997; Suh et al., 1997). Nevertheless, in support of a biological basis for a possible increased frequency of FcγRIIa–His\textsuperscript{131}, two groups found that HIT antibodies, including those that were predominantly IgG1, preferentially activated washed platelets of the His\textsuperscript{131} variant in vitro (Denomme et al., 1997; Bachelot-Loza et al., 1998). However, Brandt et al. (1995) found the opposite activation profile in platelet aggregation studies using citrated platelet-rich plasma (i.e., the Arg\textsuperscript{131} variant was preferentially activated by HIT plasmas). No consensus has emerged either among the six studies that investigated whether one of the FcγRIIa–Arg/His\textsuperscript{131} phenotypes predominated among patients with HIT: three studies show an overrepresentation of FcγRIIa–His\textsuperscript{131} (Burgess et al., 1995; Brandt et al., 1995; Denomme et al., 1997); two studies found no correlation with either variant (Arepally
et al., 1997; Bachelot-Loza et al., 1998); and one study (the largest) showed the reverse correlation (Carlsson et al., 1998). This topic is considered in detail in Section V.

F. Animal Models of HIT

One of the earliest animal models of HIT used the natural immune process of anti-idiotypic antibody production to invoke expression of HIT-IgG in mice (Blank et al., 1997, 1999). Mice immunized with HIT-IgG developed anti-idiotypic IgG that now recognized PF4–heparin. Such mice developed thrombocytopenia within 4 days of the administration of unfractionated heparin. Unfortunately, this model has limited use, as the mice did not develop thrombosis (perhaps because murine platelets lack FcIIa receptors).

Other investigators (Arepally et al., 2000) developed a murine monoclonal antibody, termed KKO, by immunizing mice with PF4–heparin. This murine IgG2b monoclonal antibody mimics HIT-IgG, as it requires both PF4 and heparin to activate human platelets through their FcyRIIa. However, besides lacking Fc receptors, another problem of the murine system is that mouse PF4 is not recognized by HIT-IgG or KKO. To overcome these problems, Reilly and colleagues (2001) produced transgenic mice that express human FcyRIIa and human PF4. In these animals, addition of KKO caused thrombocytopenia and death, including thrombosis of the lung vasculature. Thus, this murine model may prove useful to address questions of antibody titer, FcyRIIa numbers, and so on, in influencing the clinical expression of HIT.

Murine transgenic models are useful for studying other aspects of antibody-mediated thrombocytopenia. For example, McKenzie and coworkers (1999) demonstrated that transgenic mice expressing human FcyRIIa on platelets and monocytes became more thrombocytopenic than their matched wild-type littermates on being administered antimouse platelet IgG. Further, when FcR γ-chain knockout mice (which do not develop thrombocytopenia in these experiments as all FcyRI and IIIa receptors are lacking) were crossbred with FcyRIIa transgenic mice, severe immune thrombocytopenia was observed in these FcyRIIa transgenic × FcR γ-chain knockout mice.

However, when platelet-activating (anti-CD9) IgG was administered to FcyRIIa transgenic mice, even more severe thrombocytopenia resulted, compared with the previously studied antimouse platelet (nonactivating) IgG (Taylor et al., 2000). Further, severe thrombosis, shock, and death developed in the FcyRIIa transgenic mice crossed with FcR γ-chain knockout mice. Moreover, splenectomy facilitated anti-CD9-mediated shock in FcyRIIa transgenic mice. Thus, the authors concluded that phagocytosis by monocytes–macrophages of IgG-sensitized platelets may have a protective role in
preventing thrombosis. These data have implications for HIT, as there may be a balance between platelet activation by HIT-IgG (predisposing to thrombosis) and clearance of platelets by monocytes–macrophages (protecting somewhat against thrombosis).

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

G. Monocyte Fcγ Receptors in HIT

Monocytes and macrophages possess several different classes of FcγR (see Table 1), and thus may play a part in influencing the frequency and severity of both thrombocytopenia and thrombosis in HIT. One role, discussed in the previous section, involves their potential to influence the balance between platelet activation and reticuloendothelial-mediated platelet clearance in HIT. Another function recently proposed for monocytes is that of contributing to the procoagulant state in HIT, (i.e., a role posited previously for endothelial cells) (see Chap. 10). Pouplard and colleagues (2001) found that by adding HIT-IgG and PF4 (or PF4–heparin) directly to isolated monocytes or to whole blood, the monocytes produced tissue factor (TF), an effect that could be inhibited by high concentrations of heparin. Arepally and Mayer (2001) found that monocytes expressed surface TF when incubated with PF4 either in the presence of HIT-IgG or the HIT-mimicking murine monoclonal antibody, KKO. Because monocytes express sulfated proteoglycans on their surface, PF4 binding to monocytes can occur in the absence of added heparin. These studies raise the possibility that monocytes play an important role in the pathogenesis of the procoagulant state characteristic of HIT.

V. FcγRIIa POLYMORPHISMS IN DISEASE

A. Determining the FcγRIIa Polymorphism

The FcγRIIa–Arg/His131 polymorphism was first identified on the basis of functional differences effected by anti-CD3 monoclonal antibodies of the murine IgG1 subclass (Tax et al., 1983, 1984). Proliferation assays distinguished “high” and “low” responders relative to the effects of these anti-CD3 murine monoclonal antibodies on T-cell–dependent mitogenesis. Subsequently, individuals bearing the FcγRIIa–Arg131 phenotype were identified as the
“high responders” and the functional differences between the two polymorphic variants were later confirmed using other FcγRIIa-dependent assays, such as erythrocyte antigen-rosetting, phagocytosis, and platelet activation (Clark et al., 1989; Warmerdam et al., 1991; Parren et al., 1992; Salmon et al., 1992). Murine monoclonal IgG1 antibodies activate platelets of all three Arg/His131 phenotypes, but the homozygous FcγRIIa–Arg131 variant requires less murine monoclonal antibody for platelet activation to occur.

The high-affinity binding of human IgG2 to FcγRIIa results when histidine is substituted at amino acid 131 of the mature protein (Warmerdam et al., 1991). FcγRIIa–His131 has a greater affinity for human IgG2, but a lower affinity for murine IgG1. Therefore, the terms high and low responder, used historically for the effects of murine monoclonal antibodies on Arg131 and His131 FcγRIIa phenotypes, respectively, is confusing, as the opposite reaction profile is observed with human IgG2. The high/low responder terminology has been largely replaced in favor of referring simply to the amino acid polymorphism.

The FcγRIIa–Arg/His131 variant polymorphism can be determined in three ways: (a) by functional assay, such as T-cell–dependent proliferation or murine monoclonal antibody activation; (b) by specific binding using 41H16, a monoclonal antibody the Fab of which binds exclusively to the FcγRIIa–Arg131 variant; and (c) by molecular genotyping. Four DNA-based methods have been developed to genotype for the FcγRIIa–Arg/His131 nucleotide substitution (Clark et al., 1991; Osborne et al., 1994; Bachelot et al., 1995; Jiang et al., 1996; Denomme et al., 1997). In one technique, the presence of the FcγRIIa–Arg/His131 variant gene is determined using genomic DNA and a sequence-specific primer–polymerase chain reaction (PCR) assay. Two PCR reactions are necessary, each containing a common primer paired with a unique primer having different 3’-ends to detect the presence of the G or A variant nucleotide (Clark et al., 1991). This method has been modified using different sequence-specific primers (Flesch et al., 1998) or using a nested sequence-specific PCR (Carlsson et al., 1998). In a second technique, flanking primers are used to amplify a region containing the nucleotide polymorphism, followed by dot-blotting and hybridization with allele-specific, single-stranded oligonucleotide probes (Osborne et al., 1994; Burgess et al., 1995; Denomme et al., 1997). In a third technique, Bachelot and coworkers (1995) developed a denaturing gradient gel electrophoresis assay that distinguishes between the FcγRIIa–Arg/His131 variants also using flanking primers that amplify a region containing the polymorphism. Last, restriction endonuclease digestion of PCR-amplified genomic DNA has been developed using one primer immediately proximal to the polymorphic site and containing a mutation such that the polymorphism creates a restriction enzyme site for only one of the alleles (Jiang et al., 1996).
B. Influence of FcγRIIa Polymorphism in Infectious or Autoimmune Disease

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

C. FcγRIIa Polymorphism in HIT

It was logical to hypothesize that the platelet FcγRIIa–Arg/His<sup>131</sup> polymorphism would influence the clinical expression of HIT. First, platelets from normal individuals exhibit considerable variability in their activation by HIT sera (Salem and Van der Weyden, 1983; Pfueller and David, 1986; Warkentin et al., 1992). Second, many patients who form HIT antibodies during heparin treatment do not develop thrombocytopenia (Warkentin et al., 1995; Amiral et al., 1996; Suh et al., 1997). Third, the inciting role of heparin, a sulfated carbohydrate, suggested that there could be an important role for HIT antibodies of IgG2 subclass—that is, the subclass with higher affinity for FcγRIIa–His<sup>131</sup> that is predominantly formed in response to carbohydrate antigens (Herrmann et al., 1992). (However, HIT epitopes form on the protein PF4 when it undergoes conformation change bound to heparin) (see Chaps. 6–8). Consequently, it was speculated that the FcγRIIa variant distribution in HIT would differ significantly from a random control population, and especially differ from patients who did not develop thrombocytopenia during heparin treatment (Denomme et al., 1997; Bachelot-Loza et al., 1998).

The six studies investigating the role of the FcγRIIa–Arg/His<sup>131</sup> polymorphism have not yielded uniform results (Fig. 4). Three studies showed a predominance of the His<sup>131</sup> variant in patients with HIT that was significant, compared with control patients. Together with evidence that HIT antibodies preferentially activate platelets in vitro from individuals bearing the FcγRIIa–His<sup>131</sup> polymorphism (Denomme et al., 1997; Bachelot-Loza et al., 1998), it was suggested that FcγRIIa–His<sup>131</sup> predominance could reflect a greater
potential for these platelets to be activated in vivo by HIT antibodies (see Table 2). Two relatively small studies did not show any significant differences in the Arg/His\textsuperscript{131} phenotypes between HIT patients and controls. However, the largest of the six studies, involving 389 patients (i.e., more than the 260 HIT patients reported in the previous five studies combined), showed an increase in the frequency of the Arg\textsuperscript{131}, rather than the His\textsuperscript{131}, variant in patients with HIT (Carlsson et al., 1998). Moreover, these workers made the intriguing observation that the increase in Arg\textsuperscript{131} phenotype occurred only in the subset of patients whose HIT was complicated by thrombosis. These investigators proposed that reduced clearance of IgG-containing immune complexes by phagocytic cells bearing Fc\gammaRIIa–Arg\textsuperscript{131} leads to greater immune complex-dependent activation of platelets and endothelial cells, thus predisposing to thrombosis (see Table 2). Although Arepally et al. (1997) did not observe a significant increase in the Arg\textsuperscript{131} phenotype among HIT patients with thrombosis, their subset of HIT patients with thrombosis was much smaller than that reported by Carlsson (23 vs. 68

<table>
<thead>
<tr>
<th>Disease</th>
<th>Predominant Fc\gammaRIIa variant</th>
<th>Comment</th>
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<tbody>
<tr>
<td>Infections by encapsulated bacteria</td>
<td>Arg\textsuperscript{131}</td>
<td>Reduced binding of IgG2 by Fc\gammaRIIa–Arg\textsuperscript{131}-MPS cells reduces phagocytosis, conferring susceptibility to infections with bacteria bearing polysaccharide capsules (\textit{Haemophilus}, meningococcus).</td>
</tr>
<tr>
<td>Immune complex nephritis (SLE)</td>
<td>Arg\textsuperscript{131}</td>
<td>Reduced clearance of IgG-containing immune complexes by Fc\gammaRIIa–Arg\textsuperscript{131}-MPS cells leads to greater glomerular deposition of immune complexes.</td>
</tr>
<tr>
<td>HIT with thrombosis</td>
<td>Arg\textsuperscript{131}</td>
<td>Reduced clearance of IgG-containing immune complexes by Fc\gammaRIIa–Arg\textsuperscript{131}-MPS cells leads to greater immune complex-dependent activation of platelets and endothelial cells (Carlsson et al., 1998).</td>
</tr>
<tr>
<td>HIT with or without thrombosis</td>
<td>His\textsuperscript{131}</td>
<td>Increased activation by HIT-IgG1 and HIT-IgG2 of Fc\gammaRIIa–His\textsuperscript{131} platelets, without significant role for MPS cells (Denomme et al., 1997).</td>
</tr>
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</table>

Abbreviations: Arg, arginine; Fc\gammaRIIa, Fc\gammaIIa receptor; His, histidine; MPS, mononuclear phagocytic system (reticuloendothelial system); SLE, systemic lupus erythematosus.
On the other hand, when Poupland and colleagues (1999) examined the Arg/His\textsuperscript{131} gene frequency among patients who formed antibodies against PF4–heparin following cardiac surgery, they noted that platelet levels were significantly lower only in the Arg/Arg\textsuperscript{131} group, when compared with patients who did not form antibodies.

The explanation for the differences among these various studies is not readily apparent. However, a complicating aspect is noted in Fig. 4: the frequency of the His\textsuperscript{131} allele is higher in patients with HIT and thrombosis compared to those without.

Figure 4  FcγRIIa–His\textsuperscript{131} gene frequencies in six studies of HIT are shown: The first four studies were from North American centers, the last two from Europe. Although the first three studies showed predominance of His\textsuperscript{131} in patients with HIT, the last study showed predominance of Arg\textsuperscript{131} in patients with HIT complicated by thrombosis. A complicating feature is the difference in gene frequencies between certain control populations [e.g., between Denomme et al. (1997) and Carlsson et al. (1998)]. Not shown in the figure is the significant difference between control patients in the studies by Carlsson and Brandt (p = 0.013).
quency of the His\textsuperscript{131} phenotype is higher in the European controls (Bachelot-Loza et al., 1998; Carlsson et al., 1998), compared with the North American and Australian control populations (Burgess et al., 1995; Brandt et al., 1995; Denomme et al., 1997; Arepally et al., 1997), an observation consistent with population allele frequencies reported by Rascu et al. (1997). Indeed, pairwise $\chi^2$ analysis for the frequency of the His\textsuperscript{131} genotype among the various control groups shows that the control population of Carlsson’s study differs from that reported by Denomme and Brandt (see Fig. 4). The FcγRIIa–Arg/His\textsuperscript{131} polymorphism varies among populations: in whites and African Americans, the gene frequencies have roughly a 50:50 balance (Osborne et al., 1994; Lehrnbecher et al., 1999). In contrast, in the Japanese and Chinese populations, the His\textsuperscript{131} gene frequency is approximately 75% (Rascu et al., 1997; Osborne et al., 1994). It is possible that unrecognized differences in population between HIT patients and controls could be important. For example, whereas samples from HIT patients could be referred from a wider geographic area, control patients might have been obtained from a localized area. None of the six studies reported on the Arg/His\textsuperscript{131} phenotype distribution among heparin-treated patients who formed HIT antibodies, but who did not develop thrombocytopenia (i.e., the ideal control group for assessing the influence of the FcγRIIa polymorphism).

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

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10
Immune Vascular Injury in Heparin-Induced Thrombocytopenia

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I. INTRODUCTION

The most important complication of HIT is thrombosis. Clinically overt arterial or venous thrombi have been observed in 50% or more of thrombocytopenic patients with HIT in some series (see Chaps. 3 and 4), a frequency that far exceeds any other drug-induced immune platelet disorder. The propensity for thrombosis has been attributed to platelet activation through FcγIIA receptors by IgG-containing complexes that comprise heparin and platelet factor 4 (PF4) (see Chap. 9). Strong support for this notion comes from recent studies involving mice transgenic for human FcγIIA and human PF4 (Reilly et al., 2001) (see Chap. 9). However, the “nonpermissive” FcγIIA phenotype affords limited protection against thrombosis (Arepally et al., 1997; Denomme et al., 1997; Bachelot-Loza et al., 1998; Carlsson et al., 1998), perhaps because of the high incidence of IgG1 antibodies in HIT patients (Denomme et al., 1997). Furthermore, the occasional patient in whom only IgM or IgA HIT antibodies are detected (Amiral et al., 1996) suggests that additional factors, acting at the level of the platelet or elsewhere, must
contribute to the thrombotic diathesis. In addition, thrombi usually occur at a
limited number of vessel sites, typically large arteries and veins (Boshkov et
al., 1993) (see Chap. 3), even though circulating HIT antibodies and activated
platelets can be readily detected in asymptomatic patients. These consider-
ations point to injury to the local vascular milieu in influencing the clinical
expression of disease.

There are several other reasons to suspect that immune complex–
mediated injury of the vasculature may contribute to the thrombotic com-
parisons of HIT. First, the endothelium helps maintain the balance between
blood fluidity and clotting. Second, the endothelium expresses heparan
sulfate-containing proteoglycans that help regulate coagulation and contrib-
ute to the metabolism of PF4. Third, antiendothelial cell antibodies have been
identified in patients with other disorders characterized by thrombosis and
thrombocytopenia. And fourth, there is some direct evidence that patients
with HIT form antibodies that recognize heparin–PF4 complexes on the
endothelium, and thereby promote prothrombotic reactions.

II. THE ENDOTHELIUM IN HEMOSTASIS

The role of the endothelium in regulating blood fluidity and trafficking of
circulating hematopoietic cells has been the subject of recent reviews (Bus-
senge, 1996; Bombeli et al., 1997; Cadroy et al., 1997; Lüscher and Barton,
1997; Cines et al., 1998; Aird, 2003). Endothelial cells (ECs) express a variety
of factors that inhibit coagulation. These include soluble substances, such as
nitric oxide and prostacyclin (acting to inhibit platelet activation), and tissue-
type plasminogen activator (t-PA, acting to promote fibrinolysis), among
many others. Endothelial cell surface–bound molecules with anticoagulant
activity include heparan sulfate–containing proteoglycans (see later discus-
sion), thrombomodulin, complement regulatory proteins, as well as receptors
for activated C, urokinase, and plasminogen.

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

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

There is also recent evidence to indicate that protein C activation on macrovascular ECs is mediated predominantly through the protein C receptor, whereas thrombomodulin (TM) may be the dominant system in the microvasculature (Laszik et al., 1997; Fukudome et al., 1998). TM changes thrombin from a procoagulant to an anticoagulant enzyme (i.e., TM-bound thrombin activates the natural anticoagulant zymogen, protein C) (Esmon, 1989). The anticoagulant function of TM in the microvasculature may contribute to the pathogenesis of warfarin-associated venous limb gangrene that can complicate HIT. This syndrome has been attributed to the coincidence of persistent thrombin generation and acquired protein C deficiency that may occur during the first few days of anticoagulation with warfarin (Warkentin et al., 1997) (see Chaps. 3 and 12).

The behavior of ECs can also be modified during the evolution of vascular disease. For example, atherosclerotic vessels produce less nitric oxide in response to a variety of stimuli than do healthy vessels (Gryglewski, 1995). Atherosclerotic vessels may also undergo alterations in their expression of glycosaminoglycans (GAGs) (Williams and Tabas, 1995) and an increase in expression of various cell adhesion molecules (for review see Fuster et al., 1998). The binding of advanced glycation end products to specific EC receptors during normal aging and diabetes mellitus increases vascular permeability, exposing the subendothelial matrix to lipoproteins and other injurious substances (Schmidt et al., 1994). It is also likely that genetic variation in EC behavior contributes to the host response to antibody- or platelet-mediated EC injury, although the methods to identify or monitor such risk factors remain to be developed. Thus, any inquiry into the reason why only a subset of patients who develop antiheparin–PF4 antibodies develop thrombosis, or why thrombi occur at restricted vascular sites, must
take into consideration the specific attributes of a particular endothelial vascular bed.

III. IMMUNE ENDOTHELIAL CELL INJURY

Endothelial cell–reactive antibodies have been found in patients suffering from disorders characterized by vasculitis or thrombosis (for review see Meroni et al., 1996; Praprotnik et al., 2001). The best-studied example is allograft rejection, a setting in which there is extensive evidence for anti-endothelial cell antibodies (AECA), in part directed at carbohydrate antigens that regulate procoagulant activity in vitro (Saadi and Platt, 1995; Siegel et al., 1997). AECA have also been identified in patients with hyperacute and acute

Figure 1 Model of HIT antibody interactions with endothelial cells: (1) HIT antibodies bind to circulating antigen that becomes localized to platelets. (2) Platelet activation occurs after Fc receptor binding, leading to platelet granule release. ADP is released from platelet dense granules and PF4 is released from platelet α-granules. (3) Released PF4 binds to platelets and endothelial cell heparan sulfate (HS), displacing antithrombin (AT) from endothelial cells. (4) Antigen complexes on endothelial cells bind HIT antibodies. (5) HIT antibody binding to endothelial cells leads to endothelial cell activation, resulting in further platelet activation.
graft rejection, systemic lupus erythematosus (Cines et al., 1984), antiphospholipid antibody syndrome (McCrae et al., 1991), and hemolytic-uremic syndrome (Leung et al., 1988). What is curious is the extraordinary diversity of the clinical syndromes associated with AECA. Also of interest, the target cells used in most assays (i.e., endothelial cells derived from human umbilical vein [HUVEC]), are not known to be affected by immune vascular injury in the clinical setting. This suggests that the expression of the target antigen(s) is highly restricted in vivo, reflecting either regional differences in the composition of the vascular bed (microvascular or macrovascular) or, perhaps, indicating distinct responses to injury and inflammation. Alternatively, these AECA could represent a surrogate marker for other pathogenic antibodies, or the capacity of the affected vasculature is a critical determinant of whether thrombosis develops.

Several effects of AECA that could contribute to thrombosis include cell lysis or apoptosis (Bordron et al., 1998; Nakamura et al., 1998), induction of cytokine secretion and promotion of leukocyte adhesion (Del Papa et al., 1997), enhancement of vascular permeability (Saadi and Platt, 1995), acceleration of procoagulant reactions (Tannenbaum et al., 1986; Saadi et al., 1995), and reduction in the expression of heparan sulfate (Platt et al., 1991). The reason why some antibodies promote thrombosis (e.g., HIT antibodies), whereas others induce primarily a necrotizing vasculitis, remains unresolved, but may relate directly to the biological functions of heparin and PF4 (Fig. 1).

IV. HEPARAN SULFATE–CONTAINING PROTEOGLYCANS, HEPARIN, AND THE ENDOTHELIUM

The expression and anticoagulant function of heparan sulfate-type proteoglycans (HSPGs) by ECs may be central to the pathogenesis of vascular thrombosis in patients with HIT. The biochemistry and function of these GAGs and proteoglycans to which they bind has been the subject of extensive study (for review see Ruoslahti, 1988; Rosenberg et al., 1997; Esko and Lindahl, 2001); the involvement of heparan sulfate in the development of HIT is considered elsewhere (see Chap. 8). HSPGs expressed by ECs bind antithrombin in vitro and in vivo, and accelerate the inactivation of thrombin and factor Xa approximately 20-fold (Marcum et al., 1984), an effect that is biologically equivalent to 0.1–0.5 U/mL of heparin (Marcum and Rosenberg, 1987). Yet less than 1% of the HSPGs isolated from cultured ECs express anticoagulant activity (Marcum and Rosenberg, 1984). Active species are characterized by an approximately twofold enrichment in glucoronyl 3-O-sulfated glucosamine residues, compared with inactive species (Marcum and Rosenberg, 1987). Interestingly, targeted deletion of the murine 3-O-sulfo-
transferase-1 enzyme, the enzyme responsible for generating this anticoagu-

lant modification of HSPGs, does not lead to a prothrombotic phenotype
(HajMohammadi et al., 2003). The physiological mechanisms that control the

synthesis and postsynthetic modifications of HSPG remain an active area of

investigation (Forsberg and Kjellen, 2001).

Microheterogeneity in the composition of HSPG in arteries, veins,

and capillaries has been noted (Lowe-Krentz and Joyce, 1991), but the sig-

nificance of these differences is unknown. Expression of HSPG by ECs

undergoes developmental changes (David et al., 1992), and its composition

varies after the cells are exposed to thrombin (Benezra et al., 1993), homo-
cysteine (Nishinaga et al., 1993), heparin (Nader et al., 1989), wounding and

migration (Kinsella and Wight, 1986), and after induction by activated

platelets (Yahalom et al., 1984), among other stimuli. ECs also bind heparin
(for review see Patton et al., 1995), which alters EC proliferation, matrix

composition, and many other vascular functions. It has also been reported
that antithrombin is displaced from ECs by heparin, and its binding is
inhibited by PF4 (Stern et al., 1985). Whether HIT antibodies promote the
capacity of PF4 to neutralize antithrombin activity has not been reported.

V. PLATELET FACTOR 4 AND THE ENDOTHELium

The biochemistry of PF4 and its involvement in HIT is reviewed elsewhere
(see Chap. 7). The metabolism of the protein is regulated by its interactions
with the endothelium. PF4 is stored in the α-granules of platelets as a tetramer
bound to chondroitin sulfate (Barber et al., 1972). The tetramer may dis-
sociate from the glycosaminoglycan as the platelets are activated, but more
likely, dissociation occurs subsequent to binding to EC HSPG, which
contains a higher charge density. [125I]PF4 is cleared from the circulation
with an α-elimination phase approximating 2 min, which primarily represents
binding to the endothelium, and a β-elimination phase approximating 40 min,
corresponding to uptake and degradation, predominantly by hepatocytes
(Rucinski et al., 1986, 1990).

The ECs bind about 50 pmol PF4/10^5 cells (Rybak et al., 1989). Several
classes of binding sites have been identified, including a high-capacity, low-
affinity site on HSPG, as well as higher-affinity binding sites involving specific
chemokine receptors and certain coagulant proteins (see later discussion).
Binding of PF4 to the endothelium is attenuated by pretreatment with hepa-

rinate (Marcum et al., 1984), and plasma concentrations are increased 10- to
20-fold after heparin is infused intravenously (Dawes et al., 1982). Binding of
PF4 to EC GAGs is electrostatic (Wu and Cohen, 1984) and is independent of
the pentasaccharide involved in the binding of antithrombin (Loscalzo et al.,
The affinity of PF4 binding to ECs is lower than to purified heparin ($K_d = 2$ nM vs. 2–3 μM, respectively) (Rybak et al., 1989), consistent with the biochemical heterogeneity of vascular matrix. PF4 has a 10- to 100-fold greater affinity for EC HSPG than does antithrombin (Jordan et al., 1982) and markedly attenuates its antiprotease cofactor activity on intact vessels (Busch et al., 1980; Stern et al., 1985).

PF4 also binds to the chemokine receptor, Duffy (Hadley et al., 1994), which has been identified on ECs in postcapillary venules and in the splanchnic bed, even in individuals who do not express the antigen on their erythrocytes (Peiper et al., 1995). The distribution of Duffy on ECs in other vascular beds is less well studied. The binding site on PF4 for Duffy has not been deduced, and the capacity of Duffy-bound PF4 to bind heparin or HIT antibodies has not been reported. PF4 does not appear to bind to any of the other members of the chemokine receptor family (for review see Baggiolini et al., 1997), including CXCR4, the only other related receptor yet identified on ECs (Volin et al., 1998). However, PF4 exposed to leukocyte lysates is cleaved between Thr-16 and Ser-17 to yield a peptide with 30-fold greater biological activity than its parent molecule (Gupta et al., 1995). Such proteolysis may enable modified PF4 to bind to CXCR1 or R2 (formerly called IL-8R-α and -β, respectively) (for review see Baggiolini et al., 1997), but no evidence has been presented that ECs express either receptor, nor has binding of modified PF4 to CXCR4 been shown.

The CXC chemokines that contain a Glu-Leu-Arg (ELR) NH$_2$-terminal sequence induce angiogenesis, whereas those that do not, such as PF4 and γIP-10, inhibit angiogenesis (Baggiolini and Moser, 1997). The molecular basis by which PF4 inhibits angiogenesis is unclear. Considering that ECs lack CXCR1 and CXCR2, the effect of PF4 may be indirect. PF4 interrupts the binding of heparin-binding proteins, such as vascular endothelial cell growth factor (VEGF) and basic fibroblast growth factor (bFGF) to their receptors (Gengrinovitch et al., 1995; Peng et al., 1997). However, PF4 also inhibits angiogenesis stimulated by a truncated version of VEGF that lacks the heparin-binding domain (Gengrinovitch et al., 1995). Whether PF4 influences the behavior of ECs from disordered vessels in other ways, or whether the PF4-mediated signal transduction through chemokine receptors is modulated by HIT antibodies, deserves additional study.

PF4 binds preferentially to ECs at sites of angiogenesis (Hansell et al., 1995). For example, in a hamster cheek pouch model, [$^{125}$I]PF4 is taken up preferentially by ECs within the neovascularity where its binding is inhibited by the related CXC chemokine, γIP-10 (Luster et al., 1995). PF4 inhibits cell proliferation in vitro (Sharpe et al., 1990; Maione et al., 1991; Gupta and Singh, 1994) and tumor-induced neovascularization (Sharpe et al., 1990; Maione et al., 1991) in vivo. Both the heparin-binding domain (Maione et al.,
PF4 also binds to thrombomodulin (TM), a 60.3 kDa protein constitutively expressed on the surface of ECs. Binding of thrombin to TM alters its substrate specificity, such that proteolytic cleavage of protein C is accelerated 20,000-fold (Esmon, 1989). TM is posttranslationally modified by association with a chondroitin sulfate A–like GAG, which invests it with the capacity to bind cationic peptides at physiological pH. The binding of eosinophilic cationic protein, major basic protein, and histidine-rich glycoprotein to these GAG residues inhibits the function of TM, whereas the binding of PF4 (but not β-thromboglobulin or thrombospondin) increases protein C-cofactor activity 25-fold in a cell-free system (Slungaard and Key, 1994; Dudek et al., 1997). The results of one recent study suggest that PF4 may exert a physiologically relevant anticoagulant effect (Slungaard et al., 2003). Addition of PF4 to cultured endothelial cells accelerates APC generation approximately 5- to 10-fold depending on vascular origin. Injection of PF4 into primates infused with thrombin increases APC generation 2- to 3-fold and prolongs the baseline aPTT. Additional studies should clarify whether HIT antibodies interfere with the anticoagulant function of PF4 and thereby may predispose to warfarin-associated venous limb gangrene.

Recent in vitro and in vivo studies suggest potential mechanisms whereby PF4 may play a vital role in promoting atherogenesis. Human atherosclerotic lesions are invested with PF4 (Pitsilos et al., 2003). PF4 is found not only along the overlying endothelium, but also in foam cells and acellular portions of the plaque. In vitro, PF4 binds to the LDL receptor and to proteoglycans, forming ternary complexes that show limited migration into clathrin-coated pits, thereby retarding endocytosis and catabolism of LDL (Sachais et al., 2002). PF4 also binds directly to oxidized LDL, promoting foam cell formation (Nassar et al., 2003). In mice, activated platelets deposit PF4 on endothelium and monocytes, potentiating effects of P-selectin on platelet-leukocyte aggregate formation and atherosclerotic development (Huo et al., 2003). Antibodies to heparin–PF4 complexes have recently been identified as an independent predictor of myocardial infarction at 30 days in patients presenting with acute coronary ischemic syndromes (Williams et al., 2003). Thus, HIT antibodies may modify the interactions of PF4 with diseased endothelium by (1) binding to PF4/proteoglycan complexes in atherosclerotic lesions, (2) inducing formation of platelet-leukocyte aggregates (Khairy et al., 2001), or (3) binding to circulating monocytes (Pouplard et al., 2001; Arepally and Mayer, 2001), thereby increasing local inflammation and stimulating procoagulant processes.
VI. EVIDENCE FOR ENDOTHELIAL CELL ANTIBODIES IN HIT

There are limited experimental data to indicate that EC-reactive antibodies or immune complexes contribute to the development of thrombosis in patients with HIT. Over 10 years ago, one group reported that sera from essentially all patients with HIT deposit increased amounts of IgG, IgM, or IgA on HUVEC (Cines et al., 1987). Binding was reduced when the cells were pretreated with enzymes that degrade heparin or heparan sulfate, whereas addition of chondroitinase was without effect. HIT sera induced ECs to express the procoagulant tissue factor, and the expression of procoagulant activity was enhanced further in the presence of platelets. These observations were confirmed and extended by Visentin and colleagues (1994), who demonstrated that the binding of HIT antibodies of HUVEC was dependent on PF4, but not on exogenous heparin, in contrast to the requirement for both to be added for antibody binding to platelets (Fig. 2). This is consistent with the concept that PF4, released from activated platelets, can form a competent antigenic complex on the pericellular matrix of the endothelium.

The role of EC-reactive antibodies was also explored in an animal model that simulates certain aspects of HIT (Blank et al., 1997). Mice injected with IgG fractions obtained from HIT patients developed anti-idiotypic antibodies that recognized complexes between human PF4 and heparin. Furthermore, the anti-idiotypic antibodies competed with the immunizing antibodies for binding to the antigenic complex. These effects were noted when antibodies obtained from mice immunized with control IgG were studied (Fig. 3A). Additionally, mice immunized with HIT–IgG developed thrombocytopenia when exposed to heparin. Affinity-purified antiheparin–PF4 antibodies bound to murine endothelioma cells in the presence of PF4, but not β2GPI (Fig. 3B). Of interest, immunized mice did not develop overt thrombi on exposure to heparin, possibly because of insufficient circulating PF4, intrinsic differences in the balance between the procoagulant and fibrinolytic systems compared with humans, or differences in signal transduction through murine and human platelet Fcγ receptors. However, it is also possible that the difference lies in a reduced capacity of otherwise healthy mouse ECs to respond to the procoagulant stimulus induced by these antibodies, compared with the responsiveness of patients with HIT, who, in the main, comprise a more elderly population with underlying vascular disorders.

Recent studies suggest that requirements for endothelial cell activation by HIT antibodies may differ based on the vascular site. According to preliminary observations by Blank et al. (2002), anti-PF4/heparin IgG can effect activation of endothelial cells directly in microvascular sites, such as
bone marrow. However, for macrovascular endothelial cells, such as HUVECs, either antibody-mediated platelet activation (Hebert et al., 1998) or stimulation with cytokines such as TNF-α (Blank et al., 2002) appear to be necessary for activation of ECs. In this latter setting, sera or IgG from patients with HIT induced various changes in the behavior of HUVEC in the presence of platelets or TNF-α, including increased expression of E-selectin, VCAM, ICAM-1, and tissue factor, release of IL-1β, IL-6, TNF-α, and PAI-1, and adhesion of platelets or monocytes to the activated ECs. EC activation was inhibited by SR121566a (a platelet glycoprotein IIb/IIIa antagonist) and, to some extent, by apyrase and ATPγS, implicating expression of endogenous

Figure 2  Binding of IgG (A–D) and IgM (E) from the plasma of a patient with HIT to cultured human umbilical vein endothelial cells (HUVEC): (A) Plasma alone; (B) plasma plus 0.05 U/mL heparin; (C) plasma plus 10 μg/mL PF4; (D) plasma plus 0.05 U/mL heparin plus 10 μg/mL PF4; (E) binding of IgM from plasma containing 10 μg/mL PF4. Both IgG (C) and IgM (E) bound to HUVEC in the presence of PF4 alone. The binding of each was completely inhibited by heparin. (From Visentin et al., 1994.)
Figure 3  Antiendothelial antibodies in sera of mice immunized with HIT-IgG: (A) Sera from mice immunized with IgG fractions of two patients with heparin-induced thrombocytopenia, HIT-1 (□) and HIT-2 (●) or IgG from an individual not exposed to heparin, NC (■), were tested for binding to murine endothelial cells by ELISA. (B). Antiendothelial cell binding of affinity-purified anti-PF4/heparin IgG from sera of immunized mice. Affinity-purified IgG (10 μg/mL) was tested for endothelial cell binding in the absence or presence of 10 μg PF4 or β$_2$-glycoprotein-1 (β$_2$-GP I). (From Blank et al., 1997.)
platelet fibrin(ogen) and release of ADP in the process. HIT sera/IgG exerted little effect on the behavior of HUVEC in the absence of prestimulation by platelets or cytokines, even in the presence of exogenous PF4. The mechanism(s) by which platelet or cytokine activation facilitate binding of HIT antibodies to cell-associated heparin/PF4 requires further investigation.

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

The studies outlined above support the notion that platelet activation and/or an inflammatory milieu contribute to endothelial cell dysfunction, predisposing to HIT-associated thrombosis (HITT). This view of HIT as an inflammatory disorder has gained experimental support through recent findings of monocyte activation. HIT plasma or IgG stimulates monocytes to elaborate tissue factor–dependent procoagulant activity in monocytes (Pouplard et al., 2001). This procoagulant effect required small amounts of heparin to activate the monocytes in whole blood, but appeared to be heparin-independent when isolated mononuclear cells were studied, presumably by exposing cell-associated proteoglycans (Pouplard et al., 2001). Heparin-independent upregulation of monocyte tissue factor activity by HITT antibodies was confirmed in a second study using human and murine antibodies (Arepally and Mayer, 2001). In this latter study, maximal tissue factor expression was detected at 4–6 hours, suggesting that new synthesis, rather than de-encryption, was primarily responsible for increased procoagulant activity. Activation of monocytes likely requires ligation of cellular FcγRII receptors, as signaling intermediates downstream of this receptor become phosphorylated in the presence of HITT antibody (Ma and Arepally, 2002). The development of monoclonal antibodies against PF4 and PF4/heparin (Arepally et al., 2000) and the availability of a murine model of HITT should help to delineate the contribution of monocyte activation to the immune pathogenesis of thrombocytopenia and thrombosis.

VIII. PERSPECTIVE AND FUTURE DIRECTIONS

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

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Schmidt AM, Mora R, Cao R, Yan SD, Brett J, Ramakrishnan R, Tsang TC, Simionescu M, Stern D. The endothelial cell binding site for advanced glycation


I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is caused by heparin-dependent antibodies that usually recognize multimolecular complexes of platelet factor 4–heparin (PF4–H). HIT can be viewed as a clinicopathologic syndrome (Warkentin et al., 1998). This implies that a diagnosis of HIT should be based on two criteria: (1) clinically evident abnormalities, most commonly, thrombocytopenia with or without thrombosis (see Chap. 3), and (2) detection of HIT antibodies. In some ways, HIT resembles another clinicopathologic disorder, the antiphospholipid antibody (lupus anticoagulant) syndrome (Table 1).

Two major classes of assays—activation (functional) and antigen—have been developed to detect HIT antibodies (Table 2). A third (miscellaneous) group of assays will be discussed briefly at the end of this chapter.

II. ACTIVATION ASSAYS FOR HIT ANTIBODIES

A. Washed Platelet Assays

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

Preparation of Platelets for Washed Platelet Assays

1. Collect 8.4 volumes of blood from a normal donor into 1.6 volumes of acid–citrate–dextrose (ACD).
Comment. Aspirin-free normal blood donors whose platelets are known to respond well to HIT sera should be selected, as there is considerable heterogeneity to platelet activation by HIT sera among platelets obtained from different normal individuals (Warkentin et al., 1994). In Hamilton, platelets from two donors are combined. In Greifswald, platelets from four different donors selected randomly are prepared and tested individually. ABO blood group discrepancies do not affect the results of these assays (Greinacher et al., 1991).

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

Comment. Low-speed centrifugation prepares ACD-anticoagulated platelet-rich plasma (PRP). Additional ACD (111 μL/mL PRP) is added (Greifswald) to ensure that the pH of the PRP is sufficiently low (≤6.5) to prevent platelet aggregation that otherwise would occur during platelet pelleting: the platelet release reaction is triggered by close platelet contact in low calcium concentrations at physiological pH (Kinlough-Rathbone et

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**Table 2** Classification of Laboratory Tests for HIT

<table>
<thead>
<tr>
<th>Activation (functional) assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washed platelet assays</td>
</tr>
<tr>
<td>Heparin-induced platelet activation (HIPA) test: visual assessment of platelet aggregation (Greinacher et al., 1991; Eichler et al., 1999)</td>
</tr>
<tr>
<td>Serotonin release assay (SRA): quantitation of 14C-radiolabeled serotonin released from dense granules of activated platelets (Sheridan et al., 1986); chemical detection of serotonin also described (Schnell et al., 1998; Harenberg et al., 2000)</td>
</tr>
<tr>
<td>ATP release detected by luminography (Stewart et al., 1995)</td>
</tr>
<tr>
<td>Platelet microparticle assay: quantitation of platelet-derived microparticles by flow cytometry (Lee et al., 1996)</td>
</tr>
</tbody>
</table>

| Platelets in citrated platelet-rich plasma (c-PRP) |
| Platelet aggregation test (PAT): assessment of platelet aggregation using conventional aggregometry (Fratantoni et al., 1975; Chong et al., 1993a) |
| Annexin V-binding assay: quantitation by flow cytometry of annexin V binding to anionic phospholipids expressed by activated platelets (Tomer, 1997; Tomer et al., 1999) |
| Serotonin release detected by flow cytometry (Gobbi et al., 2003) |

| Antigen assays |
| Target antigen: platelet factor 4-heparin complexes |
| PF4-heparin enzyme immunoassay (PF4-H-EIA) |
| Surface-bound antigen (Amiral et al., 1992) |
| Fluid-phase antigen (Newman et al., 1998) |
| Particle gel immunoassay (Meyer et al., 1999; Eichler et al., 2001) |
| Target antigen: platelet factor 4-polyvinylsulfonate complexes |
| PF4-polyvinylsulfonate-EIA (Collins et al., 1997; Visentin et al., 2001) |
Figure 1  Schematic overview of washed platelet assays: HIT serum causes platelet activation at therapeutic (0.1–0.3 U/mL) heparin concentrations, but not in the presence of Fc receptor-blocking monoclonal antibody or high (100 U/mL) heparin concentrations. Platelet activation by HIT serum is potentiated by ADP release from platelet dense granules. Various platelet activation endpoints can be used. False-positive results can be avoided if typical reaction profiles of non-HIT platelet activation triggers are recognized [e.g., (1) residual thrombin (activation at low but not high heparin concentrations, including activation in the presence of Fc receptor-blocking monoclonal antibody; (2) immune complexes (activation at low and high heparin concentrations, both of which are inhibited by Fc receptor-blocking monoclonal antibody; and (3) thrombotic thrombocytopenic purpura (TTP) serum (variable activation in the presence of heparin that is not inhibited by Fc receptor-blocking monoclonal antibody)]. Abbreviations: ACD, acid–citrate–dextrose; ATP, adenosine triphosphate; PRP, platelet-rich plasma.
al., 1983). If the serotonin release method is used, the PRP is incubated at 37°C for 30 min with [14C]serotonin (0.1 μCi/mL of PRP added from a stock solution of 50 μCi/mL of [14C]serotonin) (Lee et al., 1996).

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

Comment. Tyrode’s buffer consists of physiological concentrations of sodium chloride (NaCl, 137 mmol/L), potassium chloride (2.7 mmol/L), calcium chloride (CaCl₂, 2 mmol/L), magnesium chloride (MgCl₂, 1.0 mmol/L), and sodium dihydrogen phosphate (NaH₂PO₄, 3.3 mmol/L); however, calcium-free and magnesium-free Tyrode’s is used in this wash step to avoid activating the coagulation factors and platelets. The low pH prevents platelets from aggregating during pelleting. Apyrase is an enzyme that degrades adenine nucleotides (i.e., accumulation of the ADP from the platelets is prevented). Azide-free bovine serum albumin (3.5 mg/mL) and hirudin (1 U/mL) are included in the wash buffer in Greifswald, but not Hamilton, although HEPES (5 mmol/L) is added to this buffer in Hamilton. Following resuspension, the platelets are incubated for 15 min at 37°C (Greifswald).

4. Pellet the washed platelets as before, and then gently resuspend the platelets into calcium- and magnesium-containing Tyrode’s buffer, pH 7.4, without apyrase or hirudin.

Comment. Following resuspension, the platelets should “rest” for 45 min at 37°C (Greifswald). The final resuspension buffer (Tyrode’s buffer at physiological pH) contains calcium (2 mmol/L) and magnesium (1 mmol/L Hamilton; 2 mmol/L Greifswald). The platelet count is adjusted to a minimum of 300 x 10⁹/L; thus, after addition of washed platelets (75 μL) to the microtiter wells containing test serum (20 μL) and heparin-buffer (5 μL in Hamilton, 10 μL in Greifswald), the final platelet concentration will be at least 215 x 10⁹/L. Apyrase must not be included in this buffer, as the ADP released during assessment of HIT-induced platelet activation is an important potentiator of the platelet Fc receptor–mediated platelet activation (Polgár et al., 1998).

Test Conditions of Heparin-Dependent Platelet Activation:
Perform Platelet Activation Studies Under Various Test Conditions

Comment. In Hamilton, sera are studied using six different reaction conditions: (1) buffer; (2) unfractionated heparin (UFH), 0.1 U/mL; (3) UFH,
0.3 U/mL; (4) UFH, 100 U/mL; (5) low molecular weight heparin (LMWH), enoxaparin, 0.1 U/mL; and (6) UFH, 0.3 U/mL plus a monoclonal antibody (IV.3) that inhibits platelet Fc receptor–mediated platelet activation. In Greifswald, routine testing is performed using (1) buffer; (2) LMWH (reviparin), 0.2 U/mL; (3) UFH, 100 U/mL; and (4) danaparoid, 0.2 U/mL to assess cross-reactivity; (5) sometimes LMWH 0.2 U/mL plus IV.3 is performed to resolve unclear results. In Greifswald, the LMWH preparation reviparin (Clivarine) is used because of its narrow molecular weight (MW) range (80% of its chains have molecular mass of 2.4–7.2 kDa, i.e., 4–12 disaccharide units) (Jeske et al., 1997); this results in more consistent formation of PF4–heparin complexes, enhancing sensitivity of the assay (Greinacher et al., 1994b). Platelets are incubated with various test and positive and negative control sera under these various reaction conditions for up to 45 min (Greifswald) or 60 min (Hamilton). The order of pipetting is important in optimizing assay results (Eichler et al., 1999) (see Table 3).

Interpretation of the Obtained Test and Control Data

Comment. Several techniques can be used to assess activation of washed platelets (see Fig. 1). The actual method of detection of platelet activation is probably less important than the technique of platelet preparation itself, including the selection of suitable platelet donors.

A positive test result is one in which heparin-dependent platelet activation occurs at therapeutic concentrations of heparin (0.1–0.3 U/mL) but is inhibited at very high (100 U/mL) heparin concentrations and in the presence of platelet Fc receptor–blocking monoclonal antibody. By assessing activation in the presence of different LMWH compounds or danaparoid, studies of in vitro cross-reactivity can be performed (discussed subsequently). It is important to ensure that control HIT sera, including one or more weak positive controls, react as expected. Given the experience from a workshop on testing for HIT antibodies (Eichler et al., 1999), we recommend exchange of weak positive control sera among laboratories for quality control.

Platelet Activation Endpoints

[14C]Serotonin release was the first activation endpoint described using washed platelets (Sheridan et al., 1986). In this method, the washed platelets are incubated with test and control serum or plasma and heparin–buffer in flat-bottomed polystyrene microtiter wells (in duplicate or triplicate), performed on a platelet shaker (shaken, not stirred). After 1 h, the reaction is halted with 100 μL of 0.5% EDTA in phosphated-buffered saline (PBS). The microtiter plates are centrifuged at 1000 g for 5 min, and 50 μL of supernatant
The order of pipetting is important. After adding serum to the microtiter plate wells, high heparin concentrations are added to the appropriate wells: this will disrupt PF4-heparin complexes that may be present in the serum. After adding washed platelets, buffer, LMWH (low concentrations), and danaparoid (for cross-reactivity testing) are added. If inhibition by monoclonal antibody IV.3 is tested, this reagent is added before addition of the washed platelet suspension. In Hamilton, the pipetting order for the SRA is (1) addition of buffer-heparin, (2) serum, and (3) platelets.

Table 3  Pipette Scheme for the HIPA Test

<table>
<thead>
<tr>
<th>Add first</th>
<th>Add second</th>
<th>Add third</th>
<th>Add last</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat-inactivated</td>
<td>UFH</td>
<td>Washed platelet</td>
<td>LMWH</td>
</tr>
<tr>
<td>patient or control</td>
<td>1050 U/mL</td>
<td>suspension</td>
<td>(2.1 anti-Xa U/mL)</td>
</tr>
<tr>
<td>serum</td>
<td>= 100 U/mL</td>
<td>(300,000 platelets/μL)</td>
<td>= 0.2 U/mL</td>
</tr>
<tr>
<td></td>
<td>(final)</td>
<td></td>
<td>(final)</td>
</tr>
<tr>
<td>Control with buffer</td>
<td>20 μL</td>
<td>75 μL</td>
<td>10 μL</td>
</tr>
<tr>
<td>Low heparin</td>
<td>20 μL</td>
<td>75 μL</td>
<td>10 μL</td>
</tr>
<tr>
<td>concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High heparin</td>
<td>20 μL</td>
<td>10 μL</td>
<td>10 μL</td>
</tr>
<tr>
<td>concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-reactivity</td>
<td>20 μL</td>
<td>75 μL</td>
<td></td>
</tr>
<tr>
<td>with danaparoid</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
fluid is transferred to tubes containing scintillation fluid for detection of 
$[^{14}\text{C}]$serotonin released during platelet activation.

Carbon-14 is a radioisotope with a long half-life (5730 years) that emits 
$\beta$-particles (electrons). Laboratories require special licenses to handle radio-
isotopes, thus limiting widespread use of this platelet-activation marker. 
However, it is also possible to quantitate serotonin by nonradioactive analysis 
(Schnell et al., 1998; Harenberg et al., 2000; Gobbi et al., 2003). Results are 
expressed as percentage of serotonin released. This is calculated based on 
comparison with maximal possible release (determined following detergent-
induced platelet lysis), adjusted for background release (determined by 
quantitating serotonin release from a sample incubated with buffer alone). 
Acceptable experiments should have less than 5% background release, with 
both buffer and negative control serum testing negative for HIT antibodies.

Aggregation of Washed Platelets. A convenient and useful activation 
endpoint—platelet aggregation—was reported in the heparin-induced plate-
let activation (HIPA) assay (Greinacher et al., 1991; Eichler et al., 1999). Test 
serum and heparin–buffer are placed in U-bottomed polystyrene microtiter 
wells containing two stainless steel spheres, and the platelets are stirred at 
approximately 500 rpm, using a magnetic stirrer. At 5-min intervals the wells 
are examined against an indirect light source: a change in appearance of the 
reaction mixture from turbidity (nonaggregated platelets) to transparency 
(aggregated platelets) is a positive result. Although the activation endpoint is 
evaluated subjectively, interobserver agreement is good. A further advantage 
of this technique is its repeated evaluation of platelet activation over time. 
Thus, strong HIT sera that cause the typical activation profile of HIT (i.e., 
activation at low, but not high, heparin concentrations) within 15–30 min are 
readily identified. In contrast, such a strong HIT serum might eventually 
cause platelet activation even at the high heparin concentration and thus 
cause an “indeterminate” reaction pattern (activation at both low and high 
heparin concentrations) if activation is assessed at a later time point only. 
Occasionally there is interference with visual interpretation (e.g., a lipemic 
serum).

Luminography. Stewart et al. (1995) reported luminography to detect 
platelet activation, using a commercially available lumiaggregometer. Aden-
osine triphosphate (ATP) is released from platelet dense granules during 
platelet activation. In the presence of luciferin-luciferase reagent, a light flash 
is generated in the presence of ATP, which is detected and quantitated. 
Another group reported similar results using a standard scintillation counter 
(Teitel et al., 1996). It is uncertain how the sensitivity and specificity of these 
assays compare with other markers of platelet activation.
Platelet-Derived Microparticle Generation. Generation of platelet-derived microparticles occurs when washed platelets are activated by HIT sera (Warkentin et al., 1994). With use of a fluorescein-labeled anti-GPIbα murine monoclonal antibody, a method for quantitating microparticles using flow cytometry was reported by Lee and coworkers (1996). Although both platelets and microparticles bind fluorescein-labeled anti-GPIbα monoclonal antibody, they can be distinguished by their size and scatter parameters using flow cytometry, with microparticles quantitated in relation to platelet numbers (Lee et al., 1996).

Heat Inactivation of Patient Serum or Plasma

To avoid thrombin-induced platelet activation in buffer containing physiological calcium, steps are taken to inactivate residual thrombin. Thus, plasma and serum must first be heat inactivated before use in these assays. Heating at 56°C for 30–45 min inactivates thrombin and complement. Fibrin and other precipitates are removed by high-speed centrifugation (8000 g for 5 min). More intense heating of serum (63°C for 20 min) forms platelet-activating immune complexes (Warkentin et al., 1994); thus, if a patient sample shows heparin-independent platelet activation (indeterminate result), another sample aliquot should be heat inactivated, and the HIT assay repeated. Often, this will result in disappearance of the initial artifact that presumably was caused by too intense heat inactivation. Serum is preferred for use in functional HIT assays in our laboratories, as serum contains more PF4, thereby facilitating initial formation of the antigen.

Biological Basis for High Sensitivity of Washed Platelets to Activation by HIT Antibodies

Table 4 lists differences between using washed platelets and platelets suspended in citrate-anticoagulated plasma to study HIT antibody-mediated platelet activation. Some of these differences may be important in explaining the greater sensitivity and specificity of washed platelets to detect HIT antibodies.

1. Baseline platelet activation, including platelet granule release, occurs during preparation of washed platelets. This enhances the platelet-binding capacity for heparin (Horne and Chao, 1989) and may also increase the availability of PF4 to form the target antigen.

2. Apyrase is used to prevent accumulation of ADP during platelet washing. This prevents platelets from becoming refractory to sub-
Table 4  Comparison Between Citrated Platelet-Rich Plasma and Washed Platelet Assays

<table>
<thead>
<tr>
<th>Technical aspects</th>
<th>Washed platelet assay</th>
<th>Platelet-rich plasma assay</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet preparation</td>
<td>High g centrifugation during washing: increased baseline platelet activation</td>
<td>Low g centrifugation: less baseline platelet activation</td>
<td>Availability of PF4 may be higher using washed platelets (greater formation of PF4-heparin antigen complexes)</td>
</tr>
<tr>
<td>Apyrase</td>
<td>Apyrase added to wash solution, but not to the final resuspension (reaction) buffer</td>
<td>No apyrase used (no wash steps)</td>
<td>Apyrase degrades ADP, and prevents its accumulation; thus, platelet refractoriness to ADP-mediated potentiation of HIT serum-induced platelet activation is avoided by apyrase</td>
</tr>
<tr>
<td>Reaction milieu</td>
<td>Physiological calcium concentration (2 mmol/L)</td>
<td>Low (micromolar) calcium owing to citrate</td>
<td>IgG-mediated platelet activation optimal with physiological calcium concentrations</td>
</tr>
<tr>
<td>IgG levels</td>
<td>Reduced IgG levels during final reaction</td>
<td>Normal plasma IgG levels</td>
<td>Reduced inhibition of Fc receptor-mediated platelet activation by IgG in washed platelet assays</td>
</tr>
<tr>
<td>Plasma protein levels</td>
<td>Reduced plasma protein levels</td>
<td>Normal plasma protein levels</td>
<td>Reduced nonidiosyncratic platelet activation by heparin using washed platelets (?)</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room temperature</td>
<td>37°C</td>
<td>Unknown significance</td>
</tr>
<tr>
<td>Reaction assessment</td>
<td>Microtiter plates</td>
<td>Conventional aggregometer</td>
<td>Many assays performed simultaneously using microtiter plates</td>
</tr>
</tbody>
</table>

See text for further details on differences between washed platelet and citrated platelet-rich plasma assays (pp. 279–282).
sequent ADP-mediated platelet activation (Ardlie et al., 1970). Empirically, apyrase grade III (Sigma) is acceptable for use: grades I and II are too impure, and grades IV and higher are expensive.

3. Physiological calcium concentrations are present when washed platelets are used. Under these conditions, ADP produces only primary platelet aggregation. However, as observed by Packham et al. (1971), traces of immunoglobulin complexes in amounts too low to cause aggregation themselves will cause secondary aggregation to occur following the addition of ADP. Recently, the importance of ADP in mediating HIT antibody-induced platelet activation has been reported by Polgár and colleagues (1998). Thus, the reaction conditions that exist when washed platelets are used appear to maximize HIT antibody-induced platelet activation because the platelets retain sensitivity to ADP-mediated platelet activation.

4. Low concentrations of IgG are present in the final washed platelet reaction mixture: there is a fivefold reduction in IgG compared with citrated platelet-rich plasma (c-PRP) assays, because only IgG from the test serum is present in the final reaction mixture. Chong and colleagues (1993a) showed that high plasma IgG levels in one platelet donor’s blood seemed to explain the discrepancy between studies using donor c-PRP (poor reactivity) and donor washed platelets (good reactivity). These and other investigators (Greinacher et al., 1994c) also observed that addition of IgG inhibits HIT serum-induced activation of washed platelets in a dose-dependent fashion.

5. Low concentrations of fibrinogen and other plasma proteins could reduce the potential for nonidiosyncratic heparin-induced platelet activation (Salzman et al., 1980; Chong et al., 1993a). In contrast, low concentrations of heparin rarely cause significant activation of washed platelets. It is possible that acute-phase reactant proteins such as fibrinogen could lead to false-positive activation assays for HIT using c-PRP.

6. Room temperature conditions are used for washed platelet assays; in contrast, c-PRP studies are performed at 37°C. Although this is a major difference between the assays, it is unknown whether there are advantages or disadvantages of performing washed platelet assays at room temperature. In Greifswald, all buffers are warmed to 37°C, and all incubation steps are performed at this temperature; only the final incubation on the microtiter plates is performed at room temperature.

7. Multiple serum–platelet reactions in microtiter plates can be performed, and even several hundred reactions studied in parallel. Quality control is thereby enhanced by the large number of control
and test reaction conditions that can be analyzed, and the long incubation period employed (up to 60 min). The incubation period in HIT assays should be at least 20–30 min (Stewart et al., 1995).

Quality Control in Washed Platelet Assays for HIT

The variable reactivity of donor platelets to HIT sera is an important issue in activation assays for HIT. It has long been recognized that inconsistent results can be obtained using these assays (Salem and van der Weyden, 1983; Pfueller and David, 1986; Warkentin et al., 1992).

Hierarchical Versus Idiosyncratic Platelet Activation by HIT Sera.

The results of a systematic investigation, summarized in Table 5, showed that both HIT sera and platelet donors exhibit variable reactivity in a hierarchical, rather than an idiosyncratic, manner. The strongest reactions were produced by strong HIT sera against strongly reactive platelet donors. All of the negative reactions occurred when the weakest sera were mixed with the weakest platelets. Importantly, no unexpected negative reactions occurred elsewhere in the 10 × 10 serum-platelet grid (see Table 5). Furthermore, the relative ranking of platelet donors appeared to be stable over time, an observation also reported by Chong and colleagues (1993a) in their study of platelet donor variability using c-PRP.

The finding of a hierarchical pattern of reactivity has important implications for quality control in diagnostic testing for HIT using activation assays. First, it indicates that platelets from certain donors who tend to respond well to HIT sera should be chosen. Second, relatively weak HIT sera should be included as positive controls (∼20–50% serotonin release, or 25-min lag time in the HIPA).

Heparin-Independent Platelet Activation: Indeterminate Results.

About 5% of test sera or plasma give an indeterminate result in an activation assay. This is defined as platelet activation that occurs at both therapeutic (0.1–0.3 U/mL) and supratherapeutic (10–100 U/mL) heparin concentrations. Often an interpretable result is obtained when the assay is repeated using another heat-inactivated aliquot. This suggests that the first result may have been an artifact caused by heat-aggregated IgG generated ex vivo. However, some serum and plasma samples repeatedly demonstrate heparin-independent platelet activation. Biological explanations include circulating immune complexes (e.g., systemic lupus erythematosus), high-titer HLA class I alloantibodies, and, possibly, other platelet-activating factors (e.g., thrombotic thrombocytopenic purpura). An antigen assay is required for further investigation when an indeterminate result is consistently obtained.
Table 5  Reactivities of Ten HIT Sera with Platelets from Ten Normal Donors

<table>
<thead>
<tr>
<th>HIT sera (S1–S10)</th>
<th>Normal platelet donors: strongest (P1) to weakest (P10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1  84.3</td>
</tr>
<tr>
<td>S1  85.4</td>
<td>++++</td>
</tr>
<tr>
<td>S2  84.4</td>
<td>++++</td>
</tr>
<tr>
<td>S3  69.1</td>
<td>++++</td>
</tr>
<tr>
<td>S4  61.0</td>
<td>++++</td>
</tr>
<tr>
<td>S5  56.4</td>
<td>++++</td>
</tr>
<tr>
<td>S6  50.7</td>
<td>++++</td>
</tr>
<tr>
<td>S7  44.1</td>
<td>++++</td>
</tr>
<tr>
<td>S8  30.1</td>
<td>++++</td>
</tr>
<tr>
<td>S9  24.2</td>
<td>++++</td>
</tr>
<tr>
<td>S10 11.3</td>
<td>++++</td>
</tr>
</tbody>
</table>

Serum samples and platelet donors are ranked from strongest to weakest (S1–S10 and P1–P10, respectively), according to the mean percentage of [14C] serotonin release when considering all 100 serum–platelet donor pairs (10 pairs corresponding to each HIT serum and each normal platelet donor). For each serum–platelet donor pair, the individual amount of serotonin release is summarized as follows: 80–100%, ++++; 60–79% release, +++; 40–59% release, ++; 20–39% release, +; <20% release, –. Overall, there is a graded pattern of reactivity among the individual reaction pairs that is hierarchical (i.e., there are no unexpected weak or strong reactions among the pairs). All negative reactions (<20% release) were found in the lower right portion of the table. Conversely, the strongest reactions (≥80% release) were found in the upper left portion of the table.

Inhibition by High Heparin Concentrations. Sheridan and colleagues (1986) first emphasized that there was a relatively specific activation profile triggered by HIT sera and plasmas: activation at therapeutic heparin concentrations (maximal at 0.1–0.3 U/mL) that progressively diminished with increasing heparin concentrations, typically falling to background activation at very high (100 U/mL) heparin concentrations. Classically, a positive test was defined as greater than 20% serotonin release at 0.1 U/mL heparin and less than 20% serotonin release at 100 U/mL heparin. These criteria should not be applied indiscriminately, however. For example, a very strong HIT serum could produce more than a 90% release at 0.1 U/mL heparin and 25% release at 100 U/mL heparin. Alternatively, a serum or plasma sample that was not adequately heat-inactivated could produce a similar reaction profile (i.e., residual thrombin is inhibited by the high, but not low, heparin concentration). The strength of reactivity caused by patient serum can be helpful: clinically significant HIT antibodies almost always cause more than 50% serotonin release using optimally reactive platelets (Warkentin et al., 2000; Warkentin and Hedde, 2003). In the HIPA test, differences in the lag time to platelet aggregation provide useful information.

Inhibition by Fc Receptor Blockade. Platelet activation by HIT antibodies is inhibited in the presence of a murine IgG2b monoclonal antibody (IV.3) that recognizes the platelet FcγIIa receptor (Kelton et al., 1988; Chong et al., 1989) and can be used to enhance test specificity.

Interpretation of Platelet Activation by HIT Serum in the Absence of Added Heparin

With activation assays, it is not uncommon for HIT serum or plasma to cause platelet activation, even in the absence of added heparin. Usually, even greater platelet activation occurs in the presence of added heparin. When strong serum-dependent platelet activation occurs with buffer and at a 0.1–0.3 U/mL heparin concentration, it is important to ensure that the other reactions (at 100 U/mL heparin, and 0.1–0.3 U/mL heparin together with Fc receptor blockade) are as expected. This is because residual thrombin could produce strong platelet activation in both the absence and presence of low heparin concentration, thereby causing the potential for a false-positive result.

There are at least two potential explanation for strong platelet activation in the absence of added heparin. First, there may be residual heparin in the sample (White et al., 1992; Pötzsch et al., 1996). However, this phenomenon can persist despite attempts to remove heparin using binding resins. Further, heparin-independent platelet activation can be a feature of serum obtained from patients with “delayed-onset HIT,” in which the presence of residual heparin is unlikely because onset of thrombocytopenia and throm-
basis begins several days after the patient’s last exposure to heparin (Warkentin and Kelton, 2001) (see Chap. 3). A second explanation is that some HIT antibodies recognize platelet-bound PF4 in the absence of an exogenous source of heparin, perhaps by PF4 bound to platelet glycosaminoglycans. Alternatively, as HIT antibodies are heterogeneous, there may be pathogenic antibody subpopulations that bind relatively well to PF4 even in the absence of heparin or heparin-like molecules (Newman and Chong, 1999; Amiral et al., 2000). This phenomenon has implications for the interpretation of tests of cross-reactivity of LMWH and danaparoid, as discussed later.

Disadvantages of Washed Platelet Assays

The major disadvantage of washed platelet assays to detect HIT antibodies is that they are technically demanding and labor-intensive. A workshop that compared a washed platelet assay (the HIPA test) and an antigen assay showed greater variability in activation assay results among the participating laboratories (Eichler et al., 1999). Washed platelet activation assays are best suited for reference laboratories assessing many HIT sera, as this facilitates acquisition of sufficient technical experience to perform the assay successfully on a consistent basis. Assay-specific disadvantages include the requirement for radioactivity (SRA), the use of a subjective, visual endpoint (HIPA), and expensive equipment (flow cytometry–based assays).

B. Activation Assays Using Citrate-Anticoagulated Blood

The first reports describing the use of normal donor c-PRP to detect platelet activation caused by HIT serum or plasma appeared in the 1970s (Rhodes et al., 1973; Fratantoni et al., 1975; Babcock et al., 1976). A ratio of serum (or plasma) to c-PRP between 0.66 and 1.0 was used (e.g., 200 μL serum added to 200–300 μL c-PRP). No standardized method has evolved, however, although a survey of 54 laboratories in France (Nguyen et al., 1994) found some practices to be more common. For example, most laboratories test patient citrated platelet-poor plasma (c-PPP) rather than heat-inactivated serum. Variable heparin concentrations are used, most commonly between 0.5 and 1.0 U/mL. The ratio of patient c-PPP to donor c-PRP is usually 1:1, and ABO discrepancies are usually ignored. About 75% of the laboratories use at least two platelet donors for diagnostic testing.

Testing for HIT Antibodies Using c-PRP

The following description of the assay taken from Chong and colleagues (1989, 1993a) has the highest reported sensitivity and specificity among c-PRP
methods. Blood is obtained from normal blood donors whose platelets respond well to serum or plasma from HIT patients, and c-PRP is prepared. Testing involves addition of 150 µL of patient heat-inactivated c-PPP or serum to 340 µL of c-PRP (final platelet concentration, 250–350 × 10^9/L) at 37°C. The platelets are monitored for a few minutes to exclude nonspecific platelet aggregation. After addition of 10 µL heparin-saline, aggregation is monitored over the next 15 min or until aggregation has occurred. A positive result is an increase in light transmission of more than 25% above baseline in the presence of therapeutic-dose heparin (0.5 U/mL) and patient serum or c-PPP and inhibition of aggregation in the presence of patient serum or plasma and supratherapeutic-dose heparin (100 U/mL). Use of such a two-point assay reduced the false-positive rate, as serum or plasma from some patients without HIT caused platelet aggregation at all heparin concentrations tested. To ensure that the platelets are functional, platelets are also tested with collagen (2 µg/mL). Details on methodology of c-PRP assays are also given elsewhere (Kapsch and Silver, 1981; Almeida et al., 1998).

Some workers report that platelets from a patient with HIT are very reactive to heparin-dependent activation by their own serum or plasma (Kappa et al., 1987; Chong et al., 1993b). Use of autologous c-PRP can sometimes be limited by the patient’s thrombocytopenia, however. Potential explanations for the high sensitivity of autologous platelets include persisting high Fc receptor expression on platelets of patients with acute HIT (Chong et al., 1993b) and baseline platelet activation (Chong et al., 1994), with the potential for higher PF4 availability.

Disadvantages of c-PRP Aggregation Assays

Problems with these assays include (1) potential for false-positive interpretation if heparin produces nonspecific aggregation of donor platelets, an effect that could be enhanced nonspecifically by proaggregatory factors in the patient serum or plasma, and (2) risk of false-negative interpretation if HIT serum–induced platelet aggregation begins even before addition of heparin.

Nonspecific activation of platelets by heparin occurs with some normal donor c-PRP (Chong et al., 1993a), rendering these donors unsuitable for diagnostic testing. It is also possible that plasma from very sick patients may be more likely to cause nonspecific aggregation of platelets in c-PRP in the presence of heparin (Goodfellow et al., 1998).

An important practical disadvantage is that only a limited number of platelet aggregation tracings can be performed using conventional aggregometers. Thus, relatively few reactions with a limited number of patient and control samples can be evaluated.
Other Assays Using Citrated-Anticoagulated Whole Blood or Platelet-Rich Plasma

Tomer (1997) reported a c-PRP activation assay for HIT antibodies in which the activation endpoint is quantitation of binding of fluorescein-labeled recombinant annexin V to platelets, as detected using flow cytometry. Annexin V, a placental protein, interacts with the prothrombinase-binding anionic phospholipids expressed on the surface of activated platelets and correlates with platelet procoagulant activity. It is uncertain whether the reaction conditions employed (e.g., 30-min incubation at 26°C) or the high sensitivity of annexin V binding (300-fold increase over baseline) overcomes the inherent limitations of sensitivity observed with other assays using c-PRP. Recently, Gobbi and colleagues (2003) developed a flow cytometry assay modeled after that of Tomer (1997), except that loss of serotonin from platelet granules was used as the platelet activation endpoint.

C. Comparison of Washed Platelet and c-PRP Activation Assays

It became evident during the mid-1980s that the sensitivity of c-PRP aggregation assays for HIT was relatively poor (Kelton et al., 1984; Pfueller et al., 1986). Favaloro and colleagues (1992) first compared the c-PRP aggregation assay with the washed platelet SRA. They observed that only 6 of 13 HIT sera or plasmas that tested positive in the SRA also tested positive in the c-PRP aggregation assay. In contrast, no sample was identified that tested positive only in the aggregation assay. Chong and colleagues (1993a) also found a higher sensitivity for the SRA method. However, considerable variability in sensitivity for HIT antibodies among the various platelet donors was seen, ranging from 39–81% (c-PRP assay) to 65–94% (washed platelet SRA).

Strong evidence in favor of a higher sensitivity for washed platelet assays was provided by direct comparison using platelets prepared and tested in parallel that were obtained simultaneously from the same platelet donors (Greinacher et al., 1994a). Only 23 of 70 HIT sera that tested positive by the HIPA assay also tested positive using c-PRP aggregation. In contrast, all but 1 of 24 sera testing positive in the c-PRP aggregation assay also tested positive in the HIPA test.

More recently, Walenga and colleagues (1999) also found a lower sensitivity of the c-PRP aggregation test compared with the SRA. In contrast, Pouplard and co-workers (1999) reported a similarly high sensitivity of the c-PRP as the SRA (91% vs. 88%), but with a lower specificity (77% vs. 100%).
III. ANTIGEN ASSAYS FOR HIT ANTIBODIES

A. Solid-Phase Enzyme Immunoassay

The solid-phase enzyme immunoassay (EIA) has been described in detail (Amiral et al., 1992; Visentin et al., 1994; Greinacher et al., 1994a; Amiral et al., 1995; Horsewood et al., 1996). Methods differ in the way that PF4–heparin complexes are coated on the microtiter wells. A general scheme is shown in Fig. 2. In this assay, stoichiometric concentrations of PF4 and heparin (e.g., 50 μL each of 20 μg/mL PF4 and 1 U/mL UFH) dissolved in phosphate buffer are added together to the wells of a microtiter plate and incubated at 4°C overnight. After washing with phosphate buffer saline–Tween 20 (PBS–Tw), the wells are “blocked” with a protein-containing solution such as PBS–Tw containing either 10% normal goat serum (NGS) or 20% fetal calf serum, followed by washing with PBS–Tw. To perform the assay, 50–100 μL of test or control plasma or serum diluted 1:50 in PBS containing 2% NGS is added to duplicate wells for 1 h at room temperature. After thorough washing with PBS–Tw, bound immunoglobulin is detected by adding alkaline phosphatase–conjugated goat antihuman immunoglobulin (e.g., affinity-purified goat antihuman IgG Fc diluted 1:1000 in PBS–Tw-2% NGS) followed by incubation for 1 h at room temperature. After thorough washing, incubation with p-nitrophenyl phosphate in 1 M diethanolamine buffer is added. After incubation in the dark, the reaction is stopped with 1 N NaOH, and absorbance is read at 405 nm using an automated microplate.

![Figure 2](image-url)
B. **PF4–Polyvinylsulfonate Antigen Assay**

Several negatively charged substances can cause the cryptic autoepitope within PF4 to become recognizable to HIT antibodies (see Chaps. 6–8). Indeed, a commercial assay for HIT using PF4 complexed with polyvinylsulfonate has been developed (Collins et al., 1997; Visentin et al., 2001). Sensitivity and reproducibility were high using polyvinylsulfonate that had been fractionated to a relatively uniform molecular weight (5000 ± 500 Da). Some technical advantages of this assay include the observation that the ratio of PF4/PVS is not critical (cf. PF4–heparin), with acceptable concentrations of PVS ranging from 0.1 to 100 μmol/L for a corresponding concentration of 10 μg/mL PF4. The antigen complex is also stable for long periods.

Table 6 compares the two commercial EIAs. In the laboratory in Greifswald, discrepant results between the two assays have been observed in about 15% of patient samples tested.

Some research laboratories perform “in-house” PF4-dependent EIAs to detect HIT antibodies. An advantage is that an EIA can be used that only detects HIT antibodies of the IgG class (Warkentin et al., 2000; Lindhoff-Last et al., 2001; Untch et al., 2002). This improves test specificity, as PF4–heparin-reactive IgA and IgM class antibodies (which are detected in the two commercial EIAs) are unlikely to cause HIT.

C. **Fluid-Phase EIA**

The fluid-phase EIA for HIT antibodies (Newman et al., 1998) is an adaption of a staphylococcal protein A antibody-capture EIA method (Nagi et al., 1993). By permitting antibody–antigen interactions to occur in a fluid phase, problems of protein (antigen) denaturation inherent in solid-phase assays are avoided.

Platelet factor 4 (5% biotinylated) is mixed with an optimal concentration of heparin, and this antigen mixture is incubated with diluted patient serum or plasma (Fig. 3). Subsequently, the antigen–antibody mixture is incubated with protein G-Sepharose in a microcentrifuge tube. Biotinylated antigen–antibody complexes become bound to the protein G–Sepharose by antibody Fc, and the complexes, are separated from unbound-antigen by centrifugation and washing. The amount of biotin–PF4–heparin–antibody complexes immobilized to the beads is measured using peroxidase substrate after initial incubation with streptavidin-conjugated peroxidase.
The fluid-phase EIA appears to have lower rate of false-positive reactions. This may be because in the solid-phase-EIA, nonspecific binding of IgG to the microtiter wells can occur. Furthermore, the cryptic antigen site of PF4 can be exposed when the molecule comes into close contact with the plastic surface, even in the absence of heparin (Newman and Chong, 1999). The fluid-phase assay avoids these problems by first precipitating all reactive IgG antibodies, then detecting the antigen specifically bound to the IgG. Thus, higher concentrations of patient serum or plasma can be tested without increasing nonspecific reactivity. The advantages of this assay in performing in vitro cross-reactivity are discussed later. Because antibody is bound using-

Table 6  Comparison of Two Commercial Antigen Assays for HIT Antibodies

<table>
<thead>
<tr>
<th></th>
<th>Asserachrom® (Stago)</th>
<th>GTI-PF4 EIA (GTI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target antigen</strong></td>
<td>PF4–heparin complex</td>
<td>PF4–polyvinyl sulfonate complex</td>
</tr>
<tr>
<td><strong>Source of PF4</strong></td>
<td>Recombinant PF4</td>
<td>PF4 purified from outdated platelets</td>
</tr>
<tr>
<td><strong>Microwell strips provided</strong></td>
<td>Six strips of eight wells, in three nonresealable pouches</td>
<td>Twelve strips of eight wells (in resealable pouches)</td>
</tr>
<tr>
<td><strong>Sample required</strong></td>
<td>Plasma (sodium citrate) 200 μL diluted 1:100 (≈ 2 μL plasma per well)</td>
<td>Serum or plasma 50 μL diluted 1:50 (≈ 1 μL serum/plasma per well)</td>
</tr>
<tr>
<td><strong>Controls supplied</strong></td>
<td>Positive and negative control lyophylate, calibrated standard</td>
<td>Positive and negative control sera</td>
</tr>
<tr>
<td><strong>Covers supplied</strong></td>
<td>One provided</td>
<td>Multiple provided</td>
</tr>
<tr>
<td><strong>Incubation times and conditions</strong></td>
<td>All at 22°C (~2 h total)</td>
<td>37°C (two steps); then 22°C (~2 h total)</td>
</tr>
<tr>
<td><strong>Plate reader settings</strong></td>
<td>492 nm</td>
<td>405 or 410 nm</td>
</tr>
<tr>
<td><strong>Detecting antibody system</strong></td>
<td>Goat anti-IgG/A/M (peroxidase-conjugated)</td>
<td>Goat anti-IgG/A/M (alkaline phosphatase-conjugated)</td>
</tr>
<tr>
<td><strong>Reaction stopping solution</strong></td>
<td>3 M sulfuric acid or 1 M hydrochloric acid</td>
<td>3 M sodium hydroxide</td>
</tr>
<tr>
<td><strong>Cutoff from negative</strong></td>
<td>Internal control reagent is used to calibrate</td>
<td>&gt;0.4 OD (assumes controls react as expected: pos ≥ 1.8, neg ≤ 0.2)</td>
</tr>
</tbody>
</table>

* The Asserachrom® assay provides lyophylized control sera, whereas handling of control sera in the GTI assay is similar to handling of the test sera/plasma.

protein G–Sepharose, IgM and IgA anti-PF4–heparin antibodies are not detected in this assay.

Wang and coworkers (1999a,b) used protein A to capture IgG antibodies from HIT patient serum. The immobilized antibodies were then incubated with normal serum (presumed to contain PF4) and fluorescence-labeled heparin. The amount of fluorescence dye bound to the protein A sepharose was used to detect HIT antibodies. Major drawbacks of this approach include the initial capturing of IgG other than HIT-IgG, as well as the unpredictable PF4/heparin ratios.

D. Particle Gel Immunoassay (ID-H/PF4 test)

This assay adopts the ID microcolumn system, in wide use for routine red blood cell serology. Known as the H/PF4–PaGIA assay (DiaMed, Switzerland), red-dyed, high-density polystyrene particles coated with PF4–heparin complexes serve as the solid phase. To perform this assay, 20 μL of these PF4–heparin–coated microbeads and 10 μL of patient serum are added to the incubation chamber of the microcolumn card. After a 5-min incubation, the card is centrifuged. In the presence of anti-PF4–heparin antibodies, the particles are agglutinated and remain at the top of the column (strong positive), or dispersed within the gel (weak positive); nonagglutinated particles precipitate with HIT-IgG.
itate to the bottom (negative) (Meyer et al., 1999; Eichler et al., 2001). The assay is technically easy, can be performed rapidly, and is readily automated. Results are read visually.

Eichler and colleagues (2001) compared this new assay with two functional assays (HIPA test; SRA) and both commercially available solid-phase PF4-dependent EIAs. In preselected samples, the H/PF4–PaGIA had a sensitivity intermediate between that of the functional and commercial antigen assays. The specificity appeared to resemble that of the functional assays.

In contrast, Risch and coworkers (2003) found many more sera to test positive using the H/PF4–PaGIA, compared to a commercial EIA (Assochrom®), among 42 patients sampled 10–18 days following cardiac surgery (69% vs. 26%). Since none of the patients had clinical evidence for HIT, this suggested the diagnostic specificity of the H/PF4–PaGIA to be far less than the solid-phase EIA. These authors did not test sera from patients with HIT, and therefore were unable to assess test sensitivity (Warkentin, 2003b).

The manufacturer’s instructions indicate that the assay is to be read as “positive” (any agglutination within the gel), “negative” (no agglutination) using neat (undiluted) serum, or “borderline.” However, when a positive or borderline test result was obtained, Alberio et al. (2003) repeated the assay with undiluted and serially diluted plasma (up to 1 in 1024) until the result was negative. The reported titer was the last positive result followed by either borderline or negative results. Patients judged clinically to have had “probable” or “highly probable/definite” HIT had antibody titers of 4 or more in 39 of 54 (72%) cases, compared with only 2 of 85 (2%) judged “unlikely” to have had HIT. Further, all 19 of the patient samples that tested positive in a c-PRP aggregation assay tested positive in the PaGIA (generally, in a titer of 8 or higher). Among all patients studied, the percentage with associated thrombotic complications increased from 8% (negative or low titer) to 55% (positive titer 4–16) to 74% (positive titer 32–256). This study suggests that reporting quantitatively the results of the HPF4–PaGIA—with a titer of 4 or more being clinically significant—may increase diagnostic usefulness.

E. Comparison of Activation and Antigen Assays

Both PF4–heparin–EIA and washed platelet activation assays have approximately equal sensitivity for clinical HIT (Greinacher et al., 1994a; Warkentin et al., 2000). For serum or plasma samples that are known to be positive by one sensitive washed platelet activation assay (e.g., SRA or HIPA), the corresponding probability of the PF4–heparin–EIA for confirming the positive result is at least 75–90% (Greinacher et al., 1994a; Aarepally et al., 1995). Conversely, a similar percentage of referred samples that test positive in the
EIA will also test positive using a washed platelet activation assay (Greinacher et al., 1994a). The sensitivity of both EIA and SRA was even higher (>90%) for detecting antibodies that caused HIT in prospectively studied postoperative patients (Warkentin et al., 2000).

Although both antigen and activation assays have similarly high sensitivity for clinical HIT, there is evidence that antigen assays have greater sensitivity for detecting HIT antibodies not associated with thrombocytopenia or other clinical events (Amiral et al., 1995; Arepally et al., 1995; Bauer et al., 1997; Warkentin et al., 2000) (Fig. 4). Stated another way, the SRA is more specific for clinical HIT than the antigen assay. The biological explanation for greater specificity of a sensitive activation assay for clinical HIT, compared with an antigen assay, could relate to the functional heterogeneity of HIT antibodies against antigenic determinants on PF4, only some of which activate platelets strongly (Amiral et al., 2000). Data reported by Visentin and colleagues (1994) also support a higher sensitivity of antigen assays for detecting HIT antibodies. These workers studied 12 HIT plasmas that tested positive in both SRA and PF4–heparin–EIA. However, at a 1:100 sample dilution, only 2 of the 12 samples still tested positive in the activation assay. In contrast, even at a 1:200 dilution, all 12 plasmas still tested positive in the EIA. Bachelot and colleagues (1998) observed that HIT plasmas that tested only weakly positive in the PF4–heparin–EIA tended to give negative washed platelet SRA results when using platelets with the least reactive FcγIIA receptor genotype, Arg131 (see Chap. 9).

The difference in sensitivity for HIT antibodies between the PF4–heparin–EIA and aggregation studies using c-PRP is considerable. Only about 33–64% of samples that test positive in the PF4–heparin–EIA also test positive using c-PRP aggregation (Greinacher et al., 1994a; Nguyen et al., 1995; Rugeri, et al., 1999). Although one laboratory reported a greater sensitivity using c-PRP aggregation than the EIA (Look et al., 1997), these workers did not employ a two-point method, and so may have observed false-positive results using the aggregation assay.

Table 7 summarizes possible explanations for discrepancies in results of activation and antigen assays for HIT.

### IV. INTERPRETATION OF HIT TEST RESULTS

It is important to incorporate clinical information into the interpretation of any laboratory result for HIT. This is because thrombocytopenia, whether or not caused by HIT, is common in hospitalized patients receiving heparin, and because nonpathogenic HIT antibodies are often detected by sensitive assays in patients who have received heparin for 5 or more days.
Several clinical scoring methods have been described to help estimate the probability of HIT independently of the HIT antibody test results (Greinacher et al., 1994a; Pouplard et al., 1997; Warkentin, 2003a; Warkentin and Heddle, 2003). Some include assessing the platelet count recovery upon stopping heparin, and so may be more useful when reviewing a case after its clinical evolution. Chapter 3 provides an example of one scoring system to estimate the pretest probability of HIT that can be applied at the time of initial diagnostic assessment.

A. Rapid Versus Typical Onset of Thrombocytopenia

Diagnostic algorithms taking into account the pretest probability of HIT are shown in Fig. 5A (activation assay screen) and 5B (antigen assay screen). We have organized the diagnostic approach based on the timing of onset of thrombocytopenia, either rapid (<5 days) or typical (≥5 days) (see Chap. 3).

In general, there are two broad pretest probabilities for patients with rapid thrombocytopenia: low and high. Patients with low pretest probability for HIT are those who have not recently been exposed to heparin (thus, they would not be expected to have circulating HIT antibodies, or to have generated them so quickly), or who have another good explanation for thrombocytopenia. (An important caveat is that sometimes a recent heparin exposure is not known to the patient or has not been documented in the medical records.) With a low pretest probability for HIT, either of the sensitive assays for HIT (washed platelet activation assay or antigen assay) can reliably rule out HIT. However, an unexpected negative result in a patient with a high pretest probability, or an unexpected positive result in a patient

Figure 4 Comparison of activation and antigen assays for HIT-IgG: analysis of prospective studies. Quantitative results of an activation assay, the SRA, are shown on the x-axis (although samples that gave <20% serotonin release are shown without reference to the actual quantitative result obtained [see box designated <20%]); quantitative results of the antigen assay (which detected only IgG anti-PF4–H antibodies) are shown on the y-axis. (A) Orthopedic surgery patients who received UFH; (B) orthopedic surgery patients who received LMWH; and (C) cardiac surgery patients who also received postoperative UFH. The arrows indicate the data points corresponding to the 15 patients who developed clinical HIT (>50% platelet count fall from the postoperative peak). The data show similarly high sensitivity of the activation and antigen assays for clinical HIT; however, the activation assay had higher specificity for clinical HIT. Most sera (13/15, 87%) from patients with clinical HIT strongly activated platelets (>80% serotonin release). (From Warkentin et al., 2000.)
Table 7  Discrepancies in Results of HIT Antibody Testing: Possible Explanations and Implications

<table>
<thead>
<tr>
<th>c-PRP aggregation assay</th>
<th>Washed platelet activation assay (SRA or HIPA)</th>
<th>PF4–H–EIA</th>
<th>Interpretation (assumes clinical picture compatible with HIT)</th>
<th>Possible explanations and implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Not HIT</td>
<td>Continue or resume heparin</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>HIT</td>
<td>Stop heparin and give alternative anti-coagulant</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Not HIT</td>
<td>False-positive aggregation assay (see text); continue or resume heparin</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>HIT caused by antibodies against “minor” antigen</td>
<td>Stop heparin and give alternative anti-coagulant</td>
</tr>
<tr>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>HIT (weak-moderate antibodies)</td>
<td>Stop heparin and give alternative anti-coagulant</td>
</tr>
<tr>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Weak HIT-IgG antibodies; IgM/IgA anti-PF4–H antibodies</td>
<td>Stop heparin and give alternative anticoagulant if clinical picture warrants; otherwise, maintain heparin</td>
</tr>
<tr>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Possible thrombin artifact or HIT caused by antibodies against “minor” antigen</td>
<td>Repeat assay with a new heat-inactivated serum aliquot; stop heparin and give alternative anticoagulant if clinical picture warrants</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>HIT (false-negative washed platelet assay)</td>
<td>Stop heparin and give alternative anticoagulant; check washed platelet activation assay method</td>
</tr>
</tbody>
</table>

Pos, positive test result; Neg, negative test result.
WASHED PLATELET ACTIVATION ASSAY SCREEN

Figure 5  (A) Diagnostic algorithm using a washed platelet activation assay as a screening test for HIT, either the serotonin-release assay (SRA) or the heparin-induced platelet activation (HIPA) test. (B) Diagnostic algorithm using PF4–H–EIA as a screening test for HIT. Abbreviations: IND, indeterminate test result; NEG, negative test result; POS, positive test result. The asterisk (*) in B indicates that a washed platelet activation assay could be useful in a patient with moderate pretest probability for HIT and a positive PF4–heparin–EIA, as a positive activation assay has a higher specificity for clinical HIT.
with a low pretest probability, should lead to repeating the test or performance of the complementary activation or antigen assay. Additionally, further clinical information should be sought (e.g., Has another explanation for the thrombocytopenia become apparent? Could the patient have had an unrecognized recent heparin exposure?).

In contrast, for patients with the typical temporal onset of thrombocytopenia (i.e., a platelet count fall that begins 5–10 days after beginning heparin treatment), we believe that, in general, there are two different pretest probabilities for HIT: moderate and high. Because HIT is a relatively common explanation for thrombocytopenia that begins during this charac-
teristic time period, it should be considered a plausible diagnosis even if another possible explanation for thrombocytopenia is identified (hence, a moderate pretest probability). In a patient without another apparent explanation for thrombocytopenia, or one in whom an unexplained new thrombotic event has occurred, the pretest probability for HIT would be considered to be high.

B. Diagnostic Interpretation of Laboratory Results

In patients with a high pretest probability of HIT who have a negative screening test, the test should be repeated and the complementary activation or antigen assay should be performed. The diagnosis of HIT is very unlikely if both activation and antigen assays are negative. In patients with a moderate pretest probability who have one or more positive tests for HIT, the final diagnosis may well rest on the overall clinical picture, rather than on the test result alone (Fig. 6). This conclusion results from two clinical realities: (1) sensitive HIT assays frequently detect clinically insignificant HIT antibodies in patients who have received heparin for more than 5 days, and (2) thrombocytopenia—whether caused by HIT or not—is common in clinical practice (see Chap. 4). There is evidence that positive washed platelet activation assays for HIT have greater diagnostic specificity for clinical HIT (Warkentin et al., 2000), especially when strong, rapid platelet activation is produced by patient serum. Regardless, these considerations underscore the importance of conceptualizing HIT as a clinicopathologic syndrome, in which both clinical information and results of HIT antibody testing are used for diagnosis.

The diagnostic usefulness of certain laboratory tests for HIT is shown in Fig. 7 (Warkentin, 2003b). Both the SRA and an in-house EIA that detect only HIT-IgG were very sensitive and specific for clinical HIT in post–orthopedic surgery patients. The diagnostic usefulness of these assays was somewhat less in a post–cardiac surgery population. For example, among post–cardiac surgery patients, the likelihood ratio of a strong-positive SRA result (90% serotonin release; see open circle in Fig. 7) is about 20. The likelihood ratio, which is defined as the extent to which a given test result alters the physician’s estimate of the pretest probability of HIT, is defined as sensitivity/1 – specificity. In this example, the corresponding likelihood ratio is 0.70/(1 – 0.965) = 20. Thus, if the physician had estimated a pretest probability of 50% (odds of 0.5:0.5), then this test result would increase the posttest probability to more than 95% (0.5:0.5 × 20:1 = 20:1, or 95.2%). In contrast, the high sensitivity of this assay to detect clinically important HIT antibodies (>95%) means that a negative test result lowers the posttest probability to less than 5%.
The diagnostic impact of such a strong-positive SRA result (90% serotonin release) is even greater in post–orthopedic surgery patients, for whom the corresponding likelihood ratio is about 85 (0.85/1-0.99). As before, a negative test result essentially rules out HIT.

Although the EIA that detects only HIT-IgG antibodies has lower diagnostic specificity than the SRA, it remains a useful assay. The likelihood ratios for a strong positive test result (e.g., optical density of 1.5) range from about 10 to 40 for post–cardiac and post–orthopedic surgery patients, respectively. Also, its high sensitivity (>95%) means that a negative test generally rules out HIT.

Figure 6  Diagnosis of HIT in doubt: Although this 71-year-old patient with moderate pretest probability of HIT had positive laboratory testing for HIT antibodies, the clinical course casts doubt on the actual role HIT played in the thrombocytopenia and thrombotic events. Thrombocytopenia began on day 5 of heparin treatment (nadir, 31 × 10^9/L; day 8), together with clinical and laboratory evidence for septicemia. Laboratory testing for HIT antibodies was strongly positive by activation assay (SRA, 90% release at 0.1 U/mL heparin, <5% release at 100 U/mL heparin) and weakly positive by PF4–heparin–EIA: O.D. = 0.709). Clinical evidence for HIT includes the symptomatic DVT and (possible) pulmonary embolism; however, the dramatic increase in platelet count during therapeutic-dose heparin treatment and the bacteremia are strong evidence for septicemia, rather than HIT, as an explanation for the thrombocytopenia.

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Thus, HIT antibody testing is among the most useful of platelet immunology assays. For comparison, Fig. 7 also shows the profile of a “noninformative assay” (see line A). This is the profile for various tests of “platelet-associated IgG” for the diagnosis of autoimmune thrombocytopenia. Certain glycoprotein-specific platelet antibody tests have operating characteristics intermediate between those for HIT and a noninformative assay. For example, the MAIPA (monoclonal antibody immobilization of platelet antigens) assay has only moderate sensitivity but high specificity for diagnosis of autoimmune thrombocytopenia (see Chap. 2).

Figure 7  Sensitivity-specificity tradeoffs for diagnosis of HIT (receiver operating characteristic curve analysis). The arrows indicate various cut-offs between positive and negative test results, e.g., the open circle indicates 90% serotonin release (post-cardiac surgery patient) using a washed platelet activation assay (serotonin release assay, or SRA). The likelihood ratio for HIT for a given positive test result can be estimated from the graph, using the formula: likelihood ratio = sensitivity/(1 - specificity). Thus, for 90% serotonin release (post-cardiac surgery), the estimated likelihood ratio is 0.7/(1 - 0.965) = 20. (From Warkentin, 2003b.)
A practical implication of Fig. 7 is that the magnitude of a positive HIT antibody test provides diagnostically useful information, with a strong positive result associated with a greater likelihood of a patient having clinical HIT than a weak positive result (Warkentin, 2003b). Similarly, if two sensitive and complementary tests for HIT antibodies (washed platelet activation assay, PF4-dependent EIA) both give negative test results, the diagnosis of HIT is virtually excluded (even in a patient with a high pretest probability).

V. IN VITRO CROSS-REACTIVITY

A. Cross-Reactivity Using Activation Assays

Cross-reactivity studies have been performed most frequently using activation assays. However, there are no standard methods for, or even a standard definition of, in vitro cross-reactivity. In one study of LMWH and danaparoid cross-reactivity, an increase in platelet activation in the presence of the drug over baseline was used to determine cross-reactivity (Warkentin, 1996). This definition was used to avoid falsely attributing cross-reactivity to drug-independent platelet activation that is produced by some patients’ sera. The reason for this definition was the common phenomenon that platelet activation can be caused by a patient’s serum even in the absence of added heparin. In the HIPA test, comparison of the lag time to aggregation can be used to judge cross-reactivity: if a sample shows platelet aggregation with heparinoid or LMWH earlier than in the presence of buffer, then cross-reactivity is present. In general, in vitro cross-reactivity with danaparoid is usually clinically insignificant (Warkentin, 1996; Newman et al., 1998) (see Chap. 14).

Comparison of c-PRP Versus Washed Platelet Assays

Sensitive washed platelet assays generally show almost 100% cross-reactivity of HIT antibodies for LMWH (Greinacher et al., 1992; Warkentin et al., 1995a). Indeed, UFH and LMWH are essentially indistinguishable in these assays. However, very different results have been reported by investigators using c-PRP assays (Makhoul et al., 1986; Chong et al., 1989; Kikta et al., 1993; Vun et al., 1996). Here, LMWH consistently shows less cross-reactivity compared with UFH. It is possible that differences in nonidiosyncratic heparin-induced platelet activation underlie these observations (see Chap. 5): UFH is more likely to result in weak platelet activation, including some PF4 release, that leads to amplification of the platelet activation response in the presence of PF4–heparin-reactive HIT antibodies. In contrast, in washed
platelet assays, IgG-mediated platelet activation, but not nonidiosyncratic heparin-induced platelet activation, occurs.

B. Cross-Reactivity Using Antigen Assays

Although it is theoretically possible to perform a solid-phase EIA to assess cross-reactivity (Amiral et al., 1995), this is complicated because the antigen has to be coated as a complex to the solid phase. This problem has been overcome in a fluid-phase EIA described by Newman and colleagues (1998). Because this assay detects binding to a defined quantity of labeled PF4-containing antigen, the assay is able to determine in vitro cross-reactivity more accurately than the solid-phase EIA. These investigators observed an in vitro cross-reactivity rate of 88% for LMWH; about half the HIT samples reacted weakly against danaparoid in their study. The fluid-phase EIA has also been used to show that the antithrombin-binding pentasaccharide, fondaparinux, does not cross-read with HIT-IgG antibodies (Warkentin et al., 2003).

VI. MISCELLANEOUS ASSAYS FOR HIT ANTIBODIES

A third group of assays detect heparin-dependent immunoglobulin binding to platelet membranes (Griffiths and Dzik, 1997). None of these assays appear to be sufficiently reliable to have gained widespread acceptance for the diagnosis of HIT. This may be related to the fundamental pathogenesis of HIT: the pathogenic platelet-activating properties of HIT antibodies appear to be mediated by relatively few antibodies. Thus, despite producing strong platelet activation, concomitant evaluation of heparin-dependent IgG binding to platelets by flow cytometry, for instance, was either no greater than background (Warkentin et al., 1994) or, in the presence of PF4, only an average of 5.6 times background (Visentin et al., 1994). Furthermore, only 7 of 12 sera exhibited any detectable IgG binding by flow cytometry, even though all 12 were positive using conventional activation or antigen assays (Visentin et al., 1994).

Assays have been developed that attempt to measure an increase in heparin-dependent platelet-bindable immunoglobulin in the presence of HIT serum (Howe and Lynch, 1985; Lynch and Howe, 1985; Gruel et al., 1991), but these whole-platelet EIA methods have been superceded by the more specific PF4–heparin–EIA described earlier. Immunofluorescence assays have also been reported (Wolf et al., 1983; Silberman and Kovarik, 1987).

A commercially available platelet-bindable IgG assay that uses anti-IgG-coated indicator red cells, known as the solid-phase red cell adherence
assay (SPRCA), has been developed (Sinor et al., 1990; Sinor and Stone, 1994; Leach et al., 1994, 1995, 1997). Platelets are coated onto U-shaped microtiter platelet wells, and either heparin-serum (immune complex method), or heparin-albumin with subsequent addition of serum after a wash step (hapten method), is added. After washing, red cells coated with anti-IgG are added to the wells. Following centrifugation, the appearance of the indicator red cells on the well bottoms is scored on a 10-point scale, ranging from negative (tight red cell button) to strongly positive (diffuse red cell binding on the well bottom). According to Leach and colleagues (1997), both immune complex and hapten reaction profiles are commonly seen with putative HIT sera (immune complex > both > hapten). However, only limited comparisons with conventional HIT assays have been performed, and the diagnostic usefulness of these assays is unknown. Disadvantages include the subjective scoring system, as well as the need to ensure that HLA and platelet-specific alloantibodies do not interfere with testing.

There have been attempts to identify specific binding of heparin-dependent antibodies to platelet glycoproteins using immunoblotting and immunoprecipitation (Lynch and Howe, 1985; Howe and Lynch, 1985; Greinacher et al., 1994d). However, no study has identified a consistent reaction profile diagnostic of HIT. There is thus no experimental evidence implicating the involvement of platelet glycoproteins as an immune target in the pathogenesis of HIT.

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Pseudo–Heparin-Induced Thrombocytopenia

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I. INTRODUCTION

A. The Concept of Pseudo-HIT

Heparin-induced thrombocytopenia (HIT) is strongly associated with life- and limb-threatening venous and arterial thrombosis, including pulmonary embolism, venous limb gangrene, and large vessel arterial occlusion. However, HIT is by no means a unique explanation for the combination of thrombocytopenia and thrombosis (Table 1).

In these pseudo-HIT disorders, thrombocytopenia usually occurs early during the course of heparin treatment. This could reflect the prothrombotic process associated with the patient’s primary diagnosis. Alternatively, heparin could exacerbate the platelet count fall by nonimmune proaggregatory effects on platelets (see Chap. 5). If the patient previously received heparin, physicians might consider HIT in the differential diagnosis of the platelet count fall.

However, one pseudo-HIT syndrome in particular closely resembles even the typical day 5–10 timing of thrombocytopenia characteristic of HIT: adenocarcinoma-associated disseminated intravascular coagulation (DIC). In these patients, the fall in platelet count begins soon after stopping heparin treatment. Because the patients usually will have received heparin for 5–10 days to treat adenocarcinoma-associated thrombosis, the timing of the onset of thrombocytopenia closely resembles immune HIT. Furthermore, the fre-
quent occurrence of new or progressive thrombosis in this setting also sug-

This chapter draws attention to those clinical disorders that can mimic and, thereby, be confused with HIT. This is not a trivial distinction: whereas heparin is contraindicated in patients with HIT, it often is the optimal treatment of patients with pseudo-HIT. Second, the close clinical parallels between HIT and certain pseudo-HIT disorders can provide insights into the pathogenesis of thrombosis. For example, the recognition that venous limb gangrene can complicate metastatic adenocarcinoma, and the clinical paral-

<table>
<thead>
<tr>
<th>Pseudo-HIT disorder</th>
<th>Pathogenesis of thrombocytopenia and thrombosis</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>DIC secondary to procoagulant material(s) produced by neoplastic cells</td>
<td>Late&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>Platelet activation by clot-bound thrombin</td>
<td>Early&lt;sup&gt;b&lt;/sup&gt; or late&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic ketoacidosis</td>
<td>Hyperaggregable platelets in ketoacidosis (?)</td>
<td>Early&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antiphospholipid antibody syndrome</td>
<td>Multiple mechanisms described, including platelet activation by antiphospholipid antibodies (?)</td>
<td>Early</td>
</tr>
<tr>
<td>Thrombolytic therapy</td>
<td>Platelet activation by thrombin bound to fibrin degradation products (?)</td>
<td>Early&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Septicemia-associated purpura fulminans</td>
<td>Symmetrical peripheral gangrene secondary to DIC with depletion of protein C and antithrombin</td>
<td>Early</td>
</tr>
<tr>
<td>Infective endocarditis</td>
<td>Infection-associated thrombocytopenia; ischemic events secondary to septic emboli</td>
<td>Early</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>Platelets susceptible to complement-mediated damage; platelet hypoproduction</td>
<td>Early</td>
</tr>
<tr>
<td>Posttransfusion purpura (PTP)</td>
<td>“Pseudospecific” alloantibody-mediated platelet destruction (exception: bleeding, not thrombosis)</td>
<td>Late</td>
</tr>
</tbody>
</table>

These pseudo-HIT disorders can mimic HIT by causing thrombocytopenia and thrombosis in association with heparin treatment. An exception is PTP, which causes bleeding, but not thrombosis; however, PTP can resemble HIT because both disorders usually occur about a week after major surgery requiring blood and postoperative heparin. The pseudo-HIT disorders can be categorized based on whether the onset of thrombocytopenia is typically “early” (<5 days) or “late” (≥5 days) in relation to the heparin.

<sup>a</sup> See Fig. 1 for an example of pseudo-HIT caused by adenocarcinoma-associated DIC.
<sup>b</sup> See Fig. 3 for early thrombocytopenia associated with pulmonary embolism.
<sup>c</sup> See Fig. 4 for late thrombocytopenia associated with pulmonary embolism.
<sup>d</sup> See Fig. 5 for early thrombocytopenia associated with diabetic ketoacidosis.
<sup>e</sup> See Fig. 6 for early thrombocytopenia caused by thrombolytic therapy.
lels with a similar syndrome in HIT patients, suggests that a common factor (coumarin anticoagulation) may play a crucial pathogenic role in both disorders (Warkentin, 2001). Likewise, similarities between HIT and the lupus anticoagulant syndrome suggest that they could also share common pathogenic mechanisms (Arnout, 1996, 2000; Gruel, 2000).

II. PSEUDO-HIT SYNDROMES

A. Adenocarcinoma

Mucin-producing adenocarcinoma is an important cause of venous and arterial thrombosis that occurs in association with thrombocytopenia. In these patients, DIC is often the explanation for the thrombocytopenia. The diagnosis is suggested by reduced fibrinogen levels (or prolonged thrombin time), elevated prothrombin time, and elevated cross-linked (d-dimer) fibrin degradation products (or a positive protamine sulfate "paracoagulation" test).

Adenocarcinoma-associated DIC can strongly resemble HIT (Fig. 1). Typically, a patient presents with idiopathic deep vein thrombosis (DVT), sometimes with mild to moderate thrombocytopenia. During treatment with therapeutic-dose unfractionated or low molecular weight heparin (LMWH) the platelet count rises, likely because of improved control of DIC by the heparin. In my experience, this often dramatic rise in the platelet count during heparin treatment of "idiopathic" DVT is a clinically useful marker for adenocarcinoma-associated DIC. During the 5- to 10-day period of heparin treatment with overlapping warfarin anticoagulation, no problems are encountered. However, there is rapid recurrence of thrombocytopenia within hours or days of discontinuing the heparin, despite apparent therapeutic anticoagulation with warfarin, during which time the patient develops new or progressive venous, or even arterial, thrombosis. Thus, the onset of thrombocytopenia and thrombosis may occur within the 5- to 10-day "window" that suggests HIT.

Venous Limb Gangrene Complicating Adenocarcinoma

The venous thrombotic events complicating adenocarcinoma include DVT, phlegmasia cerulea dolens, and even venous limb gangrene (Everett and Jones, 1986; Adamson and Currie, 1993). Clinical and laboratory parallels between HIT and adenocarcinoma suggest that, paradoxically, coumarin treatment could contribute to the pathogenesis of venous gangrene in these patients through a disturbance in procoagulant-anticoagulant balance (Warkentin, 1996, 2001). Figure 2 summarizes the proposed pathogenesis of this
syndrome from the perspective of the characteristic clinical triad of venous limb gangrene: (1) thrombocytopenia caused by HIT or adenocarcinoma-associated DIC; (2) acute DVT with acral (distal) microvascular thrombosis; and (3) a supratherapeutic international normalized ratio (INR) associated with coumarin therapy.

Venous limb gangrene appears to result from failure of the protein C anticoagulant pathway to down-regulate thrombin generation within the microvasculature (Warkentin 1996; Warkentin et al., 1997; see Chap. 3). Here, the elevated INR may represent a surrogate marker for marked reduction in functional protein C levels (by a parallel reduction in factor VII); the thrombocytopenia is a surrogate marker for uncontrolled thrombin generation associated either with HIT or adenocarcinoma (see Fig. 2). As
venous limb gangrene occurs in a limb with preceding active DVT, this suggests that local factors, such as direct extension of thrombosis, as well as exacerbation of distal thrombosis by venous stasis, contribute to large- and small-vessel thrombosis characteristic of this syndrome.

Venous thrombosis complicating adenocarcinoma, especially when complicated by DIC or severe venous ischemia or necrosis, should be treated with heparin, rather than warfarin or other coumarin anticoagulants. Reversal of warfarin anticoagulation (with vitamin K and plasma infusion, but not with prothrombin complex concentrates, as these do not contain sufficient protein C) and prompt control of DIC with heparin could salvage a limb with severe phlegmasia, or limit damage in a patient with venous gangrene. An effective agent often is LMWH (Prandoni, 1997; Lee et al., 2003). I recom-
mend intermittent monitoring using antifactor Xa levels, because some patients with heparin resistance require high doses of LMWH to achieve therapeutic anticoagulation.

Ironically, one of the problems of heparin in these patients is its efficacy: thus, if heparin is discontinued for any reason, rapid recurrence of thrombocytopenia and thrombosis can result. Figure 1 shows an example in which thrombocytopenia and pulmonary embolism occurred (day 21) when heparin was held for a few hours to permit a liver biopsy to diagnose metastatic carcinoma. I have also observed a patient with lung adenocarcinoma in whom heparin was held to permit limb amputation; postanesthesia, the patient was aphasic (intraoperative stroke). Often, recurrent thrombosis is as “malignant” as the cancer itself.

B. Pulmonary Embolism

Mild thrombocytopenia is common in patients with pulmonary embolism. Sometimes the thrombocytopenia is severe and associated with laboratory markers of DIC (Stahl et al., 1984; Mustafa et al., 1989) (Fig. 3). The thrombocytopenia presumably results from thrombin-induced platelet activation. Large thromboemboli within the high-flow pulmonary vessels may act as a reservoir for clot-bound thrombin that is relatively protected from inhibition by antithrombin-dependent inhibitors (Weitz et al., 1990). This view is indirectly supported by the observation that thrombocytopenia commonly occurs in patients with pulmonary embolism, but not in patients with DVT alone (Monreal et al., 1991; Warkentin et al., 2003a). Further, increased heparin clearance has been demonstrated in experimental pulmonary embolism (Chiu et al., 1977).

Because HIT is also strongly associated with pulmonary embolism (Warkentin et al., 1995, 2003a), a diagnostic and therapeutic dilemma results when a patient presents with pulmonary embolism and thrombocytopenia 5 or more days after surgery managed with postoperative heparin prophylaxis (Fig. 4). Initiating therapeutic heparin could have catastrophic consequences for the patient who has circulating HIT antibodies, although in sufficient doses it is effective for a patient with pulmonary embolism and DIC without HIT. Because these two possibilities cannot be readily distinguished on clinical grounds alone, one should manage such a patient with an alternative anticoagulant until the results of HIT antibody testing become available (Warkentin, 2000).

C. Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) can be associated with acute thromboembolic complications. Evidence for in vivo platelet activation was observed in one
Figure 3  Pseudo–HIT secondary to pulmonary embolism and DIC: An obese, 50-year-old man with paraplegia was admitted for recurrent hypotension. He initially received twice-daily (b.i.d.) subcutaneous (sc), unfractionated heparin (UFH) for antithrombotic prophylaxis, as the initial diagnosis was septicemia. Deep vein thrombosis (DVT) and pulmonary embolism (PE) were then diagnosed (Dx), and therapy changed to intravenous UFH, 1200 U/h. The platelet count fell over 4 days to a nadir of $30 \times 10^9$/L; danaparoid sodium (DS) was given because of concern over possible HIT (there was a remote history of previous heparin use). An echocardiogram showed large right atrial thrombus (likely representing a leg vein embolus), and the patient was transferred to a cardiac surgical center. The platelet count fall was judged too rapid to be HIT (see Chap. 3), a viewpoint supported by negative testing for HIT antibodies by serotonin-release assay (SRA) and PF4–heparin enzyme-linked immunosorbent assay (EIA). UFH administration was restarted in higher doses with antifactor Xa monitoring to overcome heparin resistance. Recurrent hypotension occurred when the right atrial thrombus embolized; full hemodynamic and platelet count recovery occurred following tissue plasminogen activator (t-PA) administration, followed by UFH, then low molecular weight heparin (LMWH), and (later) warfarin treatment. The patient was well at 3-year follow-up, without evidence of carcinoma.
Figure 4  Pseudo-HIT associated with pulmonary embolism versus HIT: (A) A patient developed a platelet count fall from 387 to $159 \times 10^9/L$ (59% fall) that began on day 7 of UFH prophylaxis following orthopedic surgery. Pulmonary embolism (PE) was diagnosed by pulmonary angiography on postoperative day 11. The platelet count fell during initial intravenous heparin therapy, rising only when sufficient UFH was given (2360 U/h) to overcome "heparin resistance" (as shown by subtherapeutic activated partial thromboplastin times, aPTTs). HIT antibodies were not detectable (day 12), either by serotonin-release assay (SRA, <5% release) or PF4–heparin–EIA (optical density, 0.149; negative, <0.450). (B) A platelet count profile similar to that seen in Fig. 4A also occurred in a patient who developed a platelet count fall from 378 to $161 \times 10^9/L$ (57% fall) that began on day 7 after cardiac surgery in which unfractionated heparin (UFH) was given for cardiopulmonary bypass (CPB). The platelet count recovered on therapeutic-dose danaparoid. Only one clinical clue pointed to the diagnosis of HIT: erythematous skin lesions at the UFH injection sites were also observed on day 7 (not shown on figure). Testing for HIT antibodies was strongly positive in the SRA (98% release at 0.1 U/mL heparin; 0% release at 100 U/mL heparin and at 0.1 U/mL heparin in the presence of Fc receptor–blocking monoclonal antibody). The similar platelet count profiles between these patients illustrate the difficulty in determining on clinical grounds whether postoperative PE is caused by HIT or not.
study of 10 patients who had elevated plasma levels of platelet factor 4 and β-
thromboglobulin during DKA that resolved following recovery (Campbell et al., 1985) Evidence for activation of coagulation includes elevated fibrin
degradation products and reduced antithrombin (Paton, 1981). Figure 5
illustrates a patient with “white clots” in the femoral artery, leading to
amputation, who was initially thought to have HIT. However, HIT antibody
testing and subsequent clinical events proved that the patient did not have
HIT as the initial explanation for this dramatic clinical presentation of
thrombocytopenia and thrombosis complicating DKA (although HIT oc-
curred later in the clinical course). I am also aware of a patient with essential
thrombocytemia who developed postoperative DKA, thrombocytopenia, and
bilateral lower limb artery thrombosis that occurred too early during
UFH prophylaxis (days 2–3) to have been caused by immune HIT. A similar
example of early-onset severe thrombocytopenia and arterial thrombosis
resulting in amputation of an arm was reported in a patient with diabetic
ketoacidosis and adult respiratory distress syndrome (ARDS) (Phillips et al.,
1994). Although the authors suggested HIT secondary to heparin “flushes” as
the diagnosis, pseudo–HIT seems more likely based upon the temporal
features of the case, as well as the negative laboratory testing for HIT
antibodies.

D. Antiphospholipid Antibody Syndrome or Lupus
Anticoagulant Syndrome

Clinical Features

Antiphospholipid antibodies can be detected either as “lupus anticoagu-
lants” or as anticoagulant antibodies (Asherson et al., 1989; Ginsberg et al.,
1995) (see Chap. 11). Antiphospholipid antibody syndrome (APLAS) is char-
acterized by increased risk for thrombosis and recurrent fetal loss; limb or
intra-abdominal vein thrombosis, cerebral venous (dural sinus) thrombosis,
nonatheromatous arterial thrombosis, cardiac valvulitis, and microvascular
thrombosis (e.g., acrocyanosis, “blue toe syndrome” digital ulceration or
gangrene, livedo reticularis) are described (Hojnik et al., 1996; Gibson et al.,
1997). Many patients have thrombocytopenia (Morgan et al., 1993; Galli et
al., 1996), which is typically mild and intermittent. The explanation for throm-
boctopenia is uncertain: some patients have platelet-reactive autoantibodies
(Galli et al., 1994; Lipp et al., 1998), but platelet-activating effects of IgG are
also suspected.

The explanation for the prothrombotic tendency of APLAS is also
elusive. A multifactorial pathogenesis is likely, because the antibodies recog-
nize complexes of negatively charged phospholipids with many different pro-
Figure 5  Pseudo-HIT during diabetic ketoacidosis (DKA), later complicated by HIT: A 27-year-old man developed rapid onset of thrombocytopenia and white clots in the left femoral artery (at a femoral artery catheter site) during management of DKA that included prophylactic-dose unfractionated heparin (UFH). HIT was suspected erroneously on the basis of a possible previous remote heparin exposure (gastric surgery 10 years earlier). The patient underwent two embolectomies as well as treatment with urokinase and intravenous (iv) danaparoid. The patient developed a second platelet count fall during danaparoid treatment that began on day 6 in relation to the initial course of UFH. Tests for HIT antibodies changed from negative (serotonin-release assay [SRA]: days 1 and 4, serotonin release <5%) to positive (days 9 and 12, serotonin release 92% and 80%, respectively). By PF4–heparin–EIA (set up to detect IgG antibodies), the day 1 sample also was negative (O.D., 0.262; negative, <0.450), the day 4 sample was weakly positive (0.804), and the day 9 and 12 samples were strongly positive (1.863 and 1.002, respectively). Although the possibility of in vivo cross-reactivity of danaparoid with the HIT antibodies is suggested by the thrombocytopenia and progression of limb ischemia, the platelet count subsequently rose during danaparoid treatment, and no additional thromboembolic events occurred. In vitro cross-reactivity was detected on the day 9, but not the day 12, blood sample.
tein cofactors, such as β₂-glycoprotein I (β₂GP I), prothrombin, protein C, protein S, and annexin V (Galli, 1996; Tripllett, 1996) (see Chap. 2). Indeed, interference with endothelial cell function, impaired fibrinolysis, disturbances in protein C anticoagulant pathway activities, and antibody-mediated platelet activation have all been described (for review see Petri, 1997; Gruel, 2000; Arnout and Vermyle, 2003).

**Parallels Between APLAS and HIT**

Table 2 lists some common features of APLAS and HIT. Both clinicopathologic disorders are characterized by thrombocytopenia, a paradoxical risk for venous and arterial thrombosis, and associated antibodies that can be detected by either functional or antigen assays (see Chap. 11): Moreover, for both APLAS and HIT, positive functional assays are more strongly associated with thrombosis than positive antigen assays (Ginsberg et al., 1995; Table 2

<table>
<thead>
<tr>
<th>Clinical Parallels Between HIT and APLAS</th>
<th>Heparin-induced thrombocytopenia (HIT)</th>
<th>Antiphospholipid antibody syndrome (APLAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombotic paradox</strong></td>
<td>Thrombosis despite thrombocytopenia</td>
<td>Thrombosis despite prolonged coagulation tests (± thrombocytopenia)</td>
</tr>
<tr>
<td><strong>Spectrum of thrombotic events</strong></td>
<td>Venous ≥ arterial thrombosis; adrenal infarction, dural sinus thrombosis</td>
<td>Venous ≥ arterial thrombosis; adrenal infarction, dural sinus thrombosis</td>
</tr>
<tr>
<td><strong>Severity of thrombocytopenia</strong></td>
<td>Mild to moderate thrombocytopenia</td>
<td>Mild to moderate thrombocytopenia</td>
</tr>
<tr>
<td><strong>Laboratory diagnosis</strong></td>
<td>(1) Platelet activation assays (e.g., serotonin release assay, heparin-induced platelet activation test); (2) platelet factor 4–heparin–EIA</td>
<td>(1) Lupus anticoagulant (i.e., prolonged phospholipid-dependent coagulation assay in presence of patient plasma); (2) β₂GP I-dependent anticardiolipin–EIA</td>
</tr>
<tr>
<td><strong>Pathogenesis</strong></td>
<td>Platelet activation by platelet Fc receptors; endothelial activation by immune injury</td>
<td>Uncertain pathogenesis: immune platelet activation and endothelial injury are possible factors</td>
</tr>
</tbody>
</table>

Further laboratory parallels between HIT and APLAS are discussed in Chap. 11.

*Abbreviations: β₂GP I, β₂ glycoprotein I; EIA, enzyme-linked immunosorbent assay.*
Warkentin et al., 2000; Galli et al., 2003). The parallels between these disorders led Arnout (1996) to hypothesize that IgG-mediated platelet activation could explain thrombosis in APLAS. Supportive experimental data include the observations that antiphospholipid antibodies enhance platelet activation induced by other agonists (Martinuzzo et al., 1993). Furthermore, Arvieux et al. (1993) observed that murine monoclonal antibodies reactive against β2GP I induced platelet activation in the presence of subthreshold concentrations of ADP and epinephrine, an effect dependent on binding to platelet FcγIIa receptors. However, other workers were unable to demonstrate enhanced platelet activation in the presence of IgG antiphospholipid antibodies (Shi et al., 1993; Ford et al., 1998) or showed no role for platelet FcγIIa receptors (Lutters et al., 2001; Jankowski et al., 2003).

Thrombocytopenia in Patients with APLAS Receiving Heparin

In retrospective studies, Auger and colleagues (1995) reported that platelet counts typically fell by about 50% in patients with chronic thromboembolic disease and the lupus anticoagulant who were treated with heparin. Neither timing of the onset of thrombocytopenia nor results of specific antigen or activation assays for HIT antibodies were reported, so it remains uncertain whether these patients had (immune) HIT. It is possible that nonidiosyncratic platelet activation caused by heparin could increase the thrombocytopenic potential of antiphospholipid antibodies in the absence of HIT antibodies. Alternatively, some patients with APLAS may have low levels of circulating HIT antibodies even in the absence of previous heparin exposure (Lasne et al., 1997; Martinuzzo et al., 1999). Recently, this view has gained some experimental support. Bourhim and colleagues (2003) showed that affinity-purified IgM anti-β2GP I from a patient with APLAS gave a positive reaction in PF4-dependent EIAs. Further, mice actively immunized with the purified IgM anti-β2GP I generated anti-β2GP I antibodies (via an idiotype–anti-idiotype mechanism) that also cross-reacted with PF4–heparin.

E. Thrombolytic Therapy

Acute thrombocytopenia is common in patients treated with streptokinase, especially when combined with heparin (Balduini et al., 1993) (Fig. 6). This could represent a direct, activating stimulus of heparin on platelets that perhaps is exacerbated by procoagulant effects of thrombolytic therapy. For example, fibrin degradation products generated by thrombolytic agents bind and protect thrombin from inhibition by heparin (Weitz et al., 1998). Such a mechanism could explain thrombocytopenia after use of any thrombolytic drug.
However, some investigators have reported that plasma containing anti-streptokinase antibodies can activate platelets through their Fc receptors in the presence of streptokinase (Vaughan et al., 1988; Lebrazi et al., 1995; Regnault et al., 2003). Thus, high-titer antistreptokinase antibodies found in some normal individuals could explain the occasional occurrence of thrombocytopenia and thrombosis following treatment with streptokinase.

**F. Septicemia-Associated Purpura Fulminans**

Septicemia complicated by DIC occasionally results in progressive ischemia and necrosis of fingers or hands and toes or feet, producing a syndrome of symmetrical peripheral gangrene also known as purpura fulminans (Knight et al., 2000). The association with DIC suggests that increased thrombin generation in vivo, together with severe consumption and depletion of natural anticoagulant factors (e.g., protein C, protein S, antithrombin), leads to

![Figure 6](image_url)
dysregulated fibrin deposition in the microvasculature. Other contributing factors can include hypotension or shock, pharmacological vasoconstriction (e.g., dopamine, epinephrine, norepinephrine) (Winkler and Trunkey, 1981; Hayes et al., 1992), vessel injury from invasive catheters, impaired hepatic synthesis of natural anticoagulants (e.g., vitamin K deficiency), postsplenectomy status, or congenital deficiency of natural anticoagulants. Rarely, purpura fulminans occurs several weeks after varicella infection, usually because of autoantibodies reactive against protein S (Smith and White, 1999).

Meningococcemia in particular is often complicated by peripheral tissue necrosis that seems to parallel the severity of protein C depletion (Fijnvandraat et al., 1995). Recent trials suggest that protein C replacement therapy improves the natural history of this infection (Smith and White, 1999; White et al., 2000). Other infections that sometimes are complicated by symmetrical peripheral gangrene include septicemia secondary to pneumococcus (Johansen and Hansen Jr., 1993), Escherichia coli (Rinaldo and Perez, 1982), Haemophilus influenzae type b (Hayes et al., 1992), and Capnocytophaga canimorsus (Kullberg et al., 1991), among others. Sometimes severe systemic inflammatory response syndromes, such as ARDS, in the absence of demonstrable infection, can be complicated by limb necrosis (Bone et al., 1976). Acquired antithrombin deficiency in such patients with ARDS could be associated with thrombosis (Owings et al., 1996).

The development of acral tissue ischemia or necrosis in a thrombocytopenic, septic patient receiving heparin may suggest HIT. Although a common therapeutic response to such a diagnostic dilemma might be to stop heparin pending results of diagnostic testing for HIT antibodies, this could result in further ischemic injury, because anticoagulants might help prevent microvascular thrombosis (White et al., 2000). Furthermore, alternative non-heparin anticoagulants could be relatively contraindicated in a patient with significant renal or hepatic dysfunction. Thus, a reasonable treatment approach might well include continued heparin if clinical judgment posited a higher likelihood of septicemia, rather than HIT, as the cause of the microvascular thrombosis.

Only a small minority of septic patients develop acral limb ischemia or necrosis. Many, however, develop thrombocytopenia, with or without laboratory evidence for DIC. The predominant explanation for increased platelet destruction in sepsis is uncertain, but appears to involve the underlying inflammatory host response (Aird, 2003a,b). Since hospitalized septic patients frequently are exposed to heparin, diagnostic confusion with HIT can result. Low protein C levels correlate with poor outcomes in sepsis (Yan et al., 2001), and recombinant human activated protein C (drotrecogin, Xigris) has been shown to reduce mortality in septic patients (Bernard et al., 2001). It is possible that this therapy might reduce risk of limb ischemia from microvascular thrombosis in this patient population. A potential dilemma is that septic
patients with severe thrombocytopenia (<30 × 10^9/L) were excluded in the clinical trials because of the bleeding potential of drotrecogin; however, as relative and absolute efficacy was greatest in the patients with the most severe sepsis, it has been suggested that otherwise eligible patients with such severe thrombocytopenia be considered as candidates for drotrecogin following platelet transfusion (Warkentin et al., 2003b).

G. Infective Endocarditis

Infective endocarditis is frequently complicated by thrombocytopenia. These patients are also at risk for septic emboli manifesting as thrombotic or hemorrhagic stroke, myocardial infarction, renal infarction, or even acute limb ischemia (de Gennes et al., 1990). Thus, the profile of macrovascular thrombosis and thrombocytopenia characteristic of HIT can be mimicked, especially as heparin is often used to anticoagulate patients with septic endocarditis (Delahaye et al., 1990). Microembolization leading to multiple small infarcts or microabscesses, in such organs as muscles, adrenal glands, and spleen, is an additional feature of endocarditis (Ting et al., 1990) that is not seen in HIT.

H. Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal myeloid disorder characterized by an acquired defect in the X-linked phosphatidylinositol glycan class A (PIG-A) gene, leading to loss of cell surface glycosylphosphatidylinositol (GPI)-anchored proteins (for review see Rosse, 1997). Loss of the complement-regulating GPI-linked surface proteins, decay-accelerating factor and membrane attack complex inhibitory factor, causes the red cells to be exquisitely sensitive to complement-mediated hemolysis. Some patients have thrombocytopenia, and an increased risk for unusual, life-threatening venous thrombotic events, such as hepatic vein thrombosis, occurs in some patients. Thus, the clinical profile of HIT potentially can be mimicked. The thrombocytopenia could be related either to decreased platelet production or to complement-mediated formation of procoagulant platelet-derived microparticles (Wiedmer et al., 1993).

I. Posttransfusion Purpura

Posttransfusion purpura (PTP) is a rare syndrome characterized by severe thrombocytopenia and mucocutaneous bleeding that begins 5–10 days after blood transfusion, usually red cell concentrates. More than 95% of affected patients are older women, in keeping with its pathogenesis of an anamnestic recurrence of platelet-specific alloantibodies in women previously sensitized
by pregnancy. Destruction of autologous platelets is believed to result from the pseudospecificity of the alloimmune response, e.g., the high-titer anti-HPA-la alloantibodies (the most frequent cause of the syndrome) probably somewhat recognize the autologous HPA-1b alloantigen (see Chap. 2).

Because both PTP and HIT typically occur about a week after surgery managed with perioperative blood transfusions and postoperative heparin prophylaxis, a diagnostic dilemma can arise (Lubenow et al., 2000). A useful clinical clue is the presence or absence of petechiae: PTP almost invariably is characterized by this hallmark of severe thrombocytopenia, whereas patients with HIT generally do not develop petechiae, even if they have very severe thrombocytopenia. The presence of high titers of platelet-reactive alloantibodies suggests PTP.

III. RECOGNITION AND TREATMENT OF PSEUDO-HIT

Many patients with pseudo-HIT can be distinguished from HIT because of the early onset of thrombocytopenia (see Table 1). Unless the patient received heparin within the past 100 days, the early platelet count fall is strong evidence against HIT (Warkentin and Kelton, 2001; Lubenow et al., 2002) (see Chap. 3). These patients with pseudo-HIT should be further anticoagulated with heparin.

However, for patients with adenocarcinoma-associated DIC, or postoperative pulmonary embolism, in whom the platelet count fall can occur after 5 days of heparin treatment, the diagnosis will be initially uncertain. As heparin can cause catastrophic complications if HIT is the underlying cause, and as alternative anticoagulants (danaparoid, lepirudin, or argatroban) are available in most countries, treatment with one of these agents before obtaining results of HIT antibody testing should be considered. For patients with adenocarcinoma without HIT antibodies, longer-term management is often more successful with LMWH or UFH than with warfarin (Prandoni, 1997; Lee et al., 2003).

A. Pseudo-HIT Complicated by HIT

Heparin-induced thrombocytopenia is a relatively common complication of heparin therapy. It may be even more common in patients who have baseline platelet activation and PF4 release, as occurs in adenocarcinoma-associated DIC or diabetic ketoacidosis. Therefore, a patient with early thrombocytopenia attributable to a pseudo-HIT disorder may subsequently develop clinically significant HIT antibodies (see Fig. 5). Another example is that of a patient with lung cancer and DVT who developed a platelet count rise during
intravenous heparin therapy, followed by recurrent thrombocytopenia and, ultimately, venous limb gangrene during anticoagulation with warfarin and ancrod (Fig. 7). In this situation, one might have expected platelet count recovery during a second course of heparin. However, an intravenous heparin challenge resulted in worsening of thrombocytopenia, and the patient had a strong positive assay for HIT antibodies. These patient cases emphasize the

Figure 7  Pseudo–HIT complicated by HIT: A 78-year-old man, with right proximal lower limb deep venous thrombosis (DVT) and thrombocytopenia, developed progressive platelet count increase during therapeutic-dose UFH treatment. Recurrent thrombocytopenia developed after UFH was stopped and when the patient was anticoagulated with warfarin. A liver biopsy on day 9 showed metastatic adenocarcinoma (primary lung neoplasm), and adenocarcinoma-associated disseminated intravascular coagulation (DIC) was diagnosed. However, a heparin challenge produced a further platelet count fall; HIT antibody testing was strongly positive (serotonin-release assay [SRA]: 88% serotonin release at 0.1 U/mL heparin; <15% release at 0 and 100 U/mL heparin). Subsequently, the patient developed new left-sided DVT, as well as venous gangrene of the left foot during treatment with warfarin and ancrod (peak INR = 3.8). Although the clinical course was initially identical with pseudo–HIT (rising platelet count on heparin therapy; abrupt platelet count fall after heparin administration was stopped), the subsequent heparin-induced fall in the platelet count, and strong positive HIT test results, indicate the patient also had HIT.
importance of a high clinical suspicion for HIT even in complex situations for which other explanations for thrombocytopenia are present. The wider availability of assays for HIT antibodies should help clinicians better diagnose and manage patients who develop thrombocytopenia and thrombosis during or shortly following heparin treatment.

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I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) presents a unique situation: heparin causes the very problems its use was intended to prevent, namely, such complications as pulmonary embolism, stroke, and limb gangrene. Furthermore, several treatment paradoxes pose serious management pitfalls (Table 1). This chapter summarizes our treatment approach, with emphasis on practical management issues. We view HIT as a syndrome of increased thrombin generation. Accordingly, we emphasize the use of rapidly acting anticoagulant drugs that control thrombin generation in HIT.

This chapter is not the outcome of a formal consensus conference, as defined elsewhere (McIntyre, 2001). Nevertheless, we have used an evidence-based approach to frame our recommendations, based upon the Sixth American College of Chest Physicians (ACCP) Consensus Conference on Antithrombotic Therapy (Hirsh et al., 2001a,b; Guyatt et al., 2001). According to these guidelines, the recommendation to use (or not use) a particular treatment is based on the trade-off between the expected benefits on the one hand, and the risks on the other. Thus, based upon the evidence, as well as our own experience, when we concluded that benefits of a particular treatment outweighed risks, we recommended the treatment. If we were quite certain the
<table>
<thead>
<tr>
<th>Treatment for HIT</th>
<th>Paradoxical effect of treatment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discontinue heparin</td>
<td>High frequency of thrombosis despite stopping heparin</td>
<td>Use an alternative, rapidly acting anticoagulant&lt;sup&gt;a&lt;/sup&gt; when heparin is stopped because of suspected HIT</td>
</tr>
<tr>
<td>Coumarin (e.g., warfarin, phenprocoumon)</td>
<td>High frequency of thrombosis; potential for warfarin-induced venous limb gangrene syndrome</td>
<td>Control thrombin generation with alternative anticoagulant&lt;sup&gt;a&lt;/sup&gt; and await partial or full resolution of HIT before starting coumarin for longer-term control of thrombosis</td>
</tr>
<tr>
<td>Low molecular weight heparin (LMWH)</td>
<td>High frequency of thrombocytopenia or thrombosis when given to patients with acute HIT</td>
<td>Although LMWH is less likely than unfractionated heparin (UFH) to cause HIT, LMWH is relatively likely to maintain or worsen acute HIT caused by UFH</td>
</tr>
<tr>
<td>Low-dose danaparoid (antithrombotic prophylaxis)</td>
<td>High frequency of thrombosis when low-dose danaparoid&lt;sup&gt;b&lt;/sup&gt; given to patients with isolated HIT</td>
<td>High (therapeutic)-dose danaparoid recommended for patients with isolated HIT (or HIT-thrombosis)</td>
</tr>
<tr>
<td>Platelet transfusions</td>
<td>May increase risk for platelet-mediated thrombosis</td>
<td>Spontaneous bleeding is uncommon even in severe HIT; thus, prophylactic platelet transfusions are relatively contraindicated</td>
</tr>
<tr>
<td>Vena cava filters</td>
<td>May increase risk for inferior vena cava thrombosis, DVT, or pulmonary embolism</td>
<td>Vena cava filters should be avoided in acute HIT; if used, concomitant anticoagulation should be given, if possible</td>
</tr>
</tbody>
</table>

<sup>a</sup> Rapidly acting alternative parenteral anticoagulants, such as danaparoid, lepirudin, and argatroban, are discussed in Table 2.

<sup>b</sup> Low-dose danaparoid (750 U two or three times a day) is approved for prevention of thrombosis in acute HIT in some jurisdictions.
evidence favored the recommendation, a grade 1 recommendation was made. If we were less certain of the trade-off between benefits and risks, a weaker recommendation of grade 2 was made.

We also assessed the methodological quality of the studies supporting the recommendations, also using the ACCP guidelines: grade A: randomized trials without important limitations; grade B: randomized trials with important limitations; grade C+: no randomized trials but observations from other trials can be unequivocably extrapolated, or there is overwhelming evidence from observation studies; and grade C: observation studies.

Regarding studies of HIT, there is only one small randomized trial (Chong et al., 2001), and this study had methodological flaws such as non-blinded assessment of treatment outcomes. Hence, we have no grade A and only one grade B recommendation. Grade C recommendations are based upon observation studies. Regarding HIT, this includes prospective cohort treatment studies with historical controls (Greinacher et al., 1999a,b; 2000; Lewis et al., 2001, 2003); case-control series (Warkentin et al., 1997); and large case series (e.g., Magnani, 1993, 1997; Warkentin and Kelton, 1996; Wallis et al., 1999). Thus, our recommendations are graded as follows, with the implications of the recommendation shown:

Grade 1B and Grade 1C+: strong recommendations; can apply to most patients in most circumstances.
Grade 1C: strong or intermediate-strength recommendation; may change when stronger evidence is available.
Grade 2C: weak recommendation; other alternatives may be equally reasonable.

As no studies have directly compared the three major treatment options for HIT (danaparoid, lepirudin, argatroban), any recommendation for use of one of these drugs does not imply any consistent advantage over one of the others. However, there are important pharmacokinetic differences among these drugs, which might very well favor one in the particular circumstances of an individual patient situation (see also Chaps. 14–19).

A. Disclaimer

There are several challenging aspects to treating patients with HIT. In particular, these patients are not clinically homogeneous: they represent a complex mix of varying initial indication for heparin, location and severity of HIT-associated thrombosis, and, not infrequently, dysfunction of one or more vital organs. This presents difficulties both for performing clinical studies as well as in the application of treatment recommendations for individual
patients. Furthermore, there are important differences among countries in the approval or availability status of certain recommended treatment approaches. The treatment recommendations we make cannot thus be indiscriminately applied to all patients with suspected HIT.

A further practical problem is that the major treatment options for HIT include relatively new and, for some physicians, unfamiliar or even unapproved anticoagulant agents. (Indeed, the approval of lepirudin by the European Union in May 1997 as a treatment for a thrombosis that complicates HIT represented the first time an anticoagulant drug obtained by recombinant technology became available for clinical use.) This presents extra challenges to physicians and also to laboratories asked to monitor anticoagulant treatment effects, as the treatment “learning curve” may occur in emergency situations. Also, immediate results of reliable laboratory tests for HIT are usually unavailable. Difficult management decisions may be needed amid diagnostic uncertainty: a diagnosis of HIT that seems obvious in retrospect may not have been so clear during its early evolution.

As an iatrogenic illness that occurs unexpectedly, often in a setting of antithrombotic prophylaxis, medicolegal aspects must be considered (see Chaps. 21 and 22). Thus, once HIT is entertained as part of a differential diagnosis, we suggest that physicians carefully document the various diagnostic and treatment considerations as events unfold.

As one of those common, rare diseases [We acknowledge Prof. R. Hull (Calgary, Canada) for his description of HIT as a “common, rare disease.”] that physicians only occasionally manage, and only rarely enter into clinical studies, we need to acknowledge that no final answer for treatment is likely to emerge. Therefore, even in this third edition, this chapter should be viewed as a basis for further discussion and study of the treatment of HIT patients.

II. NONIMMUNE HEPARIN-ASSOCIATED THROMBOCYTOPENIA

In some patients, especially those with comorbid conditions associated with platelet activation (burns, anorexia nervosa), heparin treatment can result in a transient decrease in platelet count (Burgess and Chong., 1997; Reininger et al., 1996) (see Chap. 5). Unfractionated heparin (UFH) activates platelets directly (Salzman et al., 1980), an effect observed less frequently with low molecular weight heparin (LMWH) (Brace and Fareed, 1990). Known as nonimmune heparin-associated thrombocytopenia (nonimmune HAT), this direct proaggregatory effect of heparin occurs predominantly in patients.
receiving high-dose, intravenous UFH therapy. Typically, platelet counts decrease within the first 1–2 days of treatment, and then recover over the next 3–4 days. There are no data indicating that these patients are at increased risk for adverse outcomes, including thrombosis. Indeed, it is possible that inappropriate discontinuation of heparin for nonimmune HAT could increase the risk for thrombosis owing to the underlying clinical condition for which the heparin is being given.

Recommendation. Heparin should not be discontinued in patients clinically suspected of having nonimmune heparin-associated thrombocytopenia (grade 2C).

III. THERAPY OF (IMMUNE) HIT

A. Pathogenesis of HIT: Treatment Implications

Heparin-induced thrombocytopenia is caused by antibodies that usually recognize multimolecular complexes of platelet factor 4 (PF4) and heparin. HIT can be viewed as a syndrome of in vivo thrombin generation that results from the activation of platelets, endothelium, monocytes, and coagulation pathways (Fig. 1; see color insert) (see Chaps. 5–10) (Warkentin and Kelton, 1994; Greinacher, 1995; Warkentin, 1997, 2003; Warkentin et al., 1998).

Given this model of pathogenesis, therapy for acute HIT should focus on the following issues: (1) interruption of the immune response (e.g., discontinuation of heparin); (2) rapid reduction of increased thrombin generation; and (3) treatment of HIT-associated thrombosis. In most patients with HIT, effective pharmacological therapy for thrombosis will involve an agent that rapidly controls thrombin generation, although in some situations additional adjunctive treatments may be necessary (e.g., surgical thromboembolectomy).

A newly recognized treatment issue involves patients with detectable anti-PF4–heparin antibodies, but no platelet count reduction or other clinical evidence of HIT. With increased testing for HIT antibodies, it is now clear that many patients develop HIT antibodies without developing clinical HIT (see Chaps. 4 and 11). In these patients it seems acceptable to continue heparin treatment, but to monitor the platelet counts carefully (“watch-and-wait” strategy).

Two recent retrospective studies found that patients with acute coronary syndrome who either had HIT antibodies at presentation (Williams et al., 2003) or developed HIT antibodies following heparin treatment (Mattioli et al., 2000) had a higher frequency of vascular events during follow-up that
Figure 1  Pathogenesis of HIT; a central role for thrombin generation: HIT-IgG antibodies bind to several identical epitopes on the same antigen complex, thus forming immune complexes that become localized to the platelet surface. The IgG immune complexes can cross-link the platelet FcγRIIa receptors, resulting in FcγRIIa-dependent platelet activation (Kelton et al., 1988). The GP IIb/IIIa complex is not required for platelet activation (Greinacher et al., 1994a). The activated platelets trigger a cascade of events that ultimately lead to activation of the coagulation pathways, resulting in thrombin generation. Activated platelets release their α-granule proteins (Chong et al., 1994), including PF4, leading to formation of more multimolecular PF4–heparin complexes, setting up a vicious cycle of platelet activation, triggering even more platelet activation (Greinacher, 1995). The activated platelets bind fibrinogen, recruit other platelets, and begin to form a primary clot. During shape change, procoagulant, platelet-derived microparticles are released, providing a phospholipid surface for amplifying thrombin generation (Warkentin et al., 1994). The released PF4 also binds to endothelial cell heparan sulfate, forming local antigen complexes to which HIT antibodies bind (Cines et al., 1987; Visentin et al., 1994; Greinacher et al., 1994b). Tissue factor expression on activated endothelial cells and monocytes (Arepally and Mayer, 2001; Pouplard et al., 2001) further enhances thrombin generation. (See color insert.)
ranged from 1 month to 1 year, despite the absence of apparent HIT in any patient. The implications of “subclinical” HIT antibody seroconversion on influencing subsequent cardiovascular morbidity and mortality awaits prospective study.

B. Discontinuation of Heparin for Clinically Suspected HIT

Numerous case reports describe the occurrence of new, progressive, or recurrent thromboembolic events during continued or repeated use of heparin in patients with acute HIT. Moreover, the thrombocytopenia usually persists if the administration of heparin is not stopped. Thus, all heparin treatment should be discontinued in patients strongly suspected of having HIT, and usually substituted by another anticoagulant (discussed subsequently), while awaiting results of HIT antibody testing.

Recommendation. All heparin administration should be discontinued in patients clinically suspected of having (immune) HIT (grade 1C+).

The routine use of heparin (e.g., line flushing) is pervasive in hospitals. Thus, based on our experience it can be helpful to institute methods to reduce the risk for inadvertent heparin use in hospitalized patients with HIT.

Recommendation. A clearly visible note should be placed above the patient’s bed stating “NO HEPARIN: HIT” (grade 2C).

Not infrequently, patients in whom heparin administration has been stopped because of clinically suspected HIT subsequently are found to have negative laboratory tests for HIT antibodies. In our experience, it is reasonable and safe to restart heparin therapy in these patients, provided the intervening clinical events are consistent with an alternative explanation for thrombocytopenia (see Chaps. 2, 3, and 12), and provided the laboratory has adequately excluded the presence of HIT antibodies (see Chap. 11).

Recommendation. Heparin can be safely restarted in patients proved not to have HIT antibodies by sensitive activation or antigen assay (grade 2C).

C. Anticoagulation of the HIT Patient with Thrombosis

The Need for Anticoagulation of HIT-Associated Thrombosis

Heparin-induced thrombocytopenia is a strong, independent risk factor for venous and arterial thrombosis (Warkentin et al., 1995, 2003; Girolami et al., 2003). HIT can be complicated by thrombosis in several ways: (1) a preceding thrombosis, leading to the heparin treatment that caused HIT; (2) new,
progressive, or recurrent thrombosis resulting from HIT itself; or (3) for both reasons.

For a HIT patient with thrombosis in whom heparin administration has been discontinued, there is, nevertheless, a very high risk for subsequent thrombosis. This was shown in two historically controlled prospective treatment cohort studies (Greinacher et al., 1999a,b), in which the incidence of thrombotic events ranged from 5 to 10% per patient day (see Chap. 15). This high event rate (6.1% per day in the meta-analysis) occurred after stopping heparin therapy and after laboratory confirmation of HIT, but before institution of alternative anticoagulation with lepirudin (mean period of treatment delay; 1.7 days) (Greinacher et al., 2000). This experience suggests that alternative anticoagulant therapy should not be delayed for results of HIT antibody testing in patients strongly suspected of having HIT.

Anticoagulants Evaluated for Treatment of HIT

Current treatment of HIT focuses on agents that rapidly control thrombin generation (Warkentin et al., 1998; Hirsh et al., 1998, 2001b) (Fig. 2). Table 2 lists the available evidence on efficacy for three such agents: danaparoid, lepirudin, and argatroban (listed in the order the drugs became available).

Only one randomized controlled trial for the management of HIT has been performed: this study compared danaparoid with dextran for the treatment of HIT-associated thrombosis (Chong et al., 2001; Ortel and Chong, 1998) (see Chap. 14). As the study was small and open-label, we have listed it as a level 1B, rather than level 1A, recommendation.

Recommendation. Therapeutic-dose anticoagulation with a rapidly acting anticoagulant, e.g., danaparoid (grade 1B), lepirudin (grade 1C+), or argatroban (grade 1C), should be given to a patient with thrombosis complicating acute HIT. Treatment should not be delayed pending laboratory confirmation in a patient strongly suspected of having HIT.

Pharmacological and Pharmacokinetic Considerations: Danaparoid, Lepirudin, and Argatroban

The lack of prospective comparative studies between danaparoid, lepirudin, and argatroban precludes definitive conclusions about relative efficacy and safety. However, there are several pharmacological and pharmacokinetic differences that physicians should consider when determining which drug may be preferred in an individual patient (Tables 3–5). For example, in a patient with vital organ or limb ischemia or infarction who might need urgent surgical intervention, an agent with a short half-life may be preferred. On the other hand, in a patient with venous thromboembolism in whom an uncomplicated
overlap with (longer-term) warfarin anticoagulation is anticipated, or who requires outpatient treatment by subcutaneous injections, danaparoid may have certain advantages. Argatroban (which undergoes hepatobiliary clearance) is suited for patients with renal insufficiency, as dose reduction is generally not required (cf. lepirudin). Other factors to consider include drug availability to, and prior experience of, the physician; availability and turnaround time of laboratory monitoring; and so on.

**Other Drugs that Reduce Thrombin Generation in HIT**

Other drugs with antithrombin activity described anecdotally as treatment for HIT include bivalirudin (Nand, 1993; Reid and Alving, 1994; Chamberlin et al., 1995; Campbell et al., 2000; Francis et al., 2003) and the glycosaminoglycan agent dermatan sulfate (Agnelli et al., 1994). Theoretically, the heparin-binding pentasaccharide fondaparinux should not cause HIT or cross-react with HIT antibodies (Elalamy et al., 1995; Greinacher et al., 1995; Nand et al., 1996; Reid and Alving, 1994; Chamberlin et al., 1995; Campbell et al., 2000; Francis et al., 2003).

---

**Figure 2** Thrombin generation and fibrin formation in acute HIT. (A) Thrombin generation, as assessed by thrombin–antithrombin (TAT) complexes, is markedly increased in acute HIT (mean, 55 ng/mL; normal, <4.1 ng/mL). Whereas danaparoid reduces thrombin generation in these patients, the defibrinogenating snake venom, ancrod, does not. (B) Levels of cross-linked fibrin degradation products (D-dimer) are increased in patients with acute HIT (mean, ~4–5 μg/mL; normal, <0.5 μg/mL). Whereas danaparoid reduces D-dimer levels, ancrod increases their levels. Baseline (B) samples were obtained at diagnosis of HIT and before treatment with danaparoid or ancrod; subsequent values are shown for day 1 (D1, 1–24 h postinitiation of treatment), day 2 (25–48 h), and day 3 (49–72 h). *p < 0.001, **p < 0.002. (From Warkentin, 1998.)
<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Studies</th>
</tr>
</thead>
</table>
| Danaparoid sodium (Orgaran, formerly known as Org 10172 and Lomoparan) | Mixture of glycosaminoglycans with anti-Xa activity \( \Rightarrow \) anti-IIa (antithrombin) activity | • Randomized controlled trial comparing danaparoid plus warfarin versus dextran 70 plus warfarin (Chong et al., 2001).  
• Compassionate release program (Magnani, 1993, 1997).  
• Retrospective comparison of danaparoid and ancred (Warkentin, 1996).  
• HAT-1: prospective cohort study with historical controls (Greinacher et al., 1999a).  
• HAT-2: prospective cohort study with historical controls (Greinacher et al., 1999b).  
• Meta-analysis of HAT-1 and HAT-2 studies (Greinacher et al., 2000).  
• HAT-3: prospective cohort study with historical controls (Eichler et al., 2002).  
• Meta-analysis of HAT-1, HAT-2, and HAT-3 studies (Lubenow et al., 2002a).  
• Retrospective comparison of lepirudin (prospective cohort) and danaparoid (contemporaneous controls) (Farner et al., 2001).  
• Postmarketing surveillance (Lubenow et al., 2002b).  
• Arg-911: prospective cohort study with historical controls (Lewis et al., 2001).  
• Arg-915: prospective cohort study with historical controls (Lewis et al., 2003). |
| Recombinant hirudin (lepirudin, Refludan) | Recombinant protein derived from leeches that directly inactivates thrombin |                                                                                                    |
| Argatroban (Novastan\(^a\))    | Small molecule, direct thrombin inhibitor                                              |                                                                                                    |

\(^a\) Argatroban is marketed in the United States by its generic name (with a capital A). In some other countries, it is marketed by its trademark. Novastan (see Chap. 16).
Table 3  Main Characteristics of Danaparoid Sodium

<table>
<thead>
<tr>
<th>Mechanism of action, pharmacokinetics</th>
<th>Monitoring</th>
<th>Undesirable effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalyzes the inactivation of factor Xa by AT, and of thrombin (IIa) by AT and HCII</td>
<td>Anti-Xa levels during treatment by an amidolytic assay using danaparoid reference curve</td>
<td>Cross-reactivity (XR) with HIT antibodies: in vitro XR usually not associated with adverse effects; patients should be monitored for in vivo XR (unexplained platelet count fall, progressive new TECs); in vivo XR is estimated to occur in ~3% of patients (Magnani, 1993)</td>
<td>Anticoagulant effect depends on adequate AT levels</td>
</tr>
<tr>
<td>Bioavailability after sc injection ~100%; peak anti-Xa levels, 4–5 h after injection (Danhof et al., 1992)</td>
<td>Monitoring recommended in patients with: (1) significant renal impairment; (2) body weight &lt;45 kg or &gt;110 kg; (3) life- or limb-threatening thrombosis; (4) unexpected bleeding; (5) critically ill or unstable patient</td>
<td>Bleeding complications in compassionate-release study (Ortel and Chong, 1998): fatal (0.9%), major nonfatal bleeding (6.5%); no major bleeds in RCT (Chong, 1996)</td>
<td>Does not significantly prolong the aPTT, ACT, PT/INR (does not interfere with monitoring of overlapping oral anticoagulants)</td>
</tr>
<tr>
<td>Mean plasma distribution time following iv bolus, ~2.3 h</td>
<td></td>
<td></td>
<td>Reduce dosage if serum creatinine &gt; 265 µmol/L No antidote: In case of overdosage, stop the drug and treat bleeding with blood products as indicated</td>
</tr>
<tr>
<td>Plasma t1/2 of anti-Xa activity, 17–28 h (mean, 25 h); t1/2 of anti-IIa activity, 2–4 h (Danhof et al., 1992)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ACT, activated clotting time; aPTT, activated partial thromboplastin time; AT, antithrombin; HCII, heparin cofactor II; iv, intravenous; PT/INR, prothrombin time/international normalized ratio; RCT, randomized controlled trial; sc, subcutaneous; t1/2, drug half-life.
<table>
<thead>
<tr>
<th>Mechanism of action, pharmacokinetics</th>
<th>Monitoring</th>
<th>Undesirable effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct, noncovalent, irreversible inhibitor of free and clot-bound thrombin</td>
<td>aPTT during treatment; a more precise monitoring is possible by the ECT (see Chaps. 15, 17, 19)</td>
<td>Development of antihirudin antibodies in ~40% of patients. In about 3% of patients, these antibodies enhance the anticoagulant effect of hirudin, and require a substantial dose reduction.</td>
<td>~40% of patients develop antihirudin antibodies on day 5 or later of treatment; in only ~5% of these patients is a dose reduction or increase needed; risk of anaphylactic reactions post-iv bolus</td>
</tr>
<tr>
<td>Bioavailability after sc injection, ~100%; peak effect, 2–3 h</td>
<td>Daily aPTT monitoring is recommended in all patients (see Comments re: antihirudin antibodies)</td>
<td>Anaphylactic reactions: ~0.015% (first exposure) &lt;br&gt; ~0.15% (reexposure) associated with iv bolus injection (Greinacher et al., 2003)</td>
<td>No major effect on PT/INR (Greinacher et al., 2000)</td>
</tr>
<tr>
<td>Mean plasma distribution time after iv bolus, ~2 h</td>
<td>Monitoring by ECT recommended: &lt;br&gt; 1. During cardiopulmonary bypass surgery</td>
<td>Allergic reactions: very rare &lt;br&gt; Skin hypersensitivity: very rare</td>
<td>Reduce dosage if serum creatinine &gt;120 μmol/L (see Table 3 in Chap. 15)</td>
</tr>
<tr>
<td>Mean plasma t_{1/2}: 1.3 h; t_{1/2} greatly prolonged in renal failure (~200 h in nephrectomized patients)</td>
<td>Monitoring by quantitative hirudin EIA recommended: &lt;br&gt; 1. Decreased prothrombin levels (Lindhoff-Last et al., 2000)</td>
<td>Bleeding complications in HIT patients in prospective studies: major bleeding in two prospective studies, 13.4, 17% (see Chap. 15)</td>
<td>No antidote: In case of overdosage, stop the drug and treat bleeding with blood products as indicated (hemofiltration with a high-flux membrane is a possible treatment for life-threatening bleeding)</td>
</tr>
</tbody>
</table>

**Abbreviations:** aPTT, activated partial thromboplastin time; ECT, ecarin-clotting time; EIA, enzyme immunoassay; sc, subcutaneous; iv, intravenous; t_{1/2}, drug half-life.
Table 5  Main Characteristics of the Direct Thrombin Inhibitor, Argatroban

<table>
<thead>
<tr>
<th>Mechanism of action, pharmacokinetics</th>
<th>Monitoring</th>
<th>Undesirable effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct, noncovalent, reversible inhibitor of free and clot-bound thrombin</td>
<td>aPTT during treatment; no data exist as to whether more precise monitoring at higher doses would be achieved using other methods, such as ECT</td>
<td>No reported side effects besides bleeding complications (number of patients treated is too low to rule out possibility of rare side effects)</td>
<td>Only iv use of argatroban has been tested in HIT Reduce dosage by 75% in case of liver impairment No dose reduction in renal failure No antidote; in case of overdosage or severe bleeding, stop the drug and treat bleeding with blood products as indicated Argatroban prolongs the INR and requires a strategy adopted to the INR reagent used for overlapping treatment with warfarin (see Chap. 16)</td>
</tr>
<tr>
<td>~50% of the drug is plasma protein bound; Steady state is reached 1–3 h after starting iv infusion Mean plasma $t_{1/2}$ is 40–50 min; $t_{1/2}$ is prolonged 4- to 5-fold in moderate liver impairment</td>
<td>Target INR is $&gt;4.0$ when warfarin is overlapped with argatroban (however, following discontinuation of argatroban, the usual target INR of 2.0–3.0 applies during further warfarin treatment)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steady state is reached 1–3 h after starting iv infusion Mean plasma $t_{1/2}$ is 40–50 min; $t_{1/2}$ is prolonged 4- to 5-fold in moderate liver impairment</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations:* aPTT, activated partial thromboplastin time; ECT, ecarin-clotting time; INR, international normalized ratio; iv, intravenous; $t_{1/2}$, drug half-life.
1995; Amiral et al., 1997; Turpie et al., 2001), but there is no reported experience with this agent for HIT.

D. Anticoagulation of the HIT Patient Without Thrombosis

Approximately 50% of patients with HIT do not have a new HIT-associated thrombosis at the time HIT is first clinically suspected on the basis of thrombocytopenia alone (Warkentin and Kelton, 1996; Greinacher et al., 1999a,b). In a retrospective cohort study of 62 such patients with “isolated thrombocytopenia,” the subsequent 30-day cumulative thrombotic event rate was high (52.8%) (see Fig. 2 in Chap. 4). The rate of thrombosis was similar in the two largest patient subgroups: patients treated with discontinuation of heparin therapy alone (20/36, 56%) and patients treated with substitution of warfarin for heparin (10/21, 48%). Overall, 6 of the 62 patients developed pulmonary embolism (2 fatal); another patient who died suddenly may also have had a fatal pulmonary embolism.

In a subsequent large retrospective cohort study of serologically confirmed HIT performed by Wallis and coworkers (1999), a 38% thrombotic event rate was observed in patients with isolated HIT managed by cessation of heparin. Further, early cessation of heparin was not associated with a reduction in the rate of thrombosis. Thus, the high thrombotic event rates observed in these retrospective cohort studies are consistent with the experience of the prospective treatment cohort studies that also observed a high rate of thrombosis soon after the diagnosis of HIT (Greinacher et al., 1999a,b, 2000).

The very high initial thrombotic event rates (5–10%/day over the first 1–2 days) observed in these prospective and retrospective studies suggest that many patients may have had subclinical DVT at the time that HIT was first suspected. The data support the recommendation that antithrombotic treatment for HIT be started before serological confirmation is received, even in patients without clinical evidence of thrombosis.

In a retrospective analysis of patients with isolated HIT comparing treatment with danaparoid and lepirudin, it was observed that patients who received prophylactic-dose danaparoid (750 U sc b.i.d. or t.i.d.) had a trend to a higher rate of thrombosis than patients treated with lepirudin (0.1 mg/kg b.w./h, aPTT-adjusted) (Farner et al., 2001). In contrast, patients with HIT-associated thrombosis had similar outcomes when treated with therapeutic doses of either drug. This indicates that therapeutic, rather than prophylactic, doses of danaparoid may be more effective for patients with isolated HIT (Farner et al., 2001; Warkentin, 2001).

We usually prescribe an alternative anticoagulant in therapeutic doses in this situation. Prophylactic-dose anticoagulation is a reasonable option in HIT patients judged to be at higher risk for bleeding complications, as is
regular screening for venous thrombosis without anticoagulation in a patient at very high bleeding risk. Thrombocytopenia itself should not be considered a contraindication to anticoagulation in patients with HIT, as petechiae and other spontaneous hemorrhagic manifestations are not usually seen in these patients (see Chap. 3). However, if the platelet count is less than $20 \times 10^9/L$ and bleeding signs, but not thrombosis, are observed, then alternative diagnoses such as posttransfusion purpura or other drug-dependent immune thrombocytopenic disorders should be considered (see Chap. 2).

**Recommendation.** Alternative anticoagulation with an appropriate anticoagulant, such as danaparoid, lepirudin, or argatroban, should be considered in patients with clinically suspected HIT even in the absence of symptomatic thrombosis. Anticoagulation should be continued at least until recovery of the platelet counts to a stable plateau. Patients should undergo imaging studies for lower limb DVT, especially those at highest risk for venous thromboembolism, such as postoperative patients (grade 1C+).

### E. Longer-Term Anticoagulant Management of the HIT Patient with Thrombosis

Acute HIT by itself is not an indication for longer-term anticoagulation (i.e., 3–6 months). However, HIT-associated thrombosis, or the underlying disease itself, often is. For long-term control of thrombosis, oral anticoagulants of the coumarin class (e.g., warfarin or phenprocoumon) are the treatment of choice. Generally, it takes at least 5 days of oral anticoagulant therapy before therapeutic functional hypoprothrombinemia is achieved (Harrison et al., 1997). It is important that thrombin generation be controlled in patients with acute HIT before initiation of coumarin treatment, particularly in patients with severe HIT-associated DVT, because coumarin-induced venous limb gangrene is a potential outcome (Warkentin et al., 1997) (see Chap. 3). It is our practice to postpone starting administration of coumarin anticoagulants until therapeutic anticoagulation is achieved with danaparoid, lepirudin, or argatroban and substantial platelet count recovery has occurred (at least $\geq 100 \times 10^9/L$).

**Recommendation.** The drug of choice for longer-term anticoagulation of HIT patients is an oral anticoagulant of the coumarin class (e.g., warfarin or phenprocoumon). However, in a patient with acute HIT, oral anticoagulant therapy should be delayed until the patient is adequately anticoagulated with a rapidly acting parenteral anticoagulant, and ideally not until there has been substantial platelet count recovery (at least $\geq 100 \times 10^9/L$). Oral anticoagulants should be started in low maintenance doses (e.g., $\leq 5$ mg warfarin), with at least 5 days of overlap with
the parenteral anticoagulant (including at least 2 days in the target-therapeutic range). If applicable, oral or intravenous vitamin K should be given to reverse coumarin anticoagulation in a patient recognized as having acute HIT after coumarin has been commenced (grade 1C).

Besides avoiding the risk of coumarin-induced necrosis, there are other reasons for postponing coumarin anticoagulation in a patient with acute HIT. For example, coumarin will increase a patient’s activated partial thromboplastin time (aPTT). Since the aPTT is used to monitor the anticoagulant effect of the direct thrombin inhibitors, the patient is at risk of receiving insufficient dosing of the direct thrombin inhibitor if coumarin has been given.

For unknown reasons, the direct thrombin inhibitors have varying effects upon the PT/INR (prothrombin time/international normalized ratio), which are both agent-specific and patient-specific. For example, argatroban has been reported to increase the INR more than lepirudin and bivalirudin (argatroban > bivalirudin > lepirudin) (Gosselin et al., 2003). This complicates the issue of coumarin overlap somewhat more with argatroban than the other direct thrombin inhibitors (see Chap. 16). Another reason, therefore, to postpone coumarin therapy in patients receiving concomitant therapy with direct thrombin inhibitors is that it avoids the potential for a physician to conclude incorrectly that the patient is adequately anticoagulated with coumarin (because of the greater increase in the INR during cotherapy), and thus to discontinue the direct thrombin inhibitor prematurely. Once the anticoagulant effect of the direct thrombin inhibitor has dissipated within a few hours, the circumstances favoring microvascular thrombosis (and, hence, coumarin-induced necrosis) might well be present, i.e., ongoing thrombin generation from acute HIT, warfarin-induced protein C depletion, and active deep venous thrombosis (Smythe et al., 2002; Srinivasan et al., 2003).

In case of coumarin overdose and severe bleeding during the first 3 months after an episode of HIT, prothrombin complex concentrates should only be used with extreme caution to “reverse” coumarin anticoagulation. This is because these concentrates contain heparin and have caused recurrent thrombocytopenia and thrombosis in patients with circulating HIT antibodies (Greinacher et al., 1992).

**Recommendation.** Prothrombin complex concentrates should not be used to reverse coumarin anticoagulation in a patient with acute or recent HIT unless bleeding is otherwise unmanageable (grade 2C).

F. Reexposure of the HIT Patient to Heparin

**Heparin Reexposure of the Patient with Acute or Recent HIT**

Deliberate or accidental readministration of heparin to a patient with acute or recent HIT can cause an abrupt platelet count fall, sometimes complicated
by thrombosis or acute systemic reactions (see Chap. 3). Accordingly, deliberate heparin rechallenge for diagnostic purposes is not recommended, especially because sensitive assays for HIT antibodies are available. This is a strong recommendation because the diagnostic usefulness of laboratory assays for HIT has been established in controlled studies (see Chap. 11).

**Recommendation.** Deliberate reexposure to heparin of a patient with acute or recent HIT for diagnostic purposes is not recommended. Rather, the diagnosis should be confirmed by testing acute patient serum or plasma for HIT antibodies using a sensitive activation or antigen assay (grade 1C+).

### Heparin Reexposure of the Patient with a History of Remote HIT

The HIT antibodies are usually not detectable 3 months after an episode of HIT (Warkentin and Kelton, 2001). There are few data describing the clinical and serological outcomes of patients with previously documented HIT in the remote past (arbitrarily, >3 months ago, or sooner, if HIT antibodies have disappeared). One patient who developed fatal HIT on day 15 of UFH treatment had a history of HIT complicated by thrombosis 6 years earlier (Gruel et al., 1990). However, several patients with previous remote HIT have been observed in whom repeat heparin use caused neither HIT nor HIT antibody formation (Pötzsch et al., 2000; Warkentin and Kelton, 2001).

Because there are acceptable alternative anticoagulant options for most prophylactic and therapeutic indications, both UFH and LMWH should be avoided in patients with a previous history of HIT. As discussed in the following section, however, there are special circumstances, such as cardiac or vascular surgery, during which it is reasonable to use heparin for a patient with a previous history of HIT, provided certain precautions are taken.

**Recommendation.** Heparin should not be used for antithrombotic prophylaxis or therapy in a patient with a previous history of HIT, except under special circumstances (e.g., cardiac or vascular surgery) (grade 2C).

### IV. HIT IN SPECIAL CLINICAL SITUATIONS

#### A. Cardiopulmonary Bypass or Vascular Surgery

Management of the Patient with Acute or Recent HIT

For patients with acute HIT who require heart surgery, or with recent HIT and persistence of circulating HIT antibodies, it is possible to use alternative anticoagulants during cardiopulmonary bypass (CPB) (for review, see War-
kentin and Greinacher, 2003). Three options for such patients are bivalirudin, lepirudin, and danaparoid (listed in order of preference). Unfortunately, the lack of a specific antidote, the need for special intraoperative monitoring, and other considerations mean that none is ideal for managing CPB. Another approach is to administer heparin together with a potent antiplatelet agent, e.g., tirofiban (GPIIb/IIIa antagonist) or epoprostenol (prostacyclin analogue). This special topic of managing cardiac surgery patients with acute or previous HIT is discussed in detail in Chap. 19, as well as in relation to specific anticoagulant agents in Chaps. 14–17.

Danaparoid and lepirudin have also been used to provide intraoperative anticoagulation, as well as to “flush” blood vessels during vascular surgery in patients with acute HIT.

**Recommendation.** Alternative anticoagulation should be used for heart or vascular surgery in a patient with acute or recent HIT with detectable HIT antibodies. Either bivalirudin, lepirudin, or danaparoid are appropriate alternatives for intraoperative anticoagulation, provided that appropriate, rapid-turnaround laboratory monitoring and blood product support to manage potentially severe bleeding complications are available. Another approach is to give heparin together with a potent antiplatelet agent (grade 2C).

**Management of the Patient Following Disappearance of HIT Antibodies**

The drawbacks of alternative anticoagulants for CPB provide a rationale for the use of heparin in two groups of patients with a previous history of HIT: (1) a patient with a history of HIT, but who no longer has circulating HIT antibodies detected by sensitive laboratory assay; and (2) a patient with acute or recent HIT who requires elective heart surgery. In the latter situation, it is reasonable to delay cardiac surgery until HIT antibodies become undetectable, which usually occurs in a few weeks or months (Warkentin and Kelton, 2001; Warkentin and Greinacher, 2003).

It is feasible to give UFH for cardiac or vascular surgery in a patient with a previous history of HIT, provided that HIT antibodies are not detectable at the time of surgery (Olinger et al., 1984; Smith et al., 1985; Makhoul et al., 1987; Pöttsch et al., 2000; Warkentin and Kelton, 2001; Warkentin and Greinacher, 2003). We recommend that heparin be avoided completely both before surgery (to prevent restimulation of HIT antibodies) and after surgery (thus making HIT unlikely even if HIT antibodies are reformed). Current evidence suggests that there is a minimum time to formation of clinically significant HIT antibodies of 5 days even in patients who have a previous history of HIT (Cadroy et al., 1994; Warkentin and Kelton, 2001; Lubenow
et al., 2002c). The patient should receive routine doses of UFH for the surgical procedure itself. Preoperative anticoagulation (e.g., for heart catheterization) and postoperative antithrombotic prophylaxis can be achieved with a non-heparin agent such as danaparoid (750 U b.i.d.-t.i.d.) or r-hirudin (15 mg b.i.d. s.c.) (Eriksson et al., 1997) (see Chap. 14).

**Recommendation.** In a patient with a previous history of HIT, heart or vascular surgery can be performed using heparin, provided that HIT antibodies are absent (by sensitive assay) and heparin use is restricted to the surgical procedure itself (grade 1C).

### B. HIT During Pregnancy

There are a few reports describing HIT during pregnancy (Meytes et al., 1986; Henny et al., 1986; Copplestone and Oscier, 1987; Calhoun and Hesser 1987; van Besien et al., 1991; Greinacher et al., 1993a). Danaparoid has been used in at least 31 pregnant women using dosing schedules similar to those in nonpregnant patients. Danaparoid does not cross the placenta, based on cord blood assessment (see Chap. 14).

Lepirudin, bivalirudin, argatroban, danaparoid, and fondaparinux are category B drugs, i.e., indicating absence of fetal damage in certain high-dose animal studies, but limited (if any) human data. Only one report describes the use of lepirudin during pregnancy (Huhle et al., 2000). Danaparoid and fondaparinux (Lagrange et al., 2002) do not appear to cross the placenta in low doses (Markwardt et al., 1988) and has caused embryopathy in rabbits given high doses of hirudin (Lubenow and Greinacher 2000). Further, a zebrafish model reveals that thrombin plays a role in embryogenesis (Jagadeeswaran et al., 1997). Thus, danaparoid and fondaparinux may be preferable for treatment of HIT during (early) pregnancy.

**Recommendation.** If available, danaparoid (and possibly fondaparinux) is preferred for parenteral anticoagulation of pregnant patients with HIT, or in those who have a previous history of HIT (grade 2C).

### C. Treatment of HIT in Children

There are only a few reports describing the management of HIT in children (Oriot et al., 1990; Potter et al., 1992; Murdoch et al., 1993; Klement et al., 1996; Schiffmann et al., 1997; Ranze et al., 1999) (see also Chap. 20); therefore, no clear treatment recommendations can be made. Single cases suggest that lepirudin, argatroban, and danaparoid can be used successfully.
in these children. The dosing schedules for adults (appropriately weight-adjusted for the child) can be used as a guideline, but careful monitoring is recommended.

V. ADJUNCTIVE THERAPIES

A. Medical Thrombolysis

Thrombocytopenia is not a contraindication to thrombolytic therapy in patients with HIT. Streptokinase (Fiessinger et al., 1984; Cohen et al., 1985; Bounaumeaux et al., 1986; Cummings et al., 1986; Mehta et al., 1991), urokinase (Leroy et al., 1985; Krueger et al., 1985; Clifton and Smith, 1986), and tissue plasminogen activator (t-PA) (Dieck et al., 1990; Schiffmann et al., 1997) have been used both systemically and by local infusion (Quinones-Baldrich et al., 1989). In patients at high bleeding risk, an ultra-low-dose t-PA (2 mg/h over 12 h) was successfully applied without bleeding complications (Olbrich et al., 1998). As thrombin generation is not inhibited by thrombolysis, concomitant nonheparin anticoagulation should be given, in reduced dose, until the fibrinolytic effects have waned.

Recommendation. Regional or systemic pharmacological thrombolysis should be considered as a treatment adjunct in selected patients with limb-threatening thrombosis or pulmonary embolism with severe cardiovascular compromise (grade 2C).

B. Surgical Thromboendarterectomy

Vascular surgery is often needed to salvage an ischemic limb threatened by HIT-associated acute arterial thromboembolism involving large arteries (Sobel et al., 1988). When performing vascular surgery during acute HIT, it is appropriate to maintain anticoagulation at least in the lower therapeutic range, if possible, before, during, and after surgery, until platelet count recovery. In patients with latent HIT (i.e., no longer thrombocytopenic, but with clinically significant levels of HIT antibodies still present), the intensity of anticoagulation depends on the perceived risk of vessel (or graft) occlusion. In patients at high risk of occlusion (e.g., surgery involving below-knee vessels), the patient should be therapeutically anticoagulated before vessel clamping (in addition to receiving intraoperative flushes with anticoagulant), with therapeutic anticoagulation maintained for several days after surgery. In surgery involving larger vessels, the use of intraoperative flushes alone, followed by postoperative prophylactic-dose anticoagulation, might be sufficient.
Either danaparoid or lepirudin can provide intraoperative anticoagulation. One author (AG) uses one of the following solutions to flush the vessel postembolectomy: (1) lepirudin, 0.1 mg/mL saline (one 20-mg ampule in 200 mL saline), using up to 250 mL in a normal-weight patient, and assessing the aPTT before giving more lepirudin to avoid overdosage (the lepirudin flushes thus can achieve therapeutic intraoperative anticoagulation; see pp. 425–426); (2) danaparoid, 3 anti-Xa U/mL (one 750 U ampule in 250 mL saline), using up to 50 mL in a normal-weight patient (this small flush dose is used because systemic anticoagulation is achieved by giving a 2250 U bolus of danaparoid preoperatively (see p. 377).

Recommendation. Surgical thromboembolectomy is an appropriate adjunctive treatment for selected patients with limb-threatening large-vessel arterial thromboembolism. Thrombocytopenia is not a contraindication to surgery. An alternative anticoagulant to heparin should be used for intraoperative anticoagulation (grade 1C).

C. Intravenous Gammaglobulin

In vitro, both intact IgG as well as its Fc fragments inhibit HIT antibody-induced platelet activation, an effect that depends somewhat on the method of immunoglobulin preparation (Greinacher et al., 1994a) (see Chap. 9). Case reports describe rapid increase in the platelet counts after high-dose intravenous (iv) IgG (Vender et al., 1986; Frame et al., 1989; Nurden et al., 1991; Grau et al., 1992; Prull et al., 1992; Warkentin and Kelton, 1994). The possibility that ivIgG treatment interrupts platelet activation by HIT antibodies provides a rationale for its use as an adjunct to anticoagulant therapy in certain life- or limb-threatening situations. The dose should be 1 g/kg body weight per day for 2 consecutive days.

Recommendation. ivIgG is a possible adjunctive treatment in selected patients requiring rapid blockade of the Fc receptor-dependent platelet-activating effects of HIT antibodies (e.g., management of patients with cerebral venous thrombosis, severe limb ischemia, or very severe thrombocytopenia) (grade 2C).

D. Plasmapheresis

Plasmapheresis has been associated with successful treatment outcomes in uncontrolled studies of patients with severe HIT (Vender et al., 1986; Bouvier et al., 1988; Nand and Robinson 1988; Thorp et al., 1990; Manzano et al., 1990; Brady et al., 1991; Poullin et al., 1998). Whether this is due to removal of HIT antibodies or pathogenic immune complexes, or even correction of
acquired natural anticoagulant deficiencies by normal plasma replacement, is unresolved. For example, a patient with warfarin-induced acquired protein C deficiency and severe venous limb ischemia may have benefited from correction of the protein C deficiency with apheresis using plasma replacement (Warkentin et al., 1997).

**Recommendation.** Plasmapheresis, using plasma as replacement fluid, may be a useful adjunctive therapy in selected patients with acute HIT and limb-threatening thrombosis who are suspected or proved to have acquired deficiency of one or more natural anticoagulant proteins (grade 2C).

### E. Antiplatelet Agents

**Dextran**

Dextran in high concentrations inhibits platelet function and fibrinogen polymerization. It also inhibits HIT antibody-mediated platelet aggregation (Sobel et al., 1986). However, a prospective randomized trial (Chong et al., 2001) (see Chap. 14) showed that in patients with severe HIT-associated thrombosis, dextran was less effective therapy than danaparoid. It is unknown whether dextran would provide additional clinical benefit if combined with another anticoagulant. Neither of us uses dextran for the management of HIT.

**Recommendation.** Dextran should not be used as primary therapy for acute HIT complicated by thrombosis (grade 1B).

**Acetylsalicylic Acid and Dipyridamole**

Both acetylsalicylic acid (aspirin, ASA) and dipyridamole have been used in HIT patients with variable success (Janson et al., 1983; Makhoul et al., 1986; Kappa et al., 1987, 1989; Laster et al., 1989; Gruel et al., 1991; Hall et al., 1992; Almeida et al., 1998). Sometimes the platelet count appeared to rise promptly with the application of antiplatelet therapy (Warkentin, 1997). However, HIT antibodies are potent platelet activators, and their effect cannot always be blocked in vitro by ASA or dipyridamole. These antiplatelet agents may be used as adjunctive therapy, but one should note that they can cause prolonged platelet inhibition. Because there is little information on the interactions of nonheparin anticoagulants with antiplatelet agents, in HIT combined use should probably be restricted to patients judged to be at high risk for arterial thromboembolism.

**Recommendation.** Antiplatelet agents, such as aspirin, may be used as adjuncts to anticoagulant therapy of HIT, particularly in selected pa-
Platelet Glycoprotein IIb/IIIa Inhibitors

Several platelet glycoprotein (GP) IIb/IIIa inhibitors are now available that potently block fibrinogen binding to platelets. They also can reduce thrombin generation by inhibiting the exposure of procoagulant phospholipid surfaces on platelets (Pedicord et al., 1998; Keularts et al., 1998, Hérault et al., 1998). In vitro, GP IIb/IIIa antagonists inhibit platelet aggregation (Hérault et al., 1997), endothelial cell activation (Herbert et al., 1998), and platelet microparticle generation (Mak et al., 1998) by HIT antibodies. However, Fc receptor–dependent platelet activation by HIT antibodies is independent of the GP IIb/IIIa complex (Greinacher et al., 1994a); therefore, GP IIb/IIIa inhibitors do not inhibit platelet granule release (Tsao et al., 1997; Polgár et al., 1998). As these agents do not have a direct anticoagulant effect, they probably need to be combined with an anticoagulant (danaparoid, lepirudin, or argatroban) to treat HIT. Because there are no data available on the interaction of these newer anticoagulants with the GP IIb/IIIa inhibitors, and because a synergistic effect on bleeding is likely, combined use for the management of HIT should be considered experimental. Theoretically, synthetic GP IIb/IIIa inhibitors with a short half-life could be safer than agents with a long half-life (e.g., abciximab).

**Recommendation.** GP IIb/IIIa inhibitors should be considered as experimental treatment in HIT and used with caution if combined with anticoagulant drugs (grade 2C).

### VI. CAVEATS FOR THE TREATMENT OF HIT

#### A. Low Molecular Weight Heparin

Low molecular weight heparin is less likely than UFH to cause HIT antibody formation as well as clinical HIT (Warkentin et al., 1995, 2003). Furthermore, LMWH binds less avidly to platelets than does UFH (Greinacher et al., 1993b). With functional assays employing platelet-rich plasma, several investigators reported a reduced cross-reactivity of HIT antibodies with LMWH compared with UFH (Ramakrishna et al., 1995; Slocum et al., 1996; Vun et al., 1996); however, with sensitive washed platelet functional assays, the cross-reactivity rate of LMWH is nearly 100% (Greinacher et al., 1992; Warkentin et al., 1995) (see Chap. 11).
Owing to the unavailability of other anticoagulant options during the 1980s, LMWH preparations were often used in Europe for further parenteral anticoagulation of HIT patients. No prospective cohort studies are available, but case reports (Roussi et al., 1984; Leroy et al., 1985; Vitoux et al., 1986; Gouault-Heilmann et al., 1987; Bauriedel et al., 1988; Kirchmaier and Bender, 1988) and a review (Reuter, 1987) suggest that LMWH may benefit some patients. Other case series, however, clearly show that LMWH is associated with disastrous complications in HIT patients (Horellou et al., 1984; Leroy et al., 1985; Gouault-Heilmann et al., 1987; Greinacher et al., 1992; Kleinschmidt et al., 1993). Unfortunately, no laboratory assay reliably predicts these differing treatment responses.

Treatment of HIT with LMWH is frequently unsuccessful. Of eight consecutive HIT patients who received LMWH, thrombocytopenia persisted in all, and new thromboembolic events occurred in two patients (Greinacher et al., 1992). After LMWH became available in North America, a similar experience was observed in seven HIT patients treated with LMWH (War- kentin, 1997). A recent study has also shown a relatively high risk of adverse outcomes of treating HIT with LMWH (Ranze et al., 2000).

**Recommendation.** LMWH should not be used to treat patients with acute HIT (grade 1C).

### B. Oral Anticoagulants (Vitamin K Antagonists)

Although oral anticoagulants, such as warfarin, phenprocoumon, and other coumarin agents, are an important part of the longer-term management of patients with HIT-associated thrombosis, they are ineffective, and potentially dangerous, when given as single therapy, or in combination with ancred, in patients with acute HIT (Warkentin et al., 1997) (see Chap. 3). In patients with active DVT, oral anticoagulants may cause thrombosis to progress to involve even the microvasculature, leading to coumarin-induced venous limb gangrene. This syndrome appears to result from a transient disturbance in procoagulant–anticoagulant balance: increased thrombin generation associated with HIT remains high during early warfarin treatment, while simultaneously there is severe, acquired deficiency in the natural anticoagulant protein C. Although high doses of oral anticoagulants may be more likely to cause this syndrome, even relatively low doses that produce a rise in the INR to higher than 4.0 can cause limb gangrene in some patients. Thus, warfarin and phenprocoumon should always be given in combination with an agent that reduces thrombin generation in patients with acute HIT. Furthermore, it is prudent to delay coumarin anticoagulation until the HIT is ade-
quately controlled by the parenteral anticoagulant, as judged by improved or stabilized thrombotic signs and partial or complete platelet count recovery.

Recommendation. Oral anticoagulants are contraindicated in patients with acute HIT, unless combined with an agent that reduces thrombin generation. (grade 1C+).

C. Ancrod
Ancrod is a defibrinogenating enzyme obtained from the venom of the Malayan pit viper. The thrombin-like enzyme cleaves fibrinopeptide A from fibrinogen but, in contrast with thrombin, does not proteolyze fibrinopeptide B (Bell, 1997). On the basis of uncontrolled studies, ancrod has been used successfully to treat several patients with HIT, primarily in Canada (Teasdale et al., 1989; Cole et al., 1990; Demers et al., 1991).

However, ancrod does not inhibit thrombin generation, and in HIT patients it even appears to increase thrombin generation initially (Fig. 2) (Warkentin, 1998). Animal models indicate that under special clinical circumstances, such as septicemia, ancrod contributes to enhanced fibrin deposition (Krishnamurti et al., 1993). These data could help explain why some patients have developed venous limb gangrene during combined treatment with ancrod and warfarin (Warkentin et al., 1997; Gupta et al., 1998) (i.e., increased thrombin generation during ancrod treatment could contribute to the disturbance in procoagulant–anticoagulant balance during warfarin therapy that has been hypothesized to explain venous limb gangrene) (Warkentin et al., 1997). In a retrospective nonblinded comparison, ancrod appeared to be less effective than danaparoid in one medical community (Warkentin, 1996).

The manufacturer discontinued ancrod in 2002.

D. Platelet Transfusions
Usually there is no need to treat thrombocytopenia with platelet transfusions, as patients with HIT rarely bleed spontaneously. Indeed, platelet transfusions should be avoided because the transfused platelets can be activated by the same immune mechanisms as the patient’s own platelets. Anecdotal experience describes thrombotic events soon after platelet transfusions given to patients with acute HIT (Babcock et al., 1976; Cimo et al., 1979). Several consensus conferences (Contreras, 1998; Hirsh et al., 2001b; British Committee for Standards in Haematology, 2003) stated that thrombotic thrombocytopenic purpura (TTP) and HIT are two disorders in which prophylactic
platelet transfusions are not recommended because of the risk of precipitating thrombosis.

**Recommendation.** Prophylactic platelet transfusions are relatively contraindicated in patients with acute HIT (grade 2C).

Therapeutic platelet transfusions are appropriate for patients with HIT who develop severe hemorrhage, particularly if the heparin administration has been discontinued for more than a day.

E. Vena Cava Filters

Vena cava (Greenfield) filters are sometimes used to manage patients judged to be at high risk for life-threatening pulmonary embolism. However, their use can be complicated by massive vena cava thrombosis, including the renal veins, and other serious progression of venous thromboembolism, especially if pharmacological anticoagulation is not given (Sobel et al., 1988; Jouanny et al., 1993). In our opinion, these devices should rarely be used in patients with acute HIT.

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14
Danaparoid for the Treatment of Heparin-Induced Thrombocytopenia

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I. INTRODUCTION

In patients with heparin-induced thrombocytopenia (HIT), cessation of heparin is mandatory. Thereafter, an alternative anticoagulant is usually needed for the treatment of HIT-associated venous or arterial thrombosis, for the prevention of thrombosis in isolated HIT, or for other indications (Chong, 1995; Hirsh et al., 2001; Warkentin 2001) (see Chap. 13). Currently, three antithrombotic agents, danaparoid sodium, recombinant hirudin (r-hirudin), and argatroban, have been approved in various countries for the treatment of patients with HIT. The clinical use of danaparoid in HIT patients will be reviewed in this chapter, and the other treatments in Chaps. 15 and 16. Danaparoid (Orgaran, Organon NV, The Netherlands; formerly known as Org 10172 and Lomoparan) has been used extensively to treat HIT patients. Worldwide, many thousands of patients have been successfully treated with this drug.
Chemistry, Pharmacology, Pharmacodynamics, and Pharmacokinetics

Chemistry

Although danaparoid is often referred to as a low molecular weight (LMW) heparinoid (implying that it has heparin-like activity), there are, in fact, substantial differences in the chemistry, pharmacology, and pharmacokinetics between danaparoid and both unfractionated heparin (UFH) and low molecular weight heparin (LMWH).

Danaparoid consists of a mixture of LMW glycosaminoglycans; namely, heparan sulfate (84%), dermatan sulfate (12%), and chondroitin sulfate (4%) (Meuleman, 1992). A small proportion of the heparan sulfate molecules have high affinity for antithrombin (AT, formerly known as antithrombin III) (Meuleman, 1992; Ofosu, 1992). Danaparoid has an average molecular mass of approximately 6000 Da. It does not contain heparin or heparin fragments, and differs in chemical composition from heparin in that the repeating disaccharide subunits in heparan sulfate, its principal constituent, are predominantly glucuronic acid and N-acetyl-glucosamine, whereas in heparin, they are mostly iduronic acid and glucosamine-N-sulfate (Gordon et al., 1990) (Fig. 1). Compared with LMWH, danaparoid has a lower degree of sulfation and a lower charge density. These two factors play an important role in its binding (or more precisely, its lack of binding) to plasma proteins and platelets, which is particularly relevant for the pharmacological profile of the drug (Casu, 1991) (see Chap. 8).

Figure 1 Comparison of predominant disaccharide structure of heparin with danaparoid. The low molecular weight (LMW) heparin disaccharide is mostly (left) glucosamine-N-sulfate and (right) iduronic acid, whereas danaparoid’s principal constituent, heparan sulfate, is predominantly (left) N-acetyl-glucosamine and (right) glucuronic acid. The degrees of sulfation (sulfate groups per disaccharide unit) for heparin and danaparoid are approximately 2.0–2.5 and 1.0–1.5, respectively (see Chap. 8).
Pharmacology

Danaparoid exerts its antithrombotic effects predominantly by inhibition of factor Xa; it has only minimal antifactor IIa (antithrombin) activity. Its ratio of antifactor Xa (anti-Xa) to antithrombin (anti-IIa) is 22:1 or higher, which is considerably greater than that of LMWH (2:1–4:1) and UFH (1:1) (Meuleman et al., 1982; Gordon et al., 1990; Meuleman, 1992). Its inhibition of factor Xa is mediated by AT, and its minor effect on thrombin by both AT and heparin cofactor II. Its highly selective inhibition of factor Xa confers on this drug the advantage of a linear inhibitory effect on thrombin generation and fibrin formation. Another advantage is that danaparoid does not interfere with normal platelet function (Meuleman et al., 1982; Mikhailidis et al., 1984, 1987; Meuleman, 1987). Thus, unlike UFH, danaparoid does not interfere with platelet accretion to experimental thrombi, although thrombus growth is markedly reduced by its prevention of fibrin accretion. Similarly, danaparoid has minimal effects on formation of the platelet-dependent hemostatic plug (Meuleman, 1992). These beneficial characteristics of danaparoid contribute to the high therapeutic index (i.e., favorable benefit/risk ratio) of this drug.

Pharmacodynamics and Pharmacokinetics

Danaparoid has a pharmacokinetic profile different from that of UFH or LMWH. It is well-absorbed after subcutaneous administration in humans, with its bioavailability approaching 100% (Stiekema et al., 1989; Danhof et al., 1992). In comparison, the bioavailability of LMWH is 87–92%, and that of UFH only 15–20% (Skoutakis, 1997). Danaparoid’s plasma anti-Xa levels peak 4–5 h following subcutaneous injection (Danhof et al., 1992). Unlike UFH, it is not neutralized by plasma proteins, such as platelet factor 4 (PF4) and histidine-rich glycoprotein; hence, after subcutaneous or intravenous administration, more predictable plasma levels of the drug are obtained.

Danaparoid is eliminated mainly by the kidneys. It has a relatively long plasma anti-Xa half-life ($t_{1/2}$) of about 25 h. Plasma $t_{1/2}$ values of anti-IIa activity and thrombin generation-inhibiting activity are much shorter, ranging from 2 to 4 h and 3 to 7 h, respectively (Bradbrook et al., 1987; Stiekema et al., 1989; Danhof et al., 1992). In patients with impaired renal function, the drug has a tendency to accumulate, and the dose should be reduced accordingly.

Danaparoid’s metabolism is not affected by hepatic cytochrome P-450, nor does it affect hepatic or renal handling of other drugs. It has no significant effect on the pharmacodynamics and pharmacokinetics of coumarin anticoagulants. Its pharmacokinetics are not modified by age or body weight; hence, dose adjustments are usually unnecessary in the elderly or in overweight patients (Stiekema et al., 1989; Danhof et al., 1992).
Similar to LMWH, protamine chloride only very minimally neutralizes the anticoagulant activity of danaparoid. There is no effective antidote for the drug (Stiekema et al., 1989), and in severe bleeding, the drug should be stopped and blood product replacement given, as indicated on clinical grounds. There is limited evidence that plasmapheresis can accelerate elimination of the drug (Schmahl et al., 1997), but this option may not be practical in an unstable patient.

II. CLINICAL USE OF DANAPAROID

A. Clinical Use of Danaparoid in Disorders Other Than HIT

Danaparoid has been studied for the prophylaxis and treatment of venous thromboembolism in several controlled clinical studies of routine (i.e., non-HIT) patients. These trials confirmed the efficacy of danaparoid as an antithrombotic agent and, in some trials, it was even more effective than other standard antithrombotic agents. Six prospective, randomized, controlled, and assessor-blind studies showed that danaparoid is more effective than warfarin, dextran, or low-dose UFH plus dihydroergotamine in preventing deep vein thrombosis (DVT) after total hip replacement or hip fracture surgery, and compared favorably with LMWH in patients undergoing total hip replacement surgery (Bergqvist et al., 1991; Gerhart et al., 1991; Hoek et al., 1992; Leyvraz et al., 1992; Organon report, 1994; Gent et al., 1996). In addition, there are also prospective controlled and uncontrolled studies, as well as case reports, demonstrating the efficacy of danaparoid in the treatment of DVT, acute thrombotic stroke, and disseminated intravascular coagulation complicating promyelocytic leukemia, as well as DVT prophylaxis and the prevention of fibrin deposition on the dialysis membrane during hemodialysis (Henny et al., 1983; Nieuwenhuis and Sixma, 1986; Cade et al., 1987; Biller et al., 1989; von Bonsdorff et al., 1990; Gallus et al., 1993; de Valk et al., 1995).

B. Clinical Use of Danaparoid in Patients with HIT

Danaparoid has been used extensively to treat patients with HIT (Chong and Magnani, 1992; Magnani, 1993, 1997). After the diagnosis of HIT and discontinuation of heparin administration, patients often require an alternative anticoagulant for any one of the following indications: (1) treatment of a recent or new thrombosis; (2) prophylaxis of venous thromboembolism; (3) anticoagulation for cardiopulmonary bypass (CPB) surgery or peripheral arterial surgery; (4) anticoagulation of hemodialysis or hemofiltration; (5) cardiac catheterization or coronary angioplasty; or (6) maintenance of
intravascular catheter patency. The rationale for the use of danaparoid to treat patients with HIT for these indications is that danaparoid is an effective antithrombotic-anticoagulant agent, as shown by the results of controlled trials (Skoutakis, 1997). Furthermore, it has a specific inhibitory effect on HIT antibody-induced platelet aggregation (Chong et al., 1989). Additionally, unlike LMWH, it has a low frequency of in vitro cross-reactivity with HIT antibodies (Makhoul et al., 1986; Chong et al., 1989; Greinacher et al., 1992; Kikta et al., 1993; Vun et al., 1996).

The largest clinical experience with the use of danaparoid in the treatment of patients with HIT is in the compassionate-use (named patient) program organized by the manufacturer (Organon NV, Oss, The Netherlands) (Magnani, 1993, 1997). From 1981 to 1997, over 750 patients were treated under this program for the various foregoing indications (Ortel and Chong, 1998). The duration of treatment ranged from 1 day to 3.5 years, and the posttreatment follow-up was 3 months. Interim, updated reports of this program have been published (Chong and Magnani, 1992; Magnani, 1993, 1997). The overall success rate, defined as platelet count recovery without new, progressive, or recurrent thrombosis during the danaparoid treatment period or thrombotic death during 3 months follow-up, and in the absence of any adverse effect necessitating treatment cessation, has been 91–94%, as judged by the local physician-investigators. However, as this definition does not include nonthrombotic death, the overall mortality observed in the program was 18%, including both the treatment and a 3-month posttreatment follow-up period. Most patients in this program received danaparoid for the treatment of acute thromboembolism, often in the setting of severe illness, such as renal or multisystem organ failure. Besides this compassionate-release program, other studies supporting the efficacy of danaparoid therapy for acute HIT include a randomized controlled trial comparing danaparoid with dextran (Chong et al., 2001), as well as a retrospective analysis comparing danaparoid and lepirudin (Farner et al., 2001), among other studies.

**Treatment of Venous and Arterial Thromboembolism**

Patients with HIT frequently have one or more acute thromboses, which may have occurred before the development of HIT, as a complication of HIT itself, or both (Warkentin and Kelton, 1996). Venous thrombosis complicates HIT more often than does arterial occlusion. Indeed, in the compassionate-use program, the ratio of venous to arterial thrombosis was 2:1, with some patients having both types of thrombosis (Ortel and Chong, 1998). In HIT patients, nevertheless, after cessation of UFH administration, the acute thrombosis requires continuation of antithrombotic therapy. Currently, either danaparoid, r-hirudin, or argatroban is believed to be effective in this
situation (Warkentin et al., 1998). These agents have in common the capacity to inhibit thrombin generation, either by inhibition of factor Xa (danaparoid) or by direct inhibition of thrombin (r-hirudin, argatroban).

In the compassionate-use program, it was recommended that HIT patients with acute thrombosis receive intravenous danaparoid administered as a bolus of 2500 U, followed by an infusion of danaparoid at 400 U/h for 4 h, followed by 300 U/h for 4 h, and then 150–200 U/h for at least 5 days, aiming for a plasma anti-Xa level of 0.5–0.8 anti-Xa U/mL. Table 1 describes a similar protocol that takes into account the amount of danaparoid per marketed ampule (750 anti-Xa U/ampule), as well as certain initial bolus dose adjustments based on body weight. Danaparoid is also effective when administered subcutaneously (de Valk et al., 1995): in this situation, the equivalent 24-h actual or estimated intravenous dose is given in two to three divided doses by subcutaneous injection over a 24-h period. For example, 2250 U (3 ampules) every 12 h by subcutaneous injection is approximately equal to 190 U/h by intravenous infusion given over 24 h. In the compassionate-use program, 464 patients with acute thromboembolism were treated with danaparoid, with efficacy judged to be over 90% (Ortel and Chong, 1998).

Danaparoid treatment for HIT patients also proved efficacious in a prospective, randomized, controlled clinical study (Chong et al., 2001). In this trial, HIT patients with an acute thrombosis (venous, arterial, or both) were randomized to receive either danaparoid plus warfarin, or dextran 70 plus warfarin. Dextran is a glucose polymer, with an average molecular mass of 70 kDa. It is a weak antithrombotic agent that has been used to prevent DVT in postoperative patients (Aberg and Rausing, 1978; Bergqvist, 1980). It is known to block HIT antibody-induced platelet aggregation in vitro (Sobel et al., 1986), and it has been suggested as a potentially useful drug for the treatment of HIT. The reason for its use in the control group was that dextran 70 was the only rapidly acting antithrombotic drug available for the treatment of HIT-associated thrombosis in Australia at study commencement in 1988.

The danaparoid treatment regimen was slightly different from that of the compassionate-use program. Danaparoid was given as a bolus of 2400 U, followed by an infusion of 400 U/h for 2 h, 300 U/h for 2 h, and then 200 U/h for 5 days. In the dextran 70 arm, patients received dextran, 1 L on day 1, and then 500 mL/day from days 2 to 5. In both treatment arms, the patients also received warfarin, with doses adjusted to an INR of 2–4; the warfarin was continued for 3 months. Patients were also stratified at randomization, depending on the severity of their thrombosis, using predefined criteria.

Resolution of thrombocytopenia showed a nonsignificant trend in favor of danaparoid over dextran 70. Among the patients stratified as having “mild” thrombosis, a slightly higher percentage of thromboembolic events
### Table 1  Danaparoid Dosing Schedules in HIT Patients

<table>
<thead>
<tr>
<th>Clinical indication</th>
<th>Danaparoid dosing schedule</th>
</tr>
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<tbody>
<tr>
<td>Venous thromboembolism</td>
<td>750 U sc, b.i.d. or t.i.d.</td>
</tr>
<tr>
<td>Prophylaxis (prior HIT)</td>
<td>Treatment doses (see below) may be appropriate for prophylaxis of acute HIT (see pp. 16, 348–349, 379)</td>
</tr>
<tr>
<td>Prophylaxis (acute HIT)</td>
<td>Subcutaneous administration schedule: 1500–2250 U sc b.i.d. (given almost 100% bioavailability, 2250 U sc b.i.d. is approximately equal to an iv infusion rate of 200 U/h)</td>
</tr>
<tr>
<td>Venous or arterial thromboembolism: Treatment</td>
<td>2250 U iv bolus followed by 400 U/h for 4 h, 300 U/h for 4 h, then 150–200 U/h for ≥5 days, aiming for a plasma anti-Xa level of 0.5–0.8 U/mL</td>
</tr>
<tr>
<td>(either prior or acute HIT)</td>
<td>(see pp. 16, 348–349, 379)</td>
</tr>
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</tr>
<tr>
<td>Embolectomy or other peripheral vascular surgery</td>
<td>Preoperative: 2250 U iv bolus; intraoperative flushes: 750 U in 250 mL saline, using up to 50 mL (see p. 355); postoperative: 750 U sc t.i.d. (low-risk patients) or 150–200 U/h (high-risk patients) beginning at least 6 h after surgery</td>
</tr>
<tr>
<td>Hemodialysis (on alternate days)</td>
<td>3750 U iv before 1st and 2nd dialyses; 3000 U for 3rd dialysis; then 2250 U for subsequent dialyses, aiming for plasma anti-Xa level of &lt;0.3 U/mL predialysis, and 0.5–0.8 U/mL during dialysis (see also Chap. 18).</td>
</tr>
<tr>
<td>Hemofiltration</td>
<td>2250 U iv bolus, followed by 600 U/h for 4 h, then 400 U/h for 4 h, then 200–400 U/h aiming for a plasma anti-Xa level of 0.5–1.0 U/mL (see also Chap. 18).</td>
</tr>
<tr>
<td>Cardiopulmonary bypass surgery (CPB)</td>
<td>125 U/kg iv bolus after thoracotomy; 3 U/mL in priming fluid of apparatus; 7 U/kg/h iv infusion commencing after CPB hookup, and continued until 45 min before expectation of stopping CPB (see also Chap. 19).</td>
</tr>
<tr>
<td>Cardiac catheterization</td>
<td>Preprocedure: 2250 U iv bolus (3000 U if 75–90 kg and 3750 U if &gt;90 kg)</td>
</tr>
<tr>
<td>Percutaneous transluminal coronary angioplasty (PTCA) or intra-aortic balloon pump</td>
<td>Preprocedure: bolus as per foregoing; postprocedure: 150–200 U/h for 1–2 days after PTCA (or until removal of balloon pump)</td>
</tr>
<tr>
<td>Catheter patency</td>
<td>750 U in 50 mL saline, then 5–10 mL per port, or as required</td>
</tr>
<tr>
<td>Pediatric dosage considerations</td>
<td>Prophylaxis: 10 U/kg sc b.i.d.</td>
</tr>
<tr>
<td></td>
<td>Treatment: 30 U/kg b.w., iv bolus, then 1.2–2.0 U/kg b.w./h depending upon severity of thrombosis</td>
</tr>
</tbody>
</table>

**Abbreviations:** b.w., body weight; b.i.d., twice daily; iv, intravenous; t.i.d., three times daily.

Compatibility with intravenous solutions: Danaparoid is compatible for dilution with the following solutions: saline, dextrose, dextrose–saline, Ringer’s, lactated Ringer’s, 10% mannitol.

Preparation of solution for infusion: One option is to add four ampules containing 3000 U (i.e., 750 anti-Xa U/0.6 mL ampule) of danaparoid to 300 mL of intravenous solution (i.e., a solution that comprises 10 U danaparoid per milliliter of intravenous solution: thus, an infusion rate of 40 mL/h corresponds to a dose of 400 U/h; 20 mL/h to a dose of 200 U/h, and so on.

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a Adjust iv danaparoid bolus for body weight: <60 kg, 1500 U; 60–75 kg, 2250 U; 75–90 kg, 3000 U; >90 kg, 3750 U.
treated with danaparoid (83%) improved compared with those who received dextran 70 (73%). In contrast, a substantial and significant difference in treatment outcome occurred in patients with “serious” thrombosis: 88% of danaparoid-treated thromboembolic events recovered, compared with 44% of those treated with dextran 70. These data suggest that the use of an effective anticoagulant to treat thromboembolism associated with HIT is particularly important in those with more severe disease.

Recently, treatment outcomes in patients with HIT who received either danaparoid or lepirudin have been compared (Farner et al., 2001). Although not a randomized trial, this study had important strengths. First, all patients had serologically confirmed HIT, with about 70% having thrombosis at the time of study entry. Second, all patients met the same inclusion and exclusion criteria, had similar baseline characteristics, and were treated during the same time period (25 months ending April 1996). Third, many patients were studied (danaparoid, \( n = 126 \); lepirudin, \( n = 175 \)). Furthermore, patients were subdivided into those treated with prophylactic or therapeutic doses.

This study suggests that both danaparoid and lepirudin have similar efficacy for treatment of HIT-associated thrombosis when given in therapeutic doses: the day 42 success rate was about 80% for either agent, when failure was defined as the cumulative event rate of a composite endpoint of new thrombosis, death, or limb loss. When evaluating the single endpoint of new thrombosis in those patients who received therapeutic doses of study drug, danaparoid and lepirudin also showed similar efficacy (90.6 vs. 92.1%; \( p = 0.74 \)). Moreover, safety analysis of all patients (regardless of dose received) showed significantly fewer major bleeds with danaparoid (2.5 vs. 10.4%; \( p = 0.009 \)). These data suggest that the favorable therapeutic index of danaparoid extends even to patients with a serious prothrombotic disorder such as acute HIT complicated by thrombosis.

Overlapping Oral Anticoagulants with Danaparoid

In the treatment of HIT patients, it is important to inhibit thrombin generation adequately with danaparoid until the acute thrombosis is well controlled. Furthermore, it generally takes at least 5 days of warfarin therapy before therapeutic functional hypoprothrombinemia is achieved (Harrison et al., 1997). Thus, even when oral anticoagulant therapy is begun soon after starting danaparoid, at least 5 days of danaparoid therapy are usually required. Many danaparoid-treated HIT patients also receive overlapping warfarin treatment, because oral anticoagulants are usually preferred when at least 3–6 months of further anticoagulation is indicated because of venous or arterial thromboembolism. Warfarin administration can be started safely together with danaparoid in patients who do not have severe HIT-associated thrombosis. However, in HIT patients with severe or extensive thrombosis, it
is prudent to delay administration of warfarin until the thrombotic process is controlled and substantial resolution of the thrombocytopenia has occurred. This caveat is based on the observation that warfarin may aggravate the thrombotic process during the first few days of its administration, by reducing levels of the natural anticoagulant protein C, particularly when there is uncontrolled thrombin generation (Warkentin, 1996a; Warkentin et al., 1997; Pötzsch et al., 1996) (see Chaps. 3 and 12). Warfarin does not neutralize activated coagulation factors, and thus sufficient time must pass before its antithrombotic effects are achieved through reduction in the vitamin K–dependent procoagulant factors, particularly prothrombin. Danaparoid therapy usually is discontinued when the INR is at, or near, the target therapeutic range, and provided the acute thrombosis appears controlled on clinical grounds. Danaparoid does not interfere with INR measurements during oral anticoagulant therapy.

Prophylaxis During Acute HIT

In contrast to the comparable efficacy of danaparoid and lepirudin when used in therapeutic doses to treat HIT-associated thrombosis (discussed previously), lepirudin appeared somewhat more effective than danaparoid when prophylactic-dose regimens were compared for preventing the single endpoint of new thrombosis (91.4 vs. 81.4%; \( p = 0.138 \)); this difference was larger, and reached statistical significance, when the composite endpoint (new thrombosis, limb amputation, or death) was examined (Fig. 2). However, superior efficacy of lepirudin came at a price: for patients without thrombosis at baseline (most of whom thus received prophylactic-dose therapy), lepirudin was associated with a trend to more major bleeding events than danaparoid (16.3 vs. 2.9%; \( p = 0.075 \)) (Farner et al., 2001). Kodityal and colleagues (2003) reported 5 patients who developed new thromboses while receiving relatively low doses of danaparoid (usually, 1250 U every 12 hours by subcutaneous injection).

These data support current treatment recommendations (Warkentin, 2001) (see Chaps. 1 and 13) that therapeutic doses of danaparoid are appropriate for most patients with HIT, whether they have HIT-associated thrombosis or just isolated HIT. A comparable situation exists with the two thrombin inhibitors available to treat HIT: argatroban is approved in the United States in therapeutic doses, both for HIT-associated thrombosis and isolated HIT (see Chap. 16). And, although there exists a lower-dose (prophylactic) regimen for lepirudin to manage isolated HIT (see Chap. 15), the use of anticoagulant monitoring to adjust the infusion rate means that most patients eventually receive doses that approach the therapeutic regimen (Warkentin, 2001).
Figure 2  Comparison of the outcomes of HIT patients, with and without thromboembolic complications (TEC), before the start of alternative anticoagulation; danaparoid vs. lepirudin: Time-to-event analysis of the incidences of a combined endpoint (new thromboembolic events, limb amputation, death; maximum, one endpoint per patient) up to day 42. Among patients without TEC at baseline (most of whom were treated with a prophylactic-dose regimen), there was a significantly higher incidence of the combined endpoint among patients treated with danaparoid, compared with lepirudin ($p = 0.02$, log-rank test). This suggests that the approved, prophylactic-dose regimen for danaparoid (750 U b.i.d. or t.i.d. by subcutaneous injection, without anticoagulant monitoring) may be relatively less effective for managing patients with isolated HIT, compared with the prophylactic-dose regimen for lepirudin (initial intravenous infusion rate, 0.10 mg/kg/h; subsequently, dose adjusted by aPTT). In contrast, the combined endpoint did not differ significantly between danaparoid and lepirudin for patients with TEC at baseline, suggesting that therapeutic (treatment) doses of danaparoid (see Table 1) have similar efficacy as does therapeutic-dose lepirudin (see Table 2 in Chap. 15). Also shown is the number of patients at risk on the starting day and at subsequent 7-day intervals. (From Farner et al., 2001.)
Prophylaxis of Venous Thromboembolism

Patients with a previous history of HIT may require an alternative anticoagulant to prevent venous thromboembolism if they are in a high-risk situation, such as following surgery. UFH cannot be used, particularly during the first 1 or 2 months after the onset of HIT when HIT antibodies still circulate. Thereafter, although HIT antibodies are usually undetectable, and the risk of HIT is possibly relatively low (Warkentin and Kelton, 2001), most physicians are understandably reluctant to readminister heparin in this situation.

Danaparoid is an effective and convenient drug for the prevention of venous thromboembolism in patients with prior HIT. In the compassionate-use program, 390 patients received danaparoid, 750 U by subcutaneous injection, usually twice daily for DVT prophylaxis for many postoperative settings, including following general, gynecological, neurological, cancer, and organ transplant surgery. A high rate of success was observed (Magnani, 1997; Ortel and Chong, 1998).

Prophylaxis of Arterial Thromboembolism

Danaparoid has been used to prevent arterial thromboembolism in patients undergoing various vascular operations, including peripheral artery bypass graft surgery, embolectomy, and endarterectomy. In these patients, it was given as a preoperative intravenous bolus of 2500 U, and in some it was also administered postoperatively. Given as an intravenous bolus of 2500 U immediately before the procedure, danaparoid has also been used to provide antithrombotic cover for percutaneous coronary angioplasty, with or without stenting, and for insertion of inferior vena cava filters and intra-aortic balloon devices.

Anticoagulation for Cardiopulmonary Bypass Surgery

Patients with acute HIT, or recent previous HIT with persisting HIT antibodies, may need to undergo cardiac surgery. UFH is contraindicated for these patients, necessitating an alternative anticoagulant for use during cardiopulmonary bypass surgery (CPB). After successful experiments in dogs (Henny et al., 1985a), danaparoid has been used since 1985 for CPB in these patients (Magnani, 1993; Wilhelm et al., 1996; Christiansen et al., 1998; Fernandes et al., 2000; Olin et al., 2000). A report summarizes the experience of 47 evaluable patients who underwent CPB using danaparoid (Magnani et al., 1997).

The initial recommended dosing schedule consisted of 8750 U by intravenous bolus postthoracotomy (with the bolus dose changed to 5,000 and 10,000 U for patients weighing <60 and >90 kg, respectively) plus 7500 U danaparoid given in the priming fluid of the CPB machine. Further intra-
operative booster injections were recommended up to an hourly basis, if necessary, because of fibrin clot formation, but not closer than 45 minutes to the expected end of CPB. In the 47 reported patients who received danaparoid for CPB under the compassionate-use program, the recommended dosing schedule was not strictly followed in all patients. Some received inappropriate or wrongly timed booster doses (too near to surgical closure) and others received substantially higher doses in erroneous attempts to obtain the same activated-clotting time (ACT) prolongation as with UFH, even though anticoagulant-effective danaparoid doses do not prolong the ACT (Gitlin et al., 1998).

Despite suboptimal dosing in some patients, cardiac surgery nevertheless could be successfully completed in 45 of the 47 patients (Magnani et al., 1997). In two patients, the operations had to be abandoned because of formation of “clots” in the operative field or in the CPB circuit. In another 16 patients, intraoperative clots were seen, but dissolved on booster doses of danaparoid. No patients in this series had detectable in vitro cross-reactivity; thus, intraoperative clot formation is unlikely to be attributable to a manifestation of in vivo cross-reactivity. Intraoperative clot formation did not result in clinically evident thrombosis in any of the patients.

Increased postoperative bleeding was a significant problem with the use of danaparoid for CPB (Magnani et al., 1997). Eleven (22%) of the patients suffered severe postoperative blood loss, and another 33% had mild-to-moderate bleeding. Possible reasons for the excessive bleeding included (1) excessive danaparoid dosing owing to ad hoc protocol modifications; (2) administration of danaparoid booster doses too close to the end of surgery; (3) use of blood salvage techniques that returned anticoagulant-containing blood to the patient; and (4) the long plasma half-life of danaparoid and lack of an antidote to neutralize its anticoagulant effect (Skoutakis, 1997). It is also theoretically possible that suboptimal anticoagulation during CPB would paradoxically be associated with greater postoperative bleeding, as greater in vivo thrombin generation during CPB might lead to greater postoperative blood loss from secondary hyperfibrinolysis. This issue is raised because the empirically modified protocol subsequently described may, therefore, not necessarily be associated with reduction in bleeding outcomes.

On review of this experience, it was hypothesized that unnecessarily high drug doses contributed to postoperative bleeding. Supporting evidence included the observation that no severe bleeding was observed in patients who received a total dose of danaparoid of 16,250 U or less (i.e., ≤250 U/kg) (Magnani et al., 1997). Therefore, a new dosing regimen (see Table 1) was developed that delivers no more than 232 U/kg of the drug. Continuous intraoperative danaparoid infusion is also recommended, which may reduce the need for a further drug bolus shortly before wound closure, as well as provide therapeutic drug levels throughout CPB.
Disappointingly, the new regimen does not appear to have reduced the frequency of severe postoperative bleeding. This new dosing regimen was used by Olin and coworkers (2000) to manage five patients with acute or prior HIT for CPB: four of the patients experienced prolonged bleeding requiring reexploration and extensive transfusions, and two patients had clots identified in the surgical field. Other investigators later amended the protocol to continue danaparoid until completion of CPB (rather than stopping the anticoagulant 45 min before the expected end of pump run) because of problems with clotting in the CPB circuit or in the operative field (Fernandes et al., 2000).

Advances in surgical method may permit other treatment approaches in selected patients. For example, the off-pump (“beating heart”) technique does not utilize CPB, and thus a far lower dose of danaparoid may be feasible for intraoperative anticoagulation. This approach was used successfully to perform multiple coronary artery bypass grafting in a patient with acute HIT and unstable angina (Warkentin et al., 2001). A relatively low target plasma antifactor Xa level (0.6 U/mL) was used, rather than the conventional level (>1.5 U/mL) sought during CPB (see Chap. 19).

A randomized, double-blind comparison of danaparoid (n = 34) with heparin (n = 37) for off-pump coronary artery bypass grafting in non-HIT patients showed a nonsignificant trend to greater postoperative blood loss (mean, 264 mL) but a significant increase in patients exposed to homologous blood (53% vs. 27%) with danaparoid. However, clinical outcomes appeared similar, and the authors concluded that danaparoid could be a valuable option in patients undergoing off-pump surgery when heparin is contraindicated (Carrier et al., 2002).

Other approaches for managing CPB or off-pump surgery in patients with acute or previous HIT are discussed further in Chaps. 13 and 19.

Hemodialysis and Hemofiltration

Danaparoid has been used to anticoagulate patients with HIT requiring hemodialysis or hemofiltration, in one of several clinical settings (Henny et al., 1983, 1985b). First, patients with chronic or acute renal failure undergoing regular hemodialysis with UFH occasionally develop HIT, thus necessitating use of an alternative anticoagulant for subsequent dialyses. Second, very ill patients in intensive care settings who develop HIT not uncommonly have a need for anticoagulation during hemodialysis or hemofiltration (Wester et al., 2000; Lindhoff-Last et al., 2001). Third, danaparoid is useful for patients who experience difficulty in undergoing hemodialysis or hemofiltration with UFH because of repeated deposition of fibrin on the dialysis or hemofiltration membranes (Burgess and Chong, 1997). A change from UFH to danaparoid often allows continuation of hemodialysis or hemofiltration without further
incident. This problem, which may be secondary to UFH-induced platelet aggregation and microthrombus formation, despite absence of HIT antibodies, may be a manifestation of nonimmune heparin-associated thrombocytopenia, although significant thrombocytopenia usually does not occur (Burgess and Chong, 1997).

Because danaparoid is cleared renally, the drug accumulates in the blood of patients with renal failure undergoing hemodialysis or hemofiltration with the heparinoid. A reduction in the dose of danaparoid generally is necessary for the second or third procedure after 2 or 3 days. For the first hemodialysis, an intravenous bolus of 3750 U (2250 U if the body weight is <55 kg) is usually administered. A plasma anti-Xa level should be performed before the second and subsequent dialysis. The drug dose should be appropriately reduced (usually to 2250 or 3000 U), depending on whether hemodialysis is performed daily or every second day. The aim is to maintain a plasma anti-Xa level between 0.5 and 0.8 U/mL. For hemofiltration, the dosing regimen is similar to the intravenous infusion regimen used for the treatment of venous thrombosis (see Table 1). To avoid excessive accumulation of the drug in the blood, the infusion rate may be reduced on the second or subsequent day, according to the plasma anti-Xa levels.

Reviews of danaparoid use in heparin-intolerant patients have identified 228 cases of HIT patients with acute or chronic renal failure (Magnani, in preparation; Gallus and Magnani, in preparation). Clinical outcome data show that danaparoid provides efficacious and safe anticoagulation, having been used for up to 4 years for routine hemodialysis (three times per week), and has also been found to extend the lifespan of the hemofilters (van Eps et al., 2000; Lindhoff-Last et al., 2001). Nevertheless, these patients had a relatively high mortality (26.3%) associated with severe comorbidity (the mortality was 51.2% in the subset of 77 patients requiring hemofiltration, usually in the setting of multiple organ failure).

Use in Children and Pregnant Women

Danaparoid has been used in only a very small number of pediatric and pregnant patients (Gill and Kovacs, 1997; Ranze et al., 1999, 2001; Saxon et al., 1999; Macchi et al., 2000). Its major use in these patients has been for the treatment of HIT. Sixteen children were treated with danaparoid for various indications, including maintenance of catheter patency, renal dialysis, cardiac surgery, and thrombosis. The treatment was successful in all except one patient in whom DVT occurred during danaparoid use associated with formation of HIT antibodies. In general, it was noted that children, particularly infants, often required higher doses of danaparoid than adults on a weight-adjusted basis.
Danaparoid is known to have been used in 31 pregnancies in women with HIT. All had a history of thrombosis, either acute or during previous pregnancy. Danaparoid was begun mainly in the first or third trimesters, and continued for up to 34 weeks. In 19 pregnancies no significant problems occurred. In the remaining 12, new thrombosis occurred in 6 (successfully treated by increasing danaparoid dosing in 3); one of the other 3 patients with new thrombosis developed HIT antibodies. Two maternal major bleeds occurred, one due to abruptio placenta (fatal bleeding in a Jehovah’s witness refusing blood transfusion) and the other due to placenta previa (fatal cardiopulmonary complications). In another patient danaparoid was stopped because of in vitro and in vivo evidence of danaparoid cross-reactivity. Two fetal and one infant death occurred in mothers with systemic lupus erythematosus or antiphospholipid antibody syndrome. One of these was further complicated by onset of HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), which began 6 weeks into treatment with danaparoid.

In 4 infants, testing of cord plasma revealed no anti-Xa activity. In four breast milk samples obtained from mothers continuing danaparoid postpartum, anti-Xa activity was virtually undetectable. Thus, the main antithrombotic subfraction of danaparoid does not cross the placenta, and the tiny amounts which appear in the breast milk are probably hydrolyzed in the infant’s stomach (Lindhoff-Last and Bauersachs, 2002). No increase in postpartum bleeding in danaparoid-treated pregnant mothers was reported. The doses of danaparoid used in these pregnant patients were the same as those used in nonpregnant women for the same clinical situations.

C. Laboratory Monitoring

Measurement of plasma anti-Xa levels using an amidolytic assay can be used for the laboratory monitoring of danaparoid’s anticoagulant action. The heparinoid does not significantly prolong the activated partial thromboplastin time (APTT), prothrombin time (INR), or activated-clotting time (ACT), except at very high doses. Hence, these assays cannot be used for laboratory monitoring of danaparoid. However, monitoring is not required in many clinical situations. The drug has a bioavailability of almost 100% after subcutaneous administration, and because of its lack of plasma protein interaction, predictable plasma levels are usually obtained with subcutaneous or intravenous use. However, laboratory monitoring is recommended in the following clinical settings: (1) patients with substantial renal impairment; (2) patients with unusually low or high body weight; (3) patients with life- or limb-threatening thrombosis; (4) patients with unexpected bleeding; and (5) critically ill or unstable patients.

It must be emphasized that for any assay of danaparoid-associated plasma anti-Xa activity, the standard calibration curve must be constructed...
using danaparoid, and not UFH or even LMWH (Laposata et al., 1998). Because of its lack of interaction with plasma heparin-binding proteins, danaparoid gives a dose-response relation different from these heparins, and plasma anti-Xa levels during danaparoid treatment will be overestimated if a LMWH standard curve is used. Indeed, there are differences in the stated therapeutic range among these various glycosaminoglycan anticoagulants (UFH, 0.2–0.4 U/mL by protamine titration; UFH, 0.3–0.7 anti-Xa U/mL; LMWH, 0.6–1.0 U/mL; danaparoid, 0.5–0.8 anti-Xa U/mL) (Hirsh et al., 1998; Laposata et al., 1998; Warkentin et al., 1998). In some clinical treatment settings using danaparoid, it might be advisable to aim for a lower anti-Xa level (e.g., about 0.3 U/mL for a patient judged to have a high risk of bleeding); sometimes, a higher target anti-Xa level should be sought (e.g., about 1.0 U/mL for a patient with life- or limb-threatening venous or arterial thrombosis).

Anti-Xa levels are determined using a chromogenic assay (i.e., a method similar to that performed for monitoring LMWH treatment). A standard reference curve must be constructed using various dilutions of danaparoid (e.g., 1.6, 1.0, 0.5, 0.3, and 0 U/mL danaparoid, diluted in pooled normal platelet-poor plasma). Control plasma samples are prepared by adding known quantities of danaparoid to normal pooled plasma aliquots (assuming 100% recovery of the known quantity of danaparoid added) in three different concentrations approximating treatment situations (e.g., 0.2, 0.7, and 1.25 U/mL, corresponding to low-, mid-, and high-control danaparoid levels). Aliquots stored at −70°C are stable indefinitely if used only once, without refreezing and rethawing.

D. Cross-Reactivity of HIT Antibodies with Danaparoid

As danaparoid consists of a mixture of glycosaminoglycans (mainly heparan sulfate), it is not surprising that a small percentage of antibodies from HIT patients do cross-react with the drug. The cross-reactivity rate is low (mean, 9.6%) if aggregation studies using citrated platelet-rich plasma are performed (Makhoul et al., 1986; Kikta et al., 1993; Ramakrishna et al., 1995; Vun et al., 1996), but higher if more sensitive washed platelet activation (Warkentin, 1996b; Koster et al., 2000) or a fluid-phase antigen assay (Newman et al., 1998) is used (see Chap. 11). Because activation assays are dependent on the donor platelets used for testing, we have used highly reactive donor platelets under standardized conditions using platelet aggregation, to investigate the cross-reactivity of HIT antibodies for danaparoid and LMWH. We found cross-reactivity rates of 7% with danaparoid and 83–89% with LMWH (Vun et al., 1996). However, with the sensitive fluid-phase antigen assay, we observed a higher cross-reactivity rate of 50% with danaparoid, and again, a much higher rate (88%) with LMWH (Newman et al., 1998) (Fig. 3).
Importantly, even when in vitro reactivity with danaparoid is observed, it is generally weak and quantitatively less than seen with LMWH.

The in vitro cross-reactivity of the HIT antibodies with danaparoid does not appear to be clinically significant. We have investigated the clinical significance of in vitro cross-reactivity in 21 patients treated with the LMW heparinoid (Newman et al., 1998). The 8 patients who tested positive with danaparoid by the fluid-phase enzyme immunoassay, but negative by the \([^{14}\text{C}]\)serotonin-release washed platelet assay, recovered with resolution of their thrombocytopenia and thrombosis, in a fashion similar to the 11

**Figure 3**  Cross-reactivity of HIT-IgG antibodies with PF4 complexed to heparin-like anticoagulants: The fluid-phase enzyme-linked immunoassay was used to assess the degree to which IgG present in HIT sera or plasma bound to PF4 alone, PF4-heparin, PF4-dalteparin (Fragmin), PF4-enoxaparin (Clexane), or PF4-danaparoid (Orgaran). The positive cutoff (dashed line) is 3 standard deviations above the mean (log transformed) absorbance of the normal samples (triangles) using PF4-heparin. The binding of normal antibodies is indicated by triangles. Circles indicate HIT samples that have been positive (closed circle), negative (open circle), or not tested (speckled circle) in a functional assay with the corresponding drug. (From Newman et al., 1998.)
patients who did not manifest in vitro cross-reactivity to danaparoid by both assays. Two patients tested positive by both activation and antigen assays: in 1 patient, both thrombocytopenia and pulmonary embolism resolved during danaparoid treatment. However, in the other patient, thrombocytopenia and extensive thrombosis persisted despite danaparoid therapy. It is unclear whether this unusual patient course represented a specific danaparoid treatment failure, as the patient’s subsequent clinical course was characterized by consistent failure for all of the antithrombotic therapies used (Fig. 4).

Warkentin (1996b) also evaluated the clinical significance of in vitro cross-reactivity with danaparoid in 29 HIT patients who had been treated with the heparinoid for HIT. This investigator found no difference in clinical outcomes, or in the time to platelet count recovery, between the two patient groups. However, there are isolated anecdotal reports of unfavorable clinical outcomes in HIT patients treated with danaparoid (Tandy-Poncet et al., 1995; Insler et al., 1997; Muhm et al., 1997). Cross-reactive antibodies were not always investigated in these studies. It remains possible that unrelated clinical factors might have caused the fall in the platelet count in these patients.

**Figure 4** Serial platelet counts of representative HIT patients treated with danaparoid: Solid black bar shows duration of heparin administration, striped bar indicates danaparoid therapy, open bar shows warfarin therapy. (a) Typical profile of the 11 patients who were negative in both fluid-phase EIA and functional assay. (b) Typical profile of the eight patients who were positive in the fluid-phase, but negative in a functional assay. (c) Profile of patient A, one of the two patients who were positive in both types of assay. She recovered during a short course of danaparoid. (d) Profile of patient B, the other patient positive in both assays. The profile indicates the course of HIT following transfer to a major hospital. Despite treatment with many antithrombotic agents, he eventually died following major thrombosis. (From Newman et al., 1998.)
Additionally, it may not be possible to distinguish clinical cross-reactivity to danaparoid with the natural course of a severe episode of HIT (Warkentin, 1998; Baumgärtel et al., 2000).

Overall, on the balance of current evidence, it would appear that in vitro cross-reactivity with danaparoid is not associated with adverse clinical outcomes in most patients. Accordingly, many physicians do not obtain cross-reactivity test results before instituting danaparoid therapy. This approach seems reasonable. It is based on several considerations: the overall low risk of in vivo cross-reactivity, the low predictivity of in vitro cross-reactivity testing, the lack of standardized test methods, and the potential for adverse clinical events during withholding of treatment pending the results of cross-reactivity testing. However, cross-reactivity testing should be performed in patients who develop new, progressive, or recurrent thrombocytopenia or thrombosis during treatment with danaparoid.

E. Adverse Effects

Severe bleeding, the most serious adverse effect of danaparoid, rarely occurs except in patients who are treated with very high doses of the drug, or in those who develop drug accumulation (renal failure), or who have additional hematostatic or vascular defects. However, serious bleeding occurred in a significant number of patients who had undergone CPB with danaparoid (Magnani et al., 1997; Westphal et al., 1997, Fernandes et al., 2000; Olin et al., 2000). In contrast, bleeding was not seen in the randomized trial in which HIT patients with venous or arterial thromboses received danaparoid (Chong, 1996). Compared with CPB patients, these patients underwent less intense anticoagulation and did not suffer from the additional insults of CPB and chest incision.

Skin hypersensitivity reactions have been reported with danaparoid, but these are rare (Magnani, 1993). Osteoporosis (an important complication of prolonged UFH treatment) was not detected in any danaparoid-treated patients in the compassionate-use program, even in those treated for more than 3 months. Despite the issue of in vitro cross-reactivity with danaparoid, it is noteworthy that new-onset immune-mediated thrombocytopenia has never been reported with this agent.

F. Availability of Danaparoid

Table 2 lists the countries in which danaparoid has been approved for the treatment of HIT, either with or without associated thrombosis. In some countries in which danaparoid is approved for DVT prophylaxis, physicians have the legal option to prescribe danaparoid for HIT (i.e., for “off-label” use in a nonapproved indication) (see Chaps. 19 and 20). Danaparoid is no longer
Table 2 Countries in Which Danaparoid Is Approved for Clinical Use

<table>
<thead>
<tr>
<th>Country</th>
<th>DVT prophylaxis</th>
<th>Heparin-induced thrombocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Perioperative⁶</td>
<td>Prophylaxis¹</td>
</tr>
<tr>
<td></td>
<td>Poststroke</td>
<td>Treatment</td>
</tr>
<tr>
<td>North America</td>
<td></td>
<td></td>
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<tr>
<td>Canada</td>
<td>X</td>
<td>X</td>
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<tr>
<td>United States</td>
<td>X d</td>
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<tr>
<td>Europe</td>
<td></td>
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<tr>
<td>Austria</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Belgium</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Denmark</td>
<td>X</td>
<td></td>
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<tr>
<td>Finland</td>
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<td>X</td>
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<tr>
<td>France</td>
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<tr>
<td>Germany</td>
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<td></td>
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<tr>
<td>Great Britain</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Greece</td>
<td>X</td>
<td></td>
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<tr>
<td>Ireland</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Italy</td>
<td>X</td>
<td></td>
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<tr>
<td>Luxembourg</td>
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<td>X</td>
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<tr>
<td>Netherlands</td>
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<td>Norway</td>
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<td>X</td>
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<tr>
<td>Portugal</td>
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<td>X</td>
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<td>Sweden</td>
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<td>X</td>
</tr>
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<td>Switzerland</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Australasia and Africa</td>
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<td></td>
</tr>
<tr>
<td>Australia</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Japan</td>
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<td></td>
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<tr>
<td>Korea</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>South Africa</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

a Danaparoid is no longer marketed in some of these countries, e.g., United States, Great Britain, Norway.
b Orthopedic and general surgery only (unless otherwise indicated); approval includes starting danaparoid 1–4 h preoperatively (except for U.S.).
c Approved dose of 750 U b.i.d.–t.i.d. may be too low for acute HIT (see pp. 16, 348–349, 379).
d Elective hip surgery only.
e Orthopedic and cancer surgery only.
f Approval modified to facilitate approval for HIT in Finland and Germany.
g Approved only for treatment of DIC.
marketed in some countries (e.g., United States [since April 2002], United Kingdom, Norway).

III. CONCLUSION

Danaparoid is a safe and effective anticoagulant for the prevention or treatment of venous or arterial thrombosis in HIT patients, regardless of the presence of antibody cross-reactivity with the drug. It is also efficacious as an anticoagulant for hemodialysis-hemofiltration and cardiac surgery employing CPB. With the use of danaparoid for the cardiac surgery, serious postoperative bleeding can occur.

ACKNOWLEDGMENTS

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Lepirudin for the Treatment of Heparin-Induced Thrombocytopenia

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Ernst-Moritz-Arndt University Greifswald, Greifswald, Germany

I. INTRODUCTION

With its approval by the European Medical Evaluation Agency (EMEA) in 1997 and by the U.S. Food and Drug Administration (FDA) in 1998 for the treatment of heparin-induced thrombocytopenia (HIT) complicated by thrombosis, lepirudin (Refludan, Schering AG, Berlin; licensed to Berlex Laboratories, Montville, NJ [US, Canada], and to Pharmion, Cambridge, UK [all other countries]) became the first direct thrombin inhibitor (DTI) available for this indication. The scientific literature has documented experience with lepirudin in more than 8500 patients. The total number of patients exposed to this anticoagulant (until 2003) is estimated at between 35,000 and 60,000 worldwide.

II. HIRUDIN AND ITS DERIVATIVES

A. Chemistry

Hirudin, the most potent natural thrombin inhibitor identified to date, is a 65-amino-acid polypeptide (molecular mass, approximately 7 kDa) produced by the parapharyngeal glands of the medicinal leech, Hirudo medicinalis. The NH₂ terminal part of the molecule (residues 1–39) is stabilized by three disulfide bridges integral to its function. The COOH-terminal moiety (residues 40–65) is highly acidic. In the three-dimensional structure of hirudin (Clore et al., 1987; Sukumaran et al., 1987), three areas are distinguished: a
central core (residues 3–30, 37–46, 56–57), a “finger” (residues 31–36), and a loop (residues 47–55). Hirudin is very stable at extremes of pH (1.5–13.0) and at high temperatures (>90°C). It is soluble in water, but insoluble in alcohol or acetone. The isoelectric point of hirudin is approximately 4.

Hirudins for therapeutic use are now produced by recombinant biotechnology, using the yeast Saccharomyces cerevisiae, yielding recombinant hirudin (r-hirudin). Lepirudin, a desulfatohirudin, differs from natural hirudin by lacking the sulfate group at Tyr-63 and also has an NH₂-terminal leucine residue in place of the isoleucine. Although such structural differences result in a 10-fold reduction in the dissociation constant of r-hirudin, as compared with natural hirudin, r-hirudins remain highly selective inhibitors of thrombin, with an inhibition constant for thrombin in the picomolar range (Stone and Hofsteenge, 1986).

B. Pharmacology

Lepirudin acts independently of the cofactors antithrombin and heparin cofactor II (Markwardt, 1992) and forms tight, noncovalent 1:1 complexes with thrombin. Interacting with both binding sites, lepirudin is a bivalent inhibitor of thrombin (cf. argatroban, a univalent DTI) (Fig. 1). Lepirudin inhibits all the biological activities of thrombin.

Three amino acids (residues 46–48) near the NH₂-terminus of hirudin bind to the active site cleft on thrombin, while the core of the hirudin molecule closes off the active site pocket of thrombin. The COOH-terminal tail of hirudin interacts with the fibrinogen anion-binding site, helping to block thrombin-catalyzed fibrinogen cleavage. Hirudin inhibits the feedback loop whereby thrombin enhances its own generation via activation of factors Va and VIIIa (Kaiser and Markwardt, 1986; Pieters et al., 1989). In addition to inhibiting free thrombin, hirudin inhibits clot-bound thrombin (Hogg and Jackson, 1989; Weitz et al., 1990) and thrombin bound to fibrin split products (Weitz et al., 1998). In contrast, heparin-antithrombin complexes are unable to access and inactivate clot-bound thrombin. This important difference between hirudin and heparin might explain why hirudin is more effective than heparin in dissolving mural thrombi in experimental models (Meyer et al., 1998). Hirudin shows virtually no interaction with plasma proteins (Glusa and Markwardt, 1990), and its activity is standardized in thrombin inhibitory units (TIU): 1 TIU is the amount of hirudin inhibiting 1 U of thrombin at 37°C. The specific activity of lepirudin is 16,000 TIU/mg.

C. Pharmacokinetics

Lepirudin is administered parenterally. Studies of plasma pharmacokinetics in healthy subjects reveal a two-compartment model. The initial plasma half-
Life ($t_{1/2}$) of lepirudin is 8–12 min, after which it is distributed in the extracellular space. Only 20% of lepirudin is found in the plasma, while the remaining 80% is in the extravascular compartment (Glusa, 1998). Lepirudin is not transported into the cerebrospinal fluid or breast milk (Refludan Package Insert; Lindhoff-Last et al., 2000a).

The terminal plasma elimination half-life ($t_{1/2}$) ranges from 0.8 to 1.7 h (mean, ~1.3 h, or 80 min) following intravenous (iv) injection of bolus doses of 0.01–0.5 mg/kg and 1.1–2.0 h following continuous iv infusions over 6 h. Maximum activated partial thromboplastin time (aPTT) ratios occur about 10 min after iv bolus, 3–6 h following 6-h continuous iv infusion, and 2–3 h following subcutaneous (sc) administration (Table 1). During iv infusion, therapeutic levels are usually reached within 30–60 min.

**Figure 1** Schematic representation of the thrombin molecule and its inhibition by hirudin, bivalirudin (formerly, Hirulog), hirugen, and argatroban: (1) active-site pocket; (2) fibrinogen-binding site. The active-site pocket catalyzes most of the functions of the thrombin molecule, whereas the fibrinogen-binding exosite mediates the binding of thrombin to fibrinogen. Hirudin is a 7000 Da (7 kDa) protein composed of 65 amino acids, which binds to the active-site pocket and the fibrinogen-binding exosite of thrombin, i.e., it is a bivalent direct thrombin inhibitor. Bivalirudin is a small synthetic peptide (20 amino acids) designed also to block both of these sites on thrombin. Hirugen, a synthetic peptide, mimics the binding site of fibrinogen to thrombin, thereby inhibiting binding of thrombin to fibrinogen and, therefore, fibrinogen cleavage by thrombin. The arginine derivative argatroban binds competitively to only the active binding site pocket of thrombin. Hirugen and argatroban are univalent direct thrombin inhibitors. (Adapted from Hermann et al., 1997.)
The approved dose for lepirudin in patients with HIT and acute thrombosis (with normal renal function) is an iv bolus of 0.40 mg/kg, followed by an iv infusion of 0.15 mg/kg/h (Table 2). However, in patients without massive, life-threatening thrombosis, and especially in elderly patients, I recommend that the bolus be omitted and an initial infusion rate of 0.10 mg/kg/h commenced so as to avoid overdosage in case of unrecognized renal insufficiency. The infusion rate should then be adjusted according to aPTT after 4 h.

Renal clearance (160–200 mL/min for an adult with normal body surface area of 1.73 m²) and degradation account for approximately 90% of the systemic clearance of lepirudin. The $t_{1/2}$ of r-hirudin lengthens with

**Table 1** Maximum aPTT Ratios (Mean vs. Baseline Values) After Lepirudin Administration

<table>
<thead>
<tr>
<th>Dose group (mg/kg body weight)</th>
<th>iv bolus</th>
<th>aPTT ratio (mode of application), 6 h iv infusion</th>
<th>sc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single-dose studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>1.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.02</td>
<td>1.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.04</td>
<td>1.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.05</td>
<td>ND</td>
<td>ND</td>
<td>1.2</td>
</tr>
<tr>
<td>0.07</td>
<td>2.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.10</td>
<td>2.3</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>0.15</td>
<td>ND</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>0.20</td>
<td>2.1</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>0.30</td>
<td>2.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.35</td>
<td>ND</td>
<td>ND</td>
<td>1.9</td>
</tr>
<tr>
<td>0.50</td>
<td>2.8</td>
<td>ND</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Multiple-dose studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 every 24 h</td>
<td>2.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.1 every 12 h</td>
<td>2.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.5 every 24 h</td>
<td>ND</td>
<td>ND</td>
<td>2.3</td>
</tr>
<tr>
<td>0.5 every 12 h</td>
<td>ND</td>
<td>ND</td>
<td>2.1</td>
</tr>
</tbody>
</table>

The maximum ratios of mean aPTT values versus baseline are shown. For iv bolus application, 6-h continuous iv infusion, and sc application, maximum ratios were usually reached at 0–10 min, 3–6 h, and 2–3 h after first application, respectively. This is in good correlation with the peak plasma lepirudin concentrations achieved with these different modes of application. Generally, maximum aPTT ratios increased with higher lepirudin doses, with maximum values of 2.1–2.4 × prolongation of baseline for repeated doses above 0.1 mg/kg body weight. *Abbreviations:* aPTT, activated partial thromboplastin time; iv, intravenous; ND, not done; sc, subcutaneous.
deterioration of renal function (Markwardt, 1989; Nowak et al., 1991, 1992, 1997; Vanholder et al., 1994, 1997); in nephrectomized patients, it can be up to 120 h (Wittkowsky and Kondo, 2000; Dager and White, 2001; Fischer, 2002; Shepherd, 2002).

A clinically important observation is that renal blood flow decreases during anesthesia, so that the elimination half-life is prolonged to 3–5 h. If lepirudin is used intraoperatively, the dose should be reduced by 30–50%, and close monitoring is mandatory.

With sc administration, bioavailability is nearly 100%. Dose-ranging studies have shown that its concentration in the blood reaches 0.3–0.5 μg/mL after a sc dose of lepirudin of 0.5 mg/kg and about 0.7 μg/mL after a sc dose of 0.75 mg/kg, making twice-daily injections effective (Schiele et al., 1994; Huhle et al., 2000a; Nowak, 2001). When administered sc, this drug is usually injected into an abdominal skin fold and reaches its peak concentration after 2–3 h. Lepirudin has been administered sc for long-term prophylaxis in HIT after the acute disease has been controlled (Huhle et al., 2000a). In one patient the drug was safely administered sc twice daily for 8 months for antithrombotic therapy in the setting of malignant disease. Lepirudin has been administered sc as an adjunct to streptokinase in patients with acute myocardial infarction (MI) (Neuhaus et al., 1999) and in the outpatient management of acute MI (Begelman and Deitcher, 2002).

D. Tests for Monitoring Anticoagulation

Numerous tests have been evaluated for monitoring anticoagulation produced by DTIs, ranging from the ubiquitous aPTT to the newer ecarin clotting time (ECT) and enzyme-linked immunosorbent assay (ELISA) techniques for directly measuring the hirudin concentration (Haftner et al., 2002).

The aPTT is a global coagulation assay and is the current method of choice for monitoring lepirudin therapy in most situations. In patients who require higher levels of plasma hirudin and aPTT values above ~70 s (depending on the reagent), the hirudin concentration–aPTT curve flattens, and the correlation between these parameters diminishes. Because the sensitivities of different aPTT reagents vary, each laboratory should generate its own standard curve by spiking normal pooled plasma samples with 0.25, 0.50, 0.75, 1.0, 1.25, 1.5, and 2.0 μg/mL lepirudin (Fig. 2).

Unlike global tests for measuring clotting time, the ECT monitors prolongation of clotting time caused by thrombin inhibition alone (Callas et al., 1995; Nowak and Bucha, 1996; Pötzsch et al., 1997a,b; Koster et al., 2000a; Fabrizio, 2001; de Denus and Spinler, 2002; Liu et al., 2002). Ecarin, which is obtained from snake venom, catalyzes the cleavage of prothrombin.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Bolus(^{a,b})</th>
<th>IV infusion(^{a,b})</th>
<th>Target aPTT ratio(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIT with isolated thrombocytopenia (dose regimen B in HAT trials)</td>
<td>None(^d)</td>
<td>0.10 mg/kg b.w./h(^d,e)</td>
<td>1.5–2.5 (0.5–0.8 mg/mL)</td>
</tr>
<tr>
<td>HIT and thrombosis (dose regimen A1 in HAT trials)</td>
<td>(0.40 mg/kg(^d))</td>
<td>0.15 mg/kg b.w./h(^d)</td>
<td>1.5–2.5 (0.6–1.4 mg/mL)</td>
</tr>
<tr>
<td>Thrombosis prophylaxis in patients with a history of HIT</td>
<td>15 mg sc b.i.d.(^f)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>HIT with thrombosis and concomitant thrombolysis (dose regimen A2 in HAT trials)</td>
<td>0.20 mg/kg b.w. iv(^d)</td>
<td>0.10 mg/kg b.w./h(^d)</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Renal dialysis every alternate day</td>
<td>0.10 mg/kg b.w. iv predialysis</td>
<td>—</td>
<td>2.0–2.5</td>
</tr>
<tr>
<td>Continuous venovenous hemofiltration (CVVH)</td>
<td>—</td>
<td>0.005 mg/kg b.w./h (initial rate)</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>PCI (Mehta et al., 2002); UA or acute MI without ST elevation (OASIS-2, 1999)</td>
<td>0.40 mg/kg b.w iv</td>
<td>0.15 mg/kg b.w./h</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Indication</td>
<td>Dose regimen details</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular surgery (Hach-Wunderle, 2001)</td>
<td>0.40 mg/kg b.w. iv 0.10 mg/kg/h 1.5–2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postoperative anticoagulation</td>
<td>0.10 mg/kg b.w./h 1.5–2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac surgery using CPB (dose regimen C in HAT trials) (see also Chap. 19)</td>
<td>0.25 mg/kg b.w. iv 0.20 mg/kg b.w. in the priming fluid 0.50 mg/min&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Repeat aPTT determinations should be made 4–6 h after any dose adjustment. Abbreviations: aPTT, activated partial thromboplastin time; b.w., body weight; CPB, cardiopulmonary bypass; ECT, ecarin clotting time; iv, intravenous; MI, myocardial infarction; PCI, percutaneous coronary intervention; UA, unstable angina.

<sup>a</sup> A maximum body weight of 100 kg should be used for dose calculations.

<sup>b</sup> Adjust for renal insufficiency.

<sup>c</sup> The ratio is based on comparison with the normal laboratory mean aPTT. If Actin FS or Neothromtin reagents are used, the aPTT target range is usually 1.5–3.0.

<sup>d</sup> Used in the HAT-1, HAT-2, and HAT-3 trials.

<sup>e</sup> This is the author’s recommended starting dose in all HIT patients, unless life- or limb-threatening thrombosis is present.

<sup>f</sup> Tested in a prospective, randomized trial after orthopedic surgery (Eriksson et al., 1996, 1997).

<sup>g</sup> Stop 15 min before end of CPB; put 5 mg into CPB after disconnection to avoid clotting of pump.

<sup>h</sup> The target lepirudin level pre-CPB (>2.5 μg/mL) is lower than the level sought during CPB (3.5–4.5 μg/mL) because of the addition of lepirudin to the pump priming fluid (0.2 mg/kg body weight).
to meizothrombin (Kornalik and Blombäck, 1975; Novoa and Seegers, 1980; Nishida et al., 1995). Meizothrombin is biologically similar to thrombin, except that it cleaves fibrinogen much more slowly than thrombin. The interaction of meizothrombin with hirudin, however, is similar to that of thrombin. Thus, when all the hirudin present in a blood sample has been neutralized by meizothrombin, thrombin will no longer be inhibited, and clotting will occur (see Chap. 19).

The ECT shows a linear correlation to lepirudin plasma levels over a wide range. At present this assay is recommended for monitoring anticoagulation when higher concentrations of lepirudin are used. It is mandatory for monitoring of lepirudin during cardiopulmonary bypass (CPB) surgery.

**Limitations of Functional Monitoring Tests**

Results obtained with aPTT or ECT may be inaccurate in patients whose plasma is depleted of prothrombin (e.g., severe liver disease) or in patients with fibrinogen depletion (e.g., post-thrombolysis, hemodilution during CPB) (Lindhoff-Last et al., 2000b; de Denus and Spinler, 2002). In the ECT, this can be overcome by addition of normal plasma 1:1 to the assay (Koster et al., 2000a).

ELISAs can be used to measure the concentration of lepirudin in plasma. These assays are independent of prothrombin concentrations. Plasma concentrations for lepirudin are 0.2–0.4 mg/mL for thrombosis prophylaxis, 0.5–0.8 mg/mL for isolated HIT, and 0.6–1.4 mg/mL for HIT and thrombosis.

**E. Dose Adjustments**

Generally, in lepirudin-treated patients, laboratory values to monitor the anticoagulant effect should be obtained prior to treatment, 4 h after the start of iv infusion, and 4 h after every change in dose. For most patients, the primary anticoagulation parameter used should be the aPTT, and testing should be performed at least once daily during treatment with lepirudin. If the target range is exceeded, the infusion should be stopped for 2 h and restarted at a 50% lower dose once the therapeutic range has been reached (Greinacher et al., 1999a,b). When the dose is subtherapeutic, the infusion rate should be increased by 20%.

**Figure 2** Lepirudin standard curve. This curve was generated using seven normal plasmas spiked with various concentrations of lepirudin (µg/mL) using reagent Actin FS and the BCS analyzer (Dade-Behring, Germany). Note that incremental changes in activated partial thromboplastin time (aPTT) are much smaller as the dose-response curve flattens at greater plasma lepirudin concentrations.
Renal Impairment

Lepirudin has been studied in patients with varying degrees of renal impairment. It can be used safely and effectively in reduced dose (Table 3). In case of transient renal failure close monitoring of aPTT is mandatory. To avoid overdosing due to compensated renal insufficiency, I recommend avoiding the initial lepirudin bolus (unless severe thrombosis is present) and starting the lower infusion rate of 0.10 mg/kg/h iv, with subsequent adjustments according to aPTT.

Transitioning to Warfarin

In patients with HIT, warfarin (or other coumarins) should be initiated only after platelet levels have normalized. Further, no loading dose of warfarin should be given. To cover the initial prothrombotic effects of warfarin, therapeutic levels of lepirudin (aPTT ratio = 1.5–2.5) should be maintained for at least 4–5 days after the initiation of oral anticoagulation. In case of a rapid increase in the international normalized ratio (INR), prothrombin levels should be used for dose finding.

F. Reversal/Removal of Lepirudin

Bleeding is an important and potentially severe consequence of hirudin treatment (Antman, 1994; Neuhaus et al., 1994; Frank et al., 1999). As with all DTIs, no specific antidote is available. In a patient with minor bleeding and normal renal function, stopping the drug may suffice, since the drug concentration drops quickly. However, when bleeding is life-threatening or the patient has renal failure, cessation alone may not be adequate.

Table 3  Dosing Schedule for Lepirudin in Patients with HIT and Renal Impairment

<table>
<thead>
<tr>
<th>Creatinine clearance (mL/min)</th>
<th>Serum creatinine, mg/dL (µmol/L)</th>
<th>Adjusted iv infusion rate ( % of original dose [see Table 2])</th>
</tr>
</thead>
<tbody>
<tr>
<td>45–60</td>
<td>1.6–2.0 (141–177)</td>
<td>50</td>
</tr>
<tr>
<td>30–44</td>
<td>2.1–3.0 (178–265)</td>
<td>25</td>
</tr>
<tr>
<td>15–29</td>
<td>3.1–6.0 (266–530)</td>
<td>10</td>
</tr>
<tr>
<td>&lt;15</td>
<td>&gt;6.0 (&gt;530)</td>
<td>0.005 mg/kg/h b.w. iv (adjusted for aPTT)</td>
</tr>
</tbody>
</table>

Abbreviations: aPTT, activated partial thromboplastin time; b.w., body weight; iv, intravenous.

a No initial bolus of lepirudin is given.
Hemodialysis or hemofiltration can reduce plasma levels of lepirudin (Riess et al., 1995). However, only some filters are effective, e.g., polysulfone F80 (Fresenius, Germany) (Frank et al., 1999; Bucha et al., 1999). Variable efficacy of filters in removing lepirudin could explain conflicting results (Vanholder et al., 1997). Clinical data are limited, and hemofiltration is not always a practical option in emergency situations.

Hirudin overdosage may also be treated pharmacologically by administration of desmopressin (Ibbotson et al., 1991; Butler et al. 1993; Bove et al., 1996), or von Willebrand factor (vWF), or vWF-containing factor VIII concentrates (Dickneite et al., 1996, 1998). Irami and coworkers (1995) described a patient in whom r-hirudin–induced bleeding was treated by administration of prothrombin complex concentrates, a method previously used in animal models (Diehl et al., 1995). However, since these concentrates can contain heparin, they could be dangerous for a patient with acute HIT. Recombinant FVIIa is another “panhemostatic” treatment option. Meizothrombin is another potential antidote, but it is not available for use in humans (Nowak and Bucha, 1995).

G. Clinical Use of Lepirudin

Besides its use in patients with HIT, lepirudin has been investigated extensively in controlled clinical trials for acute coronary syndromes (n > 14,000), such as MI (Antmann, 1994; Neuhaus et al., 1994) and unstable angina pectoris (Rupprecht et al., 1995; Organization to Assess Strategies for Ischemic Syndromes [OASIS-2], 1999); and in pilot studies for prophylaxis and treatment of deep venous thrombosis (Parent et al., 1993; Schiele et al., 1997). In patients undergoing dialysis (see Chap. 18) or cardiac surgery (see Chap. 19), there is observational evidence indicating safe and effective use of lepirudin.

Results of three prospective clinical trials with lepirudin and an extensive postmarketing drug monitoring study in HIT patients treated in the “real-world” setting are described in the next section.

III. CLINICAL STUDIES WITH LEPIRUDIN IN HIT

A. Three Prospective Clinical Trials: HAT-1, -2, and -3

Three prospective studies with lepirudin for HIT were designated Heparin-Associated Thrombocytopenia (HAT)-1, -2, and -3 (Greinacher et al., 1999a, b; Lubenow and Greinacher, 2002; Eichler et al., 2002). There was no approved nonheparin alternative anticoagulant during the 3 years in which the HAT studies were conducted (March 1994 to April 1996), and thus for ethical reasons, a placebo control was not appropriate. The HAT studies therefore...
included comparisons of clinical outcomes with a historical control group treated before lepirudin became available.

Meta-analysis of HAT-1 and -2 was performed to evaluate patients given lepirudin for treatment of HIT with thrombosis. A second meta-analysis of the HAT-1, -2, and -3 studies was performed to evaluate the effects of lepirudin in patients with HIT and isolated thrombocytopenia (“isolated HIT”). In addition, an observational study termed the drug-monitoring program (DMP) was carried out to determine the effects of lepirudin in a large cohort of patients treated in routine clinical settings.

Objectives

The three HAT trials examined whether lepirudin administered iv to patients with serologically confirmed HIT would safely reduce the risk of new arterial or venous thrombosis, limb amputations, and death. The laboratory objective was to determine whether the drug would allow an increase in the platelet count in thrombocytopenic patients or maintain the baseline platelet values (in nonthrombocytopenic patients), while providing effective anticoagulation. The latter was defined as a prolongation of the aPTT by 1.5- to 2.5-fold over baseline values with no more than two dose increases. (Note: If Actin FS or Neothromtin reagents were used, the aPTT target range was a 1.5- to 3.0-fold prolongation.)

Patients

Patients were eligible for study if their platelet count fell by more than 50% or to fewer than $10^5/\mu L$ or if they exhibited new thrombosis while receiving heparin. A strict criterion for study entry was laboratory confirmation of the clinical diagnosis of HIT by the heparin-induced platelet activation (HIPA) test (Greinacher et al., 1991; Eichler et al., 1999) (see Chap. 11).

Clinical outcomes included a composite endpoint (new thrombosis, limb amputation, death) as well as each individual endpoint. Clinical events that occurred between diagnosis and start of treatment with lepirudin were included, as were all clinical events that occurred up to day 14 after stopping lepirudin treatment. Clinical outcomes for lepirudin were compared with a historical control group treated conventionally by Kaplan-Meier time-to-event analysis, beginning at laboratory confirmation of HIT for lepirudin-treated patients and one day after laboratory confirmation for controls.

Laboratory response was defined as (1) the maintenance of an on-treatment aPTT ratio higher than 1.5 in at least 80% of measurements and requiring no more than two dose increases and (2) an increase in the platelet count to more than 30% from the nadir and to more than $10^5/\mu L$ by day 10 of lepirudin treatment (thrombocytopenic patients), or maintenance of normal platelet counts on days 3 and 10 (nonthrombocytopenic patients).
Historical Control Group

The historical control patients \((n = 120)\) also had a diagnosis of HIT confirmed by a positive HIPA test in our laboratory (Greinacher et al., 1999b). They were treated according to hospital protocol with danaparoid \((n = 36)\), oral anticoagulants (e.g., phenprocoumon \([n = 27]\)), no anticoagulation \((n = 23)\), or miscellaneous treatments (e.g., aspirin \([n = 5]\), low molecular weight heparin \([n = 8]\), or thrombolytics \([n = 4]\)). Incomplete data for 17 patients in the control group precluded treatment assignment.

B. HAT-1 Study

The HAT-1 study involved 82 patients with confirmed HIT: 51 patients were assigned to dose regimen A1, 5 to regimen A2, 18 to regimen B, and 8 to regimen C (Table 4) (Greinacher et al., 1999a). The median duration of treatment was 10 days (range 3–47 days) for regimen A1, 9 days (7–29 days) for A2, 15 days (2–58 days) for B, and 9 days (3–25 days) for C.

Efficacy Outcomes

Compared with the control group, the lepirudin-treated group had significantly lower rates of the combined endpoint of new thrombosis, limb amputation, and death at day 35 (25.4% vs. 52.1%; \(p = 0.014\)). This represented a 51.2% reduction in risk for the combined endpoint (Table 5). Similarly, the

Table 4  Summary of Treatment Regimens in Four Large Studies of Lepirudin for HIT

<table>
<thead>
<tr>
<th>Lepirudin treatment group</th>
<th>HAT-1</th>
<th>HAT-2</th>
<th>HAT-3</th>
<th>DMP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIT with thrombosis (regimen A1)</td>
<td>51</td>
<td>65</td>
<td>98</td>
<td>496</td>
<td>710</td>
</tr>
<tr>
<td>HIT with thrombosis treated with concomitant thrombolysis (regimen A2)</td>
<td>5</td>
<td>4</td>
<td>12</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>Prophylaxis in isolated HIT (regimen B)</td>
<td>18</td>
<td>43</td>
<td>84</td>
<td>612</td>
<td>757</td>
</tr>
<tr>
<td>CPB surgery (regimen C)</td>
<td>8</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>18</td>
</tr>
<tr>
<td>Miscellaneous (including sc dosing)</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>221</td>
<td>222</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>112</td>
<td>205</td>
<td>1329</td>
<td>1716</td>
</tr>
</tbody>
</table>

Dosing regimens A1, A2, B, and C are described in Table 2. 

**Abbreviations**: CPB, cardiopulmonary bypass; DMP, drug-monitoring program; HAT, heparin-associated thrombocytopenia trial; sc, subcutaneous.
<table>
<thead>
<tr>
<th>Study (Ref.) and endpoint&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Observation period</th>
<th>Lepirudin (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Historical control (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p-value</th>
<th>Risk reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAT-1 (Greinacher et al., 1999a)</td>
<td>Dx of HIT to d35</td>
<td>n = 71</td>
<td>n = 120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite</td>
<td>25.4</td>
<td>52.1</td>
<td>0.014</td>
<td>51.2</td>
<td></td>
</tr>
<tr>
<td>New thrombosis</td>
<td>18.4</td>
<td>32.1</td>
<td>0.270</td>
<td>42.7</td>
<td></td>
</tr>
<tr>
<td>Limb amputation</td>
<td>5.7</td>
<td>8.2</td>
<td>0.783</td>
<td>30.5</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>8.6</td>
<td>22.3</td>
<td>0.071</td>
<td>61.4</td>
<td></td>
</tr>
<tr>
<td>HAT-2 (Greinacher et al., 1999b)</td>
<td>Dx of HIT to d35</td>
<td>n = 95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite</td>
<td>30.9</td>
<td>52.1</td>
<td>0.120</td>
<td>40.7</td>
<td></td>
</tr>
<tr>
<td>New thrombosis</td>
<td>17.4</td>
<td>32.1</td>
<td>0.260</td>
<td>45.8</td>
<td></td>
</tr>
<tr>
<td>Limb amputation</td>
<td>10.0</td>
<td>8.2</td>
<td>0.430</td>
<td>-22.0</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>10.5</td>
<td>22.3</td>
<td>0.210</td>
<td>52.9</td>
<td></td>
</tr>
<tr>
<td>HAT-3 (Eichler et al., 2002)</td>
<td>Dx of HIT to d35</td>
<td>n = 191</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite</td>
<td>26.2</td>
<td>52.1</td>
<td>0.002</td>
<td>49.7</td>
<td></td>
</tr>
<tr>
<td>New thrombosis</td>
<td>9.9</td>
<td>32.1</td>
<td>&lt;0.001</td>
<td>69.2</td>
<td></td>
</tr>
<tr>
<td>Limb amputation</td>
<td>5.8</td>
<td>8.2</td>
<td>0.707</td>
<td>29.3</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>13.6</td>
<td>22.3</td>
<td>0.73</td>
<td>39.0</td>
<td></td>
</tr>
<tr>
<td>HIT with thrombosis, meta-analysis</td>
<td>Start of lep to d35</td>
<td>n = 113</td>
<td>n = 75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite</td>
<td>21.3</td>
<td>47.8</td>
<td>0.004</td>
<td>55.4</td>
<td></td>
</tr>
<tr>
<td>New thrombosis</td>
<td>10.1</td>
<td>27.2</td>
<td>0.005</td>
<td>62.9</td>
<td></td>
</tr>
<tr>
<td>Limb amputation</td>
<td>6.5</td>
<td>10.4</td>
<td>N/S</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>8.9</td>
<td>17.6</td>
<td>N/S</td>
<td>49.4</td>
<td></td>
</tr>
<tr>
<td>HIT with thrombosis, DMP</td>
<td>Start to end of lep</td>
<td>$n = 496$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------</td>
<td>-----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lubenow et al., 2002b)</td>
<td>Rx + 1d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite</td>
<td>21.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New thrombosis</td>
<td>5.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb amputation</td>
<td>5.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>10.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated HIT, meta-analysis</td>
<td>Start to end of lep Rx</td>
<td>$n = 111$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lubenow et al., 2002a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite</td>
<td>9.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New thrombosis</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb amputation</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated HIT, DMP</td>
<td>Start to end of lep</td>
<td>$n = 612$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Lubenow et al., 2002b)</td>
<td>Rx + 1d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite</td>
<td>15.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New thrombosis</td>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb amputation</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>12.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Each patient could contribute only once to the composite endpoint (any one of the individual endpoints of new thrombosis, limb amputation, or death).

*b* Number of patients eligible for comparison.

*Abbreviations: d, day; DMP, drug-monitoring program; Dx, diagnosis; lep, lepirudin; Rx, treatment.*
incidence of each of the individual endpoints at day 35 was reduced: new thrombosis (18.4% vs. 32.1%; \( p = 0.27 \)), limb amputation (5.7% vs. 8.2%; \( p = 0.78 \)), and deaths (8.6% vs. 22.3%; \( p = 0.07 \)). Causes of death were heart failure (\( n = 3 \)), sepsis (\( n = 2 \)), and multiorgan failure (\( n = 1 \)). None of the deaths was judged to be an adverse effect of lepirudin. In 88.7% of the lepirudin-treated patients, platelet counts increased to greater than 100 \( \times 10^9/L \) within 10 days (Greinacher et al., 1999a).

Compared with the historical control group, the cumulative frequency of new thrombosis, limb amputation, and death was lower in the lepirudin group at all time points after laboratory confirmation of HIT (Fig. 3). The adjusted risk ratio for lepirudin-treated patients relative to historical controls was 0.508 (95% CI 0.29–0.892).

**Safety Outcomes**

At 4 weeks the cumulative rate of bleeding events was not statistically different in the lepirudin group compared to control (39.6% vs. 35.3%; \( p = 0.60 \)). During the study, 27 patients (32.9%) experienced one bleeding event, with 11 patients (13.4%) suffering 15 major bleeding events. Of those, 8 were at invasive sites and 7 were spontaneous. There were no significant differences between groups in the frequency of bleeding events requiring transfusion (9.9% vs. 9.1%; \( p = 0.59 \)). There were no intracerebral or fatal hemorrhages in the lepirudin group.

**C. HAT-2 Study**

The HAT-2 study involved 112 patients with confirmed HIT: 65 patients were assigned to dose regimen A1, 4 to regimen A2, and 43 to regimen B (Table 4) (Greinacher et al., 1999b). The overall median duration of treatment was 11 days (range 0–104 days); for regimen A1, it was 13 days (0–104 days); for A2, 10 days (1–58 days); and for B, 8 days (1–67 days).

**Efficacy Outcomes**

The average combined outcome rate (expressed per patient-day) markedly decreased from the pretreatment period (5.1%) to the periods during (1.5%) and after (0.6%) lepirudin treatment. Platelet count recovery was achieved in 87 of 94 (92.6%) evaluable patients, with median platelet counts increasing about fourfold over the first 10 days.

Compared with the historical control group, the cumulative frequency of the composite endpoint (new thrombosis, limb amputation, death) was lower in the lepirudin group at all time points after laboratory confirmation of HIT (Table 5). At 5 weeks, the frequencies were 30.9% (95% CI 21.0–40.7)
Figure 3  Time-to-event analyses of efficacy and safety endpoints in the HAT-1 and HAT-2 studies (combined) in comparison with the historical control group. (A) Composite endpoint (new thromboembolic complication, limb amputation, or death); (B) new thromboembolic complication; (C) limb amputation; (D) death; and (E) major bleeding.
for the lepirudin group and 52.1% (95% CI 40.4–63.9) for the historical control group ($p = 0.12$). Lepirudin-treated patients fared somewhat better than historical controls at 5 weeks for the individual outcomes of new thromboses (17.4% vs. 32.1%; $p = 0.26$), and death (10.5% vs. 22.3%; $p = 0.21$) but not for limb amputation (10.0% vs. 8.2%; $p = 0.43$). The adjusted risk ratio for lepirudin-treated patients relative to historical controls was 0.709 (95% CI 0.44–1.14; $p = 0.15$). Causes of death included multi-organ failure ($n = 3$), sepsis ($n = 2$), heart failure ($n = 2$), pulmonary embolism, ventricular fibrillation, shock, and apnea ($n = 1$ each). None of the deaths were judged to be related to adverse effects of lepirudin.

Safety Outcomes

At 35 days, the cumulative frequency of bleeding events was 44.6% (95% CI 33.8–55.4) in the lepirudin group and 27.2% (95% CI 16.3–38.0) in the control group ($p = 0.0001$ by log-rank test). There were no significant differences between the lepirudin and control groups, however, in the frequency of bleeding events requiring transfusion (12.9% vs. 9.1%; $p = 0.23$). Most severe bleeding occurred at invasive sites. The frequency of serious spontaneous bleeding, including gastrointestinal hemorrhages (2.1% for lepirudin vs. 5.0% for control) and pulmonary hemorrhages (2.1% for lepirudin vs. 1.7% for control), was low and not significantly different. No cerebral hemorrhages occurred in the lepirudin group.

**D. HAT-3 Study**

A third prospective trial, HAT-3, was the largest and involved 205 patients: 98 patients were assigned to dose regimen A1, 12 to regimen A2, and 84 to regimen B (Eichler et al., 2002). Ten patients received lepirudin for CPB (regimen C), and one received lepirudin by the sc route. Fifteen patients were enrolled twice. For the efficacy parameters only the first treatment cycle was calculated. For safety analysis, especially allergic reactions, all treatment cycles were included.

Median treatment duration was 10 days over all treatment groups, 9 days (range 1–197 days) for regimen A1, 12 days (5–21 days) for regimen A2, 10 days (1–47 days) for regimen B, and 7 days (1–37 days) for regimen C.

**Efficacy Outcomes**

The results of HAT-3 confirmed the efficacy of lepirudin in HIT, as outcomes were similar to HAT-1 and HAT-2. Of the 84 patients with HIT and isolated thrombocytopenia, 9 (10.7%) experienced new thrombosis, 7 (8.3%) under-
went limb amputation, and 11 (13.1%) died. Since patients were counted only once if multiple events occurred, the incidence of the combined endpoint was 27.4% (23/84). Of the 110 patients with HIT and thrombosis (treatment regimens A1 and A2), 19 (17.3%) experienced new thromboses, 6 (5.5%) underwent limb amputation, and 16 (14.5%) died; in this group, the combined endpoint rate was 31.8% (35/98).

The average combined event-rate per day, as observed before, during, and after lepirudin treatment, supports the previous observation that heparin cessation alone may not prevent serious complications in HIT. The mean 1.7-day delay in initiating therapy for clinically suspected HIT while awaiting laboratory confirmation was associated with severe, even life-threatening clinical consequences, with an average event-rate per day of 5.6%. This dropped to 0.87% during treatment and was 0.77% after cessation of lepirudin therapy.

Compared to the historical control, from laboratory confirmation until end of the observation period, the combined endpoint was markedly reduced (26.2% vs. 52.1%; \( p = 0.002 \)) primarily due to a reduction in new thrombosis (9.9% vs. 32.1%; \( p = 0.0002 \)).

Safety Outcomes

The overall incidence of major bleeding was 19.5%, with 5 fatal outcomes (2.4%). One patient who received concomitant thrombolysis experienced intracranial hemorrhage. Of the 205 patients, 8 (3.9%)—7 in the HIT plus thrombosis group and 1 in the HIT group—experienced 11 episodes of allergic reactions to lepirudin, with 4 associated with an antibody to lepirudin. There were no cases of anaphylaxis.

E. Meta-Analysis of HAT-1 and HAT-2: Patients with HIT and Thrombosis

A meta-analysis of HAT-1 and HAT-2 was performed to determine the efficacy and safety of lepirudin in 113 patients with HIT complicated by thrombosis (Greinacher et al., 2000). As in the HAT-1, -2, and -3 studies, the risk for new thrombotic complications (per day) was highest between diagnosis of HIT and start of treatment: 34.1% of all events occurred during this pretreatment period (Fig. 4).

Efficacy Outcomes

When outcomes were assessed from the start of lepirudin treatment, the combined endpoint for new thrombosis, limb amputation, and death was
significantly lower in the lepirudin-treated patients \((n = 113)\) than in the controls \((n = 91)\) (21.3% vs. 47.8%; \(p = 0.004\)). This difference was primarily due to a reduction in the number of new thrombosis (10.1% vs. 27.2%; \(p = 0.005\)). Incidences of limb amputation (6.5% vs. 10.4%) and death (8.9% vs. 17.6%) were also lower in the lepirudin group than in the historical controls (Table 5).

**Safety Outcomes**

There were no fatal or intracranial bleeds but, compared with the controls, the cumulative incidence of bleeding was higher in the lepirudin group than in the control group (42.0% vs. 23.6%; \(p = 0.001\)), and more lepirudin-treated patients experienced bleeding requiring transfusion (18.8% vs. 7.1%; \(p = 0.02\)).

One of the more important points to emerge from the meta-analysis was the relationship of aPTT ratios with lepirudin safety and efficacy. For low aPTT ratios (<1.5), the incidence of the combined endpoint was not significantly reduced compared to the control (RR = 0.86; \(p = 0.72\)). In addition, the risk of bleeding was not significantly greater in the lepirudin group than in the control group (RR = 1.57; \(p = 0.42\)). At medium aPTT ratios (1.5–2.5), efficacy was significantly greater for the lepirudin-treated patients than for the controls (RR = 0.42; \(p = 0.009\)), but there was also an increased risk of bleeding (RR = 3.21; \(p = 0.0003\)). At higher aPTT ratios (>2.5), the efficacy of lepirudin was not enhanced, but there was an even greater risk of bleeding.

**F. Meta-Analysis of HAT-1, -2, -3: Patients with Isolated HIT**

Each of the HAT trials examined the effects of lepirudin in patients with HIT and isolated thrombocytopenia and in HIT patients with thrombosis. The

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**Figure 4** Average combined event rate (new thromboembolic complication, limb amputation, death) per day in the HAT-1 and HAT-2 studies \((n = 113)\) patients with thromboembolic complications at baseline). The bar width indicates the mean duration of the observation period (days) and is shown for three time periods: before, during, and after lepirudin therapy. The high average event rate (0.061 [6.1%] event per day during a mean period of 1.7 days) from diagnosis of HIT until start of lepirudin therapy indicates that cessation of heparin alone is insufficient to prevent HIT-associated thrombosis, thus warranting treatment with an alternative anticoagulant if HIT is strongly suspected. (From Greinacher et al., 2000.)
meta-analysis of HAT-1 and HAT-2 showed that lepirudin was effective in patients with HIT plus thrombosis. In a meta-analysis of HAT-1, -2, and -3, the safety and efficacy of lepirudin in patients with HIT in the absence of known thrombosis was demonstrated (Lubenow et al., 2002a). This meta-analysis included 111 patients treated according to dosing regimen B; of these, 17 had a history of HIT (latent HIT), not acute HIT. Patients with recent thrombosis at baseline were excluded from this analysis. Mean duration of treatment was 13.5 days (range 1–68 days).

**Efficacy Outcomes**

Of the 111 patients in this study during treatment with lepirudin, 3 (2.7%) experienced new thromboses, 3 (2.7%) underwent limb amputation, and 5 (4.5%) deaths occurred. Most of the deaths were related to underlying disease, not to HIT or treatment with lepirudin. Since patients were counted only once if multiple events occurred, the incidence of the combined endpoint was 10/111 (9.0%). The median platelet count rebounded to $150 \times 10^9$/L within 4 days of beginning lepirudin treatment.

**Safety Outcomes**

Episodes of major bleeding occurred in 16/111 (14.4%) of patients in this meta-analysis. aPTT ratios above 2.5 were associated with an increased risk of bleeding, and bleeding rates were significantly lower in patients with aPTT < 60. Nearly all patients with bleeding complications had impaired renal function. Antihirudin antibodies were detected in 36 of the 108 (33.3%) evaluable patients. There were no differences in adverse events or outcomes between patients with and without antihirudin antibodies. No anaphylaxis was observed.

**G. Postmarketing Drug Monitoring Program**

Preliminary results from 1329 patients treated with lepirudin in a drug-monitoring program (DMP) were recently made available (Lubenow et al., 2002b): 496 patients had HIT and thrombosis and 612 patients had isolated HIT. This postmarketing study evaluated the same clinical endpoints as were used in HAT-1, -2, and -3. In this DMP, lepirudin could be started immediately upon clinical diagnosis of HIT, thus avoiding the inherent delay awaiting laboratory confirmation. A total of 382 (77.0%) of the 496 patients with HIT and thrombosis were positive in the HIPA test, while 406 (66.3%) of the 612 patients with isolated HIT were positive in the HIPA test.
Efficacy Outcomes

In the routine clinical settings of the DMP, lepirudin-treated patients with isolated HIT and HIT with thrombosis had the lowest incidence of all clinical endpoints reported with any agent. The incidence of the combined clinical endpoint in the 496 patients with HIT and thrombosis was 21.9%: 26 patients (5.2%) experienced new thrombosis, 29 (5.8%) underwent limb amputation, and 54 patients (10.9%) died. The largest cause of death was multiorgan failure (23/54 patients [42.6%]), emphasizing the serious underlying medical condition of these patients. The incidence of new thrombosis in this study (5.2%) was lower than that observed in the HAT-1 and -2 meta-analysis (10.1%). This may be due to physicians’ increased clinical experience with lepirudin, as illustrated by the decision to begin lepirudin treatment immediately upon clinical diagnosis of HIT, thereby improving efficacy and safety outcomes.

The combined endpoint of new thrombosis, limb amputation, and death occurred in 96 (15.7%) of the 612 patients with isolated HIT; 13 patients (2.1%) experienced new thrombosis, 8 (1.3%) underwent limb amputation, and 75 patients (12.3%) died. These event rates are the lowest reported for any agent used to treat HIT. As seen in the group of patients with HIT plus thrombosis, the largest cause of death in this group was multiorgan failure (39/75 patients, 52.0%).

The overall mortality rate due to new thrombosis in the group of 1108 patients treated with regimen A1 or B (thus, excluding patients receiving “miscellaneous” treatments) (see Table 4) was low (15 patients, or 1.4%). Efficacy variables in the DMP were even more favorable than those seen in the meta-analyses of the HAT studies. This DMP thus confirms the efficacy of lepirudin in routine clinical practice for both the prophylaxis and the treatment of thromboembolism in patients with HIT.

There were no differences in the mean infusion rates in patients with HIT and thrombosis (0.12 mg/kg/h) and those with isolated HIT (0.11 mg/kg/h) in the DMP. As lepirudin dose is adjusted based on aPTT, the major difference between the two regimens is the initial bolus in HIT patients with acute thrombosis. However, as discussed earlier, in my view the bolus should be avoided in most situations to prevent overdosing.

Safety Outcomes

In the DMP incidence of bleeding was greatly decreased when compared to the HAT clinical trials. In the group of 496 patients with HIT plus thrombosis, there were 27 (5.4%) major bleeding episodes, and among the 612 patients with isolated HIT, 36 (5.9%) had major bleeding. Allergic reactions were re-
ported in 4 (0.8%) patients in the HIT plus thrombosis group and in 1 (0.2%) patient with isolated HIT. No anaphylaxis was reported.

The decreased incidence of bleeding events in the DMP most likely is attributed to physicians’ greater experience with administering lepirudin and monitoring its effects.

H. Comparison with Other Treatments for HIT

Mortality rates in patients with HIT have been approximately 20–30% for more than a decade (King and Kelton, 1984; AbuRahma et al., 1991; Warkentin and Kelton, 1996; Nand et al., 1997). Notably, these rates are two to three times higher than those observed in the HAT studies. The HAT trials demonstrate the necessity for prompt initiation of alternative anticoagulation following the discontinuation of heparin. In addition to lepirudin, other drugs with antithrombin activity (e.g., argatroban) or antifactor Xa activity (e.g., danaparoid) may be appropriate for further parenteral anticoagulation in patients with HIT (see Chaps. 13, 14, 16, 17).

Comparisons of the results of the various clinical trials of agents used to treat HIT need to be interpreted with caution, since there have been no direct comparative trials and the studies employed are of somewhat different designs. Trials of lepirudin and argatroban, however, utilized similar clinical endpoints and historical controls for comparison. The most obvious differences between the lepirudin and the argatroban trials are (1) the need for laboratory confirmation of HIT in the lepirudin trials; (2) treatment duration, which was consistently longer than 10 days in the lepirudin-treated patients but less than 6 days in the argatroban-treated patients (potential to increase apparent efficacy and also bleeding with lepirudin); (3) the observation period, which started at the time of diagnosis in lepirudin-treated patients compared with the time of treatment initiation in the argatroban trials (potential to underestimate the efficacy of lepirudin); and (4) a considerable proportion of patients in the historical control group of the HAT trials had been treated with danaparoid (potential to underestimate the efficacy of lepirudin). To allow a more direct comparison, we reanalyzed the data of the HAT trials as per the argatroban trials, i.e., analyzing those events occurring from start of active treatment only (Table 5).

The rates for the combined endpoint were consistently lower in the lepirudin trials than in the two argatroban studies. They were 9.0% in the HAT-1, -2, -3 meta-analysis and 15.7% in the DMP during treatment with lepirudin, as compared to 25.6% and 28.0% in the two argatroban trials, for patients with isolated HIT. (However, the observation period for argatroban also included a follow-up period, whereas the lepirudin data were obtained during the treatment period only.) For patients with HIT and thrombosis, the
combined endpoint occurred in 21.3% (meta-analysis, including the post-treatment follow-up period) and 21.9% (DMP, treatment period only) with lepirudin, and in 43.8% and 41.5% (including the follow-up period) of patients treated in the two argatroban trials.

Because of the often-critical condition of the patient population investigated, the rate of deaths observed for both DTIs is not likely to be attributable to treatment failure and may vary considerably with different patient populations. As the argatroban trials included many patients who most likely did not have HIT, the death rate associated with HIT might have been overestimated, as non-HIT patients with a decrease of platelet count are often very sick (e.g., septicemia, disseminated intravascular coagulation). Death rates were 4.5% (meta-analysis) and 12.3% (DMP) for patients with isolated HIT treated with lepirudin, but 18.1% and 23.1% in those treated in the two argatroban trials. In patients with HIT complicated by thrombosis, death rates with lepirudin were 8.9% (meta-analysis) and 10.9% (DMP), and 18.0% and 23.1% in the argatroban trials.

Limb amputation occurred in patients with isolated HIT in 2.7% (meta-analysis) and 1.3% (DMP) when treated with lepirudin, and in 1.9% and 4.2% in those treated with argatroban. The amputation rates were higher in HIT with thrombosis; 6.5% (meta-analysis) and 5.8% (DMP) in those treated with lepirudin, and 11.1% and 14.8% in those treated with argatroban.

There was also a difference in the incidences of new thrombosis, which is most likely the most important parameter for assessment of an alternative anticoagulant in HIT. In those with isolated HIT, it was 2.7% (meta-analysis) and 2.1% (DMP) with lepirudin, and 6.9% and 5.8% in those treated with argatroban. In HIT with thrombosis it was 10.1% (meta-analysis) and 5.2% (DMP) for lepirudin, and 14.6% and 13.1% for argatroban-treated patients.

The risk of major bleeding seems to be higher in lepirudin-treated patients when comparing total numbers. In those with isolated HIT, it was 14.4% (meta-analysis) and 5.9% (DMP), but 3.1% and 5.3% in those treated with argatroban. In patients with HIT and thrombosis, maj or bleeding occurred in lepirudin-treated patients in 18.8% (meta-analysis) and 5.4% (DMP) and in 11.1% and 6.1% of the patients in the argatroban trials. However, the differences in treatment duration are important for assessing the bleeding risk. In fact, when calculating the risk for major bleeds per treatment day, for HIT patients with thrombosis it was 0.020 major bleeds per patient day during active treatment in the HAT trials and 0.019 in the argatroban trials, respectively.

Because there are no prospective data comparing lepirudin and danaparoid for treatment of HIT, we retrospectively compared 126 danaparoid-treated patients with 175 lepirudin-treated patients who fulfilled the same inclusion and exclusion criteria (Farner et al., 2001). In the patients with HIT
without thromboembolic complications at baseline, a time-to-event analysis showed that the cumulative risk of the combined endpoint was higher in danaparoid-treated patients than in the lepirudin-treated patients ($p = 0.02$ by log rank test; hazard ratio [HR] = 2.9 [95% CI 1.1–7.6]; $p = 0.027$). This was due primarily to an increased incidence of new thromboembolic complications (20% [95% CI 8.4–36.9] for danaparoid vs. 6.3% [95% CI 1.3–17.2] for lepirudin; $p = 0.087$). Of note, patients with isolated HIT usually received only prophylactic-dose danaparoid. In contrast, HIT patients with thrombosis at baseline, and who were therefore treated with a therapeutic-dose regimen of danaparoid, had a similar outcome as patients receiving lepirudin ($p = 0.913$).

The major conclusions of these comparisons are: (1) HIT patients seem to benefit from a longer treatment period with an alternative anticoagulant, with 10 days better than 5 days; (2) the prophylactic-dose regimen of danaparoid (750 U sc two or three times daily) approved in the European Union for HIT with isolated thrombocytopenia appears to be suboptimal.

I. Antibody Formation

Because hirudin is a protein obtained from a nonhuman species, lepirudin can induce antibody production in humans. Antibodies are induced by both iv therapeutic-dose and sc prophylactic-dose use (Greinacher et al., 2003a). Antihirudin antibodies have been detected in 44–74% of patients treated with lepirudin (Huhle et al., 1998; Song et al., 1999; Eichler et al., 2000). Of 196 HIT patients treated with lepirudin for 5 or more days, 44% developed antihirudin antibodies of the IgG class (Eichler et al., 2000). These antibodies were not associated with an increase in thrombin-antithrombin (TAT) complexes (Fig. 5). None of these patients developed allergic reactions to lepirudin. Antibody formation occurred as early as day 4 and peaked at days 8–9 (Eichler et al., 2000).

Antilepirudin antibodies can extend the half-life of lepirudin (Liebe et al., 2002), most likely by reduced renal filtration of lepirudin-antilepirudin complexes (Fig. 6); in about 2–3% of patients with antilepirudin antibodies, an inhibitory effect is seen (Huhle et al., 2001; Fischer et al., 2003).
biological effects of antilepirudin antibodies on anticoagulation can be easily compensated by changes in the lepirudin dose. Thus, ongoing daily aPTT measurements are recommended during lepirudin treatment, even when stable anticoagulation has been observed during the first 5 days.

**J. Allergic Reactions**

Lepirudin administration during prospective studies in patients with HIT was associated with a low incidence of allergic events, as well as during the much larger clinical trials in patients with acute coronary syndromes. Among the adverse events reported were eczema, rash, pruritus, hot flushes, fever, chills, urticaria, bronchospasm, cough, stridor, dyspnea, angioedema (variably of the face, tongue, larynx), and injection-site reactions. Any causal relationship of lepirudin to these adverse events is unclear.

To date, of 35,000–60,000 patients treated with lepirudin, nine patients were judged to have had severe anaphylaxis in close temporal association with

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**Figure 5** Thrombin-antithrombin (TAT) complex concentrations in relation to antihirudin antibody formation. TAT complex concentrations did not differ between antihirudin antibody–positive (○, solid lines) and antihirudin antibody–negative (●, dotted lines) patients (median and 25 and 75% quartiles are given).
lepirudin use (Greinacher et al., 2003b). All reactions occurred within minutes of iv bolus lepirudin administration, with four fatal outcomes (three acute cardiorespiratory arrests, one hypotension-induced MI). In these four cases, a previous uneventful treatment course with lepirudin was identified (1–12 weeks earlier). In an additional patient with nonfatal anaphylaxis (who did not receive a bolus), we found high-titer IgG antilepirudin antibodies. Since lepirudin has been used in approximately 35,000 patients, the risk of anaphylaxis is estimated at 0.015% (5/35,000) in first-exposure and 0.16% (4/2500) in reexposed patients (assuming 7.5% reexposure frequency). We and others (Bircher et al., 1996) demonstrated high titer antihirudin antibodies of the IgG class, but not of the IgE class in patients with hirudin-associated anaphylaxis. IgG-dependent anaphylaxis likely is Fc receptor-mediated and

Figure 6  This 53-year-old woman was admitted to the hospital because of an ankle fracture. She received low molecular weight heparin for 10 days, but was switched to unfractionated heparin because of a distal deep vein thrombosis (DVT). Ten days later she presented with proximal DVT, pulmonary embolism, and a rapid fall in platelet count from more than 200 to 12 × 10^9/L. She was switched to intravenous (iv) lepirudin (schedule A1). After normalization of platelet counts, she received overlapping oral anticoagulation (phenprocoumon), with lepirudin stopped when the INR reached 2.0. Antihirudin antibodies were first detected on day 7; at the same time, the aPTT increased despite a stable hirudin dosage of 0.05 mg/kg b.w. per hour.
related to infusion dose. Thus, besides reducing bleeding risk, avoiding iv bolus administration of lepirudin should also reduce the risk of severe anaphylactic reactions.

IV. LEPIRUDIN TREATMENT IN OTHER CLINICAL SETTINGS

A. Acute Coronary Syndromes and Percutaneous Coronary Intervention

Due in part to their ability to inhibit clot-bound thrombin, DTIs have also been investigated as anticoagulants for acute coronary syndromes (ACS) and percutaneous coronary intervention (PCI). Lepirudin was examined in large numbers of patients with unstable angina or suspected acute MI without ST segment elevation in the OASIS-1 (OASIS Investigators, 1997) \( n = 909 \) and OASIS-2 \( n = 10,141 \) trials (OASIS Investigators, 1999). These trials concluded that lepirudin is superior to heparin in preventing ischemic outcomes. A meta-analysis of 11 ACS trials involving over 35,000 patients revealed a 15% reduction in death or MI when bivalent DTIs (lepirudin or bivalirudin) were used to treat ACS patients, compared with heparin (DTI Trialists’ Collaborative Group, 2002). A retrospective subset analysis of the OASIS 2 trial examined the benefit of lepirudin in 117 ACS patients undergoing PCI within the first 72 hours (Mehta et al., 2002). Lepirudin was superior to heparin in reducing the risk of death or MI at 96 hours \( p = 0.036 \) and 35 days \( p = 0.02 \). Based on this evidence, lepirudin should be considered a treatment option in ACS patients with HIT.

B. Cardiopulmonary Bypass and Vascular Surgery

Lepirudin was initially used to manage CPB patients in the HAT studies (Riess et al., 1995, 1996) but has also been used successfully by other investigators for CPB (Warkentin and Greinacher, 2003). It is now accepted that lepirudin is a suitable alternative for anticoagulation during CPB in patients with acute HIT, provided that ECT monitoring is performed (Koster et al., 1998, 2000a,b; Johnston et al., 1999; Follis and Schmidt, 2000; Latham et al., 2000; Longrois et al., 2000). Neither the activated clotting time (ACT) nor the aPTT is appropriate for monitoring r-hirudin plasma levels in such high-dose situations (see Chap. 19).

Koster and colleagues (2000b) used lepirudin instead of heparin in 57 patients who had clinically diagnosed HIT and required CPB. The primary diagnoses included coronary artery disease \( n = 27 \), including 8 cases of MI,
valvular heart disease \( (n = 14) \), combined coronary artery and valvular disease \( (n = 9) \), thoracic aortic aneurysms \( (n = 4) \), ventricular septal defect resulting from infarction \( (n = 2) \), and atrial tumor \( (n = 1) \). In that study, anticoagulation was monitored with ECT, and lepirudin was maintained in the range of 3–4 \( \mu g/mL \). The dose requirement for CPB was 0.016–0.035 \( \mu g/kg/min \), with concurrent 24-h blood drainage of 50–2200 mL. Elimination of the drug at the conclusion of CPB was augmented through modified zero-balanced ultrafiltration and forced diuresis. However, drug removal was dependent on the prevailing renal function. Four patients with impaired renal function showed prolonged elimination and bleeding. Of the 57 patients, 54 achieved full recovery and showed no signs of thromboembolism over a 6-month follow-up. Three patient deaths were unrelated to perioperative management.

For patients undergoing vascular surgery, the dosage of lepirudin should be adjusted for the risk for reocclusion (Hach-Wunderle, 2001). In patients with a low risk of reocclusion (e.g., in the aortic, iliac, and carotid arteries), a bolus of 0.4 mg/kg (reduced in case of renal insufficiency) is given just before the vessel is clamped and is followed postoperatively by either an aPTT-adjusted infusion of 0.1 mg/kg/h or 15 mg injected sc b.i.d. In patients with an increased risk for enhanced reocclusion (e.g., undergoing calf-vessel reconstruction or bypass), a preoperative bolus of lepirudin (0.4 mg/kg [less in case of renal impairment]) should be administered, followed by a postoperative infusion of 0.15 mg/kg/h, aPTT-adjusted, for at least 3–4 days. For intraoperative flushing of the vessel during vascular surgery, up to 250 mL (0.1 mg/mL solution) of lepirudin can be used. As patients with acute HIT are at high risk for new thromboembolic complications, therapeutic levels of anticoagulation should be achieved before surgery and maintained after surgery, at least until platelet counts are normalized (see Chap. 13).

C. Hemodialysis

Hirudin was the first anticoagulant to be used for hemodialysis, as performed by Haas (1924) in Germany. Because native hirudin preparations were crude and supply of leeches insufficient, hirudin was replaced by heparin to prevent clotting during dialysis. Currently, more published reports describe lepirudin for hemodialysis than the other DTIs (see Chap. 18).

Management of these patients requires careful dosing and frequent monitoring. HIT patients with transient renal failure are difficult to manage with lepirudin, because substantial dose adjustments are necessary, depending on the extent of renal failure. To reduce bleeding risk, we prefer administering a continuous iv infusion, starting at 0.005 mg/kg/h, with adjustments
made according to the aPTT, while others use intermittent iv boluses of 0.005–0.01 mg/kg (Fischer et al., 1999; Kern et al., 1999).

D. Lepirudin in Pregnancy

Data on the treatment of HIT during pregnancy are limited. In general, the use of lepirudin during pregnancy is not recommended, as it crosses the placenta. Zebrafish experiments indicate that thrombin has an important role in early embryogenesis and that inhibition by lepirudin may cause cell regulation defects (Jagadeeswaran et al., 1997). Experiments in rabbits showed a fetal hirudin plasma concentration that was 1/60 that of the maternal concentration (Markwardt et al., 1988), and embryotoxic effects were seen in rabbits at high, but not low, doses (30 vs. 1–10 mg/kg/day, respectively) (Berlex Laboratories, data on file).

Reports on the use of lepirudin in pregnancy are sparse (Lindhoff-Last and Bauersachs, 2002). A pregnant woman with systemic lupus erythematosus who was treated with dalteparin developed HIT at week 25. Her platelet count dropped from 230 to $5 \times 10^9/L$, after which she was treated with lepirudin (15 mg sc twice daily), with aPTT and ECT used to monitor her dosage. Following delivery by cesarean section, she experienced no postpartum bleeding complications, and treatment with lepirudin was continued for several weeks thereafter (Huhle et al., 2000b). Another pregnant woman with lupus anticoagulant and HIT was successfully treated for 36 weeks with lepirudin.

A case report described a breastfeeding woman diagnosed with HIT who was treated with sc lepirudin, 50 mg twice daily (Lindhoff-Last et al., 2000a). No lepirudin was detected in her breast milk, although plasma levels were within therapeutic range. Neither bleeding nor thrombosis occurred in mother or infant.

Lepirudin and danaparoid are each classified by the FDA as pregnancy category B, based on limited animal data. However, danaparoid does not cross the placenta, and it has been used for prophylaxis and therapy of HIT during pregnancy (Greinacher et al., 1993; Dager and White, 2002) (see Chaps. 13 and 14).

E. Lepirudin in Children

Although rare in children, HIT is important in the differential diagnosis of thrombocytopenia or unexplained thrombosis in the presence of heparin administration (Ranze et al., 1999). Because of the rarity of HIT and its clinical heterogeneity in pediatric patients, it is difficult to design a standard-
ized dosage protocol for lepirudin. Accordingly, current therapeutic recommendations are based on anecdotal experience. Given that children usually have normal renal function, the short half-life of lepirudin presents an advantage in the event of bleeding complications or the need for invasive procedures. However, the dose required may range between 0.05 and 0.22 mg/kg/h, depending on comorbidity and renal function (Schiffmann et al., 1997; Deitcher et al., 2002; Nguyen et al., 2003) (see Chap. 20).

V. CONCLUSION

The r-hirudin lepirudin is a DTI that provides rapid and effective anticoagulation and significantly reduces the risk of thrombosis in patients with HIT, including those with isolated thrombocytopenia. Fewer than 10% of all patient groups with HIT developed a new thrombosis after start of active treatment. The drug also reduced risk of limb amputation and death.

Published data on lepirudin include more than 8500 treated patients. Of these, about 1500 patients were treated for HIT (the largest experience with a DTI). An additional 7300 patients received lepirudin for ACS and PCI.

Lepirudin is given parenterally by iv infusion or sc injection. Recommended lepirudin dosage schedules have been established (Table 2). Lepirudin has a short half-life, which presents an advantage if invasive surgical procedures are indicated. However, its elimination strongly depends on renal function. Bolus dosing should be avoided, especially in elderly patients, to avoid overdosing. Lepirudin can be used safely and effectively in patients with renal impairment by appropriate dosing according to serum creatinine and regular monitoring. Lepirudin also allows for a safe and uncomplicated transition to warfarin.

The most common adverse event in the prospective clinical trials was bleeding. No antidote exists for the DTIs. Excess lepirudin can be removed by hemofiltration, but clinical data are limited. Daily monitoring of aPTT is recommended with dosage adjustments made as needed to maintain the target aPTT value. Routine monitoring with ECT should be performed in high-dose situations, such as those required during CPB.

Besides the 399 patients with HIT treated in prospective trials, an additional 1329 patients received lepirudin for HIT in a postmarketing surveillance study. Data on these patients, collected under routine clinical conditions, showed the lowest incidence of the clinical endpoints of death, new thrombosis, and amputations, with risk reductions exceeding those reported in the prospective clinical trials. Even more importantly, the incidence of major bleeding was low. These differences support the assumption that outcomes in patients with HIT can be substantially improved by immediately stopping heparin and starting lepirudin when HIT is strongly sus-
pected on clinical grounds, without awaiting results of antibody testing, and that the bleeding risk has been reduced substantially as physicians have learned to handle this agent.

The experience with lepirudin in the prospective trials and the DMP has now been extended to about 35,000–60,000 patients, including HIT as well as ACS, PCI, CPB, and deep vein thrombosis. This far exceeds the available data on any other therapy in HIT and also gave insights in the frequency of rare adverse effects associated with lepirudin treatment, such as anaphylaxis. The results of these trials and the DMP demonstrate that lepirudin is highly effective in reducing the risk of the potentially devastating complications of HIT.

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16
Argatroban Therapy in Heparin-Induced Thrombocytopenia

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I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is an immune-mediated syndrome characterized by thrombocytopenia, which can be isolated or associated with thrombotic events (see Chaps. 1–4). Complications of HIT span a spectrum of venous and arterial thromboembolic events, including deep venous thrombosis, pulmonary embolism, myocardial infarction, thrombotic stroke, and limb artery occlusion requiring amputation (Warkentin, 2003). The elimination of all heparin sources and the initiation of alternate anticoagulation are recommended for treating patients with HIT (Hirsh et al., 2001). Not infrequently, thrombosis is the first manifestation of HIT. Even among patients with isolated thrombocytopenia who are managed by heparin cessation alone, approximately 25–50% develop new thrombosis (Warkentin and Kelton, 1996; Wallis et al., 1999; Lewis et al., 2003). Furthermore, many patients with HIT require ongoing anticoagulation for underlying medical conditions.

Two direct thrombin inhibitors, argatroban and r-hirudin (lepirudin) (see Chap. 15), are approved in the United States for use as anticoagulants in patients with HIT. The specific indications of these agents differ somewhat.
Lepirudin is indicated as anticoagulation for patients having HIT with associated thromboembolic disease in order to prevent further thromboembolic complications (Refudan™ Prescribing Information, U.S., 1998). Argatroban is indicated as an anticoagulant for prophylaxis or treatment of thrombosis in patients with HIT (Argatroban Prescribing Information, U.S., 2002). In other words, while both agents are approved for treating HIT patients with thrombosis, only argatroban is approved for managing HIT patients with isolated thrombocytopenia. Argatroban is also approved in Canada as an anticoagulant for patients with HIT who, in the opinion of their attending physician, require anticoagulation, and in the United States as an anticoagulant for patients with, or at risk for, HIT undergoing percutaneous coronary intervention (PCI).

In this chapter, the clinical pharmacology of argatroban is reviewed, together with its clinical utility as an anticoagulant in patients with HIT. Historically, argatroban was initially known worldwide as “MD-805” and then under the trademark “Novastan.” In 2000, the U.S. Food and Drug Administration disallowed the trademark Novastan in the United States because of potential similarities with other named products. Hence, argatroban (generic name) is now marketed in the United States without a trademark and under the name “Argatroban” (with a capital A). However, the trademark Novastan continues to be used in some other countries.

II. ARGATROBAN
A. Chemical Description

Argatroban is a synthetic direct thrombin inhibitor derived from L-arginine (Okamoto and Hijikata, 1981; Kikumoto et al., 1984). Its chemical structure is shown in Fig. 1. Argatroban (molecular weight, 526.66) consists of a mixture of 21-(R) and 21-(S) stereoisomers in a ratio of approximately 65:35 (Rawson et al., 1993). There is no interconversion between these stereoisomers.

B. Clinical Pharmacology

Mechanism of Action

Argatroban is a potent and selective inhibitor of thrombin (Okamoto and Hijikata, 1981; Kikumoto et al., 1984). Argatroban was developed using the approach of rational drug design through the mimicry of substrates of thrombin. It displays an inhibitory constant ($K_i$) of 0.04 μmol/L for thrombin and has little or no effect on related serine proteases ($K_i$ values of 5 μmol/L for
trypsin, 210 μmol/L for factor Xa, and 800 μmol/L for plasmin) (Kikumoto et al., 1984). Argatroban exerts its anticoagulant effects in the absence of any cofactor by inhibiting thrombin-catalyzed or -induced reactions, such as fibrin formation, the activation of factors V, VIII, and XIII, and platelet aggregation (Okamoto and Hijikata-Okunomiya, 1993).

Argatroban is 500-fold more potent than r-hirudin in its relative ability to inhibit clot-bound versus free thrombin (Berry et al., 1994). The lower molecular weight of argatroban, compared with hirudin, may allow it better accessibility to thrombin incorporated within the clot. The ability to inhibit effectively clot-bound thrombin may be of particular benefit in treating hypercoagulable states such as HIT and also in reducing extension of existing thromboses.

Structural studies have shown that argatroban binds tightly to thrombin by inserting the dual hydrophobic moieties on its arginine backbone into deep clefts near the thrombin active site (Banner and Hadvary, 1993). Thus, physiological substrates of thrombin are sterically hindered from access to the catalytic pocket of thrombin. Figure 2 (see color insert) shows a model of the interaction between argatroban and thrombin. This interaction is reversible, unlike the irreversible interaction between r-hirudin and thrombin (Chap. 15). The combination of reversible binding and a short elimination half-life (see next subsection) may improve the ability to control anticoagulation in the intensive care setting.
Distribution, Metabolism, and Excretion

Argatroban distributes mainly in the extracellular fluid, as evidenced by a steady state volume of distribution of 174 mL/kg (Swan and Hursting, 2000). It is 54% serum protein-bound (Tatsuno et al., 1986).

Unlike other commercially available direct thrombin inhibitors, argatroban undergoes no significant renal clearance. The main route of metabolism is hydroxylation and aromatization of the 3-methyltetrahydroquinoline ring in the liver (Izawa et al., 1986). In vitro, the human liver microsomal cytochrome P450 3A4/5 (CYP3A4/5) catalyzes the formation of each of the four known metabolites. In plasma, unchanged argatroban is the major component, while the primary metabolite (M1), which has three- to fivefold less activity than argatroban, is present at concentrations that are 0–20% of that of the parent drug (Ahsan et al., 1997). The other metabolites have not been detected in plasma or feces and are found only in very low quantities in urine. These data, together with the lack of effect of erythromycin, a potent CYP3A4/5 inhibitor, on argatroban pharmacokinetics (Tran et al., 1999) suggest that CYP3A4/5-mediated metabolism is not an important pathway in vivo.

The plasma clearance rate of argatroban is approximately 5.1 mL/min/kg for infusion doses up to 40 µg/kg/min in healthy volunteers (Swan et al., 2000). Its elimination half-life is 39–51 min (Swan and Hursting, 2000),

Figure 2  Model of the interaction between argatroban and thrombin. (See color insert.)
which is somewhat less than the 1–2 h half-life of r-hirudin (Vanholder et al., 1997). Argatroban is excreted primarily in the feces, presumably by biliary secretion.

Pharmacokinetic–Pharmacodynamic Relationship

The pharmacokinetic and pharmacodynamic profiles of intravenously administered argatroban are consistent with an anticoagulant agent that is predictable, has a fast onset of action, and is rapidly eliminated (Swan and Hursting, 2000; Swan et al., 2000).

The anticoagulant effects of argatroban are routinely monitored using the activated partial thromboplastin time (aPTT). Higher levels of anticoagulation, such as that required during interventional procedures, are monitored using the activated clotting time (ACT). Argatroban also increases in a dose-dependent fashion the prothrombin time (PT)/International Normalized Ratio (INR), thrombin time (TT), and ecarin clotting time (ECT) (Nagasaki et al., 1981; Clark et al., 1991; Walenga et al., 1999a; Swan et al., 2000; Sheth et al., 2001). High-performance liquid chromatography (Rawson et al., 1993; Walenga et al., 1999a) and liquid chromatography/tandem mass spectrometry (Tran et al., 1999) methods for measuring plasma argatroban are described but are not practical (or needed) for routine clinical monitoring.

Immediately upon starting argatroban infusion, anticoagulant effects are produced as plasma argatroban concentrations begin to rise. Steady-state levels of both drug and anticoagulant effect typically are attained within 1–3 h (faster when a loading bolus is administered) and maintained with low intra- and intersubject variability until the infusion is discontinued or the dosage adjusted. Plasma drug concentrations increase proportionally with doses up to 40 μg/kg/min and are well correlated with steady-state anticoagulant effects. The relationship at steady state between argatroban dose up to 10 μg/kg/min, plasma argatroban concentration, and anticoagulant effect (aPTT) is shown in Fig. 3. On stopping infusion, plasma argatroban concentrations decline rapidly (half-life of 39–51 min), and anticoagulant effects return to pretreatment values with similar effect half-lives (Swan et al., 2000).

Special Populations

Age, gender, and renal function exert no clinically significant effects on the pharmacokinetics or pharmacodynamics of argatroban. Patients with moderate hepatic impairment (Child–Pugh score > 6), compared with healthy volunteers, have an approximate fourfold decrease in drug clearance (to 1.5 mL/min/kg) and an approximate threefold increase in elimination half-life (to 152 min) (Swan and Hursting, 2000). Owing to the decreased clearance, a
fourfold downward adjustment in argatroban dosage is required for individuals with moderate hepatic impairment. No adjustment in initial argatroban dosage is needed for patients with renal impairment. In contrast, r-hirudin requires dosage adjustment for patients with renal impairment but not for patients with hepatic impairment (Refludan™ Prescribing Information, U.S., 1998). In patients without hepatic dysfunction undergoing PCI, the pharmacokinetic values of argatroban are similar to those reported in healthy volunteers, and argatroban clearance is unaffected by age, gender, or race (Cox et al., 2003).

Drug-Drug Interactions

No pharmacokinetic or pharmacodynamic drug interactions have been demonstrated between argatroban and aspirin (Clarke et al., 1991), erythromycin (Tran et al., 1999), acetaminophen, digoxin, or lidocaine (Inglis et al., 2002). In practice, argatroban coadministered with these frequently used medications should require no dosage adjustments.

No pharmacokinetic interactions have been demonstrated between argatroban and warfarin (Brown and Hursting, 2002). However, because
Argatroban is a direct thrombin inhibitor, the concomitant use of argatroban and warfarin results in prolongation of the PT/INR beyond that produced by warfarin alone (Hursting et al., 1999; Sheth et al., 2001). Cotherapy compared with warfarin monotherapy exerts no additional effect on vitamin K–dependent factor X levels (Sheth et al., 2001). Hence the previously established (“traditional”) relationship between INR and bleeding risk is altered during combination therapy. Guidelines for monitoring the transition from argatroban to warfarin anticoagulation are presented in Section IV.

Argatroban and a variety of drugs, including the glycoprotein (GP) IIb/IIIa antagonists abciximab, eptifibatide, and tirofiban, have been evaluated for chemical or physical/visual compatibility at concentrations commonly used in practice. This is important for supporting their simultaneous administration via Y-site injection. Argatroban and eptifibatide or tirofiban are chemically and physically compatible (Patel, 2002). Argatroban and abciximab (Patel, 2002), fentanyl citrate, midazolam hydrochloride, morphine sulfate, dopamine hydrochloride, dobutamine hydrochloride, phenylephrine hydrochloride, atropine sulfate, hydrocortisone sodium succinate, metoprolol tartrate, diphenhydramine hydrochloride, verapamil hydrochloride, norepinephrine bitartrate, or diltiazem hydrochloride (Hartman et al., 2002) are physically/visually compatible; their chemical stability remains to be established.

C. Other Distinguishing Features

Lack of Cross-Reactivity with HIT Antibody

In contrast with low molecular weight heparin and danaparoid, the direct thrombin inhibitors, including argatroban, hirudin, and bivalirudin, bear no resemblance to heparin, do not cross-react with HIT antibodies, and have not been associated with potentiation of HIT (Walenga et al., 1996).

Lack of Drug-Specific Antibody

Prolonged or repeated exposure to argatroban does not result in the generation of antibodies that alter its anticoagulant activity (Walenga et al., 2002). That is, there is no enhancement or suppression of anticoagulant response among individuals receiving prolonged or repeated administration. This has been shown in healthy volunteers, HIT patients, and HIT patients undergoing PCI, and in the postmarketing safety surveillance of over 4800 patients treated in Japan between 1991 and 1998 with argatroban anticoagulation (Walenga et al., 2002). In contrast, approximately 50% of r-hirudin (lepirudin)-treated patients develop drug-specific antibodies (Song et al., 1999; Eichler et al., 2000) (see Chap. 15), and levels of these antibodies increase...
upon reexposure to lepirudin (Harenberg et al., 2000). Among those who develop anti-hirudin IgG antibodies during treatment, the anticoagulant activity of lepirudin is mildly enhanced in approximately 45% of patients and suppressed in approximately 6% of patients, requiring careful monitoring and probable dosage adjustment (Eichler et al., 2000).

D. Reversal of Argatroban

Argatroban has a gentle dose-response relationship that offers a wide margin of safety during dose titration (see Fig. 3). However, as with any anticoagulant, bleeding is a major safety concern. Excessive anticoagulation, with or without bleeding, may be controlled by discontinuing argatroban or decreasing its infusion dose. Anticoagulant parameters generally return to baseline within 2–4 h after discontinuation of argatroban (Swan et al., 2000; Swan and Hursting, 2000). This reversal takes longer (at least 6 h and up to more than 20 h) in patients with hepatic impairment (Swan and Hursting, 2000).

There is no specific antidote to argatroban available. If life-threatening bleeding occurs and excessive plasma levels of argatroban are suspected, argatroban should be discontinued immediately, and the patient should be provided symptomatic and supportive therapy. Argatroban can be cleared, albeit slowly, using high flux dialysis membranes (Murray et al., 2003), which suggests a possible means to facilitate its removal if needed urgently. Recombinant factor VIIa has been suggested as a possible pharmacologic agent for treating severe bleeding in this setting (Alving, 2002), although this remains to be evaluated.

E. Clinical Use of Argatroban

In addition to studies conducted in patients with HIT, argatroban has been evaluated in patients with acute myocardial infarction (Théroux, 1997; Jang et al., 1999; Vermeer et al., 2000), unstable angina pectoris (Gold et al., 1993), peripheral arterial obstructive disease (Matsuo et al., 1995), or stroke (Kobayashi and Tazaki, 1997; LaMonte, 2003), and in patients undergoing PCI (Herrman et al., 1996; Jang et al., 2003), and patients with unstable angina pectoris (Gold et al., 1993) or hemodialysis (Murray et al., 2003). In each of these populations, argatroban produces predictable anticoagulant effects and is generally safe and well tolerated. In Japan, argatroban is approved for use in nonlacunar stroke, chronic arterial occlusion, and hemodialysis of patients with acquired or congenital antithrombin deficiency. Argatroban is also approved in Korea for use in chronic arterial occlusion and acute cerebral thrombosis. A prospective, controlled study of argatroban with tissue plasminogen activator in acute ischemic stroke is currently underway in the United States.
In a study in patients with unstable angina (Gold et al., 1993), cessation of argatroban was associated with “rebound” thrombin generation, as measured by increased plasma levels of thrombin–antithrombin complex (TAT) at 2 h after infusion cessation compared with the pretreatment baseline values, and early dose-related recurrence of angina. However, there was no rebound in plasma levels of fibrinopeptide A, which is a marker of thrombin activity. Also, since argatroban displaces thrombin from antithrombin (Eidt et al., 1989) and since argatroban is largely eliminated within 2 h of cessation of infusion, an increase in TAT at the end of the infusion is reasonably expected (Willerson and Casscells, 1993). Furthermore, reactivation of angina after the termination of heparin occurs (Théroux et al., 1992), and heparin had been administered in this study within 4 h of argatroban therapy. Rather than true “rebound” phenomena, the renewed angina after argatroban infusion may simply represent its recurrence after withdrawal of thrombin inhibition in some refractory patients (Willerson and Casscells, 1993). Notably, all patients studied (n = 43) were free of angina during argatroban therapy.

III. ARGATROBAN THERAPY OF HIT

A. Overview of Studies

The efficacy and safety of argatroban anticoagulant therapy in patients with clinically diagnosed HIT has been evaluated in the following prospective, multicenter, open-label studies:

- ARG-911, a historical controlled study
- ARG-915, a follow-on study that also used the historical control group from ARG-911 as comparator
- ARG-915X, a Phase III extension of study ARG-915 that allowed physicians continued access to argatroban while it was under regulatory review

Study ARG-911 has been reported in full (Lewis et al., 2001). Topline data from study ARG-915 (without its extension) as well as safety summaries from ARG-911 plus ARG-915 appear in the product’s labeling information (Argatroban Prescribing Information, U.S., 2002). Outcomes of patients with acute HIT from study ARG-915 plus its extension, together simply referred to as “Argatroban-915,” have also been reported in full (Lewis et al., 2003). Across these studies, 754 patients received argatroban therapy on 809 separate occasions (Lewis et al., 2000).

When these studies were conducted between 1995 and 1998, no approved alternative agent was available for use as an active comparator, and a randomized, placebo-controlled design was deemed unethical; thus historical controls were used for comparison. The studies were similar in design with
regard to objectives, inclusion and exclusion criteria, the argatroban dosing regimen, and assessments. In each study, patients were assigned at enrollment to one of two prospectively defined study arms: HIT (with isolated thrombocytopenia) or HIT with thrombosis (also referred to as “HIT with thrombosis syndrome” or “HITTS”). The overall study design is presented in Fig. 4.

Study Objectives

The objective of study ARG-911 was to evaluate the use of argatroban as an anticoagulant for the prophylaxis of thrombosis in HIT patients and the treatment of HIT patients with thrombosis. Similarly, the objective of studies ARG-915 and ARG-915X was to evaluate the safety and efficacy of argatroban in HIT patients, with or without thrombosis, requiring anticoagulation.

Study Population

Adult patients were eligible if they had a clinical diagnosis of HIT with or without thrombosis. HIT was defined as a platelet count $<100 \times 10^9/L$, or a 50% decrease in the platelet count after initiation of heparin therapy, with no apparent explanation other than HIT. Patients with a documented history of a positive HIT antibody test who needed anticoagulation were also eligible for the HIT study arm in the absence of thrombocytopenia. Patients were excluded if they had an unexplained aPTT greater than 2 times control at baseline, documented coagulation disorder or bleeding diathesis unrelated to HIT, a lumbar puncture within the prior 7 days, or a history of previous

---

**Figure 4** Schematic of the study design for ARG-911, ARG-915, and ARG-915X. Patients with a clinical diagnosis of HIT with or without thrombosis were eligible. The starting dose of argatroban, 2 μg/kg/min, was titrated to achieve an aPTT 1.5–3.0 times the baseline aPTT (not to exceed 100 s). Outcomes over a 37-day period were compared with those of a historical control group.
aneurysm, hemorrhagic stroke, or recent (within 6 months) thrombotic stroke unrelated to HIT. Reentry of patients into studies ARG-915 and ARG-915X was allowed, although outcomes from initial entries only were included in the primary analyses to avoid potential bias.

The historical control group of ARG-911 consisted of patients at the participating centers who met the same inclusion–exclusion criteria for the study and who were seen prior to the initiation of the study. Controls were treated according to the local standard of practice at the time of HIT diagnosis, with typical treatments being heparin discontinuation and/or oral anticoagulation (Lewis et al., 2001).

Treatment

The treatment group received an initial dose of argatroban 2 μg/kg/min via continuous intravenous infusion. The aPTT was measured at least 2 h later, and dosage was adjusted (up to 10 μg/kg/min, maximum) until the aPTT was 1.5–3 times the baseline aPTT value (not to exceed 100 s). The aPTT was measured daily and 2 h after each dosage adjustment. Patients remained on argatroban for up to 14 days, until the underlying condition resolved or appropriate anticoagulation was provided with other agents.

Assessments

The primary efficacy assessment was a composite endpoint of all-cause death, all-cause amputation, or new thrombosis within a 37-day study period. Additional analyses included the evaluation of event rates for the components of the composite endpoint and death due to thrombosis. Secondary efficacy endpoints included the achievement of adequate anticoagulation (i.e., an aPTT >1.5 times baseline) and resolution of thrombocytopenia (i.e., platelet count >100 × 10^9/L or ≥1.5 times baseline by study day 3).

Major bleeding was defined as overt and associated with a hemoglobin decrease ≥2 g/dL, that led to a transfusion of ≥2 units, or that was intracranial, retroperitoneal, or into a major prosthetic joint. Other overt bleeding was considered minor.

B. ARG-911

In study ARG-911, 304 patients having clinically diagnosed HIT (n = 160) or HIT with thrombosis (n = 144) received argatroban at a mean dose of 2.0 μg/kg/min for an average of 6 days. This study also enrolled 193 historical controls (HIT, n = 147; HIT with thrombosis, n = 46). Although not required for enrollment, laboratory confirmation of HIT antibodies occurred in 57% of the argatroban-treated patients and 77% of controls; the remaining individuals were either never tested or had a negative result (Lewis et al., 2001).
Efficacy

As seen in Table 1, the composite endpoint was reduced significantly in argatroban-treated patients versus controls with HIT (25.6% vs. 38.8%, \( p = 0.014 \)). In HIT with thrombosis, the composite endpoint occurred in 43.8% of argatroban-treated patients compared with 56.5% of controls (\( p = 0.13 \)). Significant between-group differences by time-to-event analysis of the composite endpoint favored argatroban treatment in HIT (\( p = 0.010 \), hazard ratio = 0.60; 95% CI, 0.40–0.89) (Fig. 5a) and HIT with thrombosis (\( p = 0.014 \), hazard ratio = 0.57; 95% CI, 0.36–0.90) (Fig. 5b).

Argatroban therapy, compared with controls, significantly reduced death due to thrombosis in each study arm (HIT, \( p = 0.005 \); HIT with thrombosis, \( p < 0.001 \)). There were no between-group differences in all-cause mortality. The incidence of amputation (as the most severe outcome) was similar between groups. Argatroban therapy also significantly reduced the percentage of patients experiencing new thrombosis in each study arm (HIT, \( p < 0.001 \); HIT with thrombosis, \( p = 0.044 \)).

Argatroban-treated patients achieved therapeutic aPTTs generally at first measure (i.e., within 4–5 h of starting therapy) and maintained these levels throughout infusion. Resolution of thrombocytopenia occurred by day 3 in 53% of argatroban-treated patients with HIT and 58% of patients having HIT with thrombosis. Compared with controls, argatroban-treated patients had a significantly more rapid rise in platelet counts.

Safety

Major bleeding occurred in 6.9% (21/304) of argatroban-treated patients, compared with 6.7% (13/193) of historical controls. In each group, there were two fatal bleeding events. One patient experienced a fatal intracranial hemorrhage 4 days after discontinuation of argatroban and following urokinase and warfarin therapy; one historical control also experienced a fatal intracranial hemorrhage. Minor bleeding rates were similar between the groups (41%). The most common adverse events among argatroban-treated patients with HIT or HIT with thrombosis, respectively, were diarrhea (11%) and pain (9%).

C. Argatroban-915

A total of 418 patients with acute HIT (\( n = 189 \)) or HIT with thrombosis (\( n = 229 \)) were prospectively treated with argatroban in study ARG-915 or its extension (together referred to as “Argatroban-915”) (Lewis et al., 2003). The mean argatroban dose was 1.8 \( \mu \text{g/kg/min} \), and the mean duration of therapy
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 147)</th>
<th>Argatroban (n = 160)</th>
<th>p</th>
<th>Control (n = 46)</th>
<th>Argatroban (n = 144)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite endpoint&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57 (38.8)</td>
<td>41 (25.6)</td>
<td>0.014</td>
<td>26 (56.5)</td>
<td>63 (43.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>Components by severity&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death (all causes)</td>
<td>32 (21.8)</td>
<td>27 (16.9)</td>
<td>0.31</td>
<td>13 (28.3)</td>
<td>26 (18.1)</td>
<td>0.15</td>
</tr>
<tr>
<td>Amputation (all causes)</td>
<td>3 (2.0)</td>
<td>3 (1.9)</td>
<td>1.00</td>
<td>4 (8.7)</td>
<td>16 (11.1)</td>
<td>0.79</td>
</tr>
<tr>
<td>New thrombosis</td>
<td>22 (15.0)</td>
<td>11 (6.9)</td>
<td>0.027</td>
<td>9 (19.6)</td>
<td>21 (14.6)</td>
<td>0.49</td>
</tr>
<tr>
<td>Death due to thrombosis</td>
<td>7 (4.8)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
<td>7 (15.2)</td>
<td>1 (0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any new thrombosis&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33 (22.4)</td>
<td>13 (8.1)</td>
<td>&lt;0.001</td>
<td>16 (34.8)</td>
<td>28 (19.4)</td>
<td>0.044</td>
</tr>
</tbody>
</table>

<sup>a</sup> All-cause death, all-cause amputation, or new thrombosis within 37-day study period.

<sup>b</sup> Severity ranking: all-cause death > all-cause amputation > new thrombosis; patients with multiple outcomes counted once.

<sup>c</sup> Patient counted only once if multiple events occurred.

Source: Lewis et al., 2001.
Figure 5  Time to first event for the composite endpoint through day 37 in study ARG-911. Significant differences in favor of argatroban therapy were detected in (a) the HIT study arm (argatroban group, $n = 160$; historical controls, $n = 147$) and (b) the HIT with thrombosis study arm (argatroban group, $n = 144$; historical controls, $n = 46$). (Data from Lewis et al., 2001.)
was 6 days. Comparisons were made with 185 historical controls with acute HIT with or without thrombosis (obtained from ARG-911).

**Efficacy**

Efficacy results (Table 2) were confirmatory and supportive of those from ARG-911. There were significant improvements in the composite endpoint for argatroban-treated patients versus controls among those with HIT (28.0% vs. 38.8%, \( p = 0.04 \)) or HIT with thrombosis (41.5% vs. 56.5%, \( p = 0.07 \)). Argatroban treatment was significantly favored, compared with control, by time-to-event analysis of the composite endpoint in HIT (\( p = 0.02 \), hazard ratio = 0.64, 95% CI, 0.43–0.93) or HIT with thrombosis (\( p = 0.008 \), hazard ratio = 0.56, 95% CI, 0.36–0.87).

Consistent with ARG-911, the positive benefits on the composite endpoint were driven in main part by significant reductions in new thrombosis (\( p < 0.001 \) in each study arm) (Table 2). There were no significant between-group differences in all-cause mortality or amputation. Argatroban therapy significantly reduced the incidence of death due to thrombosis in patients having HIT with thrombosis (\( p = 0.008 \)).

Similar, predictable aPTT responses occurred in patients with HIT or HIT with thrombosis. The target aPTT was typically achieved by first assessment, and mean aPTT values remained generally constant throughout the infusion. Platelet counts recovered more rapidly in argatroban-treated patients than controls (\( p < 0.001 \) for each study arm).

**Safety**

Major bleeding rates were not different between argatroban-treated patients and controls in either study arm (Table 2). Twenty-four (5.7%) argatroban-treated patients experienced major bleeding, including a single fatal event in a patient hospitalized for rectal bleeding and who received urokinase. No patient experienced an intracranial hemorrhage. Minor bleeding rates were not different between the groups and were similar to those in ARG-911.

**D. Patients with a History of HIT Requiring Acute Anticoagulation**

Across the studies of argatroban in HIT, 76 patients with a serologically confirmed history of HIT and requiring acute anticoagulation were prospectively administered argatroban on a total of 87 occasions (Matthai et al., 2001). The most common admitting diagnoses were deep venous thrombosis or pulmonary embolism, chest pain or acute coronary syndrome, and arterial peripheral vascular disease. A therapeutic aPTT was achieved in 95% of the
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Control (n = 139)</th>
<th>Argatroban (n = 189)</th>
<th>p^d</th>
<th>Control (n = 46)</th>
<th>Argatroban (n = 229)</th>
<th>p^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite Endpoint^a</td>
<td>54 (38.8)</td>
<td>53 (28.0)</td>
<td>0.04</td>
<td>26 (56.5)</td>
<td>95 (41.5)</td>
<td>0.07</td>
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<tr>
<td>Death (all causes)^b</td>
<td>29 (20.9)</td>
<td>36 (19.0)</td>
<td>0.78</td>
<td>13 (28.3)</td>
<td>53 (23.1)</td>
<td>0.45</td>
</tr>
<tr>
<td>Death due to thrombosis</td>
<td>6 (4.3)</td>
<td>1 (0.5)</td>
<td>0.04</td>
<td>7 (15.2)</td>
<td>6 (2.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Amputation (all causes)^b</td>
<td>4 (2.9)</td>
<td>8 (4.2)</td>
<td>0.57</td>
<td>5 (10.9)</td>
<td>34 (14.8)</td>
<td>0.64</td>
</tr>
<tr>
<td>New thrombosis^b</td>
<td>32 (23.0)</td>
<td>11 (5.8)</td>
<td>&lt;0.001</td>
<td>16 (34.8)</td>
<td>30 (13.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Major bleeding^c</td>
<td>12 (8.6)</td>
<td>10 (5.3)</td>
<td>0.27</td>
<td>1 (2.2)</td>
<td>14 (6.1)</td>
<td>0.48</td>
</tr>
<tr>
<td>Minor bleeding^c</td>
<td>57 (41.0)</td>
<td>59 (31.2)</td>
<td>0.08</td>
<td>19 (41.3)</td>
<td>87 (38.0)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

^a All-cause death, all-cause amputation, or new thrombosis within 37-day study period.

^b Outcome categories are not mutually exclusive; within a given category, a patient is counted only once if >1 event.

^c Patients with >1 event are counted only once.

^d Significance level of p < 0.05 for the primary endpoint (composite) and bleeding and p < 0.0125 for secondary endpoints (components of composite, and death due to thrombosis).
patients. Adverse outcome rates were significantly less than those of patients with active HIT (e.g., composite endpoint rates of 16.1% vs. 36.3%, \( p < 0.001 \)) and were comparable with rates that have been reported for similar patients without HIT receiving standard therapy. Among 42 treatment courses in 40 patients with a history of HIT who had fully recovered from their initial episode of HIT, had a normal platelet count, and had no exposure to heparin or other parenteral anticoagulants during their hospitalization, none had a bleeding or new thromboembolic event (Matthai et al., 2004). Argatroban therefore provided effective anticoagulation, upon initial and repeat exposure, in patients with a history of HIT requiring acute anticoagulation for a variety of indications.

E. Argatroban Reexposure

Across the prospective studies of HIT, 55 patients underwent therapy with argatroban on more than one occasion. The argatroban dosing and duration were similar between these patients (repeat group) and patients upon their first exposure (initial group, \( n = 754 \)). Event rates in the repeat group were less than with those in the initial group for the composite endpoint (20% vs. 34%), new thrombosis (3.6% vs. 11.1%), and major bleeding (3.6% vs. 6.6%). The patients reexposed to argatroban had no allergic reactions or apparent differences, relative to the initial group, in adverse experiences. Hence, argatroban is well tolerated upon reexposure, while providing effective antithrombotic therapy for HIT (Lewis et al., 2000).

F. Discussion of Prospective Studies of Argatroban in HIT

Consistently in these studies, argatroban therapy, compared with historical controls, produced significant benefits in clinical outcomes in patients having HIT with or without thrombosis. Specifically, argatroban, relative to control, was effective in reducing the composite of death, amputation, or new thrombosis, lowering mortality from thrombosis and preventing new thrombotic events—without increasing bleeding.

Patients were entered into these studies having a clinical diagnosis of HIT, and laboratory confirmation of HIT was not required for their treatment. This study design simulated the “real world” of managing HIT, wherein it may be important to initiate alternative anticoagulation in a clinically suspected HIT patient prior to laboratory confirmation of HIT (due perhaps to limited availability and/or a long turnaround time for the laboratory test). In ARG-911, HIT antibodies were demonstrated in the majority of patients and controls. However, individuals with a clinical diagnosis of HIT were also studied who lacked serologically confirmed HIT antibodies and in whom
thrombocytopenia may have been due to sepsis, cancer, or other causes. Argatroban therefore is an effective antithrombotic agent in clinically diagnosed, albeit not always laboratory-confirmed, HIT. Argatroban also provided effective anticoagulation in patients with a history of HIT who required acute anticoagulation for a variety of indications.

Across the studies, the overall major bleeding rate was 6.6% for argatroban-treated patients, similar to that (6.7%) of the historical control (Lewis et al., 2000). No patient experienced an intracranial hemorrhage while on argatroban therapy. By comparison, the major bleeding rate for lepirudin-treated HIT patients is 14–17% (Greinacher et al., 1999a,b). However, these direct thrombin inhibitors remain to be compared directly, and conclusions about their relative safety profiles cannot be reached.

These studies supported the approval of argatroban in the United States as an anticoagulant for the prophylaxis or treatment of thrombosis in patients with HIT, and the protocol-specified dosing regimen used in the studies was adopted as the recommended dosing schedule. The dosing regimen evaluated was initially selected in consideration of the established relationships between the aPTT and adequate, safe levels of anticoagulation. Prolongation of the aPTT to 1.5 times control is the typical minimum target for heparin anticoagulation in the prevention and treatment of thromboembolic disease, and downward titration of heparin is recommended when aPTTs are approximately 3 times control (Hirsh, 1991). Also, aPTTs greater than 100 s are associated with significant increases in major hemorrhage when another direct thrombin inhibitor, hirudin, is used as adjunctive therapy to thrombolysis (Antman, 1994). Hence, an argatroban dose was selected for study that was anticipated to prolong the aPTT 1.5–3 times baseline (i.e., 2 μg/kg/min, up to 10 μg/kg/min), and also a maximum aPTT of 100 s was recommended to help ensure patient safety. The mean steady-state aPTT in healthy subjects receiving argatroban 10 μg/kg/min is 86 s (Swan et al., 2000). That finding contributed to the selection of 10 μg/kg/min as a reasonable upper dose limit for prolonging the aPTT to no more than 3 times control, without exceeding 100 s. Although these studies did not evaluate argatroban at doses greater than 10 μg/kg/min, experience in healthy volunteers (Swan et al., 2000) and HIT patients undergoing PCI indicates that doses up to 40 μg/kg/min may be used safely to achieve higher levels of anticoagulation, if desired.

IV. PRACTICAL ASPECTS OF ARGATROBAN DOSING AND MONITORING

A. Duration of Therapy

Guidelines for treating patients with HIT (Hirsh et al., 2001) recommend that anticoagulation with an alternative parenteral agent such as argatroban
should be continued at least until platelet count recovery. This approach should help avoid thrombotic events resulting from premature discontinuation of anticoagulation in a patient with profound hypercoagulability secondary to HIT. In patients with HIT in study Argatroban-915, mean platelet counts were >100 × 10⁹/L after 2 days of therapy and >150 × 10⁹/L after 4 days of therapy (Lewis et al., 2003).

B. Dosing and Dosage Adjustments

For either the prophylaxis or treatment of thrombosis in HIT patients, the recommended initial dose of argatroban is 2 µg/kg/min (Table 3). Because argatroban clearance is reduced in patients with hepatic impairment, a reduced initial dose, i.e., 0.5 µg/kg/min, is recommended for patients with at least moderate hepatic impairment, e.g., a Child-Pugh score >6 (Pugh et al., 1973). Retrospective studies suggest that routine liver function tests, such as serum bilirubin and alkaline phosphatase, may be useful for refining individualized dosing in patients with hepatic dysfunction (Pippis et al., 2002; Smith et al., 2002); however, this requires further investigation. No initial dosage adjustment is required in patients with renal impairment, which is an important advantage in managing patients with renal dysfunction.

The initial dose should be adjusted, as needed, to achieve a target aPTT 1.5–3 times the baseline value. The choice of aPTT reagent does not materially affect assessment of argatroban therapy (Francis and Court, 2002). Approximately 1 in 6 patients in study ARG-911 maintained their initial argatroban dose for the duration of therapy, indicating that dosage adjustment is often unnecessary (Verme-Gibboney and Hursting, 2003). When dosage adjustment is necessary, the patient’s current dose, aPTT, and clinical status (e.g., hepatic function) should be considered. A reasonable increment for most patients is 0.5 µg/kg/min. Smaller increments (e.g., 0.2 µg/kg/min) are appropriate when dosing is already reduced for reasons such as hepatic impairment (Verme-Gibboney and Hursting, 2003). It has been suggested that downward dosage adjustments required in some patients may be associated with decreased liver perfusion (Reichert et al., 2003). At substantially higher argatroban doses, such as used during PCI, increments of 5 µg/kg/min are recommended (see Sec. V.A).

C. Conversion to Warfarin Anticoagulation

Warfarin is not recommended as sole anticoagulant therapy during acute HIT. For patients with HIT requiring long-term oral anticoagulation, the initiation of warfarin should be delayed until substantial recovery of the platelet count has occurred (at least > 100 x 10⁹/L), the patient is adequately
Table 3  Dosing Schedules for Argatroban Treatment of Patients with HIT (Approved Indications)

<table>
<thead>
<tr>
<th>Clinical use</th>
<th>Bolus&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IV infusion&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Monitoring and adjusting therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis or treatment of thrombosis&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>—</td>
<td>2 µg/kg/min (For hepatically impaired patients, reduce initial dose.&lt;sup&gt;d&lt;/sup&gt; Patients with renal insufficiency require no initial dosage adjustment.)</td>
<td>Dose adjusted (not to exceed 10 µg/kg/min) to achieve steady state aPTT 1.5–3.0 times the baseline value (not to exceed 100 s)&lt;sup&gt;e,f,g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percutaneous coronary intervention (PCI)&lt;sup&gt;b,h,i&lt;/sup&gt;</td>
<td>350 µg/kg (given over 3–5 min)</td>
<td>25 µg/kg/min</td>
<td>Infusion dose adjusted (15–40 µg/kg/min) to achieve an ACT 300–450 s; additional bolus doses of 150 µg/kg may be given as needed&lt;sup&gt;j,k&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: HIT, heparin-induced thrombocytopenia; IV, intravenous; aPTT, activated partial thromboplastin time; ACT, activated clotting time; PCI, percutaneous coronary intervention.

<sup>a</sup> Based on patient’s body weight.

<sup>b</sup> Includes patients with active HIT who have isolated thrombocytopenia or associated thrombosis, as well as patients with a documented history of HIT who are no longer thrombocytopenic but require anticoagulation.

<sup>c</sup> Argatroban is approved in the United States as an anticoagulant for prophylaxis or treatment of thrombosis in patients with HIT, and in Canada as an anticoagulant in patients with HIT who, in the opinion of their attending physician requires anticoagulation.

<sup>d</sup> For patients with moderate hepatic impairment, an initial dose of 0.5 µg/kg/min is recommended.

<sup>e</sup> The aPTT should be checked at least 2 h after the initiation of argatroban or any dosage change.

<sup>f</sup> For patients in studies ARG-911 and ARG-915, the mean ± SEM dose of argatroban was 1.9 ± 0.1 µg/kg/min.

<sup>g</sup> For transferring a patient to warfarin anticoagulant therapy: After substantial resolution of thrombocytopenia, initiate warfarin therapy using the expected daily dose of warfarin (do not use a loading dose) while maintaining argatroban infusion. At least 5 days of warfarin therapy are required to lower functional prothrombin concentrations to a therapeutic, steady state level. For monitoring the conversion to warfarin during coadministration of argatroban at doses up to 2 µg/kg/min, see text and Fig. 7.

<sup>h</sup> Argatroban is approved in the United States as an anticoagulant in patients with or at risk for HIT undergoing PCI. Argatroban has not been evaluated in hepatically impaired patients undergoing PCI. These recommendations do not consider the combination use of argatroban with glycoprotein IIb/IIIa antagonists, wherein lower doses of argatroban (e.g., 250–300 µg/kg bolus followed by infusion of 15 µg/kg/min) have been shown to provide effective anticoagulation with an acceptable bleeding risk (Jang et al., 2003).

<sup>i</sup> Includes percutaneous transluminal coronary angioplasty (balloon angioplasty), stent implantation, and atherectomy; oral aspirin 325 mg should be given 2–24 h prior to PCI.

<sup>j</sup> The ACT should be checked 5–10 min following the initial bolus dose and after any additional bolus dose or change in the infusion rate. In studies ARG-216, ARG-310, and ARG-311, the majority of patients required only one bolus dose during the interventional procedure, and the mean ± SEM dose of argatroban was 23.1 ± 0.7 µg/kg/min.

<sup>k</sup> After the procedure, the sheaths should be removed no sooner than 2 h after discontinuing argatroban and when the ACT is <160 s.
anticoagulated with an alternative parenteral agent such as argatroban, and the patient is clinically improving (Hirsh et al., 2001). Because warfarin should be avoided during acute HIT (Hirsh et al., 2001; Smythe et al., 2002; Srinivasan et al., 2003), if warfarin has already been initiated before HIT is diagnosed, it may be prudent in some circumstances to administer vitamin K to reverse warfarin’s effects. During the transition period from argatroban to warfarin anticoagulation, both agents are used concurrently, and their overlap should be continued for at least 4–5 days. A loading dose (> 5 mg) of warfarin should not be used; rather, warfarin should be initiated using modest, anticipated daily maintenance doses (≤ 5 mg), with careful monitoring. Ideally, the INR should be within the target therapeutic range for at least the last 2 days of overlap (discussed subsequently). During the overlap period, argatroban is monitored using the aPTT. These guidelines are important to ensure continuous anticoagulation and avoid prothrombotic effects of initiating warfarin during acute HIT, e.g., warfarin-induced venous limb gangrene or skin necrosis syndromes (see Chap. 3).

As mentioned in Section II, because argatroban is a direct thrombin inhibitor, concomitant use of argatroban and warfarin prolongs the PT/INR beyond that produced by warfarin alone (Hursting et al., 1999; Sheth et al., 2001). In the clinical studies of argatroban therapy in HIT, the majority of patients transferred to warfarin therapy for continued anticoagulation and there was no evidence of systematic underdosing or overdosing of warfarin (Hiatt et al., 2001). The method of transition from argatroban to warfarin was not specified in the protocols, and a variety of approaches were employed. Warfarin was generally started at the expected daily maintenance dose and continued for 3–4 days. In one method, the argatroban infusion was then discontinued for 4 h, if deemed clinically acceptable, prior to the INR being checked in the absence of argatroban effect. If the INR was in the therapeutic range, argatroban was permanently discontinued, and warfarin therapy was continued. In order to improve this procedure and avoid the possibility that the patient would be unprotected before the desired level of warfarin anticoagulation was achieved, a study in healthy subjects was conducted to develop guidelines for monitoring this transition (Sheth et al., 2001).

Results from Sheth et al. (2001) indicate that the relationship between the INR on cotherapy and the INR on warfarin monotherapy can be used to interpret the INR during the transition period. Specifically, INRs on cotherapy increase linearly with INRs on warfarin monotherapy. The slope of this relationship is sensitive to the argatroban dose and the thromboplastin reagent used, particularly, its International Sensitivity Index (ISI). For argatroban doses of 1–2 μg/kg/min, prediction errors for monotherapy INRs from cotherapy INRs are sufficiently low (±0.4 units) to allow for clinically reliable estimations of a monotherapy INR from a cotherapy INR. However,
at argatroban doses greater than 2 μg/kg/min, monotherapy INRs cannot be reliably predicted from cotherapy INRs.

Figure 6 presents the relationships between INRs on cotherapy and INRs on warfarin monotherapy, for argatroban doses of 1–2 μg/kg/min and thromboplastin reagents with ISIs between 0.88 and 2.13. For each of these combinations of argatroban dose and thromboplastin, a cotherapy INR greater than 4 is predicted to be related to a monotherapy INR between approximately 2.0 and 3.0, i.e., in the therapeutic range for warfarin mono-

**Figure 6** Relationships between the INR during warfarin/argatroban cotherapy and the INR during warfarin monotherapy, by argatroban dose and the International Sensitivity Index (ISI) of the thromboplastin reagent used. Two sets of lines are superimposed due to similarity in slope and intercept. Prediction errors are ± 0.4 INR units.
therapy. In the United States, commercially available thromboplastins with ISI values greater than 2.2 are rare. In general, therefore, following at least 4–5 days of coadministration of warfarin and argatroban at doses up to 2 μg/kg/min, argatroban can be discontinued when the cotherapy INR is greater than 4 (and ideally has been for 2 days). Upon cessation of argatroban, it would be prudent to check the INR 4–6 hours later, when the effect of argatroban is negligible, to ensure an actual therapeutic value reflective of warfarin monotherapy. For coadministration of warfarin and argatroban at doses greater than 2 μg/kg/min, the argatroban dose should be temporarily (4–6 h) reduced to 2 μg/kg/min. Then the procedure described above for predicting the warfarin monotherapy INR from the cotherapy INR at doses up to 2 μg/kg/min can be followed. These guidelines are summarized in Fig. 7.

Of additional note, factor assays that are insensitive to argatroban interference, such as the two-stage chromogenic factor X assay (Hoppen-
D. Conversion to Phenprocoumon or Acenocoumarol Anticoagulation

The pharmacologic interactions between argatroban and the oral anticoagulants phenprocoumon or acenocoumarol are comparable to those described for argatroban and warfarin. Guidelines for the conversion from argatroban to phenprocoumon or acenocoumarol are similar to those for the conversion to warfarin (Breddin et al., 2002).

V. ARGATROBAN FOR HIT PATIENTS IN SPECIAL CLINICAL CIRCUMSTANCES

A. Percutaneous Coronary Intervention

Argatroban is the only agent approved in the United States as an anticoagulant for patients with, or at risk of, HIT who undergo PCI.

Clinical Studies

Argatroban has been evaluated in three multicenter, open-label prospective studies in patients with HIT undergoing PCI, including percutaneous transluminal coronary angioplasty, stent implantation, or rotational atherectomy. The studies (ARG-216, ARG-310, and ARG-311) were similar in design with respect to eligibility criteria, argatroban dosing regimen, and main outcome assessments, and their pooled analysis has been reported (Lewis et al., 2002).

Among these studies, 91 patients with HIT underwent 112 PCIs on argatroban anticoagulation (Lewis et al., 2002). Patients received 325 mg oral aspirin 2–24 h before PCI. In the catheterization laboratory, patients received intravenous argatroban at 25 μg/kg/min (initial bolus dose of 350 μg/kg) titrated to achieve an ACT of 300–450 s during PCI (mean infusion dose, 23 μg/kg/min). Additional bolus doses of 150 μg/kg to achieve or maintain the target ACT were allowed, though usually not needed. Target ACT values were achieved typically within 10 min of initiating argatroban and were maintained throughout the infusion. When argatroban was discontinued after the procedure, ACTs rapidly returned to baseline.

Primary efficacy endpoints were subjective assessment of the satisfactory outcome of the procedure and adequate anticoagulation, which occurred in 94.5% and 97.8%, respectively, of patients undergoing their initial PCI.
with argatroban ($n = 91$) (Table 4). Death (no patients), myocardial infarction (4 patients), and revascularization at 24 h after PCI (4 patients) occurred in 7 (7.7%) patients. Other efficacy endpoints were also consistent with argatroban enabling a satisfactory outcome (Table 4). One patient (1%) experienced major periprocedural bleeding (nonfatal retroperitoneal hemorrhage). No unsatisfactory outcomes occurred during repeat PCIs with argatroban ($n = 21$; mean separation of 150 days from the initial PCI). Overall, the clinical outcomes compared favorably with those reported historically for heparin anticoagulation during PCI.

In a separate multicenter prospective study of 101 patients (including 1 patient with HIT) undergoing PCI, reduced doses of argatroban were evaluated in combination with the GPIIb/IIIa antagonists abciximab ($n = 99$) or eptifibatide ($n = 2$) (Jang et al., 2003). Patients received argatroban as an initial bolus of 250 μg/kg followed by an infusion of 15 μg/kg/min, and additional boluses of 150 μg/kg were allowed, if necessary, to achieve a target ACT of 275–325 s. This target ACT was reached in 94 patients. Death (no patients), myocardial infarction (3 patients), and urgent revascularization at 30 days (2 patients) occurred in 3 (3%) patients. Two additional patients had cardiac symptoms and elevated troponin without significant creatine kinase elevation. There were 2 major bleeding events (1 retroperitoneal, 1 groin

### Table 4  Efficacy Assessments in HIT Patients Undergoing PCI Using Argatroban Anticoagulation

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Initial group/Total ($n$) (%)</th>
<th>Repeat group/Total ($n$) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satisfactory outcome of procedure$^a$</td>
<td>86/91 (94.5%)</td>
<td>21/21 (100%)</td>
</tr>
<tr>
<td>Adequate anticoagulation$^a$</td>
<td>89/91 (97.8%)</td>
<td>21/21 (100%)</td>
</tr>
<tr>
<td>Lack of major acute complications$^b$</td>
<td>89/91 (97.8%)</td>
<td>21/21 (100%)</td>
</tr>
<tr>
<td>Angiographic success$^c$</td>
<td>86/88 (97.7%)</td>
<td>20/20 (100%)</td>
</tr>
<tr>
<td>Clinical success$^d$</td>
<td>86/88 (97.7%)</td>
<td>20/20 (100%)</td>
</tr>
</tbody>
</table>

$^a$ Primary, subjective outcomes.

$^b$ No death, emergent coronary artery bypass graft surgery, or Q-wave myocardial infarction during argatroban infusion or 24 h of its cessation (or discharge, whichever came first).

$^c$ Final stenosis of <50% in at least one lesion attempted, for patients with angiographic data available.

$^d$ Angiographic success plus the lack of major acute complications.
hematoma). Hence, argatroban in combination with a GPIIb/IIIa antagonist provides adequate anticoagulation with an acceptable bleeding risk. Additional studies are underway to refine this dosing regimen (e.g., evaluating a greater initial bolus dose of 300 μg/kg).

Argatroban Dosing and Monitoring During PCI

Dosing recommendations for patients with, or at risk for, HIT undergoing PCI (Table 3) are based on those used during studies ARG-216, ARG-310, and ARG-311 (Lewis et al., 2002). Argatroban should be started at an infusion dose of 25 μg/kg/min and a bolus of 350 μg/kg given over 3–5 min. The ACT should be checked 5–10 min after the bolus dose is completed. If the ACT is >300 s, the PCI may proceed. If the ACT is <300 s, an additional bolus dose of 150 μg/kg should be given and the infusion dose increased to 30 μg/kg/min. If, however, the ACT is >450 s after the initial bolus, then the infusion dose should be reduced to 15 μg/kg/min. After any additional bolus or dosage adjustment, the ACT should be checked again after 5–10 min to confirm the patient attained a therapeutic ACT. During a prolonged procedure, additional ACTs should be obtained every 20–30 min. For patients requiring anticoagulation after the procedure, argatroban infusion may be continued at a reduced dose such as that recommended for the prophylaxis or treatment of thrombosis in HIT.

These dosing recommendations do not take into consideration the possible combination use of GPIIb/IIIa antagonists. In that setting, lower doses of argatroban are prudent, e.g., bolus dose of 250–300 μg/kg followed by an infusion dose of 15 μg/kg/min (Jang et al., 2003).

High doses of argatroban should be avoided in HIT patients who require PCI and have clinically significant hepatic disease, including laboratory evidence such as aspartate aminotransferase or alanine aminotransferase at least three times the upper limit of normal. Argatroban use during PCI has not been studied in such patients (Lewis et al., 2002; Jang et al., 2003).

A practical issue associated with monitoring heparin anticoagulation during PCI is that ACTs from the most commonly used automated systems (the Hemochron and HemoTec systems) are not interchangeable (Avendano and Ferguson, 1994). In contrast, this is not an issue with argatroban anticoagulation. Argatroban equally prolongs the Hemochron ACT and HemoTec ACT (Iqbal et al., 2002), and investigators in the PCI trials effectively used whichever ACT method was available at their sites to monitor anticoagulation.

In reported studies of argatroban anticoagulation use during PCI (Lewis et al., 2002; Jang et al., 2003), sheaths were removed when the ACT was less than 160 s.
B. Peripheral Intervention

Case reports describe the successful use of argatroban anticoagulation in patients with HIT during renal stent implant (Lewis et al., 1997) and carotid stent implant (Lewis et al., 1998). The argatroban dose and target ACT values were the same as those recommended for PCI in the absence of GPIIb/IIIa inhibition.

C. Argatroban in Hemodialysis

Argatroban has been used successfully for anticoagulation during hemodialysis in patients with HIT (Matsuo et al., 1988, 1990; Koide et al., 1995; Reddy et al., 2002; Mihindu et al., 2002), including a patient with comorbid hepatic failure (Dager and White, 2003) (see also Chap. 18). In the latter case, argatroban effectively prevented clotting in the dialyzer circuit, and consistent with reduced argatroban clearance in patients with hepatic impairment, the aPTT and INR were slow to recover after stopping argatroban. The effective use of argatroban for the maintenance of catheter or graft patency between dialysis treatments has also been reported (Mihindu et al., 2002). Although guidelines are available for argatroban use in dialysis-dependent patients with HIT at a major medical center (O'Shea et al., 2003), the safety and efficacy of argatroban in patients with HIT undergoing hemodialysis have not been fully evaluated in a clinical trial.

Argatroban administration by bolus alone, infusion alone, or bolus plus infusion has been evaluated in a prospective cross-over study of 12 patients with end-stage renal disease undergoing chronic hemodialysis (Murray et al., 2003). Target ACTs during dialysis were 140–180% of the baseline value. The most satisfactory intradialysis anticoagulation was achieved using a steady-state infusion of argatroban (2 µg/kg/min begun ~4 h before dialysis), or a 250 µg/kg bolus dose at the start of dialysis followed by a continuous 2 µg/kg/min infusion. In 38 separate hemodialysis sessions, no dialysis membrane required changing, and one (2.6%) session was shortened owing to circuit clotting that occurred after 3 h of hemodialysis. There were no bleeding events. Although confirmatory studies are required, it is anticipated that similar dosing regimens may be adequate for inpatients with HIT already at steady-state argatroban levels or outpatients with a history of HIT who require hemodialysis.

In support of the general safety of argatroban as an anticoagulant during hemodialysis in patients with HIT, 54 argatroban-treated patients with HIT underwent hemodialysis in studies ARG-911, ARG-915, and ARG-915X. These protocols made no recommendations regarding argatroban dosing during hemodialysis. For the patients who did, versus did not, undergo...
hemodialysis, there were no significant between-group differences in either the primary efficacy endpoint or major bleeding.

Argatroban is approved in Japan as an anticoagulant for hemodialysis in patients with congenital or acquired antithrombin deficiency. The recommended infusion dose for argatroban in antithrombin-deficient patients undergoing hemodialysis (Novastan Prescribing Information, Japan, 1998) is generally similar to that which has been used for HIT patients undergoing hemodialysis (Matsuo et al., 1988, 1990; Koide et al., 1995).

D. Stroke

The effect of argatroban anticoagulation on stroke in HIT has been retrospectively evaluated using case records from studies of argatroban for HIT (LaMonte et al., 2003). Stroke occurred in 3.0% of 1005 individuals with HIT and was a significant predictor of death in argatroban-treated patients and historical controls (odds ratio ≥3 for each group). Almost all strokes (33/35, 94%) were ischemic, consistent with the prothrombotic nature of HIT. Stroke occurred most often in females, in those with a traditional risk factor for stroke, in patients with more severe thrombocytopenia, and within 2 weeks of HIT presentation. Compared with controls, argatroban therapy for HIT was associated with reduced frequency of new stroke (1.8% vs. 4.7%, $p = 0.032$) and stroke-associated mortality (1.0% vs. 3.1%, $p = 0.036$). These benefits were achieved without increased intracranial or major bleeding. These data highlight the importance of considering HIT in the differential diagnosis when stroke occurs, particularly in hospital in-patients.

In a randomized, double-blind clinical study of patients treated with argatroban vs. placebo within 12 h of ischemic stroke onset, there were no significant between-group differences in intracranial hemorrhage or major bleeding rates (LaMonte, 2003). Although not conducted in patients with HIT, the study further supports the safety of argatroban anticoagulation in patients with stroke.

As mentioned previously, argatroban is approved in Japan for use in nonlacunar stroke and in Korea for use in acute cerebral thrombosis.

E. Cardiopulmonary Bypass Surgery or Extracorporeal Membrane Oxygenation

Cardiopulmonary bypass (CPB) surgery using argatroban anticoagulation has been performed successfully in dogs (Walenga et al., 1997) and pigs (Ahmad et al., 2001). In addition, there have been case reports of HIT patients in whom argatroban anticoagulation has been used successfully during off-pump coronary artery bypass surgery (Arnoletti and Whitman, 1999; Ide et al.,
2001; Kieta et al., 2003; Ohno et al., 2003), and during (Edwards et al., 2003) or immediately before and after CPB (Lubenow et al., 2003). Levels of anticoagulation used during off-pump coronary bypass surgery tend to parallel those used during angioplasty. However, in the experience to date with argatroban anticoagulation in HIT patients in this setting, infusion doses have been generally similar to those recommended for the prophylaxis or treatment of thrombosis in HIT, with target ACTs > 200 s (Ide et al., 2001; Ohno et al., 2003) or twice the baseline value (Kieta et al., 2003). A consistently safe and effective dose to support CPB surgery in humans has not been established.

In vitro studies indicate that argatroban is at least as effective as heparin in preventing thrombin generation in extracorporeal membrane oxygenation (ECMO) circuits (Yonekawa et al., 2002). The successful use of argatroban anticoagulation in ECMO has been described, including an adult patient with HIT (Johnston et al., 2003) and two neonates, including one in whom ECMO was continued for 78 days (Kawada et al., 2000). In each case, ACTs were typically maintained > 200 s, although further study is required to establish dosing recommendations.

F. Other Hypercoagulability States

Antithrombin (AT) deficiency is a congenital hypercoagulability disorder wherein an alternative to heparin may be required. Argatroban (2 μg/kg/min) has been used successfully in a patient with burn-related severe acquired AT deficiency who failed heparin (Gorman et al., 2001). However, no formal studies have been conducted. As mentioned, argatroban is approved in Japan as anticoagulation for hemodialysis in patients with congenital or acquired AT deficiency.

The effective use of direct thrombin inhibitors, including argatroban, has been described in patients with disseminated intravascular coagulation (DIC), including patients with low levels of AT or with suspected HIT (Kumon et al., 1984; Mukundan and Zeigler, 2002). The data, albeit limited, provide evidence that argatroban can improve DIC, and also that DIC in a patient with HIT should not preclude use of argatroban.

G. Argatroban in Pregnant, Nursing, or Postpartum Women

Argatroban anticoagulation in pregnant or nursing women has not been studied. Teratology studies in rats reveal no evidence of impaired fertility or fetal harm due to argatroban (Argatroban Prescribing Information, 2002). Because animal reproductive studies are not always predictive of human response, it is recommended that the drug be used during pregnancy only if clearly needed.
Argatroban is detected in rat milk (Iida et al., 1986). It is unknown whether argatroban is excreted in human milk, although many drugs are. Hence, it is recommended that a decision be made either to discontinue nursing or discontinue the drug.

H. Argatroban in Geriatric or Pediatric Patients

The pharmacokinetic parameters of argatroban are similar between young adult and elderly volunteers (Swan and Hursting, 2000), and no dosage adjustment is required for the elderly. Further, the effectiveness of argatroban for HIT was not influenced by patient age (Lewis et al., 2001). Across the pivotal studies evaluating argatroban for HIT, patient age ranged from 17 to 91 years.

Data on argatroban use in pediatric patients are limited. Argatroban anticoagulation has been used successfully in at least three pediatric patients with HIT for the prophylaxis or treatment of thrombosis or cardiac catheterization (Boshkov et al., 2002, 2003) and at least two neonates during ECMO (Kawada et al., 2000). A study to establish the pharmacokinetics of argatroban in children is underway in the United States.

VI. CONCLUSION

Argatroban, a synthetic direct thrombin inhibitor, is an effective anticoagulant with a predictable dose-response effect. This agent offers several theoretical advantages as an anticoagulant for patients with HIT: it inhibits free and bound thrombin, it does not cross-react with HIT antibodies, and its anticoagulant effects are rapidly active and also rapidly reversible. Further, upon prolonged or repeated administration, argatroban is well tolerated, with no alteration in anticoagulant response and no induction of drug-specific antibodies.

In clinical studies, argatroban therapy, compared with historical controls, improves outcomes of HIT, particularly thrombosis and its sequelae, without increasing bleeding risk. Argatroban also provides safe and effective anticoagulation in patients with a history of HIT requiring acute anticoagulation. No intracranial hemorrhage has occurred during argatroban infusion in over 900 patients with HIT, including many with stroke, who have received argatroban during clinical trials. These benefits are achieved when argatroban is administered intravenously at 2 μg/kg/min, titrated to achieve an aPTT 1.5–3.0 times baseline. Although no initial dosage adjustment is required for patients with renal impairment, an initial dose of 0.5 μg/kg/min is recommended for hepatically impaired patients. Also in clinical studies, argatroban at higher doses (25 μg/kg/min, titrated to achieve an ACT
of 300–450 s) provides safe and adequate anticoagulation in HIT patients
during PCI. Lower doses of argatroban in combination with a GPIIb/IIIa
antagonist also provide safe and adequate anticoagulation during PCI. The
recommended dosing schedules for the approved indications of argatroban in
the United States (i.e., as an anticoagulant for the prophylaxis or treatment of
thrombosis in patients with HIT and during PCI for patients with or at risk
for HIT) are summarized in Table 3.

Although data are limited, patients with HIT have also successfully un-
dergone hemodialysis, off-pump coronary bypass surgery, and ECMO using
argatroban anticoagulation. Furthermore, although studied in patients with-
out HIT, argatroban dosing regimens tailored to meet the needs of the inpa-
tient or outpatient safely provide intradialysis anticoagulation. Argatroban
therefore offers a versatile therapeutic option for the management of patients
with HIT in diverse clinical settings.

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Bivalirudin for the Treatment of Heparin-Induced Thrombocytopenia

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I. INTRODUCTION

The treatment for heparin-induced thrombocytopenia (HIT) has undergone major changes over the past decade. Until recently, clinicians had few alternatives for treating this potentially devastating syndrome. Fortunately, with the development of several new anticoagulants, physicians have a number of novel treatment options. These include the heparinoid danaparoid (see Chap. 14) and the direct thrombin inhibitors (DTIs), including recombinant hirudin (r-hirudin) (e.g., lepirudin) (see Chap. 15) and the small molecule DTI argatroban (see Chap. 16) (Alving, 2003; Chong, 2003; Warkentin, 2003). More recently, another DTI, bivalirudin (Angiomax), has been approved for use in percutaneous coronary angioplasty (PCI). This anticoagulant also has been used “off-label” for treatment of HIT.

Bivalirudin is a hirulog, i.e., one of a group of drugs designed from the structure of hirudin (analogue of hirudin). It was developed in the early 1990s by the Biogen Corporation (Cambridge, MA), and was originally known as BG8967 or Hirulog. The U.S. Food and Drug Administration (FDA) mandated a name change to avoid confusion with Humalog (recombinant human insulin) when The Medicines Company (Parsippany, NJ) acquired licensure for bivalirudin in 1997. The name was then changed to Angiomax.

Bivalirudin has also been used with favorable results in several “on-pump” and “off-pump” cardiac surgery cases in patients with HIT. A recently completed clinical trial in New Zealand compared bivalirudin with heparin (with protamine reversal) in non-HIT patients requiring off-pump...
coronary artery bypass (OPCAB) surgery (Merry et al., 2004). It is currently under investigation (phase II and III multicenter trials) as an alternative anticoagulant for both on-pump and off-pump cardiac surgery. EVOLUTION, a randomized trial, will compare bivalirudin with heparin (and protamine reversal) in non-HIT patients, and CHOOSE (not randomized) will use bivalirudin as an alternative anticoagulant for patients with HIT.

II. BIVALIRUDIN

A. Chemistry

Bivalirudin is a small synthetic 20-amino-acid peptide that is a specific and reversible inhibitor of thrombin (Parry et al., 1994) (Fig. 1). Although it is an analogue of hirudin, its amino acid sequence is considerably shorter. Bivalirudin unites a carboxy-terminal segment of 12 amino acids (dodecapeptide) derived from native hirudin (residues 53–64) to an active site-binding tetrapeptide sequence (d-Phe-Pro-Arg-Pro) at its amino terminal (Maraganore et al., 1990; Nawarskas and Anderson, 2001; White and Chew, 2002). Four glycine residues bridge these two segments together. The amino-terminal segment has a high affinity and specificity for binding to the active site of thrombin (Fareed et al., 1999; Sciulli and Mauro, 2002), while the carboxy terminal

![Figure 1](image-url) The structure of bivalirudin. (Top) Bivalirudin is comprised of 20 amino acids, with an N-(amino-)terminal d-Phe-Pro-Arg-Pro (F-P-R-P) region that binds with high affinity to the active site region of thrombin; a (gly)₄ (“spacer”) region; and a C-(carboxy-)terminal Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu dodecapeptide (N-G-D-F-E-I-P-E-E-Y-L) that binds to the fibrinogen-binding region (exosite 1) of thrombin. The 11 C-terminal amino acids (shaded circles) correspond exactly to the 53- to 64-amino-acid sequence of lepirudin. Highly-specific, non-competitive binding between bivalirudin and thrombin results. (Not shown is the heparin-binding region [exosite 2] of thrombin.) However, proteases (including other thrombin molecules [not shown]) can cleave the Arg₃-Pro₄ of bivalirudin, leading to loss of antithrombin activity. (Bottom) Initially, there is bivalent binding of bivalirudin to thrombin, as shown. Following cleavage at Arg₃-Pro₄, the N-terminal sequence of bivalirudin no longer binds to thrombin, leaving the residual C-terminal dodecapeptide with greatly reduced binding affinity for exosite 1 of thrombin. Thus, the bivalirudin remnant transforms to a competitive inhibitor of thrombin. Other substrates, e.g., fibrinogen, can compete with, and displace, bivalirudin, thus allowing thrombin to resume its prohemostatic functions. Abbreviations: Arg (R), arginine; Asn (N), asparagine; Asp (D), aspartic acid; Glu (E), glutamic acid; Gly (G), glycine; Ile (I), isoleucine; Leu (L), leucine; Phe (F), phenylalanine; Pro (P), proline; Tyr (Y), tyrosine.
Bivalirudin in HIT Treatment

Bivalirudin binds reversibly to thrombin

Proteolytic cleavage

Identical to residues 53–64 of lepirudin

Binds to fibrinogen-binding region of thrombin (exosite 1)

Thrombin

Fibrinogen

C-terminal dodecapeptide

Proteolytic cleavage

+ H$_3$N– (Gly)$_4$ active site region

Binds to active site

+ H$_3$N– (Gly)$_4$ fibrinogen-binding region

Identical to residues 53–64 of lepirudin

Binds to fibrinogen-binding region of thrombin (exosite 1)
binds to the fibrinogen recognition site of thrombin at exosite 1 (Thiagarajan and Wu, 1999; Reed and Bell, 2002). One difference between bivalirudin and hirudin is that the binding of bivalirudin to the active site of thrombin is transient, whereas with lepirudin, irreversible thrombin-hirudin complexes are formed (Weitz and Hirsh, 1998; Nawarskas and Anderson, 2001).

Bivalirudin is produced by solid-phase peptide synthesis (Maraganore et al., 1990). Its molecular mass is 2180 Da. Bivalirudin has no structural similarity to heparin.

B. Pharmacology

Bivalirudin is a bivalent DTI, i.e., it binds two distinct regions of thrombin: the active (catalytic) site and the fibrinogen-binding site. Moreover, like lepirudin and argatroban, bivalirudin binds to both free (soluble) and clot-bound (fibrin-bound) thrombin. It forms a 1:1 stoichiometric complex that neutralizes thrombin during coagulation and thrombus formation (Maraganore and Adelman, 1996). Thus, bivalirudin inhibits proteolytic cleavage of fibrinogen, thrombin-mediated activation of factors V and VIII, and thrombin-induced platelet activation.

Bivalirudin (unlike lepirudin) is a reversible inhibitor of thrombin (Fig. 1). It acts initially as a noncompetitive inhibitor, rendering thrombin inactive. Circulating proteases (including other thrombin molecules) slowly cleave bivalirudin near the amino-terminal end (between arg₃-pro₄), thus eventually releasing the amino-terminal segment from the active site region of thrombin (Bates and Weitz, 1998; Carswell and Plosker, 2002; Reed and Bell, 2002; Sciulli and Mauro, 2002). This allows thrombin to resume catalytic function.

As mentioned, bivalirudin also inhibits thrombin by the binding of its carboxy-terminal segment to the fibrinogen-binding site on thrombin. This occurs at the same time that the amino-terminal segment attaches to the active site, thus resulting in dual blockage with complete inhibition of thrombin’s multiple activities (Sciulli and Mauro, 2002). Once the amino-terminal moiety of bivalirudin is cleaved, however, the carboxy-terminal region acquires low-affinity, weakly competitive binding properties. Fibrinogen can now displace the bivalirudin remnant from thrombin and align itself over the active site to be converted to fibrin (Parry et al., 1994).

Bivalirudin is not inactivated by platelet factor 4, nor does it require any cofactor for its activity. It does not bind to red blood cells or proteins other than thrombin.

C. Pharmacokinetics

Bivalirudin has predictable pharmacokinetics, and exhibits a linear dose-response relationship when given by the intravenous (iv) route to healthy
volunteers with normal renal function. Its half-life is approximately 25–36 min (Fox et al., 1993; Robson, 2000; Robson et al., 2002). Peak bivalirudin plasma concentrations after a 15-min iv infusion are related to dose and occur within 5 min of completing the infusion (Fig. 2).

Bivalirudin has a volume of distribution of 0.24 L/kg and a clearance rate of approximately 3.4 mL/min/kg (Fox et al., 1993). It is cleared from plasma by both renal mechanisms and cleavage by plasma proteases. Bivalirudin undergoes glomerular filtration, secretion in the proximal convoluted tubule, and reabsorption in the distal convoluted tubule. The peptides are then further degraded within the intracellular lysosomes (Robson, 2000; Robson et al., 2002). In a study by Fox and colleagues (1993), only 20% of bivalirudin was recovered in the urine.

Clearance of bivalirudin is accomplished predominately by proteolytic cleavage within plasma and elsewhere and accounts for approximately 80% of the drug’s metabolism (Fox et al., 1993; Scatena, 2000; Robson et al., 2002; Warkentin and Greinacher, 2003). Indeed, proteolysis of bivalirudin appears to result mainly from (nonbivalirudin-inhibited) thrombin, thus providing a mechanism of degradation that is independent of specific organ function (Bates and Weitz, 2000; Koster et al., 2002b). This results in degradation to individual amino acids and small, inactive peptide fragments (Carswell and Plosker, 2002).

Patients with renal insufficiency need dose adjustments for bivalirudin, according to their degree of impairment (Table 1). In a study of 45 patients with normal to severe renal disease, Robson (2000) found that patients with normal kidneys (glomerular filtration rate [GFR] >90 mL/min) and mildly impaired renal disease (GFR = 60–89 mL/min) had similar renal clearance levels and required no dose adjustments. The clearance rate was reduced by 45% in individuals with moderate renal impairment (GFR = 30–59 mL/min) and by 68% in persons with severe renal impairment (GFR <30 mL/min). In dialysis-dependent patients, the clearance rate was reduced by 77% (Robson, 2000; Robson et al., 2002). Dose adjustments are thus recommended for patients with moderate or severe kidney dysfunction and for individuals on dialysis (Irvin et al., 1999; Robson, 2000; Robson et al., 2002). The half-life of bivalirudin in patients with severe renal impairment is prolonged (about 1 h). In dialysis patients, the half-life is approximately 3.5 h (Nawarskas and Anderson, 2001).

D. Pharmacodynamics

Bivalirudin produces an immediate effect after iv administration. It causes prolongation of the prothrombin time (PT)/international normalized ratio (INR), activated clotting time (ACT), the activated partial thromboplastin time (aPTT), and the thrombin time (TT) (Fox et al., 1993; Lidon et al., 1993;
Sharma et al., 1993; Topol et al., 1993). Although there is some interindividual variability, a dose of bivalirudin given as an infusion of 0.2 mg/kg/h increased the aPTT from 27 to 62 s in one study, while an infusion rate of 1.0 mg/kg/h resulted in an average aPTT of 98 s in another group of patients (Lidon et al., 1993).

The INR is also prolonged somewhat during bivalirudin infusion. In 54 healthy volunteers, a dose of 0.05–0.6 mg/kg of bivalirudin given over 15 min iv increased the INR to between 1.25 and 2.43 (Fox et al., 1993). In a study by Lidon and coworkers (1993), the PT was prolonged to between 12 and 16 s with a dose of 0.2 mg/kg/h, while Francis and colleagues (2003; and unpublished data) recently reported the mean INR to be 1.51 (range 1.26–2.08) in 40 patients with suspected HIT treated with bivalirudin. Two recent abstracts also mention a slight prolongation in the INR (Bufton et al., 2002a,b). Although the increase in the INR seems not to be as great as with the DTI argatroban, physicians should be aware of DTI-coumarin interactions during overlapping therapy (see Chap. 13).

Bivalirudin decreases fibrinopeptide A levels (a marker of fibrinogen cleavage) in patients with coronary artery disease (Cannon et al., 1993; Ren et al., 1997). It may also increase the bleeding time in some patients (Topol et al., 1993).

Bivalirudin does not inhibit platelet activation or aggregation directly, but it has been shown to inhibit thrombin-mediated platelet aggregation without affecting adenosine 5'-diphosphate (ADP) or collagen-mediated platelet activation (Weitz and Maraganore, 2001; Wiggins et al., 2002; Wittkowski, 2002) (Fig. 3). This antiplatelet effect may make bivalirudin more useful than heparin in the platelet-rich environment of acute coronary syndromes, where both platelet activation and thrombin formation play significant roles.

The anticoagulant effects of bivalirudin reverse rapidly, with coagulation times returning to baseline within 1–2 h after stopping the infusion (Fox et al., 1993).

E. Dosage

Bivalirudin is approved for iv administration only. It has not yet been approved for use in HIT, and therefore no dosing guidelines for this indication

Figure 2 Prolongation of the activated partial thromboplastin time (aPTT) by increasing doses of bivalirudin. Bivalirudin was given by iv infusion over 15 min to five groups (4 subjects each) in doses ranging from 0.05 mg/kg per 15 min up to 0.6 mg/kg per 15 min. Each series of data points represents the mean of four study subjects. (From Fox et al., 1993.)
are established (Dager and White, 2002). In trials to date, however, several dosing regimens have been reported. In three patients with venous and arterial thrombosis treated with bivalirudin for HIT, Chamberlin and associates (1994) used doses ranging from 0.05 to 0.2 mg/kg/h. Their goal was to maintain a therapeutic aPTT greater than 50 s. Bufton and coworkers (2002a) used an average dose of 0.27 mg/kg/h in one patient who received bivalirudin for over 2 months.

Francis and colleagues (2003; and unpublished data) have used bivalirudin in 40 patients with clinically suspected HIT. Only two patients were given iv boluses. Initial infusion rates usually ranged from 0.15 to 0.20 mg/kg/h; the overall mean infusion rate was 0.165 mg/kg/h (maximum 0.38 mg/kg/h). The target aPTT was a 1.5- to 2.5-fold prolongation of the therapeutic aPTT.

Table 1  Bivalirudin Dosage Adjustments for Percutaneous Coronary Intervention Based on Renal Function

<table>
<thead>
<tr>
<th>Renal function (glomerular filtration rate, mL/min)</th>
<th>Bivalirudin clearance (mL/min/kg)</th>
<th>Half-life (min)</th>
<th>Initial bolus (mg/kg)</th>
<th>Infusion rate for 4 h (mg/kg/h) (% reduction in infusion dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal renal function (≥90 mL/min)</td>
<td>3.4-4.6</td>
<td>25</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Mild renal impairment (60–89 mL/min)</td>
<td>3.4-4.9</td>
<td>22</td>
<td>1</td>
<td>2.5 (no adjustment)</td>
</tr>
<tr>
<td>Moderate renal impairment (30–59 mL/min)</td>
<td>2.5-2.7</td>
<td>34</td>
<td>1</td>
<td>2.0 (20% reduction)</td>
</tr>
<tr>
<td>Severe renal impairment (10–29 mL/min)</td>
<td>1.5-2.8</td>
<td>57</td>
<td>1</td>
<td>1.0 (60% reduction)</td>
</tr>
<tr>
<td>Dialysis-dependent patients</td>
<td>1.0</td>
<td>210</td>
<td>1</td>
<td>0.25 (90% reduction)</td>
</tr>
</tbody>
</table>

Source: Data shown upon which bivalirudin dosing recommendations (for PCI) are based are available in Robson (2000) and Robson et al. (2002).

are established (Dager and White, 2002). In trials to date, however, several dosing regimens have been reported. In three patients with venous and arterial thrombosis treated with bivalirudin for HIT, Chamberlin and associates (1994) used doses ranging from 0.05 to 0.2 mg/kg/h. Their goal was to maintain a therapeutic aPTT greater than 50 s. Bufton and coworkers (2002a) used an average dose of 0.27 mg/kg/h in one patient who received bivalirudin for over 2 months.

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Figure 3  The effect of bivalirudin on thrombin-induced platelet aggregation. Bivalirudin completely inhibits thrombin-induced platelet aggregation at concentrations about 1/500 that of therapeutic doses achieved during PCI, without significant effect on platelet aggregation by collagen or adenosine diphosphate (ADP). (From Wittkowsky, 2002.)
baseline aPTT value. Thus, a reasonable regimen might be to start at 0.15 mg/kg/h (no initial bolus), with subsequent adjustments according to aPTT.

The dose recommended in the “Anticoagulant Therapy with Bivalirudin to Assist in the Performance of Percutaneous Coronary Intervention in Patients with Heparin-induced Thrombocytopenia” (ATBAT) trial was a bolus of 1.0 mg/kg followed by an infusion of 2.5 mg/kg/h for 4 h. This dose was later changed to a bolus of 0.75 mg/kg followed by a 1.75 mg/kg/h infusion over 4 h, based on data from the CACHET and REPLACE-1 trials (Mahaffey, 2001; Lincoff et al., 2002a).

Bivalirudin is approved for PCI in patients with unstable angina. Currently, the recommended dose for patients with (near) normal renal function is a bolus of 1.0 mg/kg followed immediately by a continuous 4-h infusion at 2.5 mg/kg/h (Sciulli and Mauro, 2002). The bolus is given just prior to angioplasty. After completing the 4-h infusion, additional bivalirudin may be given at a rate of 0.2 mg/kg/h for up to 20 h.

Bivalirudin infusion should be reduced 20% in patients with moderate renal impairment and by 60% in patients with severe renal impairment (GFR <30 mL/min) (Robson et al., 2002). In dialysis-dependent patients the dose is reduced by 90% and ideally should be given when the patient is off dialysis (Sciulli and Mauro, 2002) (Table 1).

Allie et al. (2003) used doses similar to the modified ATBAT trial dose for percutaneous transluminal angioplasty (PTA) of the renal and iliac arteries. Following a 0.75 mg/kg iv bolus, bivalirudin was subsequently given by infusion (1.75 mg/kg/h infusion) until completion of the procedure. Bivalirudin is not FDA approved for this indication.

For patients given bivalirudin for OPCAB, Merry and colleagues (2004) selected a 0.75 mg/kg bolus followed by a 1.75 mg/kg/h infusion. In the multicenter CHOOSE and EVOLUTION trials for cardiac surgery (see Sec. IV.C), for those patients undergoing OPCAB surgery the same dosing will be given, with the option to increase or decrease the infusion in 0.25 mg/kg/h increments (or to administer additional 0.1–0.5 mg/kg boluses) to maintain the ACT over 300 s.

For cardiac surgery using cardiopulmonary bypass (CPB), i.e., on-pump surgery, about two- to threefold greater levels of anticoagulation are required, compared with OPCAB. In the multicenter CHOOSE (HIT patients) and EVOLUTION (non-HIT patients) trials, for patients undergoing CPB, an iv bolus dose of 1.0 mg/kg will be given, followed by a 2.5 mg/kg/h iv infusion, and 50 mg bivalirudin will be added pre-CPB to the pump circuit volume. (For details regarding this protocol, including important technical considerations for the cardiac surgeon and cardiac anesthesiologist, see Chap. 19; see also Warkentin and Greinacher, 2003.)
**F. Administration**

Bivalirudin is administered iv and produces a rapid anticoagulant effect (Fig. 2). In several small trials, however, it has also been given by subcutaneous (sc) injection. In contrast to its rapid clearance following iv injection, its anticoagulant effects are sustained for several hours following sc administration (Fox et al., 1993) (Fig. 4). The peak anticoagulant effect occurred between 1 and 2 h after sc administration in a study of human volunteers, with detectable plasma levels measured up to 6 h postinjection. The aPTT was prolonged from 150 ± 19.4% to 176 ± 19.4% of the baseline value, and the INR increased from 1.18 ± 0.05 to 1.48 ± 0.17 (Fox et al., 1993). Urinary excretion of the drug was complete by 8–12 h.

A number of drugs commonly used in patients undergoing PCI have been tested for Y-site compatibility with bivalirudin. Testing was for short-term mixing, rather than longer-term interactions (4 h). Drugs found to be compatible with bivalirudin included abciximab, dexamethasone, digoxin, diphenhydramine, dobutamine, dopamine, epinephrine, eptifibatide, esmolol, furosemide, heparin, lidocaine, morphine, nitroglycerin, potassium chloride, sodium bicarbonate, tirofiban, and verapamil (The Medicines Company, 2001; Reed and Bell, 2002). Table 2 lists nine drugs found to cause haze formation or gross precipitation, which thus should not be administered in the same line as bivalirudin.

Drug-drug interaction studies have been performed with the thienopyridine derivative ticlopidine, the glycoprotein (GP) IIb/IIIa inhibitors abciximab, eptifibatide and tirofiban, and low molecular weight heparin and unfractionated heparin (Reed and Bell, 2002). No pharmacodynamic interactions occurred between bivalirudin and these agents. In patients undergoing PCI, use of bivalirudin in conjunction with heparin, warfarin, or thrombolytic therapy has been associated with increased risk of bleeding (The Medicines Company, 2001). Aspirin was associated with a mild increase in bleeding times in patients receiving bivalirudin infusions when compared to placebo. These changes were not felt to be clinically significant (Fox et al., 1993).

**G. Monitoring**

The PT/INR, ACT, aPTT, and TT all rise linearly with increases in the dose of bivalirudin. The ACT is generally used to monitor bivalirudin in patients undergoing PCI, while the aPTT has been used in patients treated for HIT and other non-PCI indications. Currently, the ECT (see Chap. 19) is recommended for monitoring during on-pump cardiac surgery (CPB). Dosing in PCI generally aims to maintain the ACT above 300 or 350, while in patients...
undergoing aPTT monitoring, the target range generally is a 1.5- to 2.5-fold increase in the baseline aPTT (Chew et al., 2001).

The ACT and aPTT have limitations, and questions regarding their adequacy for monitoring DTI therapy remain (Pötzsch et al., 1997; Koster et al., 2000, 2003a; Despotis et al., 2001; de Denus and Spinler, 2002). As a result, several new tests have been developed for monitoring these anticoagulants.

Cho and colleagues (2003a) have used a thrombin inhibitor management (TIM) point-of-care test based upon the ECT for monitoring the anticoagulant effects of bivalirudin. This test (TIM-ECT) was developed by PharmaNetics, Inc. (Morrisville, NC) and evaluated in 64 consecutive patients who underwent nonemergent PCI. The study compared the TIM-ECT to two available ACT methods with an antifactor IIa assay for monitoring bivalirudin anticoagulation. In their study, the TIM-ECT correlated better with bivalirudin levels than either of the ACT methods.

Koster and colleagues (2003a) and Pötzsch and coworkers (1997) have also questioned the validity of using the ACT for monitoring DTI therapy. For cardiac surgery requiring CPB, they recommend intraoperative monitoring utilizing the ECT. In a case report, the ECT was maintained between 400 and 450 s (Koster et al., 2003a). A close relationship between the ECT and bivalirudin concentrations was noted, but not with the ACT. However, Merry et al. (2004) have suggested that the ACT can be used for off-pump procedures when using lower bivalirudin concentrations.

As with other anticoagulants, monitoring is not reliable if the patient has a lupus anticoagulant, low fibrinogen levels, elevated fibrinogen-fibrin degradation products, or if the plasma contains heparin (Reid and Alving, 1993). In these situations, other tests including high-performance liquid chromatography, immunoassays, and chromogenic assays may be superior. Although such assays have been used to measure levels of various DTIs (Griessbach et al., 1985; Bichler et al., 1991; Spannagl et al., 1991; Walenga et al., 1991), these assays are not widely available.

Reid and Alving (1993) developed a quantitative thrombin time (QTT) in which bivalirudin (or hirudin) levels are measured using patient plasma (or whole blood) mixed with human fibrinogen solution, with the clotting time measured after adding human thrombin. The concentration of bivalirudin (or hirudin) is then determined by comparison with a standard curve that is generated by adding known concentrations of bivalirudin to pooled normal plasma.

Figure 4  Prolongation of the activated partial thromboplastin time (aPTT) by administration of subcutaneous bivalirudin. Three groups of study subjects containing four subjects each were given an increasing dose of sc bivalirudin. Each line represents the mean value of four study subjects. (From Fox et al., 1993.)
H. Reversal

There is no specific antagonist to bivalirudin. If renal function is normal, bivalirudin is eliminated rapidly, and its anticoagulant effect clears within a few hours after discontinuing the infusion. Kaplan and Francis (2002) have suggested that recombinant factor VIIa and desmopressin may be of benefit if bleeding occurs. Bivalirudin can be removed by hemodialysis (Irvin et al., 1999).

Koster and colleagues (2003b) recently demonstrated that large amounts of bivalirudin can be removed by hemofiltration and plasmapheresis. They utilized five different hemofilters in an in vitro study (conditions mimicking CPB) and observed a correlation between pore size and elimination rate. In their study, 65% of bivalirudin was removed using a hemofilter with a large pore size (65,000 Da) (Mintech Hemocor HPH 700, Minneapolis, MN), an amount comparable to that eliminated with a plasmapheresis filter system (69%). This represents a 50% improvement over the amount of lepirudin that can be removed through filtration (moreover, lepirudin filtration correlates poorly with pore size). These authors suggest that hemofiltration using appropriate filters may be useful for routine management of patients who receive bivalirudin for cardiac surgery.

I. Adverse Effects

Bleeding is the major adverse effect of bivalirudin and occurs more commonly in patients with renal impairment. Injection site pain has been reported in individuals given sc bivalirudin (Fox et al., 1993). Mild headache, diarrhea,

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Table 2  Drugs Incompatible with Bivalirudin

<table>
<thead>
<tr>
<th>Drug</th>
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<tr>
<td>Alteplase</td>
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<tr>
<td>Amiodarone hydrochloride</td>
</tr>
<tr>
<td>Amphotericin B</td>
</tr>
<tr>
<td>Chlorpromazine hydrochloride</td>
</tr>
<tr>
<td>Diazepam</td>
</tr>
<tr>
<td>Prochlorperazine edisylate</td>
</tr>
<tr>
<td>Reteplase</td>
</tr>
<tr>
<td>Streptokinase</td>
</tr>
<tr>
<td>Vancomycin hydrochloride</td>
</tr>
</tbody>
</table>

nausea, and abdominal cramps have also been reported (Fox et al., 1993). In the Hirulog Angioplasty Study (HAS) (now known as the Bivalirudin Angioplasty Trial [BAT]), the most frequent adverse effects included back pain, nausea, hypotension, pain, and headache. Approximately 5–10% of patients reported insomnia, hypertension, vomiting, anxiety, dyspepsia, bradycardia, abdominal pain, fever, nervousness, pelvic pain, and pain at the injection site (Bittl et al., 1995; Sciulli and Mauro, 2002) (Table 3).

### III. CLINICAL USE OF BIVALIRUDIN (NON-HIT PATIENTS)

#### A. Treatment of Deep Vein Thrombosis

Bivalirudin is not approved for the treatment of venous thromboembolism. However, it has been evaluated in animal models of venous and arterial thrombosis.
bosis and in one study involving humans. In a rat model of venous thrombosis using injections of tissue thromboplastin combined with stasis, the administration of bivalirudin demonstrated a dose-dependent interruption of thrombus formation (Maraganore et al., 1991).

Ginsberg et al. (1994a) studied iv and sc injections of bivalirudin in 10 patients with calf-vein thrombosis to determine if single injections could inhibit thrombin generation in a sustained fashion. Prothrombin fragment (F1 + 2) levels were used as an index of thrombin generation. Significant reductions in F1 + 2 levels were noted at 6 h postinjection, but by 24 h levels had increased significantly. These workers speculated that higher doses, more frequent sc injections, or prolonged infusion was required to achieve ongoing inhibition.

B. Prevention of Deep Vein Thrombosis

Bivalirudin has been evaluated for prevention of deep vein thrombosis (DVT) in patients undergoing hip or knee surgery. In a phase II, open-label, dose-optimization study of 222 patients, sc bivalirudin was given beginning 12–24 h postoperatively for up to 14 days or until hospital discharge (Ginsberg et al., 1994b). Five dose regimens were used, ranging from 0.3 mg/kg twice a day to 1.0 mg/kg three times a day (Table 4). Patients were evaluated for the occurrence of symptomatic DVT or pulmonary embolism (PE) within 72 h of discontinuing bivalirudin, and assessment of distal or proximal DVT by venography was performed on day 14 or just prior to discharge. Two patients suffered PE while three patients had major bleeding. The rate of DVT ranged from 59% in the lowest-dose regimen to only 17% in the highest-dose regimen (1.0 mg/kg three times a day). Proximal DVT occurred in only 2% of patients in the highest-dose regimen. Bleeding rates were low (<5%) with all regimens.

C. Percutaneous Coronary Intervention

Bivalirudin has been studied for several cardiology indications, including most prominently percutaneous coronary intervention (PCI), but also other nonintervention cardiac situations (Table 5). Bivalirudin has been approved by the FDA for use in patients with unstable angina undergoing PCI. To date, over 80,000 patients have been treated with bivalirudin, with nearly 12,000 subjects enrolled in comparative trials with heparin for PCI. Bivalirudin is a safe and effective alternative to heparin in this patient population.

The first clinical study using bivalirudin for coronary angioplasty was reported by Topol and coworkers (1993) in a multicenter, open-label, dose-
finding trial of 258 patients. The encouraging results led to larger studies of patients requiring urgent angioplasty because of unstable or postinfarction angina, the Hirulog (bivalirudin) Angioplasty Study (HAS) (for review, see Nawarskas and Anderson, 2001). The primary endpoint was in-hospital death, myocardial infarction (MI), or abrupt vessel closure within 24 h of initiating PCI, or rapid clinical deterioration of cardiac origin. In the original publication, no statistically significant difference in the primary endpoint was noted between bivalirudin and heparin (Bittl et al., 1995), causing the sponsor (Biogen) to abandon further drug development.

Subsequently, The Medicines Company reanalyzed the trial data (including an additional 214 patients analyzed by intention-to-treat principle who were not included in the per-protocol analysis initially reported). In this study, renamed as Bivalirudin Angioplasty Trial (BAT), the frequency of secondary endpoints (including death or MI, and major hemorrhage) were found to be significantly reduced with bivalirudin. Bivalirudin was at least as effective as heparin in preventing ischemic complications in patients who underwent angioplasty for unstable angina and included fewer episodes of

---

Table 4

<table>
<thead>
<tr>
<th>Efficacy or safety endpoint</th>
<th>Bivalirudin dosing regimen</th>
<th>0.3 mg/kg every 12 h</th>
<th>0.6 mg/kg every 12 h</th>
<th>1.0 mg/kg every 12 h for 3 d, then 0.6 mg/kg every 12 h</th>
<th>1.0 mg/kg every 8 h (high-dose regimen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>54</td>
<td>40</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>Overall DVT rate</td>
<td>10 (59%)</td>
<td>23 (43%)</td>
<td>16 (40%)</td>
<td>7 (35%)</td>
<td>8 (17%)</td>
</tr>
<tr>
<td>Proximal DVT rate</td>
<td>7 (41%)</td>
<td>9 (17%)</td>
<td>6 (15%)</td>
<td>4 (20%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>0</td>
<td>2 (4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Major bleeding</td>
<td>0</td>
<td>1 (2%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Minor bleeding</td>
<td>0</td>
<td>2 (4%)</td>
<td>0</td>
<td>1 (5%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Venous thrombosis was documented by bilateral venography or by the occurrence of pulmonary embolism. Of the 222 patients enrolled in the study, 177 patients had technically adequate bilateral venography or clinically documented pulmonary embolism and were considered in the analysis of efficacy. Major bleeding was defined as a fall in hemoglobin level of >2 g/dL or transfusion of >2 units of blood. All other clinically overt bleeding was classified as minor.

Abbreviations: DVT, deep vein thrombosis.

a Significantly lower overall DVT rate compared with the first four regimens combined: 8/46 (17%) vs. 56/131 (43%); p < 0.05

b Significantly lower proximal DVT rate compared with the first regimens combined: 1/46 (2%) vs. 26/131 (20%); p < 0.01.

Source: Ginsberg et al., 1994b.
major hemorrhage, retroperitoneal bleeding, and need for blood transfusion (Topol et al., 1993; Bittl et al., 1995, 2001; Campbell et al., 2000a; Antman and Braunwald, 2001).

CACHET (phases A, B, and C) evaluated the combination of bivalirudin plus the provisional use of a GP IIb/IIIa inhibitor (abciximab) in comparison to heparin and abciximab in patients undergoing balloon angioplasty and stenting. Bivalirudin was found to be safe and effective with stents and was associated with a lower combined incidence of death, MI, revascularization or major hemorrhage at 7 days (Nawarskas and Anderson, 2001; Lincoff et al., 2002b; Sciulli and Mauro, 2002).

### Table 5  Major Clinical Studies Using Bivalirudin in Cardiac Patients (PCI and Non-PCI Indications)

<table>
<thead>
<tr>
<th>Study acronym or description</th>
<th>Trial (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCI indications</strong></td>
<td></td>
</tr>
<tr>
<td>Dose-finding study</td>
<td>Multicenter, open-label study (Topol et al., 1993)</td>
</tr>
<tr>
<td>HAS</td>
<td>Hirulog (Bivalirudin) Angioplasty Study (Bittl, 1995; Bittl et al., 1995)</td>
</tr>
<tr>
<td>BAT</td>
<td>Bivalirudin Angioplasty Trial (Bittl et al., 2001)*</td>
</tr>
<tr>
<td>CACHET</td>
<td>Comparison of Abciximab Complications with Hirulog Ischemic Events Trial (Lincoff et al., 2002b)</td>
</tr>
<tr>
<td>REPLACE-1</td>
<td>Randomized Evaluation in PCI Linking Angiomax to Reduced Clinical Events (REPLACE)-1 Trial (Lincoff et al., 2002a)</td>
</tr>
<tr>
<td>REPLACE-2</td>
<td>Randomized Evaluation in PCI Linking Angiomax to Reduced Clinical Events (REPLACE)-2 Trial (Lincoff et al., 2003)</td>
</tr>
<tr>
<td>Angiomax in Practice Registry</td>
<td>Cho et al. (2003b)</td>
</tr>
<tr>
<td><strong>Non-PCI (unstable angina or acute MI)</strong></td>
<td></td>
</tr>
<tr>
<td>TIMI-7</td>
<td>Thrombin Inhibition in Myocardial Ischemia-7 (Fuchs and Cannon, 1995)</td>
</tr>
<tr>
<td>HERO-1</td>
<td>Hirulog Early Reperfusion/Occlusion-1 (White et al., 1997)</td>
</tr>
<tr>
<td>HERO-2</td>
<td>Hirulog Early Reperfusion/Occlusion-2 (White, 2001)</td>
</tr>
</tbody>
</table>

* Bittl et al. (1995) reported the first study (combining two randomized, controlled trials) comparing bivalirudin against heparin for PCI; this study, subsequently called the Bivalirudin Angioplasty Trial (BAT), was later reanalyzed (including data from an additional 214 patients) (Bittl et al., 2001).
In the REPLACE-1 trial, heparin was compared to bivalirudin in patients undergoing coronary stenting with any one of the GP IIb/IIIa inhibitors (at the discretion of the physician) in 1056 patients. The combined endpoint of death, MI, or revascularization showed a trend toward a reduction in bivalirudin-treated patients at 48 h (Lincoff et al., 2002a).

The REPLACE-2 trial was a randomized, double-blind, active-controlled trial of 6010 patients who received bivalirudin with provisional use of GPIIb/IIIa blockage or heparin with planned GP IIb/IIIa inhibition. Bivalirudin was found to be superior to heparin alone and as effective as heparin plus GP IIb/IIIa inhibition for ischemic protection (Lincoff et al., 2003). A significant reduction in the incidence of bleeding and thrombocytopenia were also noted.

Bivalirudin may be a suitable substitute for heparin in patients with chronic renal disease who require PCI because its clearance is primarily determined by proteolysis and not by renal excretion (Robson et al., 2002). ACT monitoring is recommended in patients with chronic renal disease. The dose of bivalirudin should be reduced in accordance with the degree of renal impairment (Table 1) (Robson, 2000; Robson et al., 2002).

D. Unstable Angina and Acute Myocardial Infarction

Some of the largest experience with bivalirudin is with patients who have had an acute myocardial infarction (MI) or unstable angina. Two open-label, uncontrolled trials were performed to evaluate the efficacy and tolerability of bivalirudin in patients with unstable angina. Sharma et al. (1993) utilized a 5-day infusion of bivalirudin in patients with unstable angina. Their primary endpoints included death, development of an MI, or the need for coronary intervention. Lidon and coworkers (1993) studied 55 patients in patients with unstable angina in a dose-ranging study. As a result of favorable findings in these two trials, the Thrombin Inhibition in Myocardial Infarction (TIMI) 7 trial comparing four different doses of bivalirudin in combination with aspirin was performed in over 400 patients (Fuchs and Cannon, 1995). These trials suggested that there is a role for bivalirudin in the management of unstable angina.

A number of trials have evaluated the concomitant use of bivalirudin in patients who received streptokinase and aspirin for an acute MI. Lidon et al. (1994) compared bivalirudin to heparin in 45 patients who suffered an acute MI, while Theroux and colleagues (1995) utilized this same strategy in 68 patients. Higher early patency rates and a lower incidence of serious hemorrhage were noted (Nawarskas and Anderson, 2001).

The Hirulog Early Reperfusion/Occlusion (HERO) trial randomized 412 patients with acute MI to receive low-dose bivalirudin, high-dose bivali-
rudin, or heparin (White et al., 1997). Bivalirudin was found to be more effective than heparin in producing early patency rates at a reduced risk for bleeding.

The HERO-2 trial randomized 17,073 patients who received streptokinase to heparin or bivalirudin for 48 h in patients who presented with an acute ST-elevation MI. Bivalirudin did not reduce mortality compared to heparin, but was associated with a 30% reduction in repeat MI, without significant increase in severe or life-threatening bleeding (White, 2001).

A meta-analysis by the Direct Thrombin Inhibitor Trialists’ Collaborative Group (2002) based on individual patients’ data reported on 11 studies (35,970 patients) receiving either heparin or DTI therapy (relative number of patients treated: hirudin > bivalirudin > argatroban > inogatran > efegatran). Overall, DTI therapy appeared to be superior over heparin for the prevention of MI in patients with acute coronary syndromes (although the larger number of patients treated with hirudin meant that this DTI contributed most to the overall result reported). Bivalirudin was associated with a 56% reduction in major bleeding risk.

E. Percutaneous Transluminal Angioplasty

There is limited experience using bivalirudin in the performance of PTA involving the renal or other peripheral arteries. Allie et al. (2003) performed 180 renal and 75 iliac artery PTAs for patients with severe arterial disease using bivalirudin as the only anticoagulant. Procedural success was achieved in 100% of patients, and no adverse thrombotic events were reported. The authors did note a decrease in sheath removal time, time to ambulation, and length of hospital stay. A decrease in vascular access complications was seen. Bivalirudin was felt to be a safe and reasonable alternative.

F. Off-Pump Coronary Artery Bypass Surgery

Merry et al. (2004) compared bivalirudin to unfractionated heparin for OPCAB surgery in a semi-open label (surgeon-blinded), prospective study of 100 patients (half receiving bivalirudin). The primary endpoint was 12-h blood loss, and secondary endpoints were ischemic complications and coronary artery patency at 12 weeks. No deaths were reported. The ACT took longer to return to normal after stopping bivalirudin, when compared to the heparin group (which received protamine reversal). Total blood loss was similar in both groups, however. An intriguing (and potentially important) finding was that graft patency was improved in the patients receiving bivalirudin.
G. Pregnancy and Nursing Mothers

No evidence for impaired fertility or harm to the fetus has been attributed to bivalirudin in teratogenicity studies performed on rats and rabbits using higher doses than recommended for human use (The Medicines Company, 2001). There are no well-controlled studies in pregnant women. Caution is advised when giving bivalirudin to nursing women, as it is not known whether bivalirudin crosses the placenta or whether it is excreted in breast milk (Carswell and Plosker, 2002).

H. Other Potential Uses

Bivalirudin has also been studied in animal models for its potential role in both surgical and interventional fields. Its antithrombotic effects were first studied in a baboon carotid endarterectomy model (Kelly et al., 1992). In later studies using endarterectomized rats, significant decreases in platelet deposition with bivalirudin were shown using $^{111}$Indium-labeled platelets (Hamelink et al., 1995) and scanning electron microscopy (Jackson et al., 1996).

Bivalirudin has also been studied for prevention of vascular restenosis in a rat carotid artery injury model. Xue and associates (2000, 2001) found that bivalirudin reduced platelet deposition on denuded intima. Platelet-derived growth factor levels were also decreased following bivalirudin infusion. The authors suggested that balloon catheter injury–induced neointima formation might be suppressed by bivalirudin.

Bivalirudin has been administered to rabbits following balloon injury and reduces vascular restenosis in the femoral artery of angioplasty-injured, diet-induced atherosclerotic rabbits (Sarembock et al., 1996). These studies support the possible role of thrombin in restenosis.

In contrast to the above study, Kranzhofer et al. (1999) administered bivalirudin to rabbits over 3 days immediately after balloon injury to the abdominal aorta and right iliac artery. Markers of inflammation, including intercellular adhesion molecule-1, macrophage colony-stimulating factor, tumor necrosis factor, and interleukin-1β, were examined by immunohistochemistry. These workers found that bivalirudin did not acutely reduce vascular smooth muscle cell proliferation or inflammation postangioplasty. They did not rule out other mechanisms by which thrombin inhibition could prevent restenosis.

Bivalirudin has also been shown to reduce thrombin-generated increase in levels of plasminogen activator inhibitor-1 (PAI-1) in cultured baboon aortic smooth muscle cells (Ren et al., 1997). Elevated levels of PAI-1 have been found in patients with coronary artery disease (Hamsten et al., 1985; Francis et al., 1988; Sakata et al., 1990), and numerous authors have sug-
gested their role in the development of atherosclerosis and thrombosis (Ren et al., 1997). Bivalirudin may potentially prevent intravascular thrombogenesis through inhibition of thrombin-induced PAI-1 production (Ren et al., 1997; Shen et al., 1998).

Bivalirudin has also been studied in a rat model of endotoxemia and found to increase survival rate in one (but not the other) study (Cicala et al., 1995; Itoh et al., 1996). Bivalirudin reduced endotoxin-induced thrombocytopenia, leukopenia, and fibrinogen consumption, suggesting a possible future therapeutic role in sepsis (Cicala et al., 1995).

IV. BIVALIRUDIN FOR THE TREATMENT OF HIT

A. Miscellaneous Studies

Data on the use of bivalirudin in the treatment of HIT is limited. Chamberlin et al. (1994) reported three patients who received bivalirudin for HIT. One patient was treated for 8 days due to bilateral lower extremity DVTs and recurrent PE with a positive heparin-induced platelet aggregation test, while the other two patients received bivalirudin for arterial ischemia due to HIT. One patient required an above-the-knee amputation and was given bivalirudin (for 12 days) to prevent loss of the other limb, while the other patient had worsening peripheral arterial disease and underwent angioplasty of his right superficial femoral artery using bivalirudin anticoagulation.

In another study, a total of 39 patients with HIT were treated with bivalirudin (Berkowitz, 1999a; Campbell et al., 2000a; Gladwell, 2002). Seventeen patients had acute HIT, while 22 had previous HIT. Patients were treated for a variety of indications (Table 6). There were 4 deaths (10%), all due to complications from HIT. Revascularization was successful in all but one patient (94%) who had PCI. The one failure was attributed to an unapproachable lesion. Two of the patients required intra-aortic balloon pumps, while another two underwent successful coronary artery bypass surgery. Bleeding complications were usually minor.

At the recent Congress of the International Society on Thrombosis and Haemostasis (ISTH), Francis and colleagues (2003; and unpublished data) presented their experience using bivalirudin to treat 40 patients with suspected HIT (32 tested positive for HIT antibodies; 16 had thrombosis preceding bivalirudin treatment). The mean bivalirudin infusion rate (using a target aPTT 1.5- to 2.5-fold greater than baseline) was 0.165 mg/kg/h (maximum 0.38 mg/kg/h). Bivalirudin was given for a mean of 8.7 days, with transition to warfarin (mean overlap 5 days) performed in 35 of 40 patients. The authors noted minimal increase in the INR on bivalirudin alone (mean increase 0.31). Minor bleeding was seen in only a few patients. Antithrom-
botic efficacy was believed by the authors to be acceptable, but detailed results are forthcoming.

There is one case report of long-term (2-month) use of bivalirudin to treat serologically confirmed HIT complicated by recurrent left leg ischemia and arterial thrombosis while on low molecular weight heparin (Bufton et al., 2002a). The patient received a continuous infusion of bivalirudin (22 mg/h) using a CADD pump.

Table 7 summarizes theoretical advantages of bivalirudin as a treatment for HIT.

### B. Bivalirudin for Percutaneous Coronary Intervention

The ATBAT trial has recently been completed (Campbell et al., 2000b; Mahaffey et al., 2003). This was a prospective, open-label study to evaluate the safety and efficacy of bivalirudin in patients with acute HIT or a past history of HIT undergoing PCI. The primary endpoint was major bleeding within 48 h after completion of the bivalirudin infusion (1.0 mg/kg/h iv bolus followed by 2.5 mg/kg/h by iv infusion for 4 h). This dose was later changed to a 0.75 mg/kg/iv bolus followed by a 1.75 mg/kg/h infusion for 4 h. Secondary endpoints included event rates for components of the primary endpoint and the ACT, aPTT, and platelet counts (at baseline, pre-PCI/post-PCI, and prior to discharge). Clinical success was defined as procedural success without death, emergency bypass surgery, or q-wave MI. Only one of 52 patients required a blood transfusion (1 U), and procedural and clinical success

<table>
<thead>
<tr>
<th>Indication</th>
<th>Number treated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percutaneous coronary intervention (PCI)</td>
<td>17 (44)</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>5 (13)</td>
</tr>
<tr>
<td>Coronary artery bypass grafting</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Pulmonary embolism and deep vein thrombosis</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Intra-aortic balloon pump</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Cardiac catheterization</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Deep vein thrombosis</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Pulmonary thromboendarterectomy</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Aortic reconstructive surgery</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Femoral bypass grafting</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
</tr>
</tbody>
</table>

*Source: Campbell et al., 2000a.*
were achieved in 98% and 96% of the patients, respectively. There were no abrupt closures, nor was thrombus formation reported during or after PCI. One patient died of cardiac arrest about 46 h after successful PCI.

C. Bivalirudin for Cardiac Surgery

Bivalirudin has been used off-label for cardiac surgery in a number of patients with acute or previous HIT. Both “on-pump” and “off-pump” experience has been reported. Except for a recent trial comparing bivalirudin with heparin (Merry et al., 2004), experience has been anecdotal.

Off-Pump Coronary Artery Bypass Surgery

Spiess et al. (2002) recently reported a case of OPCAB surgery using bivalirudin for intraoperative anticoagulation in a patient with HIT. Bivalirudin was chosen over hirudin because of the patient’s renal insufficiency. The patient received a 0.75 mg/kg bolus followed by a continuous infusion of 1.75 mg/kg/h. Monitoring was performed using both the ACT and the ECT. The infusion rate was increased to 2.0 mg/kg/h when the ACT fell below 300 s.

Table 7 Theoretical Advantages of Bivalirudin for Treatment of HIT

<table>
<thead>
<tr>
<th>Feature of bivalirudin</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short half-life (25–36 min)</td>
<td>Avoids need for initial iv bolus; rapid reversal of anticoagulation (useful if patient develops bleeding or if used for intraoperative anticoagulation)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Predominant enzymic metabolism</td>
<td>Minor renal excretion (20%) means that risk of overdosing in renal failure less than with lepirudin; less risk of postoperative bleeding (compared with lepirudin) if used for intraoperative anticoagulation (in case of postoperative renal insufficiency)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Minimal effect on PT/INR</td>
<td>Simplifies transition to oral anticoagulation (compared with argatroban)</td>
</tr>
<tr>
<td>Low immunogenicity</td>
<td>Reduced risk of allergy and anaphylaxis (compared with lepirudin)</td>
</tr>
</tbody>
</table>

Abbreviation: iv, intravenous.

<sup>a</sup> Possible disadvantages of a short half-life include need for frequent sc administration (e.g., three or four times daily) and rapid loss of anticoagulation (with risk of rebound thrombosis) if prematurely discontinued in patient with acute HIT.

<sup>b</sup> Possible disadvantage of enzymic metabolism includes loss of anticoagulant action in stagnant blood (implications for cardiac anesthesiology) (see Chap. 19).
The patient required 2 units of packed red blood cells intraoperatively and underwent surgical reexploration 8 h postoperatively because of increased chest tube drainage. No bleeding was found, and the patient’s recovery was otherwise uneventful.

**On-Pump (Cardiopulmonary Bypass) Cardiac Surgery**

Vasquez et al. (2002) utilized bivalirudin for anticoagulation during CPB in a patient with infective endocarditis in whom HIT was suspected. Bivalirudin was administered as a 1.25 mg/kg bolus followed by a continuous iv infusion of 2.75 mg/kg/h, with the dose adjusted upwards to maintain an ACT of between 500 and 600 s. The patient required 4 units of packed red blood cells and recovered uneventfully. No clot formation was observed in the CPB circuit, and postoperative bleeding was considered acceptable.

Davis and coworkers (2003) reported the use of bivalirudin for a patient with a history of HIT who required CPB for aortic valve replacement and coronary artery bypass grafting. The authors used a 50 mg bolus of bivalirudin followed by an infusion of 1.5–1.75 mg/kg/h and found that adequate anticoagulation was obtained. The patient required 3 units of packed red blood cells within the first 6 h postoperatively, plus 7 units of fresh frozen plasma, 2 sets of platelets, and an additional unit of packed red blood cells within the first 24 h after surgery. The recovery was otherwise uneventful, although the authors did note clot formation in the cell-saving device and recommended adding bivalirudin to the pump circuit when using this device. The ACT steadily declined after stopping the bivalirudin infusion.

Koster et al. (2003a) reported the use of bivalirudin in a patient with HIT, a history of severe anaphylactic reaction to heparin, and renal insufficiency in a patient requiring repeat open-heart surgery. They used the ECT for monitoring anticoagulation during CPB. The authors gave the patient a 1.5 mg/kg bolus of bivalirudin followed by 2.5 mg/kg/h continuous infusion. The infusion was increased to 5.0 mg/kg/h to maintain the ECT between 400 and 450 s. The patient required 2 units of red blood cells and 4 units of plasma and recovered uneventfully.

**Current Studies of Bivalirudin During Cardiac Surgery**

Based on these reports, and buoyed by the experience with bivalirudin in OPCAB surgery from New Zealand (Merry et al., 2004), four phase II and III multicenter trials utilizing bivalirudin in patients with HIT (CHOOSE) and comparing bivalirudin to heparin with protamine reversal (EVOLUTION) are underway. The rationale for using bivalirudin in these settings includes its direct thrombin inhibition without the requirement of a cofactor, its rapid, dose-dependent prolongation of the ACT, its short half-life, lack of structural
similarity to heparin (thus, no cross-reactivity with PF4/heparin-dependent antibodies), avoidance of protamine use (and its potentially-severe adverse reactions), no need for dose reduction in mild renal impairment, and an ability to “reverse” its anticoagulant effect through hemofiltration, all of which make it a drug potentially superior to heparin in cardiac surgery. Further, there is the potential to avoid HIT antibody formation and, consequently, postoperative HIT.

V. ANTIBIVALIRUDIN ANTIBODIES

Bivalirudin is a relatively small polypeptide and thus is expected to lack significant antigenicity (Fenton et al., 1998). In a study of plasma samples from 7 patients, no evidence for antibody formation (IgG, IgM, or IgE) was found (Fox et al., 1993) with plasma samples obtained at 7 and 14 days after iv administration. There was also no evidence for changes in the pharmacokinetics or pharmacodynamics of bivalirudin in their study. One patient exhibited antibody titers of greater than 1:2,000 in the assay prior to administration of bivalirudin, although no explanation was given.

In another review of 494 bivalirudin-treated patients from 9 different studies, 11 subjects initially tested positive for antibivalirudin antibodies (Berkowitz, 1999b). However, 9 of these were found to be false positives on repeat testing. The remaining two (who could not be retested) did not develop any allergic or anaphylactic reactions. In clinical trials of bivalirudin performed from 1993 to 1995, only 1 of 3639 patients (0.03%) experienced an allergic reaction considered by the investigator to be related to study drug.

Since bivalirudin shares an 11-amino-acid sequence with hirudin, it is at least theoretically possible that patients with antilepirudin antibodies resulting from treatment with lepirudin could cross-react with bivalirudin. Recently, Eichler and colleagues (2004) found that 22 of 43 (51%) sera containing antilepirudin antibodies showed reactivity in vitro against bivalirudin. This suggests that if bivalirudin is used in patients previously treated with lepirudin, extra caution should be used, e.g., careful anticoagulant monitoring, as antilepirudin antibodies sometimes influence pharmacokinetics.

VI. COST ANALYSIS WITH BIVALIRUDIN

Bivalirudin is the only anticoagulant associated with lower rates of both ischemic and bleeding complications compared to heparin in studies of PCI. These complications are associated with increased morbidity and mortality and also higher costs (Lauer, 2000; Compton, 2002). Bivalirudin may also be
associated with shorter hospital stay, use of fewer closure devices, lower incidence of hematoma formation, earlier sheath removal, and more selective use of the GP IIb/IIIa inhibitors. Potential savings are also possible in patients treated for HIT by reducing its devastating and costly thrombotic complications.

VII. CONCLUSION

Bivalirudin is a unique new anticoagulant with a number of potential applications. The FDA has approved it for use in PCI. It has extensive experience in patients with unstable angina and MI. Data are accumulating on using bivalirudin for HIT, and it may prove especially useful as an alternative anticoagulant in patients with acute HIT requiring cardiac surgery (Warkentin and Greinacher, 2003). Additionally, based on the OPCAB experience from New Zealand (Merry et al., 2004), bivalirudin has the potential to become the anticoagulant of choice for heart surgery (and thus avoid HIT in the post–cardiac surgery setting). Its short half-life, unique metabolism (enzymic) and low immunogenicity provide it with distinct advantages over other DTIs. In addition, its reversible thrombin inhibition may be associated with decreased bleeding risk. Finally, although there are no antidotes available, the potential for reversibility with hemofiltration (which can be used routinely in the postcardiac surgery setting) adds to its attractiveness.

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I. HEPARIN-INDUCED THROMBOCYTOPENIA IN HEMODIALYSIS PATIENTS

Because unfractionated heparin (UFH) is the major anticoagulant in hemodialysis (HD), it is important to define the potential role of heparin-induced thrombocytopenia (HIT) in contributing to morbidity and mortality in patients with dialysis-dependent renal failure. Of 154 patients newly treated with HD, 6 (3.9%) were clinically suspected of having developed HIT because of a fall in the platelet count accompanied by clotting of the dialyzer and extracorporeal circuit (Yamamoto et al., 1996). The clinical diagnosis was confirmed by the detection of HIT antibodies in all but one patient. Only one patient developed organ damage from thrombosis (myocardial infarction and stroke). All six patients were switched to an alternative anticoagulant and did not suffer from thromboembolic events in the follow-up period. Compared with the incidence of HIT of 2.7% found in 332 hip surgery patients treated with UFH (Warkentin et al., 1995), the incidence of HIT in acute hemodialysis patients thus appears to be similar, regardless of the underlying cause of renal dysfunction (Finazzi and Remuzzi, 1996).

Greinacher and colleagues (1996) performed a cross-sectional study of 165 patients undergoing hemodialysis using UFH and identified 7 (4.2%) patients as having HIT antibodies using a sensitive activation assay for HIT; however, there was no difference in the incidence of thromboembolic events in HIT antibody-positive patients, when compared with HIT antibody-
negative patients. They considered alternative anticoagulants to be justified only if clinical symptoms of HIT occurred.

Similar findings were reported that used an antigen assay for HIT antibodies (platelet factor 4 [PF4]–heparin enzyme immunoassay [EIA]) in four other cross-sectional studies of HD patients. In patients undergoing HD using UFH, the frequency of HIT IgG antibodies varied: 0% (0/45) (de Sancho et al., 1996), 2.3% (3/128) (Boon et al., 1996), 2.8% (2/170) (Sitter et al., 1998) and 6% (3/50) (Luzzatto et al., 1998). For patients undergoing HD using low molecular weight heparin (LMWH), the frequency in one study was 0.3% (1/133) (Boon et al., 1996). Thrombocytopenia was usually not observed in patients who formed HIT antibodies, and none of the patients developed bleeding or thrombosis.

Tentative conclusions suggested by these studies are that only a few patients who form HIT antibodies in association with HD develop clinical events and that these are more likely to be clotting of the dialyzer and extracorporeal circuit than symptomatic thrombosis affecting the patient. It is also possible that the risk of clinical HIT is higher in patients undergoing short-term HD (the population studied by Yamamoto et al., 1996) than in patients in the long-term phase of HD (as per the remaining studies). Anecdotal case reports of HIT complicating HD also seem frequently to include patients undergoing short-term HD (Matsuo et al., 1989; Hall et al., 1992; Nowak et al., 1997; Gupta et al., 1998).

II. CLINICAL PRESENTATION OF HIT IN HEMODIALYSIS PATIENTS

The diagnosis of HIT and respective management decisions should be primarily based on clinical criteria (Lewis et al., 1997). A further consideration in HD patients is that the procedure of HD itself is associated with a relative decrease in platelet count, even when so-called biocompatible dialyzer membranes are used (Beijering et al., 1997; Schmitt et al., 1987). Furthermore, the fall in platelet count in HD patients developing HIT may be only moderate (Matsuo et al., 1997).

The occurrence of fibrin formation, or even frank clotting of the extracorporeal circuit, despite apparent sufficient anticoagulation, should lead to a strong suggestion of possible HIT (Koide et al., 1995). One of the most serious complications, occlusion of vascular access (the “Achilles’ heel” of HD), may also indicate HIT, and it has been described both for native fistulae as well as prosthetic grafts (Hall et al., 1992; Laster et al., 1989). However, vascular
access thrombosis is not frequently associated with HIT. Of 88 HD patients prospectively evaluated for the presence of HIT antibodies, 18 (20%) had a prior history of access thrombosis, but only one patient (1.1%) without a history of graft thrombosis tested positive for HIT antibodies (O'Shea et al., 2002). Severe skin necrosis, even in the presence of normal platelet count, has been reported in association with the presence of HIT antibodies in patients after both short- and long-term HD (Bredlich et al., 1997; Leblanc et al., 1994).

Rarely, patients can develop HIT after years of regular long-term maintenance HD. Tholl et al. (1997) reported on a patient developing HIT following surgery after 9 years of long-term intermittent HD performed with UFH. In this patient, an anaphylactic reaction to heparin, accompanied by a platelet count fall, led to the diagnosis of HIT. It is possible that the surgery itself contributed to HIT antibody formation, as the highest reported rates of HIT are in postoperative patients receiving UFH (see Chap. 4).

Unfortunately, HD complications associated with HIT are not very specific. Thus, the clinician must consider other factors that could compromise patency of the extracorporeal circuit (e.g., low blood flow, high ultrafiltration rate, excess turbulence within the circuit, or foam formation with blood-air interfaces in the drip chambers). The quality of the vascular access plays a crucial role in this. Other patient-related factors include low arterial blood pressure, high hematocrit, and the need for intradialytic blood transfusion or lipid infusion (Hertel et al., 2001). In addition to insufficient anticoagulation, these factors should be ruled out first as the underlying causes of clotting within the extracorporeal circuit before HIT is considered in the differential diagnosis. Given the long-term implications of labeling HD patients as having HIT, laboratory testing for HIT antibodies should be performed when HIT is clinically suspected (O'Shea et al., 2003).

III. MANAGEMENT OF HEMODIALYSIS IN HIT PATIENTS

A. Discontinuation of Heparin Treatment

As HIT is frequently associated with potentially life-threatening thrombotic events (Warkentin et al., 1995; Warkentin and Kelton, 1996), discontinuation of heparin treatment and initiation of adequate alternative anticoagulation is generally considered mandatory (Warkentin et al., 1998). Thus, heparin must not be added to any flushing solution, and no heparin-coated systems can be used. Indeed, heparin flushes and heparin-coated devices can both initiate and sustain HIT (Moberg et al., 1990; Kadidal et al., 1999).
B. Unsuitable Approaches

Low Molecular Weight Heparin

LMWH is not recommended as an alternative anticoagulant. In vitro tests for HIT antibodies show a high degree of cross-reactivity between UFH and LMWH (Greinacher et al., 1992b; Vun et al., 1996). Furthermore, in vivo cross-reactivity manifesting as persistent or recurrent thrombocytopenia or thrombosis during LMWH treatment of HIT appears to be common (Greinacher et al., 1992a; Morello et al., 1984; Rouss et al., 1984). Because non-heparin anticoagulants are available, LMWH should not be used even if in vitro cross-reactivity is reported to be negative.

Regional Heparinization

Regional heparinization is defined as application of heparin at the inlet of the extracorporeal circuit and its neutralization by protamine at the outlet of the circuit. However, its use in HIT is problematic because of the potential for heparin “contamination” of the patient, as well as for heparin “rebound anticoagulation” (recurrence of heparin anticoagulation owing to shorter half-life of protamine compared with heparin) (Blafox et al., 1966). Moreover, direct injurious effects of protamine on the clotting cascade can occur. Consequently, this regimen is not recommended for HD of patients with HIT.

Aspirin

Acetylsalicylic acid has been used as an antiplatelet agent together with continued anticoagulation with UFH for HD of patients with HIT (Hall et al., 1992; Janson et al., 1983; Matsuo et al., 1989). This approach is not recommended for several reasons: (1) protection against heparin-induced platelet activation may be incomplete or absent, as aspirin's effects on blocking the thromboxane-dependent pathway of platelet activation does not reliably inhibit platelet activation by HIT antibodies (Kappa et al., 1987; Polgar et al., 1998); (2) the bleeding risk of uremic patients is increased; and (3) theoretically, it may lead to induction of persistently high levels of HIT antibodies.

Hemodialysis Without Anticoagulant

Hemodialysis without an anticoagulant (Romao et al., 1997) is not adequate for maintenance HD. Without anticoagulation, the artificial surfaces become coated, first by plasma proteins, followed by adhesion and activation of platelets, with accompanying activation of the coagulation cascade (Basmadjian et al., 1997). This will markedly reduce dialysis quality in removal of fluid and solutes long before clotting of the circuit is visible. Moreover, this ap-
proach may aggravate HIT. However, in patients at high risk of bleeding (e.g., owing to hepatic disorders or multiorgan failure, or those requiring surgery), temporary hemodialysis without anticoagulant may be appropriate.

C. Adequate Anticoagulants for Hemodialysis in HIT Patients

Patients with renal failure show plasma hypercoagulability as well as uremic platelet defects, both of which can be worsened by HD (Ambühl et al., 1997; Sreedhara et al., 1995; Vecino et al., 1998). Therefore, selection of an appropriate anticoagulant in HD patients who also suffer from HIT is difficult.

Reports on specific anticoagulant strategies in HIT are anecdotal. Large studies, especially those comparing different anticoagulant regimens, are lacking. Therefore, no treatment recommendations based on level A or B evidence can be provided. Furthermore, because UFH is the routine anticoagulant in use for HD, considerable additional time, effort, and costs are usually required to manage a new anticoagulant for HD, especially during initial use. Ideally, therefore, a center should try to gain experience with a single appropriate alternative anticoagulant for management of these difficult patients. Fear of inducing bleeding should not be used to justify undertreatment, with the potential risk for thrombotic complications.

Danaparoid Sodium

Danaparoid sodium (Orgaran, formerly known as Org 10172) is the alternative anticoagulant that has been most widely used for management of HD in patients with HIT (Chong and Magnani, 1992; Greinacher et al., 1992a, 1993; Henny et al., 1983; Magnani, 1993; Neuhaus et al., 2000; Ortel et al., 1992; Roe et al., 1998; Tholl et al., 1997; Wilde and Markham, 1997). However, danaparoid has been withdrawn from certain markets, such as the United States and the United Kingdom (see Chap. 14). Some of its characteristics require specific attention:

1. The anticoagulant activity of danaparoid can be monitored only by measurement of antifactor Xa levels based on a danaparoid calibration curve; however, many laboratories do not routinely perform these assays. Except for an emergency situation, such as when HIT is strongly suspected and danaparoid is the only available alternative, HD should not be performed without monitoring the antifactor Xa activity to evaluate the dose required for adequate anticoagulation. Once the optimal dose is identified, it can often be used without alteration for several subsequent HD sessions, provided no bleeding or inappropriate clotting occurs and no surgical intervention is scheduled. Periodic measurement of antifactor Xa activity to validate the appropriate
dosage of danaparoid is recommended. For maintenance HD without complications, single determination of pre-HD antifactor Xa activity probably suffices. If there are concerns about adequate or excess anticoagulation, then monitoring of levels at three time points is appropriate (e.g., 30–60 min pre-HD, 30 min after beginning HD, and just before completion).

2. Regarding the pharmacokinetics of danaparoid, renal excretion accounts for approximately 40–50% of total plasma clearance; accordingly, diminished clearance of antifactor Xa activity occurs in hemodialysis patients (Danhof et al., 1992). The elimination half-life of the antifactor Xa activity (about 24 h in healthy individuals) (Danhof et al., 1992) may reach as high as 4 days (unpublished observations of the author). Thus, significant antifactor Xa levels can be detected in patients undergoing HD with danaparoid even during the interdialytic interval. Whether this yields clinical benefit, such as decreased risk of thrombosis or greater maintenance of vascular access, is unknown. An increase in interdialytic bleeding episodes has not been reported.

3. Given its pharmacokinetics, danaparoid is given by initial bolus in intermittent HD, which normally is sufficient to prevent clotting within the extracorporeal circuit during the procedure. Danaparoid anticoagulation may also be required in critically ill patients on continuous renal replacement therapy. In 13 consecutive intensive care patients clinically suspected to have HIT, danaparoid was administered by initial bolus followed by continuous infusion (Lindhoff-Last et al., 2001). This regimen was sufficient to prevent clotting within the extracorporeal circuit both in continuous venovenous hemofiltration (8 patients) and in continuous venovenous hemodialysis (5 patients), respectively (Lindhoff-Last et al., 2001). Thromboembolic complications did not occur. Despite a mean danaparoid infusion rate of approximately 140 U/h, which is markedly reduced compared to the recommendation of the manufacturer, major bleeding was observed in 6 of 13 patients (which could be explained by disseminated intravascular coagulation in 5 patients). However, HIT was confirmed by antibody detection in only 2 patients. Thrombocytopenic patients not having the prothrombotic state of acute HIT likely are at increased bleeding risk. Therefore, dosing of danaparoid in intensive care patients should be based on the individual patient’s risk of bleeding versus thrombosis. With regard to invasive procedures, the long half-life of danaparoid should be considered.

4. No antidote to danaparoid exists. Recently, we evaluated hemofiltration as a potential means to rapidly reduce danaparoid plasma concentration. Whereas five different high-flux hemodialyzer membranes did not allow for danaparoid filtration, a plasmapheresis membrane was capable of removing danaparoid from the blood compartment (unpublished results). Hence, plasmapheresis may be a way to reduce danaparoid levels in situations of
overdosing or bleeding. Again, careful dosing of danaparoid is important to avoid bleeding.

5. HIT antibodies potentially cross-react with danaparoid. Although the respective clinical risk has been claimed to be less than 5% (Warkentin et al., 1998), individual patients, nevertheless, may be severely threatened if this condition occurs. This may be especially true for maintenance HD patients, who would be exposed to danaparoid repeatedly. As positive in vitro cross-reactivity is of uncertain clinical significance (Warkentin, 1996; Wilde and Markham, 1997; Newman et al., 1998), attention should focus on platelet count monitoring. A further fall in platelet count, or new fibrin deposits and clot formation within the extracorporeal circuit after application of danaparoid, may indicate clinically relevant cross-reactivity. To differentiate in vivo cross-reactivity from “under-anticoagulation” owing to insufficient dosage, determination of antifactor Xa levels and HIT antibody cross-reactivity studies are needed.

Table 1 lists dose recommendations for use of danaparoid for HD as provided by the manufacturer. The recommendations should be considered as guidelines and not followed uncritically in any individual patient. If applied with appropriate care, danaparoid provides adequate anticoagulation for HD of HIT patients with a favorable benefit/risk ratio, even during long-term use.

Recombinant Hirudin (r-Hirudin)

Native hirudin was the first anticoagulant used for HD over 75 years ago (Haas, 1925). In recent years, interest in its use for HD has redeveloped because of the availability of recombinant preparations, as well as the clinical need for managing patients with HIT. A preparation of r-hirudin, lepirudin (Refudan or HBW023), has been used successfully in humans for anticoagulation of both intermittent (Bucha et al., 1999a; Nowak et al., 1992, 1997; Steuer et al., 1999; Vanholder et al., 1994; Van Wyk et al., 1995) and continuous HD (Fischer et al., 1999; Schneider et al., 2000; Saner et al., 2001; Vargas Hein et al., 2001).

For use of r-hirudin anticoagulation in HD, some aspects should be specifically addressed. For further information on recombinant hirudin in renal insufficiency, the reader is referred to a recent review (Fischer, 2002):

1. As there is repetitive exposure to r-hirudin when used for regular, intermittent HD, immunogenicity of r-hirudin is of particular interest. Initially, r-hirudin appeared to be a weak immunogen (Bichler et al., 1991). However, recent studies revealed frequent development of antihirudin antibodies (AHAb) in patients receiving lepirudin for more than 5 days (Huhle et al., 1999, 2001; Song et al., 1999; Eichler et al., 2000). In addition, allergic reactions to r-hirudin were reported (Huhle et al., 1998; Eichler et al., 2000).
Table 1  Alternative Anticoagulation for Hemodialysis and Hemofiltration of Patients with HIT

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dialysis Procedure</th>
<th>Bolus</th>
<th>Continuous infusion</th>
<th>Monitoring parameter</th>
<th>Target range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danaparoid sodium (Orgaran)</td>
<td>Intermittent HD (every 2nd day)</td>
<td>Before first 2 HDs</td>
<td>3750 (2500)</td>
<td>Anti-Xa activity 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subsequent HD</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Predialytic anti-Xa activity (U/mL)</td>
<td>0.5–0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.3</td>
<td>3000 (2000)</td>
<td>Anti-Xa activity 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3–0.35</td>
<td>2500 (1500)</td>
<td>Anti-Xa activity 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35–0.4</td>
<td>2000 (1500)</td>
<td>Anti-Xa activity 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.4</td>
<td>0</td>
<td>Anti-Xa activity 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intermittent HD (daily)</td>
<td>1st HD</td>
<td>3750 (2500)</td>
<td>Anti-Xa activity 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2nd HD</td>
<td>2500 (2000)</td>
<td>Anti-Xa activity 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subsequent HD</td>
<td>See above</td>
<td>Anti-Xa activity 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Continuous HD/HF</td>
<td>Initial bolus</td>
<td>2500 (2000)</td>
<td>Anti-Xa activity 0.5–1.0</td>
<td></td>
</tr>
<tr>
<td>Lepirudin (Refudan)</td>
<td>Intermittent HD (every 2nd day)</td>
<td></td>
<td></td>
<td>aPTT ratio 2–2.5</td>
<td></td>
</tr>
<tr>
<td>Continuous HD/HF</td>
<td>Initial bolus</td>
<td>0.08–0.1</td>
<td>—</td>
<td>Hirudin conc. 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Next 4 h</td>
<td>—</td>
<td>600 (600)</td>
<td>Anti-Xa activity 0.5–1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subsequently</td>
<td>—</td>
<td>400 (400)</td>
<td>Anti-Xa activity 0.5–1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>200–600 (150–400)</td>
<td>Anti-Xa activity 0.5–1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argatroban (Novastan)</td>
<td>Intermittent HD (every 2nd day)</td>
<td></td>
<td></td>
<td>aPTT ratio 1.5–3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial bolus</td>
<td>0,1</td>
<td>—</td>
<td>Hirudin conc. 0.5–0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subsequent boluses</td>
<td>0.01</td>
<td>0.01</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alternatively</td>
<td>0.005–0.01</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Many of the approaches listed have not been formally studied, and none is approved. Treatment examples are given based on a limited number of cases successfully treated with the respective regimen. The different anticoagulants thus cannot be uncritically applied in the dosages given here. The choice of anticoagulant should depend on the experience of the center and the anticoagulant monitoring available. Doses for danaparoid as given by the manufacturer.
Abbreviations: HD, hemodialysis; HF, hemofiltration; conc., concentration; Xa, factor Xa; aPTT, activated partial thromboplastin time.

Monitoring the condition of the dialyzer after a HD session as well as the time required for termination of bleeding of the fistula should also be included.

Dosage for bolus given in anti-Xa units (U).

Dosage in parentheses for patients with body weight <55 kg.

Target range given in anti-factor Xa U/mL.

Peak activity determined after about 30 min of HD; this level is not required throughout the whole HD session.

If fibrin deposition in the dialyzer or clots in the extracorporeal circuit occur, give bolus of 1500 anti-Xa U.

Dosage given in anti-Xa U/h (infusion).

Maintenance dosage dependent on actual anti-Xa activity; determination every 12 h (provided that no bleeding or clotting occurs).

Dosage given in mg/kg body weight for hemodialysis performed with polysulfone high-flux hemodialyzers.

The dosage required to reach the target range may vary, e.g., owing to residual renal function or the type of dialyzer used (see text).

If larger doses are needed to achieve the target range or to avoid clotting of the extracorporeal circuit, changing to another type of dialyzer may be helpful.

The aPTT ratio is determined using the mean of the laboratory normal range; according to the literature, alternative tests such as ecarin-clotting time or chromogenic assays appear superior for monitoring.

It is unclear which test is best suited to monitor anticoagulation with r-hirudin, as no test has been prospectively evaluated in HD patients.

A peak aPTT of 300 s should not be exceeded.

Determination in plasma by chromogenic assays.

Target range given in µg/mL.

The agent has not been formally studied in continuous HD procedures.

This approach has been successfully performed in several patients in our center without adverse events.

Dosage given for anuric patients; in case of polyuria a higher dosage may be required; the daily dosage may vary significantly among patients.

As patients requiring continuous procedures often are at increased risk of bleeding, a lower aPTT is preferred (50–70s).

To be initially controlled every 4–6 h to avoid overdosage, especially in patients at risk of bleeding.

In our experience, a continuous infusion is more often associated with bleeding events.

Dosage given in mg/kg body weight/h.

In the United States, argatroban is marketed under the name Argatroban.
r-Hirudin is increasingly used for alternative anticoagulation in HD. Here, repetitive application of r-hirudin in patients on an intermittent maintenance HD regimen is likely to favor both induction and boosting of an immune response against the drug. As prospective studies evaluating sufficient numbers of HD patients on r-hirudin anticoagulation for the generation of AHAb are lacking, the incidence of AHAb and related adverse clinical events in this patient population remain to be elucidated.

Studies of HIT patients treated with lepirudin suggest that AHAb sometimes reduce renal lepirudin clearance (Huhle et al., 1999; Eichler et al., 2000). Indeed, marked reduction of renal lepirudin clearance due to monoclonal AHAb has recently been demonstrated in rats with normal renal function (Fischer et al., 2003). This was accompanied by a significant increase of both maximal plasma concentration and area under the curve of the alternative anticoagulant when compared to non–AHAb-treated animals. In chronic renal failure patients undergoing HD this may not be an issue. However, even small reductions in residual renal function have been shown to account for relevant prolongation of r-hirudin decay in plasma (Bucha et al., 1999a; Vanholder et al., 1997). Further reduction of renal r-hirudin clearance due to AHAb thus may influence r-hirudin dosing in these patients.

In acute renal failure requiring HD treatment for a prolonged period, reduction of renal r-hirudin clearance attributable to AHAb may be more relevant. Here, in patients suffering from multiorgan failure, the r-hirudin dosage required for sufficient anticoagulation was reduced significantly compared with the dosage needed in patients with normal renal function. In addition, r-hirudin dosage varied markedly depending on the residual renal function (Fischer et al., 1999). AHAb are likely to reduce further the amount of r-hirudin required, and thus may complicate anticoagulation in this challenging patient population.

The animal study also showed a significant decrease in the volume of distribution of lepirudin at steady state in the presence of AHAb (Fischer et al., 2003). Hence, even if further reduction of renal r-hirudin clearance owing to AHAb was negligible, major alterations in r-hirudin plasma concentration could still occur.

2. There remains debate as to which laboratory parameter is best suited for monitoring r-hirudin treatment. Initial studies addressing this in HD patients yielded conflicting results (Vanholder et al., 1994, 1997; van Wyk, 1995). However, it now appears that the ecarin clotting time (ECT) (Nowak and Bucha, 1996) and chromogenic substrate assays (Griessbach et al., 1985) measure the r-hirudin plasma concentration with adequate precision over a wide concentration range and correlate well with each other (Hafner et al., 2000, 2002). However, as these tests are often not available, monitoring of r-hirudin anticoagulation is usually performed with the acti-
vated partial thromboplastin time (aPTT). A meta-analysis of two lepirudin treatment trials for HIT revealed the optimal aPTT ratio for reducing clinical thromboembolic complications to be between 1.5 and 2.5, which was associated with only a moderately increased bleeding risk (Greinacher et al., 2000). Control of r-hirudin treatment by the aPTT is problematic: there is considerable assay variability among patients and different aPTT reagents (Nurmohamed et al., 1994; Hafner et al., 2000; Lubenow and Greinacher, 2000). In contrast to the foregoing tests, correlation between aPTT and plasma r-hirudin concentration is not linear over a broad concentration range. Instead, linear correlation is observed only with r-hirudin concentrations up to 0.5 μg/mL (Nowak and Bucha, 1996), a concentration often insufficient for HD. Above this concentration, the correlation between aPTT and r-hirudin concentration is poor (Nowak and Bucha, 1996; Hafner et al., 2000), especially for aPTT values of more than 70 s (Lubenow and Greinacher, 2000). Nevertheless, because of its wide availability, aPTT monitoring of r-hirudin treatment is likely to remain common. If available, ECT or chromogenic assays are preferred.

3. The elimination of r-hirudin is markedly prolonged in renal impairment. Nowak and colleagues (1992) reported elimination half-lives of up to 316 h in dialysis patients. Vanholder and coworkers (1997) found a prolongation of r-hirudin half-life by a factor of 31 in HD patients compared with healthy controls. Both studies showed a correlation between the residual creatinine clearance and the r-hirudin clearance, in that a minor improvement in creatinine clearance resulted in a shorter elimination half-life of r-hirudin. This was confirmed in a recent study of HD patients repetitively anticoagulated with r-hirudin (Bucha et al., 1999a). As with HD patients treated with danaparoid, r-hirudin–treated patients remain anticoagulated during the interdialytic interval (Nowak et al., 1997). Because various organs may metabolize hirudin (Grötsch and Hropot, 1991), other factors affecting metabolic clearance of hirudin may be present in patients with end-stage renal failure.

In patients suffering from acute renal failure, further deterioration or partial recovery of renal function frequently occurs (Fischer et al., 1999). Hence, r-hirudin anticoagulation should be closely monitored in these patients for timely dose adjustments. Preferably, r-hirudin should be given in repeated small boluses, rather than administered continuously, to minimize bleeding risk (Fischer et al., 1999; Kern et al., 1999). For the same reason, use of r-hirudin permeable high-flux hemodialyzers for patients with HD-dependent acute renal failure is recommended, especially as the patients often need vessel punctures, biopsies, or surgical interventions.

Given the prolonged half-life of r-hirudin in renal impairment, use of polyethylene glycol-hirudin (molecular mass 17 kDa), which has an even
greater elimination half-life compared with uncoupled r-hirudin (Pöschel et al., 2000), does not seem appropriate for HD, as bleeding risk likely would be increased.

4. Pharmacokinetics of r-hirudin are also influenced by the type of dialyzer used. The pharmacology of r-hirudin (molecular mass ~7 kDa; volume of distribution 0.2–0.25 L/kg b.w.; low protein binding) should favor its elimination by high flux hemodialyzers with a nominal cutoff point of approximately 60 kDa. Indeed, recent studies confirm high-flux hemodialyzers to be permeable to r-hirudin, whereas most of the low-flux hemodialyzers tested appear to be r-hirudin–impermeable (Bucha et al., 1999b; Frank et al., 1999, 2002; Benz et al., 2000; Koster et al., 2000). However, high-flux hemodialyzers vary considerably in their capacity to filter r-hirudin (Fischer, 2002; Willey et al., 2002). Further, a specific type of hemophan low-flux dialyzer has been reported to show high permeability for r-hirudin (Nowak et al., 1997), whereas a specific type of polysulfone high-flux dialyzer, with a cutoff point of approximately 50 kDa, did not filter r-hirudin from the circulation (Vanholder et al., 1997). Thus, knowledge of the actual filtration characteristics for r-hirudin of a given type of hemodialyzer improves safety of treatment with r-hirudin in HD.

5. r-Hirudin overdosing or unexpected drug accumulation can lead to severe bleeding (Fischer et al., 2000; Kern et al., 1999; Müller et al., 1999). In this situation, r-hirudin can be removed from the circulation using hemofiltration (Bauersachs, 1999; Fischer et al., 2000). However, several hours may be needed to lower r-hirudin plasma levels by 50%, even at high ultrafiltration rates. Thus, careful r-hirudin dosing is of utmost importance. In the presence of AHAb, hemofiltration may no longer suffice to eliminate r-hirudin (Fischer et al., 2003). Here, plasmapheresis may be the only means to clear r-hirudin from the circulation. Preliminary studies in animals suggest that a possible future treatment might be use of certain AHAb with r-hirudin–neutralizing capacity (Liebe et al., 2001).

Table 1 lists dosing recommendations for use of lepirudin for HD. The recommendations should be considered as guidelines and not followed uncritically in any individual patient. In summary, r-hirudin is a valid alternative anticoagulant for HD procedures in HIT patients, but it should be used with caution and careful monitoring.

Argatroban

Argatroban (Novastan; MD-805) is a potent arginine-derived, synthetic, catalytic site-directed thrombin inhibitor lacking antiplatelet and antifibrinolytic activities (Koide et al., 1995; Matsuo et al., 1992). This agent is approved in the United States and Canada as a treatment for HIT (see Chaps. 1, 13, and
16). It does not cross-react with HIT antibodies. Apart from an even better relative ability to inhibit fibrin-bound versus soluble thrombin (Berry et al., 1996; Lunven et al., 1996), the principal advantages of argatroban over heparin are similar to r-hirudin (Markwardt, 1991; Matsuo et al., 1992). However, argatroban is metabolized primarily by the liver, and its half-life is only moderately extended in patients with renal insufficiency.

After argatroban proved to be a valuable anticoagulant in HD (Matsuo et al., 1986), it was applied successfully to HIT patients undergoing this procedure (Koide et al., 1995; Matsuo et al., 1992). In the studies ARG-911, ARG-915, and ARG-915X, no differences in the primary efficacy endpoint or in bleeding were observed in 54 HD patients compared to non-HD patients being treated with comparable doses of argatroban. A recent prospective crossover study of 12 maintenance HD patients showed three different argatroban dosing regimens (bolus alone, infusion alone, or bolus plus infusion) to be safe and well tolerated (Murray et al., 2003) (see also Chap. 16). Argatroban also proved effective and safe in HD patients with antithrombin deficiency (Ota et al., 2003). Whether anticoagulation with argatroban alone is always sufficient to prevent clotting in the extracorporeal circuit is unclear: in one HD patient treated with argatroban, marked spontaneous platelet aggregation occurred, perhaps due to HIT together with additional platelet activation known to occur in HD (Koide et al., 1995). Because platelet aggregation could not be suppressed by argatroban alone in this patient, aspirin was added to achieve patency of the extracorporeal circuit.

As nonspecific inactivation of argatroban may occur in blood, periodic monitoring of its anticoagulant activity is recommended (Matsuo et al., 1992); for example, by measuring the aPTT (Koide et al., 1995; Matsuo et al., 1992) or the ECT (Berry et al., 1998).

Argatroban appears to be at least as well suited as r-hirudin for anticoagulation of HIT patients requiring HD. Its predominant hepatic elimination theoretically favors argatroban for alternative anticoagulation in renal failure.

Oral Anticoagulation

For HIT patients requiring long-term anticoagulation, orally active agents are usually given. Although coumarins interfere with the clotting cascade, fibrin formation within the extracorporeal circuit is not always sufficiently blocked. In these cases, additional low-dose intravenous anticoagulation with UFH is usually given for regular maintenance HD. However, in HIT patients requiring HD, alternative low-dose anticoagulation has not been formally studied. The need for additional intravenous anticoagulation depends on the increase of the INR, which should be checked regularly before HD. Priming
of the extracorporeal circuit by addition of a compatible anticoagulant to the filling solution with subsequent washout before start of the respective HD session may be of value in diminishing the risk of “overanticoagulation.”

D. Other Approaches

Dermatan Sulfate

Dermatan sulfate is a natural glycosaminoglycan that selectively inhibits both soluble and fibrin-bound thrombin through potentiation of endogenous heparin cofactor II. It does not interfere with platelet function. Dermatan sulfate has been used successfully to anticoagulate patients with HIT (Agnelli et al., 1994), and has also been applied successfully as an anticoagulant for HD (Boccardo et al., 1997).

Nafamostat Mesilate

Nafamostat mesilate (FUT-175), a synthetic nonspecific serine protease inhibitor with a short half-life, has been evaluated for regional hemodialysis in patients at risk of bleeding (Akizawa et al., 1993). It has also been applied occasionally to HIT patients on HD (Koide et al., 1995). However, owing to significant clot formation at the dialyzer outlet, despite a twofold prolongation of aPTT, reported both in HIT and non-HIT patients (Koide et al., 1995; Matsuo et al., 1993; Takahashi et al., 2003), this anticoagulant cannot currently be recommended for HD of HIT patients.

Prostacyclin

Prostacyclin (PGI2, epoprostenol), a potent antiplatelet agent with a short half-life, has been evaluated both as a substitute for, and as an adjunct to standard heparin for HD of patients with acute or chronic renal insufficiency (Turney et al., 1980; Smith et al., 1982; Samuelsson et al., 1995). Adverse effects, such as nausea, vomiting, and hypotension, can be avoided by dose reduction, use of bicarbonate- instead of acetate-containing dialysate, or infusion of the drug at the inlet of the extracorporeal circuit. Because of its mode of action, prostacyclin cannot inhibit activation of coagulation during HD (Rylance et al., 1985; Novacek et al., 1997). Moreover, in a HIT patient receiving continuous venovenous HD, prostacyclin was unable to suppress platelet consumption effectively after heparin had been re instituted, owing to a false-negative platelet aggregation assay (Samuelsson et al., 1995). Prostacyclin does not seem to be a suitable antithrombotic agent for HD in HIT. Whether it may be a useful adjunct in selected cases remains to be clarified.
Regional Citrate Anticoagulation

Anticoagulation by regional citrate is based on the concept of inhibition of clotting by chelation of ionized calcium, and it was first developed as an alternative anticoagulant regimen in HD patients at risk of bleeding (Pinnick et al., 1983). Metabolic alkalosis, hypernatremia, alterations in calcium homeostasis, and hyperalbuminemia are reported side effects that are generally manageable (Ward and Mehta, 1993; Flanigan et al., 1996; Janssen et al., 1996). Regional citrate anticoagulation is a valuable approach in experienced centers. Recently, efficient and safe long-term citrate anticoagulation in a HIT patient over a period of 9 months was reported (Unver et al., 2002). Regional citrate anticoagulation is a treatment option only in patients with a history of HIT as it does not suppress the prothrombotic state in acute HIT.

IV. SUMMARY

An alternative anticoagulant is required for HD in patients with HIT. Appropriate agents would appear to be danaparoid sodium; r-hirudin derivatives, such as lepirudin; or argatroban, as these appear to be able to suppress clot formation without substantially increasing the bleeding risk. As these results are based on experience with a limited number of patients, larger prospective trials are needed to define the best treatment options in this setting. Even today, though, HIT should no longer be a life-threatening problem for patients requiring dialysis.

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Hemodialysis in HIT

525


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Immediate cessation of, and avoidance of reexposure to, heparin are important principles underlying the management of patients with immune-mediated heparin-induced thrombocytopenia (HIT) (Chong and Berndt, 1989; Warkentin et al., 1998). Because further antithrombotic therapy is often necessary for these patients, several alternative anticoagulant strategies have been developed (see Chaps. 13–17). However, patients with HIT who require cardiac surgery present special problems. Considerable activation of the hemostatic system results when blood is exposed to the artificial surfaces of the cardiopulmonary bypass (CPB) pump used for most heart surgery, making high-dose anticoagulation mandatory (Edmunds, 1993; Slaughter et al., 1994). Heparin is the current anticoagulant of choice for CPB, and there is relatively little experience with other forms of anticoagulation in this patient setting. Moreover, any alternative anticoagulant considered for HIT patients should ideally meet certain requirements. First, the agent should be effective in minimizing activation of coagulation during CPB. Second, a rapid and simple method of monitoring its anticoagulating effects should be available to
avoid inappropriate under- or overanticoagulation. Finally, rapid and complete reversibility of the anticoagulating effects is important to minimize postoperative bleeding complications. Unfortunately, no existing agent meets all of these requirements.

II. ALTERNATIVE STRATEGIES FOR CPB ANTICOAGULATION

A variety of approaches to perform CPB anticoagulation in HIT patients has been reported, including the use of danaparoid sodium, the thrombin inhibitors lepirudin, bivalirudin, and argatroban, the defibrinogenating enzyme ancrod, and antiplatelet agents, such as aspirin, tirofiban, and iloprost. These antiplatelet agents were used to prevent platelet activation when heparin was used in a patient with acute HIT or a history of previous HIT. Experience with a planned reexposure to heparin to permit CPB in patients with a previous history of HIT, but who no longer have detectable HIT antibodies at the time of the subsequent heparin reexposure, will also be discussed.

A. Danaparoid Sodium

The efficacy of danaparoid sodium for CPB anticoagulation was first shown in a dog model (Henny et al., 1985). Subsequently, this agent was used for patients with HIT who needed heart surgery (Doherty et al., 1990; Magnani, 1993; Wilhelm et al., 1996). In a retrospective analysis Magnani and coworkers (1997) summarized the experience in 53 patients with HIT who underwent CPB using danaparoid for anticoagulation. The patients included in this study generally received an intravenous (iv) bolus of 8750 antifactor Xa (anti-Xa) units (U) of danaparoid after thoracotomy. The CPB circuit was primed with 7500 U. During CPB, booster iv injections (1500 U) were to be administered up to once hourly if there was visually apparent clot or fibrin formation. Plasma levels of anti-Xa activity generally were not monitored during CPB.

With this fixed-dosing schedule, “clots” in the operative field, as an indicator of inadequate anticoagulation, were observed in 18 (34%) patients. One patient, reported elsewhere, developed near-fatal thrombosis of the CPB circuit during weaning from bypass (Grocott et al., 1997). Severe postoperative bleeding, defined as more than 20 U of blood transfused, was noted in 11 (20%) patients. As a result of these data, the authors recommended a modified treatment regimen that included a priming dose of 3 U/mL, a weight-adjusted postthoracotomy iv bolus dose of 125 U/kg body weight (b.w.), and a constant iv infusion of 7 U/kg b.w. per hour started immediately
after institution of the CPB, and stopped 45 min before the expected end of CPB. Thus, for a 70-kg person undergoing an operation with a CPB time of 2 h and a priming volume of 1500 mL, a total dose of approximately 13,860 U danaparoid, or 198 U/kg, is recommended by the authors.

However, this revised protocol was developed empirically, with adjustments made based on some of the complications observed using the fixed-dose protocol (Grocott et al., 1997). Even though the revised protocol means that many patients would receive a lower dose of danaparoid than with the earlier fixed-dose regimen, this might not lead to reduced bleeding outcomes. Paradoxically, less effective anticoagulation during CPB could lead to more thrombin generation during the procedure potentially leading to even greater postoperative bleeding because of secondary hyperfibrinolysis, even if the postoperative danaparoid levels are not high. Indeed, Insler and colleagues (1997) reported a patient receiving danaparoid for CPB who first developed clots in the operative field and arterial filter of the CPB, followed by severe postoperative bleeding requiring surgical reexploration. Regardless of the explanation for excessive bleeding, even a weight-modified treatment regimen bears the risk of under- or overanticoagulation, if the anticoagulant effect is not monitored.

Therefore, we developed a danaparoid-dosing schedule for CPB with dose adjustments made according to the results of anti-Xa measurements (Table 1). Unfortunately, although both the activated clotting time (ACT) and the activated partial thromboplastin time (aPTT) are prolonged by the higher plasma levels of danaparoid used during CPB, there is no acceptable linear correlation (Gitlin et al., 1998). Because only the plasma anti-Xa levels correlate linearly with the plasma levels of danaparoid, we based our schedule on anti-Xa levels. Similar to the protocol recommended by Magnani and coworkers (1997), the iv bolus and priming dose are adjusted to body weight and priming volume, respectively. After beginning CPB, plasma anti-Xa levels should be maintained at 1.5 ± 0.3 U/mL. The continuous infusion is stopped 30 min before the expected end of bypass. However, in our experience, even if such an anti-Xa–adjusted danaparoid treatment regimen is used, increased postoperative bleeding is a problem. Possible explanations for the increased postoperative bleeding include an ongoing anticoagulant effect of danaparoid (half-life, approximately 17 h) for which there is no pharmacological antagonist (Meuleman, 1992), as well as the incomplete inhibition of thrombin generation during CPB, potentially leading to increased postoperative hyperfibrinolysis.

In all, CPB anticoagulation with danaparoid can lead to successful outcomes. About three quarters (36 of 47; 77%) of the patients reported by Magnani and coworkers (1997) were alive 6 weeks after cardiac surgery with danaparoid. Nevertheless, the disadvantages of danaparoid, including
its long-lasting anticoagulant activity that cannot be neutralized, and the significant difficulties in monitoring its anticoagulant effects in an operating room setting, render danaparoid a “second-choice” anticoagulant in HIT patients requiring cardiac surgery.

B. Recombinant Hirudin

Recombinant hirudin (r-hirudin), an anticoagulant naturally produced by the salivary gland of the leech (*Hirudo medicinalis*), is now approved in most countries for clinical use by the iv route. Hirudin is a single-chain polypeptide of 65 amino acids (7000 Da) that forms a tight 1:1 stoichiometric complex with thrombin, thereby occupying the putative fibrinogen-binding site and blocking the catalytic site of thrombin. As a result, all of the thrombin-catalyzed
procoagulant reactions, such as conversion of fibrinogen to fibrin, activation of coagulation factors V, VIII, and XIII, and thrombin-induced platelet activation, are inhibited. Although two hirudins are approved (lepirudin, desirudin), data on the use in cardiac surgery are only available for lepirudin.

Because of its potent anticoagulant effect, r-hirudin has been studied as an anticoagulant for use in open heart surgery in both dogs (Walenga et al., 1991) and pigs (Riess et al., 1997). In both animal models, effective CPB anticoagulation could be achieved by administration of r-hirudin as a bolus injection (1 mg/kg b.w.) followed by a continuous infusion of 1 mg/kg b.w., started after initiation of CPB, and continuing until end of CPB. In humans, however, recovery of hirudin in the plasma following body weight–adjusted dosing shows a high interindividual variability (Koza et al., 1993). Therefore, a fixed-dose protocol for r-hirudin in the CPB setting bears the risk of both inadequate anticoagulation and overdosing. Although the latter is complicated by excessive and potentially fatal postoperative bleeds, the former may result in the occurrence of thromboembolic complications while on pump, including catastrophic total pump occlusion.

To establish a treatment schedule that is adjusted to the individual’s response to hirudin, we investigated different monitoring systems for hirudin plasma levels. Several in vitro and in vivo experiments demonstrated that the ACT and aPTT were not sufficiently sensitive to monitor hirudin plasma levels (Pötzsche et al., 1997). However, reliable results were obtained by using the whole blood ecarin clotting time (ECT) (Pötzsche et al., 1997).

Ecarin is a prothrombin-activating enzyme, derived from the venom of the snake *Echis carinatus*, that activates prothrombin to an intermediate product, meizothrombin (Nishida et al., 1995). Meizothrombin expresses only moderate clotting activity, but is fully reactive toward, and thus inhibited by, hirudin. As a result, in r-hirudin–containing plasma, meizothrombin forms stable 1:1 complexes with r-hirudin. Only when hirudin is neutralized does clotting become initiated, either by meizothrombin or subsequently generated thrombin. Ecarin is available from commercial sources.

Table 2 outlines the whole blood ECT method, which we perform using the KC10a coagulometer (Pötzsche et al., 1997). The method is easily adapt-

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Whole Blood Ecarin Clotting Time</th>
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<tbody>
<tr>
<td>50 µL citrate-anticoagulated whole blood to be analyzed</td>
<td>+ 50 µL standard normal human plasma</td>
</tr>
<tr>
<td><em>Incubate for 1 min at 37°C</em></td>
<td>+ 50 µL ecarin solution (20 U/mL) containing 0.025 M calcium chloride</td>
</tr>
<tr>
<td><em>Determination of the clotting time</em></td>
<td></td>
</tr>
</tbody>
</table>
able to any other coagulometer. A calibration curve is constructed by using citrate-anticoagulated whole blood spiked with r-hirudin to achieve final concentrations of 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 μg/mL. A reliable ECT required adequate prothrombin levels, which can be reduced in severely ill patients and/or by hemodilution after beginning CPB. This problem can be overcome by mixing patient blood with normal human plasma (1:1).

In the United States and Canada, there is the additional option to use a commercial ECT method available from PharmaNetics (Morrisville, NC) by way of a “humanitarian device exemption” (H990012) for the specific situation of CPB when heparin is contraindicated because of HIT (Koster et al., 2000a). The assay is performed using a point-of-care methodology (Thrombolytic Assessment System [TAS]; manufactured by PharmaNetics and marketed as Rapidpoint Coag by Bayer Diagnostics, Toronto, ON, and Tarrytown, NY). Practical issues include the time required for obtaining the indemnification agreement and institutional review board approval (U.S.) or patient-specific regulatory approval (Canada) (Warkentin and Greinacher, 2003).

Critical levels of r-hirudin during the CPB operation were established in an in vitro CPB setting and in a first series of HIT patients undergoing cardiac surgery (Pötzsch et al., 1993; Riess et al., 1995, 1996). Clot formation in the CPB apparatus was seen at levels of r-hirudin below 1.8 μg/mL, and increasing levels of fibrinopeptide A (an indicator of thrombin-mediated fibrinogen cleavage) occurred at r-hirudin plasma levels less than 2.0 μg/mL. Based on these results, the therapeutic level of r-hirudin during CPB was set between 3.5 and 4.5 μg/mL. Higher intraoperative levels of r-hirudin could be complicated by a higher postoperative bleeding risk, especially because no antidote is available.

A treatment protocol based upon the ECT-monitoring of hirudin levels is given in Table 3. The data obtained from ten patients with HIT, treated with r-hirudin for heart surgery, demonstrated that stable r-hirudin plasma levels in the range from 3.5 to 5.0 μg/mL could be obtained using the ECT-adjusted treatment schedule (Fig. 1a). Because of the relatively short half-life of r-hirudin of approximately 1 h, plasma levels of r-hirudin declined rapidly after stopping its infusion (Fig. 1b). However, in renally impaired patients, r-hirudin can accumulate, leading to postoperative bleeding (Koster et al., 2000b).

To date, the clinical data demonstrate that r-hirudin is a suitable alternative for anticoagulation of CPB in HIT patients. The ECT provides adequate monitoring and allows an adjusted treatment schedule with apparently minimal risk for thrombotic problems on pump. Because of the relatively short half-life, plasma levels of r-hirudin decline rapidly after stopping its infusion. As hirudin is almost completely eliminated by the kidney in humans, patients with impaired renal function may require hemofiltration to reduce plasma levels of r-hirudin.
Bivalirudin

Bivalirudin (Angiomax; see Chap. 17) is a 20-amino-acid synthetic peptide modeled after hirudin. It consists of two peptide fragments connected by a tetraglycine spacer that recognize thrombin’s fibrinogen binding site (exosite I) and its catalytic site. Unlike lepirudin, this bivalent interaction with thrombin is reversible once plasma enzymes (including thrombin itself) cleave the arg3-pro4 bond on bivalirudin. Its short half-life (25 min) and predominant enzymic elimination might be advantageous for use in CPB. As with lepirudin, the ECT is recommended for intraoperative monitoring during CPB (Koster et al., 2003), although anecdotal success (and some failure) using ACT monitoring exists. For off-pump cardiac surgery (which

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Table 3  Treatment Protocol for r-Hirudin (Lepirudin) Anticoagulation During CPB

<table>
<thead>
<tr>
<th>Initial lepirudin dosing (pre-CPB)</th>
<th>0.25 mg/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial iv lepirudin bolus:</td>
<td>0.25 mg/kg body weight</td>
</tr>
<tr>
<td>Initiate continuous iv infusion*:</td>
<td>30 mL/h (0.5 mg/min.)</td>
</tr>
<tr>
<td>Lepirudin added to priming solution:</td>
<td>&gt;2.5 μg/mL before start of CPB</td>
</tr>
<tr>
<td>Target lepirudin plasma levels:*b</td>
<td>If &lt;2.5 μg/mL, give additional bolus (10 mg)</td>
</tr>
</tbody>
</table>

Lepirudin dosing and monitoring while on CPB

| Frequency of lepirudin level monitoring: | every 15 min using ECT |
| Intraoperative dose adjustments, based on ECT: | |
| Lepirudin plasma level | Dosing modification |
| >4.5 μg/mL | Reduce infusion rate by 10 mL/h |
| 3.5-4.5 μg/mL | No change in infusion rate |
| <3.5 μg/mL | Increase infusion rate by 10 mL/h |

Special steps toward end of CPB

Stop lepirudin infusion 15 min before anticipated end of CPB.

After disconnection of CPB, administer 5 mg hirudin to the heart–lung machine to avoid clot formation.

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CPB, cardiopulmonary; iv, intravenous.

* 50 mg of lepirudin are dissolved in 50 mL 0.9% sodium chloride

b The target lepirudin level pre-CPB (>2.5 μg/mL) is lower than the ones sought during CPB (3.5–4.5 μg/mL) because of the addition of lepirudin to the pump circuit volume (0.2 mg/kg body weight).

C. Bivalirudin and Argatroban

Bivalirudin
Figure 1  Course of free r-hirudin concentrations in HIT patients \((n = 10)\) treated with hirudin before, (a) during, and (b) after CPB. Free r-hirudin was measured using a chromogenic thrombin-based assay as described. (From Pötzsch et al., 1997.)
requires only one-third to one-half the usual concentrations of anticoagulant
compared with CPB), the ACT can be used (Merry et al., 2004).

Bivalirudin has been used successfully off-label for anticoagulation
during off-pump and on-pump cardiac surgery in patients with acute or
previous HIT (Spiess et al., 2002; Vasquez et al., 2002; Davis et al., 2003).
In addition, bivalirudin compared favorably in a randomized trial against
heparin in non-HIT patients undergoing off-pump surgery (Merry et al.,
2003). Bivalirudin was therefore evaluated in a 20-patient pilot study (on-
pump, non-HIT), and is currently under investigation in a phase III multi-
center pivotal trial (in comparison with UFH) for on- and off-pump cardiac
surgery in patients with and without HIT.

An investigational, ECT-adjusted treatment protocol is outlined in
Table 4 (Warkentin and Greinacher, 2003). Bivalirudin’s enzymic metabolism
presents certain practical issues and limitations. Surgical techniques that
allow blood to lie stagnant should be avoided, since local bivalirudin levels
will decrease due to its metabolism by proteases, produced in blood exposed
to wound or foreign surfaces, leading to local clot formation. The presence
of visible thrombus in an area of stagnation, such as in the pericardial cavity,
should not be interpreted by the surgeon as indicative of the need for
additional anticoagulation, as this may only reflect local bivalirudin metab-
olism and not correlate with plasma levels. If blood cardioplegia is used, the
blood should be directly sourced from the circuit, and (after mixing with the
cardioplegia solution) immediately reinfused into the coronary system. For
the same reason, assessment of graft blood flow and testing for leakage should
preferably be performed with albumin and saline solutions or, alternatively,
using blood taken directly from the patient and used immediately for this
assessment. Because hypothermia somewhat reduces the proteolysis of
bivalirudin, the patient’s core temperature should be maintained close to
37°C after coming off CPB (or after completing the final anastomoses in off-
pump procedures) and care taken to maintain body temperature during the
early postoperative period.

Following separation from CPB, the risk that the circuit may clot
rapidly may be even higher than with lepirudin, due to bivalirudin’s shorter
half-life and ongoing metabolism. Thus, provision to continue to recirculate
pump blood following separation from bypass is made by adding a cross-limb
in the bypass circuit at the time of setup, which remains clamped until coming
off bypass. Following clamping of the venous line, this limb is opened and the
contents recirculated. Within 10 min of separation from bypass, in case the
patient might need to return to bypass support, a 50 mg bolus of bivalirudin
should be added to the circuit to prevent clotting, and a 50 mg/h bivalirudin
infusion into the bypass circuit should also be started and continued until
such time as it is clear the patient will not require urgent return to CPB. Once
Table 4  Treatment Protocol for Bivalirudin Anticoagulation During CPB (Under Investigation)

<table>
<thead>
<tr>
<th>Initial bivalirudin dosing (pre-CPB)</th>
<th>Initial iv bivalirudin bolus: 1.5 mg/kg body weight</th>
<th>and initiate continuous iv infusion: 2.5 mg/kg/h (42 μg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bivalirudin added to pump circuit volume:</td>
<td>50 mg</td>
<td>&gt;10 μg/mL before start of CPB if &lt;10 μg/mL, give additional bolus (0.25 mg/kg) and increase infusion rate by 0.25 mg/kg/h</td>
</tr>
<tr>
<td>Target bivalirudin plasma level:</td>
<td>&gt;10 μg/mL</td>
<td>10–15 μg/mL (400–500 s)</td>
</tr>
<tr>
<td>&lt;10 μg/mL (&lt;400 s)</td>
<td>Give additional bolus (0.25 mg/kg) and increase infusion rate by 0.25 mg/kg/h</td>
<td></td>
</tr>
</tbody>
</table>

Bivalirudin dosing and monitoring while on CPB

<table>
<thead>
<tr>
<th>Initial iv bivalirudin bolus: 1.5 mg/kg body weight</th>
<th>and initiate continuous iv infusion: 2.5 mg/kg/h (42 μg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bivalirudin added to pump circuit volume:</td>
<td>50 mg</td>
</tr>
<tr>
<td>Target bivalirudin plasma level:</td>
<td>&gt;10 μg/mL</td>
</tr>
<tr>
<td>&lt;10 μg/mL (&lt;400 s)</td>
<td>Give additional bolus (0.25 mg/kg) and increase infusion rate by 0.25 mg/kg/h</td>
</tr>
</tbody>
</table>

Special steps at end of CPB

(A) Within 10 min of stopping bivalirudin infusion: first reinfuse appropriate portion of pump volume to patient, and then give 50 mg bivalirudin bolus to the circuit to prevent clotting; start an infusion of 50 mg/h into the circuit only, and continue to recirculate; any subsequent reinfusion of remaining pump volume to patient should be processed through a cell saver (which removes >90% of bivalirudin); or

(B) Promptly empty remaining pump volume into cell-saving device (replacing the pump contents with crystalloid), thus avoiding need for postseparation bivalirudin boluses to circuit; process blood for reinfusion with cell saver to remove bivalirudin.

CPB, cardiopulmonary bypass; ECT, ecarin clotting time; iv, intravenous.

a Up-to-date information on protocol amendments are available from the manufacturer of bivalirudin (“1-800-ANGIOMAX”; The Medicines Company, Parsippany, NJ).

b The target bivalirudin concentration (10–15 μg/mL) corresponds to an ecarin clotting time (ECT) of 400–500 s using the RapidPoint Coag (Bayer); with other ECT methods, the bivalirudin concentration should be determined using a calibration curve.

postseparation bivalirudin dosing to the circuit has commenced, any remain-
ing pump volume contents intended for reinfusion to the patient should first be processed using a cell-saving device (“cell saver”), thus washing away most of the bivalirudin. Another approach is simply to drain rapidly the contents of the pump into a cell saver following separation from bypass and replace the pump contents with crystalloid, and wash the blood, thus avoiding the possibility of pump clotting without the need to administer additional bivalirudin into the pump.

Argatroban

Argatroban is a specific thrombin inhibitor derived from L-arginine. It is a small molecule (532 Da) that binds reversibly to thrombin. It has a half-life of about 40–50 min in normal humans. The potential of argatroban to be an effective anticoagulant in patients with HIT has been documented by the studies of Lewis and coworkers (1997a,b, 2001, 2003) (see Chap. 16). Although argatroban has been a successful anticoagulant in a CPB model, only limited information is available on its use in humans for this purpose. Furukawa and coworkers report a patient who successfully underwent CPB anticoagulation with argatroban (Furukawa et al., 2001). Argatroban was administered as a bolus injection of 0.1 mg/kg followed by a continuous infusion at 5–10 μg/kg/min. The ACT was used for monitoring.

D. Platelet Inhibition as a Strategy to Permit Heparinization for CPB

Another approach described to manage CPB in a patient with HIT is to combine full heparinization with one or more antiplatelet agents. Several groups of investigators have used iloprost for this situation (Kappa et al., 1985; Long, 1985; Palmer Smith et al., 1985; Addonizio et al., 1987; Kraenzler and Starr, 1988), following the original observation by Olinger and colleagues (1984) that iloprost inhibited heparin-dependent platelet activation in the presence of HIT serum. Iloprost is a stable analogue of prostacyclin; thus, it stimulates adenylate cyclase, resulting in increased platelet cAMP levels, which prevents platelet activation by various platelet agonists, including HIT antibodies.

Recently this approach has experienced a resurgence with epoprostenol sodium (Flolan), a freeze-dried preparation of prostacyclin itself (Mertzlufft et al., 2000; Aouifi et al., 2001). Epoprostenol is approved for use in patients with primary pulmonary hypertension. Its very short half-life (6 min) means that continuous iv infusion is necessary. Complete inhibition of heparin-dependent platelet aggregation by HIT antibodies is generally achieved by doses ranging from 15 to 30 ng/kg/min. One protocol that does not perform
intraoperative monitoring of platelet aggregation gradually increases epoprostenol infusion (in 5 ng/kg/min increments made at 5-min intervals) until the target rate (30 ng/kg/min) is reached, whereupon standard-dose UFH anticoagulation is commenced (Aouifi et al., 2001). The epoprostenol infusion is continued until 15 min following reversal of UFH with protamine. The major adverse effect is vasodilatation, leading to severe hypotension that requires intraoperative vasopressors.

Pretreatment of patients with more conventional antiplatelet agents, such as aspirin and dipyridamole, followed by heparin use, has been used successfully in patients with a documented previous history of HIT (Makhoul et al., 1987). However, such an approach is controversial for a patient with acute HIT, because in vitro activation of platelets by HIT antibodies is not reliably inhibited by these relatively weak antiplatelet agents (Kappa et al., 1987).

Koster and coworkers (2000c, 2001a,b) combined the short-acting GP IIb/IIIa inhibitor tirofiban with heparin for anticoagulation during CPB for patients with HIT and renal insufficiency; pre- and postoperative anticoagulation with lepirudin was used. Tirofiban is given 10 min before standard-dose UFH as a 10 mg/kg bolus followed by 0.15 mg/kg/min continuous infusion. The tirofiban infusion is stopped 1 hour before end of surgery. UFH is neutralized with protamine as per usual. Using this treatment protocol, no thromboses occurred. However, in patients with severe renal impairment, tirofiban persists in the circulation and can cause major bleeding refractory to platelet transfusions: three such cases led the manufacturer to discourage use of this off-label protocol (Warkentin and Greinacher, 2003). In such patients, extracorporeal elimination of tirofiban (e.g., ultrafiltration at the end of CPB or modified zero-balanced ultrafiltration after CPB) might be required.

E. Other Anticoagulant Strategies

The thrombin-like snake venom ancrod (Arvin) is a defibrinogenating agent that cleaves fibrinopeptide A, but not fibrinopeptide B, from fibrinogen. This results in formation of fibrinogen–fibrin polymers into an unstable configuration that is susceptible to rapid degradation by plasmin. Ancrod has been used as a treatment for HIT (Cole and Bormanis, 1988; Demers et al., 1991), including as an alternative anticoagulant for cardiac surgery requiring CPB (Zulys et al., 1989; Teasdale et al., 1989). The recommended initial dose is usually 70 U in normal saline, administered slowly, over at least 6–12 h.

There are some important disadvantages of using ancrod for cardiac surgery. First, ancrod must be given slowly, because a very rapid infusion can lead to life-threatening intravascular fibrin deposition. Thus, it is not appropriate for emergency situations. Second, it is difficult to determine accurately
the fibrinogen level at the recommended target fibrinogen range (0.2–0.5 g/L). Furthermore, it is uncertain what fibrinogen level is required, if any, to prevent clinically important fibrin formation during CPB. Third, reversal of “anticoagulation” requires a blood product, fibrinogen concentrates (Europe) or cryoprecipitate (North America), to replace fibrinogen. Finally, ancrod does not inhibit thrombin generation and has even been associated with increased thrombin generation in some clinical settings, such as acute HIT (Warkentin, 1998). It is possible that this could lead to thrombotic or post-CPB hemorrhagic complications when used for the management of acute HIT. All of the considerations suggest that ancrod is not a suitable alternative to heparin in the setting of CPB surgery.

III. USE OF HEPARIN FOR CPB IN PATIENTS WITH A REMOTE HISTORY OF HIT

An intriguing option for patients with a history of HIT, but in whom persisting HIT antibodies can no longer be detected, is to consider reexposure to heparin for CPB, and to avoid heparin completely both before surgery (e.g., at heart catheterization) and in the postoperative period. This approach has been used successfully by some physicians (Makhoul et al., 1987; Pötzsch et al., 2000; Selleng et al., 2001; Warkentin and Kelton, 2001), and it is based on the following rationale. First, HIT antibodies are transient, and they usually are not detectable after 100 days following an episode of HIT (see Chap. 3). Thus, no immediate problems would be expected in a patient without residual HIT antibodies whose previous episode of HIT was “remote” (i.e., more than several months before the need for heart surgery). Second, it appears that a minimum of 5 days are required before clinically significant levels of HIT antibodies are generated following any episode of heparin treatment (Warkentin and Kelton, 2001). In the event that a recurrent immune response to platelet factor 4–heparin is induced by reexposure to heparin during CPB, it is unlikely that the newly generated HIT antibodies will contact exogenously administered heparin. As a consequence, platelet activation by HIT antibodies should not occur, and thus the thrombotic risk should not be increased. We reported 10 patients with a documented history of HIT, but no detectable HIT antibodies at the time of the proposed surgery, who thus underwent CPB anticoagulation with heparin (Pötzsch et al., 2000). In none of the 10 patients was a thromboembolic complication or prolonged thrombocytopenia observed. Further, no increase in HIT antibody concentrations occurred during a 10-day follow-up period. These data are in contrast to reports of a rapid “anamnestic” immune response in HIT. However, there is evidence that these episodes represent acute-onset HIT in a patient who has residual circulating
HIT antibodies resulting from a recent episode of HIT, rather than a rapid recurrence of HIT antibodies because of immune memory caused by a remote exposure to heparin (Warkentin and Kelton, 2001).

As outlined in Fig. 2, we recommend that HIT antibody–negative patients with a history of HIT who require CPB for heart surgery should be treated according to established heparin protocols. The use of heparin should be restricted to the operative period itself; if necessary, postoperative anticoagulation should be achieved with an alternative anticoagulant (see Chaps. 13–16).

Testing for HIT antibodies in patients with a history of HIT before anticipated heparin reexposure at heart surgery should be performed using one or more sensitive tests (see Chap. 11) if this approach is to be considered. Particularly in cardiac surgical centers where there is limited experience with nonheparin anticoagulation for CPB, risk-benefit considerations favor a brief use of heparin for these patients. For example, a patient who developed near-fatal CPB circuit thrombosis during danaparoid anticoagulation had had HIT 11 years earlier and had no detectable HIT antibodies at the time danaparoid was used (Grocott et al., 1997).

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**Figure 2** Algorithm for decision making for alternative anticoagulation in HIT patients.
IV. ACUTE HIT BEFORE AND AFTER HEART SURGERY

There are several reasons why a patient with HIT might require urgent heart surgery, including the result of life-threatening thrombotic complications of HIT affecting the heart (e.g., acute coronary insufficiency or myocardial infarction; removal of intracardiac thrombus), or because HIT has complicated the course of a critically ill patient receiving heparin before anticipated heart surgery (e.g., while awaiting a heart for cardiac transplantation, or during use of an intra-aortic balloon pump). The latter group of patients appear to have a relatively high risk of developing HIT (Walls et al., 1992). A suitable alternative for patients who require urgent coronary revascularization during acute HIT might be the use of an off-pump (“beating-heart”) strategy. This surgical technique requires lower levels of anticoagulation. Therefore, the dose of the nonheparin anticoagulant could be markedly reduced, which could reduce postoperative bleeding. Such an approach using danaparoid as heparin substitute was recently reported by Warkentin and colleagues (2001). The authors found a minimum plasma anti-Xa level of 0.6 U/mL sufficient to perform surgery off-pump.

Studies of the frequency of HIT antibody formation (Visentin et al., 1996; Bauer et al., 1997; Warkentin et al., 2000) following heart surgery suggest that as many as 15–50% of patients form HIT antibodies, using an enzyme immunoassay that detects IgG antibodies that recognize platelet factor 4–heparin complexes (see Chap. 4). With the washed platelet serotonin-release assay, HIT antibodies are detected in 13–20% of patients (Bauer et al., 1997; Warkentin et al., 2000). However, despite this high rate of seroconversion, only about 1–3% of patients who receive further postoperative anticoagulation with unfractionated heparin develop HIT. Currently, there is no convincing evidence that patients who form HIT antibodies in the absence of thrombocytopenia are at increased risk for thrombosis (Bauer et al., 1997; Trossaërt et al., 1998; Warkentin et al., 2000). However, postoperative cardiac surgical patients who develop clinical HIT appear to be at increased risk for both venous and arterial thrombotic events (Walls et al., 1990; van Dyck et al., 1996; Pouplard et al., 1999).

V. DECISION MAKING FOR ANTICOAGULATION IN HIT PATIENTS

Given these data and clinical experience, an algorithm has been developed to assist in determining the need for alternative anticoagulation for CPB in HIT patients (Fig. 2). After the decision to avoid use of heparin in the CPB setting, the important remaining question is, which strategy should be chosen? Because each of the different approaches described here provides specific
advantages and limitations, it is not possible to recommend one treatment regimen that is applicable for each patient. The algorithm shown in Figure 3 considers typical clinical settings in HIT patients and is constructed to support the decision in finding which of the different approaches is best suited for the individual patient.

REFERENCES


Figure 3  Treatment strategies for HIT patients requiring alternative anticoagulation for cardiopulmonary bypass. The estimated risk of bleeding is highest for azotemic patients receiving hirudin, intermediate for patients receiving danaparoid, and lowest for patients with normal renal function receiving hirudin or bivalirudin. Abbreviations: CPB, cardiopulmonary bypass; ECT, ecarin clotting time.


with heparin-induced thrombocytopenia type II. J Cardiothorac Vasc Anesth 2000a; 14:249–252.


Long RW. Management of patients with heparin-induced thrombocytopenia re-


Heparin-Induced Thrombocytopenia in Children

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I. INTRODUCTION

Heparin-induced thrombocytopenia (HIT) can occur in children, with the potential for severe venous and arterial thrombotic complications (Table 1). Unlike adults, few data exist regarding pediatric HIT. Only 68 children have been reported with HIT between 1990 and 2003, including 12 new cases added in this chapter involving our medical center for diagnosis and/or treatment (Table 2).

II. PATHOPHYSIOLOGY

Studies of the pathophysiology of HIT have been performed using adult blood. In our laboratory, pediatric and adult HIT sera react similarly in various in vitro assays. Therefore, it seems reasonable to infer that the pathophysiology of HIT in children resembles that in adults (see Chaps. 5–10).

III. FREQUENCY

Only four studies have addressed the frequency of HIT in children. Spadone and coworkers (1992) collected cases of suspected HIT in a neonatal intensive care unit (ICU) between 1988 and 1990. Of 1329 newborns enrolled, about 70% received unfractionated heparin (UFH): either 0.5–1.0 IU/mL added to
central venous or peripheral/umbilical artery catheters or via flushing of peripheral venous catheters (10 IU/mL UFH-saline every 4 h). In 34 (3.7%) newborns, HIT was suspected because the platelet count fell to less than 70 × 10^9/L or because of new thromboembolic events. In 14 of these 34 infants, HIT antibodies were detected by platelet aggregation assay (incidence 14/930 = 1.5%). However, this study has several limitations. It is an observational study without a defined protocol. Differentiating HIT from other causes of thrombocytopenia or thrombosis is difficult. Further, the specificity of platelet aggregation testing for HIT antibodies may be low in ICU patients (see Chap. 11).

Table 1  Clinical Complicationsa of HIT in Children (n = 68)

<table>
<thead>
<tr>
<th>Complication</th>
<th>Percentage</th>
<th>Case numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iliac vein</td>
<td>16.2</td>
<td>21, 24, 27, 32, 38, 39, 42, 43, 48, 53, 60</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>13.2</td>
<td>21, 27, 34, 35, 36, 43, 48, 54, 58</td>
</tr>
<tr>
<td>Inferior vena cava</td>
<td>11.8</td>
<td>9, 24, 35, 38, 43, 48, 51, 58</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>10.3</td>
<td>6, 32, 37, 49, 51, 65, 66</td>
</tr>
<tr>
<td>Progression of deep-vein thrombosis</td>
<td>5.9</td>
<td>34, 40, 42, 44</td>
</tr>
<tr>
<td>Subclavian vein</td>
<td>5.9</td>
<td>17, 36, 38, 55</td>
</tr>
<tr>
<td>Calf vein</td>
<td>5.9</td>
<td>32, 35, 36, 48</td>
</tr>
<tr>
<td>Superior vena cava</td>
<td>5.9</td>
<td>12, 13, 54, 57</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>2.9</td>
<td>62, 64</td>
</tr>
<tr>
<td>Rare: renal vein, arm veins, intracranial veins, pulmonary vein, shunt</td>
<td>&lt;2</td>
<td>9, 10, 7, 45, 44</td>
</tr>
<tr>
<td>Arterial thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral artery</td>
<td>4.4</td>
<td>11, 23, 59</td>
</tr>
<tr>
<td>Iliac artery</td>
<td>2.9</td>
<td>2, 16</td>
</tr>
<tr>
<td>Foot arteries</td>
<td>2.9</td>
<td>23, 30</td>
</tr>
<tr>
<td>Rare: renal artery</td>
<td>&lt;2</td>
<td>54</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracardiac thrombi</td>
<td>5.9</td>
<td>4, 19, 25, 37</td>
</tr>
<tr>
<td>Bleeding</td>
<td>5.9</td>
<td>21, 40, 47, 63</td>
</tr>
<tr>
<td>Clots in dialyzer</td>
<td>4.4</td>
<td>6, 65, 66</td>
</tr>
<tr>
<td>Neurological deficits</td>
<td>2.9</td>
<td>36, 41</td>
</tr>
<tr>
<td>Rare: subdural hematoma</td>
<td>&lt;2</td>
<td>61</td>
</tr>
<tr>
<td>thrombosis of pulmonary valve</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Patients may have had more than one complication. Case numbers refer to Table 2.
In a retrospective cohort study in a pediatric ICU, 57 patients developed arterial and/or venous thrombosis among 612 children treated with UFH for more than 5 days (Schmugge et al., 2002). In 14 children (2.3%), HIT was suspected based on thrombosis and a platelet count below $150 \times 10^9/L$ (or platelet fall exceeding 50%) occurring after 5 or more days of UFH use. In six patients (1.0%), HIT antibodies were demonstrated by platelet factor 4 (PF4)–dependent enzyme immunoassay (EIA) using adult cutoff values for a positive assay result (Table 2). The eight other patients with clinically suspected HIT had antibody levels below adult cutoff (Table 2). Eleven of the 14 patients had received UFH following cardiac surgery. Four were newborns and five others were also under one year of age (mean age 6.5 months).

Newall et al. (2003) retrospectively collected cases of HIT in a tertiary pediatric hospital. During the 2-year study, 116 patients received UFH over a 7-day period (25 reexposures). HIT was suspected in 4 patients who received therapeutic-dose UFH and developed a platelet count fall of more than 85% of the preheparin value. Three of the patients were tested for HIT antibodies, with one positive result (incidence $1/116 = 0.9\%$).

Between 1999 and 2002 we performed a prospective, randomized, double-blind, placebo-controlled trial in a neonatal ICU to assess the impact of intravenous (i.v.) UFH on the patency of peripheral venous catheters (Klenner et al., 2003b). Of 213 eligible infants, 108 received UFH (0.5 IU/mL) and 105 received saline for at least 5 days. None developed HIT or HIT antibodies (assessed by EIA). This suggests that the incidence of HIT is lower in neonatal ICU patients than previously reported. As mentioned, one reason for this difference could be false-positive results in the platelet aggregation test performed in an earlier study (Spadone et al., 1992). Another reason could be differences in patient population. For example, we did not enroll neonates following cardiac surgery, whereas Schmugge and coworkers (2002) noted that most of their neonates/infants with HIT had received UFH after cardiac surgery.

IV. CLINICAL PRESENTATION

Table 2 summarizes 68 children with HIT. Thirteen (19.1%) were newborns, 22 (32.4%) were children aged 3 months to 3 years, 11 (16.2%) were between 4 and 11 years of age, and 22 (32.4%) ranged in age from 12 to 18 years (Fig. 1). In newborns and young children (under 4 years of age), HIT usually occurred after cardiac surgery ($28/35 = 80\%$). In contrast, among 22 children aged 12 years or older, HIT complicated the use of UFH given because of preceding thrombosis in 13 (59.1%) patients, and following use of antithrombotic prophylaxis in 5 (22.7%); only one older child had undergone cardiac surgery.
Table 2  Characteristics of Children with HIT

<table>
<thead>
<tr>
<th>No.</th>
<th>Gender, age</th>
<th>Diagnosis, procedure</th>
<th>Platelet nadir (× 10^9/L)</th>
<th>Thrombocytopenia (TP) and thrombotic complication(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Confirmed HIT (clinical criteria present, laboratory test positive); all children received UFH except case 45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M, nb</td>
<td>Preterm, sepsis</td>
<td>&lt;100</td>
<td>TP</td>
</tr>
<tr>
<td>2</td>
<td>F, nb</td>
<td>Heart surgery</td>
<td>191</td>
<td>Iliac artery</td>
</tr>
<tr>
<td>3</td>
<td>M, nb</td>
<td>Norwood I, hypoplastic heart</td>
<td>36</td>
<td>TP</td>
</tr>
<tr>
<td>4</td>
<td>M, nb</td>
<td>ECMO</td>
<td>&lt;1</td>
<td>TP, ventricular thrombus</td>
</tr>
<tr>
<td>5</td>
<td>F, nb</td>
<td>Tetralogy of Fallot, heart surgery</td>
<td>45</td>
<td>TP</td>
</tr>
<tr>
<td>6</td>
<td>F, nb</td>
<td>Aortic stenosis, hypoplastic left ventricle, heart surgery</td>
<td>NA</td>
<td>TP, clots in circuit, PE, lung hemorrhage, renal vein</td>
</tr>
<tr>
<td>7</td>
<td>F, nb</td>
<td>CHD, heart surgery</td>
<td>48</td>
<td>TP, clot in shunt</td>
</tr>
<tr>
<td>8</td>
<td>F, 2m</td>
<td>Tetralogy of Fallot, premature, heart surgery, NEC</td>
<td>34</td>
<td>Vena cava, renal vein</td>
</tr>
<tr>
<td>9</td>
<td>M, 3m</td>
<td>Tricuspid valve atresia, Blalock-Taussig shunt</td>
<td>34</td>
<td>Vena cava, renal vein</td>
</tr>
<tr>
<td>10</td>
<td>M, 5m</td>
<td>Hypoplastic left heart, heart surgery, ECMO</td>
<td>34</td>
<td>Vena cava, renal vein</td>
</tr>
<tr>
<td>11</td>
<td>F, 6m</td>
<td>Heart surgery</td>
<td>46</td>
<td>Fem artery, DVT, TP</td>
</tr>
<tr>
<td>12</td>
<td>M, 8m</td>
<td>Tetralogy of Fallot, heart surgery</td>
<td>46</td>
<td>TP, clot in superior vena cava</td>
</tr>
<tr>
<td>13</td>
<td>M, 10m</td>
<td>Heart surgery</td>
<td>46</td>
<td>TP, vena cava</td>
</tr>
<tr>
<td>14</td>
<td>M, 10m</td>
<td>Preterm, VACTERL-syndrome</td>
<td>45</td>
<td>TP</td>
</tr>
<tr>
<td>15</td>
<td>M, 12m</td>
<td>Renal failure, tetralogy of Fallot</td>
<td>NA</td>
<td>TP</td>
</tr>
<tr>
<td>16</td>
<td>M, 13m</td>
<td>CHD, renal agenesis, heart surgery</td>
<td>46</td>
<td>TP, iliac artery</td>
</tr>
<tr>
<td>17</td>
<td>F, 15m</td>
<td>Heart surgery</td>
<td>123</td>
<td>TP, SC</td>
</tr>
<tr>
<td>18</td>
<td>M, 15m</td>
<td>CHD, heart surgery</td>
<td>10</td>
<td>TP</td>
</tr>
<tr>
<td>19</td>
<td>F, 17m</td>
<td>Acute myocarditis</td>
<td>80</td>
<td>TP, intracardiac</td>
</tr>
<tr>
<td>20</td>
<td>M, 23m</td>
<td>Hemofiltration</td>
<td>NA</td>
<td>TP</td>
</tr>
<tr>
<td>21</td>
<td>F, 2y</td>
<td>Fontan operation</td>
<td>55</td>
<td>TP, DVT</td>
</tr>
<tr>
<td>22</td>
<td>M, 2y</td>
<td>Fontan operation</td>
<td>73</td>
<td>TP, heparin resistance</td>
</tr>
<tr>
<td>23</td>
<td>M, 3y</td>
<td>Tricuspid, pulmonary valve atresia, Fontan operation</td>
<td>40</td>
<td>Fem artery, foot gangrene</td>
</tr>
<tr>
<td>24</td>
<td>M, 4y</td>
<td>Double-inlet left ventricle, Fontan operation</td>
<td>25</td>
<td>TP, vena cava, DVT</td>
</tr>
<tr>
<td>25</td>
<td>F, 4y</td>
<td>Cardiomyopathy, heart transplant</td>
<td>16</td>
<td>TP, intracardiac, bleeding</td>
</tr>
<tr>
<td>26</td>
<td>F, 4y</td>
<td>Lung disease, mechanical ventilation</td>
<td>50</td>
<td>TP</td>
</tr>
<tr>
<td>27</td>
<td>F, 7y</td>
<td>Cardiomyopathy</td>
<td>71</td>
<td>TP, DVT</td>
</tr>
<tr>
<td>28</td>
<td>F, 8y</td>
<td>Turner’s syndrome, sinus vein thrombosis</td>
<td>190</td>
<td>TP, heparin resistance</td>
</tr>
<tr>
<td>29</td>
<td>F, 9y</td>
<td>DVT, APS</td>
<td>82</td>
<td>TP</td>
</tr>
<tr>
<td>30</td>
<td>M, 10y</td>
<td>Leg artery thrombosis, PS def</td>
<td>39</td>
<td>TP, foot arteries</td>
</tr>
<tr>
<td>31</td>
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<td>(Nguyen et al., 2003)</td>
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<td>S</td>
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<td>6</td>
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<td>† bleeding (Boshkov et al., 2003a)</td>
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<td>7</td>
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<td>†</td>
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<td>8</td>
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<td>(Boshkov et al., 2003a)</td>
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<tr>
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<td>(Murdoch et al., 1993)</td>
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<td>(Ranze et al., 2001)</td>
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<td>(Schmugge et al., 2002)</td>
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<td>(Severin et al., 2002a)</td>
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<td>(Porcelli et al., 2003)</td>
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<td>(Deitche et al., 2002)</td>
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<td>26</td>
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<td>27</td>
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<td>(Deitche et al., 2002)</td>
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<td>(Severin et al., 2002b)</td>
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<td>(Bocquet et al., 1999)</td>
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<td>Thrombocytopenia (TP) and thrombotic complication(s)</td>
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<tr>
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<td>DVT, vena cava, TP</td>
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<tr>
<td>36 M, 13y</td>
<td>Closure of Blalock-Taussig shunt</td>
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<td>TP, stroke, DVT, SC vein</td>
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<tr>
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<td>38 M, 13y</td>
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<td>55</td>
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<td>39 M, 13y</td>
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<td>PE, vena cava, APS</td>
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<td>TP, progressive thrombosis</td>
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<td>Fem artery, mitral valve tumor</td>
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<td>TP, amaurosis fugax</td>
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<td>No TP</td>
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<td>43 M, 15y</td>
<td>DVT, PS def</td>
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<td>DVT, vena cava</td>
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<td>44 F, 15y</td>
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<td>Progressive thrombosis</td>
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<td>46 M, 16y</td>
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<td>48 M, 17y</td>
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<td>DVT, vena cava</td>
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</tr>
<tr>
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<td>DVT, PS def</td>
<td>?</td>
<td>DVT, PE</td>
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<tr>
<td>50 F, 18y</td>
<td>Trauma</td>
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<td>TP</td>
<td></td>
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<tr>
<td>51 M, 18y</td>
<td>Ulcerative colitis, DVT</td>
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<td>PE, vena cava</td>
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**B: Probable Hit (clinical criteria present, laboratory test borderline)**

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<tr>
<th>No.</th>
<th>Gender, age</th>
<th>Diagnosis, procedure</th>
<th>Platelet nadir (× 10^9/L)</th>
<th>Thrombocytopenia (TP) and thrombotic complication(s)</th>
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<tr>
<td>52 M, nb</td>
<td>Transposition of great vessels</td>
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**C: Unlikely HIT (laboratory test negative)**

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<th>Thrombocytopenia (TP) and thrombotic complication(s)</th>
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<tr>
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<td>Outcome (Ref.)</td>
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<td>Amp forefoot (Schiffmann et al., 1997)</td>
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<td>DS</td>
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<td>r-tPA, DS, OAC</td>
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<td>ASA</td>
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<td>DS</td>
<td>S (Sauer et al., 1998)</td>
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<td>Lep, OAC</td>
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<td>DS</td>
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<tr>
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<td>HIT screen</td>
<td>Platelets</td>
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<tr>
<td>64</td>
<td>EIA</td>
<td>Unknown</td>
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</table>
Four patients developed HIT during low-dose UFH given for catheter patency (cases 1, 14, 47, 52). Hemodialysis or hemofiltration accounted for UFH use in four (5.9%) patients (cases 20, 33, 65, 66). In 17 of the 68 patients, the laboratory test for HIT was negative or not performed (cases 53–68, inclusive).

The most frequent manifestation of HIT in the 68 children was a decrease in platelet count (85.3%). HIT was associated with thromboembolic complications in about two thirds of the patients, most commonly involving iliac and femoral veins, the inferior vena cava, and pulmonary embolism (Table 1). Less commonly, intracardiac thrombi or neurological events occurred, or clotting of the dialyzer. Only about 10% (7/68) of patients developed arterial thrombosis. Thus, there is a strong preponderance of venous thrombosis in pediatric HIT.

Eight (11.8%) of the 68 children died (cases 4, 6, 7, 24, 25, 52, 57, 61) and three required amputations (cases 23, 30, 35). In four children, only partial recanalization of thrombosed veins occurred (cases 29, 34, 36, 43).

This summary does not include the 14 newborns reported by Spadone and colleagues (1992). These workers primarily observed arterial thrombosis, with at least 11 (78.6%) developing aortic thrombosis (one infant died without imaging studies). Two newborns with thrombosis had normal platelet counts. Eleven (78.6%) survived, the remaining three developing mesenteric ischemia. Arterial thrombosis likely was related to umbilical artery catheters.

### Table 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Gender, age</th>
<th>Diagnosis, procedure</th>
<th>Platelet nadir (\times 10^9/L)</th>
<th>Thrombocytopenia (TP) and thrombotic complication(s)</th>
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<td>68</td>
<td>F, 17y</td>
<td>Prophylaxis</td>
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</table>

*Abbrev: †, died; Amp, amputation; APS, antiphospholipid syndrome; Arg, argatroban; ASA, aspirin (acetylsalicylic acid); CHD, congenital heart disease; DS, danaparoid sodium; DVT, deep-vein thrombosis; ECMO, extracorporeal membrane oxygenation; EIA, PF4-dependent enzyme immunoassay; F, female; fem, femoral; HIPA, heparin-induced platelet activation test; Lep, lepirudin; +Lep, switch to lepirudin after suspected or confirmed cross-reactivity to danaparoid; LMWH, low molecular weight heparin; m, month; M, male; MTHFR, methylene tetrahydrofolate reductase; NA, not available; nb, newborn; NEC, necrotizing enterocolitis; OAC, oral anticoagulants; PE, pulmonary embolism; PS def, protein S deficiency; r-tPA, recombinant tissue-plasminogen activator; S, survived; SC, subclavian; TP, thrombocytopenia; UAC, umbilical artery catheter; UFH, unfractionated heparin; UK, urokinase; XR, confirmed cross-reactivity with danaparoid; XR?, suspected cross-reactivity with danaparoid; y, year.
**Heparin-Induced Thrombocytopenia in Children**

<table>
<thead>
<tr>
<th>No.</th>
<th>Test for HIT antibodies</th>
<th>Treatment</th>
<th>Outcome (Ref.)</th>
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<td>LMWH, DS</td>
<td>S (Neuhaus et al., 2000)</td>
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<td>66</td>
<td>HIPA, EIA</td>
<td>LMWH, DS</td>
<td>S (Neuhaus et al., 2000)</td>
</tr>
<tr>
<td>67</td>
<td>Not done</td>
<td>DS</td>
<td>S (Weigel et al., 1999)</td>
</tr>
<tr>
<td>68</td>
<td>None</td>
<td>None</td>
<td>S (Boon et al., 1994)</td>
</tr>
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**Figure 1**  Reasons for preceding heparin therapy in children with HIT. Among the various age groups, the reasons for heparin therapy that led to HIT varied considerably: whereas newborns and infants usually developed HIT after cardiac surgery, among teenagers HIT more often complicated the use of heparin during treatment of thrombosis.
(used in all but one of the 14 neonates). In adults, intravascular catheters are a risk factor for HIT-associated thrombosis (Hong et al., 2003), but whether the arterial thrombi observed by Spadone et al. indeed were HIT-related is unclear.

V. LABORATORY TESTING

As in adults, no data exist to justify routine screening for HIT antibodies during heparin use in children. Laboratory tests for HIT should be used to exclude or confirm clinically suspected HIT. During UFH therapy, platelet counts should be monitored regularly (see Chap. 4), particularly between days 5 and 14 of heparin use, the time when over 90% of HIT patients develop their platelet count fall (see Chap. 3).

For laboratory testing, functional and antigen tests are available (see Chap. 11). Commercial antigen assays (EIA) are often used and are especially appropriate for neonates and infants because small blood volumes are needed (<100 µL vs. >1 mL for many platelet activation assays). However, the appropriate cutoff level that defines a positive EIA result suitable for children is debated. In a retrospective study, Schmugge and coworkers (2002) investigated cutoff levels for children using a commercial EIA (Asserachrom, Stago). Among 612 children, HIT was suspected in 14 because of thrombocytopenia and thrombosis. Positive test results (using the adult cutoff) were seen in 6 of the 14 patients. In the remaining 8 children with suspected HIT, test results ranged from 26 to 80% of the adult cutoff level, i.e., levels that were higher than among controls (with wide overlap).

A retrospective analysis performed by Risch and colleagues (2003) of the same 612 pediatric ICU patients initially reported by Schmugge and coworkers (2002) addressed whether there was an association between anti-PF4/heparin antibody levels (measured by EIA) and thrombosis. Ten patients who developed thrombosis without thrombocytopenia constituted the study group and were compared with 19 matched controls with neither thrombosis nor thrombocytopenia. All 29 subjects had lower antibody levels than the cutoff level recommended for adults. However, median assay results were significantly higher in the thrombosis patients than in controls (51% vs. 23% of the manufacturer’s cutoff; \( p = 0.004 \)). The authors concluded that there might be an association between anti-PF4/heparin antibody levels and thrombosis, even in the absence of thrombocytopenia or a positive test result (by conventional criteria).

However, in our prospective, randomized, double-blind trial, we screened 108 newborns receiving UFH for more than 5 days and 105 controls using the PF4/polyvinyl sulfonate EIA (GTI, Brookfield) (Klenner et al., 2003).
None of the infants developed HIT antibodies using the adult cutoff [UFH group: mean optical density (OD), 0.020; maximum, 0.328; saline group: mean OD, 0.019, maximum, 0.239]. Minor changes in OD (increase > 0.100) occurred in six patients (three in each group) (Fig. 2). Therefore, these minor increases in OD are unlikely to be related to UFH use and could represent a nonspecific increase in antibody levels in ill patients (acute phase reaction). Among the subjects receiving placebo, all OD values were below 0.400 (the accepted adult cutoff value), suggesting that this level is also appropriate for neonates.

The limitations of antigen assays observed in adults likely also apply to children. Thus, in 5–10% of cases, the antigen assay could be false-negative.

Figure 2  Six neonates with rising absorbance levels in platelet factor 4–dependent enzyme immunoassay (EIA). Six of 213 neonates participating in a randomized, double-blind trial comparing heparin with normal saline for maintenance of peripheral venous catheter patency developed a rise in absorbance of more than 0.100 optical density (OD) units by PF4/polyvinyl sulfonate EIA (GTI, Brookfield, WI). All OD values were less than the positive adult cutoff (0.400 OD units). No differences were observed between patients receiving heparin (solid lines) compared to patients receiving saline (dotted lines). As the maximum OD in saline controls was 0.239, the 0.400 cutoff seems appropriate also for pediatric patients. (Klenner et al., 2003b.)
if HIT antibodies recognize a non–PF4-dependent antigen (Greinacher et al., 1994) (see Chaps. 6 and 7). Thus, a functional test for HIT should be performed when HIT remains strongly suspected despite a negative EIA. However, when the pretest probability of HIT is low, a negative antigen test usually excludes HIT.

VI. THERAPY OF PEDIATRIC HIT

Numerous case reports describe the occurrence of new or recurrent thromboembolic events during continued or repeated use of heparin in adult patients with acute HIT. Further, thrombocytopenia usually persists if heparin is not stopped. Thus, all heparin should be discontinued in patients strongly suspected of having HIT, including heparin “flushes,” heparin-coated catheters, and heparin-containing blood products (Severin et al., 2002b) (see Chap. 13). Because HIT is a prothrombotic (“hypercoagulability”) state with high risk of thromboembolic complications, alternative anticoagulation is usually required after stopping heparin. In adults, danaparoid, lepirudin, and argatroban have been studied prospectively. For children, experience with these agents is anecdotal and heterogeneous (Table 2). Danaparoid use has been reported in 27 patients (with additional aspirin, thrombolysis, or lepirudin given in some cases). In two children, danaparoid was stopped because of cross-reactivity, with further anticoagulation with lepirudin. Twelve patients received lepirudin (one combined with thrombolysis and oral anticoagulants). Five children were treated with low molecular weight heparin (LMWH), and one with warfarin and aspirin. One infant received aspirin, two were given argatroban plus aspirin, and three were treated with argatroban alone. Eleven children received oral anticoagulants. In five children, no anticoagulant was given.

A. Danaparoid

Danaparoid (see Chap. 14) is a mixture of low molecular weight glycosaminoglycans that catalyze the inactivation of factor Xa (FXa) by antithrombin. It has minimal anti-factor IIa activity. Dosing schedules for adults (appropriately weight-adjusted for the child) can be used for guidance. For antithrombotic prophylaxis, 10 IU/kg body weight given twice daily by subcutaneous injection is recommended. For therapeutic anticoagulation in pediatric HIT patients, an initial i.v. bolus of 30 IU/kg is followed by continuous infusion of 1.2–2.0 IU/kg/h (Monagle et al., 2001). The anti-FXa level should be measured during treatment for optimal dosing. Target levels of anti-FXa activity are 0.4–0.6 IU/mL for standard and 0.5–0.8 IU/mL for higher danaparoid doses (Severin et al., 2002b).
Based on experience in adults, LMWH should not be used to treat acute HIT in children (see Chap. 13).

B. Lepirudin

Lepirudin (see Chap. 15) is a direct inhibitor of free and clot-bound thrombin through noncovalent, irreversible binding. In adults with HIT complicated by thrombosis, the approved dose is an initial bolus of 0.4 mg/kg followed by continuous i.v. infusion (0.15 mg/kg/h) adjusted by activated partial thromboplastin time (aPTT). The usual target aPTT ratio should be 1.5–2.5 times the normal laboratory mean aPTT. Dosing in children is based on anecdotal experience. Schiffmann et al. (1997) gave a bolus of lepirudin (0.2 mg/kg) and a continuous infusion (ranging between 0.1 and 0.7 mg/kg/h) adjusted by aPTT. Severin et al. (2002b) achieved therapeutic anticoagulation with a continuous infusion of 0.1 mg/kg/h in a 15-year-old boy, and with an infusion rate of about 0.15 mg/kg/h in an 8-year-old girl. In an 11-year-old girl, 0.15–0.22 mg/kg/h was given. In a premature infant, Nguyen and coworkers (2003) gave a 0.2 mg/kg bolus followed initially by 0.1 mg/kg/h infusion rate; the dose was adjusted daily based on the aPTT, and 0.03–0.05 mg/kg/hr provided adequate anticoagulation. Since pharmacokinetics depend largely on renal function, we recommend starting lepirudin with an i.v. infusion of 0.1 mg/kg/h (if renal function is normal) and to adjust the dose according to aPTT 4 h later, without initial bolus. This minimizes both risk of overdosing and anaphylaxis (see Chap. 15).

C. Argatroban

Argatroban (see Chap. 16) is a synthetic direct thrombin inhibitor that binds reversibly to the active site of thrombin. In adults, the recommended initial dose of argatroban is 2 μg/kg/min given by continuous i.v. infusion and adjusted by aPTT (target range, 1.5–3 times the baseline aPTT). Safety and efficacy of argatroban in pediatric patients have not been established. Argatroban has been used in neonates (Boshkov et al., 2003a,b). In one newborn, an initial bolus (200 μg/kg) was followed by continuous i.v. infusion (7.5 μg/kg/min). A 5-month-old infant was also treated with an initial bolus (250 μg/kg) with subsequent infusion (10 μg/kg/min).

D. Coumarin

Oral anticoagulants of the coumarin class (warfarin, phenprocoumon) are not appropriate for therapy of acute HIT (see Chap. 13). HIT patients are at relatively high risk of developing coumarin-induced skin necrosis and venous limb gangrene syndromes (see Chap. 3). Therefore, coumarin should be
delayed until the patient is adequately anticoagulated with danaparoid, lepirudin, or argatroban, and platelet counts have substantially recovered.

VII. PREVENTION OF HIT IN CHILDREN

Since the pivotal trial in adult orthopedic patients (Warkentin et al., 1995), it has become clear that LMWH induces HIT less frequently than does UFH. In children, HIT appears to occur most often among the very young following cardiac surgery and among adolescents given UFH to treat spontaneous thrombosis (Table 2). Data from Pouplard and colleagues (1999) suggest that HIT also could occur less often if LMWH rather than UFH is used for anti-thrombotic prophylaxis post–cardiac surgery. This approach should be investigated in children.

Similarly, in the second group of at-risk pediatric patients (adolescents with thrombosis), it seems plausible that the frequency of HIT would be reduced if LMWH is given instead of UFH. Pharmacokinetic studies of

<table>
<thead>
<tr>
<th>Study/Ref.</th>
<th>Number of patients</th>
<th>New thromboembolic event</th>
<th>Bleeding</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td>Therapeutic doses</td>
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<td></td>
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<tr>
<td>Massicotte et al., 1996</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
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<td>4</td>
</tr>
<tr>
<td>Nohe et al., 1999</td>
<td>38</td>
<td>0</td>
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</tr>
<tr>
<td>Massicotte et al., 2003a</td>
<td>36</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>249</td>
<td>4</td>
<td>62</td>
</tr>
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<td>2</td>
</tr>
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<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
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<td>46</td>
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<td>5</td>
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<td>10</td>
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<td>11</td>
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LMWH in infants and children have been conducted for enoxaparin and reviparin. With both drugs, therapeutic anti-FXa levels are achieved 3–4 hours following the first subcutaneous injection and are similar in both infants and older children (Albisetti and Andrew, 2002; Massicotte et al., 2003c).

The safety and efficacy of prophylactic and therapeutic doses of LMWH (enoxaparin, reviparin, dalteparin) in children have been evaluated in clinical trials for a variety of conditions. LMWH is safe and effective for anticoagulation of infants and children of varying age (Table 3) (Roschitz et al., 2002). Based on adult experience, substituting UFH with LMWH whenever possible will likely reduce the risk of HIT in children. However, no data exist in children to support this supposition.

VIII. SUMMARY

HIT appears to be rare in children. The incidence depends somewhat on patient age and indication for heparin. Two major pediatric at-risk groups are apparent: newborns/infants after cardiac surgery (incidence ~1%), and adolescents treated with UFH for spontaneous thrombosis. HIT can be life-threatening even in children (~12% mortality). Venous thrombosis is the most frequent HIT-associated complication in children. For laboratory confirmation of HIT, antigen assays are most appropriate. Although there are conflicting data on the optimal laboratory cutoff for antigen assays, a randomized, double-blind clinical trial suggests that the cutoff level established in adults is also appropriate for children. There are no prospective studies of alternative anticoagulants in children with HIT. Most available data are for lepirudin and danaparoid. By substituting LMWH for UFH whenever possible, the risk of HIT may be similarly reduced in children as it is in adults.

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Heparin-Induced Thrombocytopenia in Children


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I. INTRODUCTION

Litigation is a not uncommon occurrence when a patient has suffered harm because of heparin-induced thrombocytopenia (HIT) due to alleged physician or nursing “malpractice” (more properly termed professional negligence). One reason may be the general increase in medicolegal actions (Mello et al., 2003) including those involving issues of thrombosis and antithrombotic therapy, especially in the United States (McIntyre, 2001). Regarding HIT in particular, one explanation could be the legal concept of *res ipsa loquitur* (“the thing speaks for itself”), i.e., the situation of a patient who enters the hospital for elective surgery and leaves with an amputated limb may itself “speak” persuasively to a jury that someone somehow must have been at fault for such a tragic outcome to have occurred (Kelton, 1998). Further, the patient may
have been unaware of such a potential catastrophic outcome, as physicians usually do not specifically include HIT and its attendant complications when obtaining informed consent. The tendency for a finding of substandard care to occur more frequently when harm is severe (“outcome bias”) exists even though outcome per se is a poor index of blameworthiness (Caplan et al., 1991; Runciman et al., 2003).

Another factor is that HIT is a “common, rare disease” (see p. 338), suggesting that although many thousands of patients probably suffer HIT-related thrombotic complications each year in the United States, few physicians encounter enough patients to develop proficiency in its diagnosis and management. This is of particular relevance to HIT, where the use of standard, common-sense treatment approaches, such as discontinuing heparin and commencing oral anticoagulants, can paradoxically lead to catastrophic outcomes, e.g., warfarin-induced venous limb gangrene (see Chaps. 3 and 13). Further, there is an emerging consensus that appropriate medical treatment for HIT should include use of an alternative agent such as danaparoid, lepirudin, or argatroban (see Chaps. 13–19). However, many physicians are relatively unfamiliar with these newer drugs, some of which might not even be stocked in the hospital pharmacy (and thus not be quickly available), or for which special monitoring assays required in special circumstances may not be available (e.g., anti-factor Xa level; ecarin clotting time) (see Chaps. 14 and 15).

Few medical centers have access to timely diagnostic testing for the pathogenic HIT antibodies. Further, there exists a variety of assays, with varying diagnostic usefulness (see Chap. 11). Plus, even a positive test for HIT does not necessarily establish the diagnosis, given the high frequency of subclinical HIT antibody seroconversion in certain patient populations (see Chaps. 4 and 11). Physicians often must make crucial decisions to give or withhold anticoagulants amidst considerable diagnostic uncertainty.

Thrombocytopenia is a very common event in hospitalized patients, and it is often inconsequential (e.g., perioperative hemodilution) or otherwise expected in the context of the patient’s presenting illness (e.g., septicemia) or its management (e.g., hemodilution/platelet consumption secondary to intra-aortic balloon pump or heart surgery utilizing cardiopulmonary bypass). Yet, in a patient who develops HIT, often in the setting of these other potential explanations for thrombocytopenia, the initial mild or moderate platelet count decline that first signaled the onset of HIT could very well be overlooked, but will later be highlighted during the glaring retrospection of a medicolegal action.

The purpose of this chapter is to discuss the medicolegal impact of clinical practice guidelines, and to discuss some specific issues that can arise in HIT from the perspective of the U.S. medicolegal system. Chapter 22 discusses medicolegal aspects of HIT from a European viewpoint.
II. U.S. MEDICOLEGAL SYSTEM

The key issue in a medicolegal case is whether the physician failed to uphold the perceived “standard of care” in the context of the facts presented and expert opinions expressed; and whether such failure, if established, was a substantial contributing factor in bringing about the plaintiff’s injury. Unless the legal action is dropped, or an out-of-court settlement is reached, the action generally progresses to the courtroom (with or without prior judicially overseen medical review, in some states), where it usually is heard and decided by a jury.

The “standard of care,” as it applies to a malpractice case, generally refers to how a qualified and reasonably competent practitioner of the same generalist or specialist class would perform acting under the same or similar circumstances. The standard of care is developed for the jury through the interaction of expert witnesses and the lawyers for plaintiff and defendant sides (McIntyre, 2001). Depending upon the state, this interaction can consist of experts’ opinions expressed through written reports, and/or as testimony expressed in response to questioning that primarily is conducted by the opposing attorney (deposition).

Given the progressively accumulating scientific data base, there is controversy as to whether a jury system is appropriate for ever more complex medical issues (McIntyre, 2001; Mello et al., 2003). The jury system may function reasonably well when the failure of the physician is clear (e.g., removal of the wrong leg). But as the requirement for judgment on the part of the physician increases, especially in a disorder such as HIT with complex diagnostic and treatment dilemmas, the jury may increasingly find the medical issues in particular difficult to understand. Even for interested physicians and scientists, it can be challenging to follow the exponential expansion of the scientific literature on HIT, with its implications for an evolving standard of care based upon this new information. The standard of care to be applied to an individual case must consider the available information at the time the treatment was applied, which can differ significantly from the standard at the time of the subsequent medicolegal action itself. Further, diagnostic uncertainty in specific cases of HIT, as discussed earlier, can be considerable, with the implication that the standard of care to be applied to that case becomes increasingly ill-defined and uncertain as well.

While the summary of the medicolegal process put forth above tends to make the U.S. system appear quite fair and orderly, there are those who believe the process has serious flaws. On the medical side, the increasing emphasis on scientifically sound evidence as the basis for the development of a “standard of care” for medical practice may have improved the quality of the medical care in the United States, as judged by such entities as Physician
Review and Evaluation Boards. However, treatment guidelines developed in part by the consensus conference process have not resulted in either a decline in the number of malpractice cases or the size of dollar awards to plaintiffs, awards that at times appear to be massively inappropriate and costly to a medical system currently staggering under cost burdens.

A principal reason for this cited repeatedly by critics is the alleged financial motivation of plaintiffs’ attorneys, who may receive one-third of a multimillion dollar award. These large fees reflect the “contingency” system, whereby the legal fees and costs are based upon a percentage of the gain realized for the client. Such contingency fees make access to legal services available even to an indigent plaintiff, but tend to emphasize legal actions with the potential for large awards. Attempts at curtailing huge awards have so far failed, and the cost burden reflected in the form of rising insurance costs is ultimately passed on to consumers or their employers.

Another aspect of the medical malpractice system in the United States appears to be the willingness of some expert witnesses to do or say whatever is necessary to win the case for the client on whose behalf they are testifying. The Honorable Richard Thornburgh, former U.S. Attorney General under President Ronald Reagan, and frequent commentator on legal aspects of important social problems, has used the term “junk science” to apply to the “testimony of expert witnesses hired not for their scientific expertise, but for their willingness, for a price, to say whatever is needed to make the client’s case” (Thornburgh, 1998). The expert witness in a malpractice action is often well paid, perhaps receiving several thousand dollars for a few hours work, and it is difficult not to believe that, at least in some circumstances, it is that expert’s preference that the client on behalf of whom he is testifying would go on to win the case.

In practice, it is the role of the jury to determine whether or not the practitioner’s actions met the requirements of the “standard of care.” An expert witness with impressive credentials and a striking presence can have a powerful effect on the jury’s decision, by simply indicating that, in his opinion, the practitioner failed to meet the required “standard of care.” This effect may be disproportionately increased in a complex case involving considerable physician judgment.

The “burden of proof” required for the plaintiff to prevail in a malpractice action is an important additional determinant of outcome. Unlike criminal cases, where the jury must be convinced “beyond a reasonable doubt” (i.e., theoretically, at least, to a 99.999% level of certainty) that the individual on trial is guilty, in a medical malpractice trial, the “burden of proof” is much lower, requiring only that the jury determine that the physician, based on “a preponderance of the evidence,” i.e., “more likely than not,” breached the standard of care; and that such breach was a “substantial
factor” (or alternate terminology, depending upon the state), in causing the damages or injuries alleged by the plaintiff. Depending upon the jurisdiction, the experts need to express their opinions “based on a reasonable degree of medical probability” or “based upon a reasonable degree of medical certainty” or similar such wording.

III. CLINICAL PRACTICE GUIDELINES AND HIT

There are at least seven clinical practice guidelines that discuss one or more aspects of platelet count surveillance, diagnosis, or management of HIT, including the guidelines summarized in this book (Olson et al., 1998; Hirsh et al., 1998, 2001; Greinacher and Warkentin, 2000, 2001; Warkentin, 2002; Greinacher et al., 2003; see Chap. 13). Although the major purpose of clinical practice guidelines is to enhance quality of care, it is also possible that their existence could contribute to medicolegal risk if recommendations are not followed (McIntyre, 2001). Of course, it is not possible for any consensus conference or practice guideline to deal explicitly with all of the complex issues that can arise in any individual case. Thus, there is a major role for physician judgment in the context of the individual case itself (McIntyre, 2001). Additionally, as Olson (1995) states, “Readers themselves must assess the quality and validity of consensus statements as they do all literature.” Finally, it remains unproven whether clinical practice guidelines have a positive impact on patient care (McIntyre, 2001).

Clinical practice guidelines also can demonstrate that the scientific basis for many recommendations is preliminary, incomplete, or even contradictory. Increasingly, attempts are being made formally to grade the strength of the recommendations. The increasing sophistication of the grading systems has paralleled the growing scope of the recommendations themselves, as shown by the evolution of the grading systems used by the successive Consensus Conferences on Antithrombotic Therapy held under the aegis of the American College of Chest Physicians (Sackett, 1989; Cook et al., 1992, 1995; Guyatt et al., 1998, 2001). The significance of the strength of a recommendation is that a strong scientific basis theoretically would render more difficult the defense of a deviation of practice from a guideline based upon such strong evidence (McIntyre, 2001).

Regarding HIT, most of the recommendations are based upon observational data (level C evidence), rather than randomized clinical trials that might lead to higher grade recommendations. Nevertheless, it is possible for a level 1 recommendation to be derived from observational data, provided that the evidence is convincing and widely accepted.
IV. SPECIFIC MEDICOLEGAL ISSUES IN HIT

A. Informed Consent

HIT often occurs in patients who receive heparin for “routine” antithrombotic prophylaxis. For example, the highest frequency of HIT (3–5%) has been reported in postoperative orthopedic patients receiving unfractionated heparin (UFH) (Warkentin et al., 1995, 2003; see Chap. 4). HIT also is fairly common (1–3%) after cardiac or vascular surgery if postoperative UFH prophylaxis is also given. In these situations, which often involve elective surgery, the process of informed consent routinely includes important perioperative and postoperative complications, including severe consequences such as death or disability from thrombosis, bleeding, infection from blood transfusion, etc. However, in our experience, it appears that most physicians do not specifically list thrombosis secondary to HIT when obtaining consent. One obvious explanation is that there is something inherently contradictory about informing a patient that heparin can sometimes cause severe clots, while at the same time educating the patient that the heparin is prescribed to prevent clots. Thus, the argument raised is that the overall “net” effect of heparin is prevention of thrombosis, and so the issue of HIT is irrelevant. Another factor is that in most jurisdictions, there is no specific requirement to obtain consent for an approved medication, although specific informed consent is required in the context of an experimental therapy involving an unapproved agent. There is no consensus in the medical community whether informed consent should include specific mention of HIT and its complications.

B. Platelet Count Surveillance

It is a truism that for a thrombocytopenic disorder the platelet count values, and the interpretation of these results, take on a central role. Few guidelines exist, however, as to how frequently platelet counts should be measured in patients receiving heparin. A recommendation from the College of American Pathologists (CAP) Conference XXXI stated, “In patients receiving adjusted-dose and full-dose heparin, monitor for and evaluate HIT by performing platelet counts pretreatment and, at least, on alternate days for 14 days beginning on day 4 of therapy in the naive patient and beginning on day 1 in patients with prior heparin exposure” (Olson et al., 1998).

Recent data, however, suggest that these recommendations should be reconsidered. For example, HIT occurs most often in postoperative orthopedic patients receiving nonadjusted low- or intermediate-dose unfractionated heparin (10,000–15,000 U per day in divided doses) (Warkentin et al., 1995, 2000, 2003; Ganzer et al., 1997; Funk et al., 2000). Presumably, at least alternate-day monitoring should apply to these patients as well. Conversely,
HIT appears to be very rare in medical patients receiving therapeutic-dose low molecular weight heparin (LMWH); perhaps, platelet count monitoring—especially in an out-patient setting—is not warranted. Also, the role of prior heparin exposure in the timing of onset of HIT has recently been clarified: a rapid fall in platelet count seems to occur only in patients who have recently been exposed to heparin (within the past 100 days) (Warkentin and Kelton, 2001). Thus, for a patient with “remote” heparin exposure, platelet count monitoring for HIT presumably could begin on day 4 of heparin treatment. This information has been incorporated into new recommendations regarding platelet count surveillance that takes into account the risk of HIT in various clinical situations (Warkentin, 2002; Greinacher et al., 2003; see also Chap. 4).

Another central issue in some medicolegal cases involving HIT is the interpretative response of the physician to a particular platelet count profile. Thrombocytopenia is very common in hospitalized patients, with only a small minority of thrombocytopenic patients receiving heparin actually having HIT. Unfortunately, the severity of the platelet count fall is usually not helpful in distinguishing HIT from other medical problems. For instance, about 10–15% of patients with serologically proven HIT never develop “thrombocytopenia,” as conventionally defined (platelet fall to less than 150 × 10^9/L or by > 50%). Further, the median platelet count nadir in HIT is about 60 × 10^9/L, and there are many other illnesses that cause such a moderate degree of thrombocytopenia. Although a 50% or greater fall in the platelet count from the postoperative peak is a fairly sensitive and specific marker for HIT in surgical patients (Warkentin et al., 2003), other conditions (e.g., septicemia) also can cause a similar platelet count decline. Regardless of the scientific “definition” of thrombocytopenia applicable to HIT, there is the potential for considerable disagreement in a medicolegal action as to whether a particular platelet count sequence should have been considered indicative of HIT, and at what point in time along the platelet count sequence this possibility should have been considered.

C. Diagnosis of HIT

Although many cases of HIT can be diagnosed with accuracy on clinical grounds, there are others in which the diagnosis is not certain, and laboratory confirmation or refutation is important. Moreover, availability of laboratory testing is variable and may not have been obtained in a patient, or may have given results that are at odds with the clinical diagnosis. For example, some tests have limited sensitivity (e.g., platelet aggregation assays), so a negative test does not necessarily exclude HIT. Further, the transience of HIT antibodies means that the diagnosis cannot usually be established even when sensitive tests are performed using blood samples obtained several months
after recovery from acute HIT (see Chap. 3). As discussed in Chap. 12, some disorders so closely mimic HIT on clinical grounds as to merit the designation, “pseudo-HIT.” These disorders can cause “experts” on opposite sides to disagree fundamentally on whether HIT was even present. Ironically, certain of the pseudo-HIT disorders also have a high risk for thrombosis or fatal outcomes. Issues of turnaround time and diagnostic usefulness of particular assays can also be an issue in some medicolegal actions, given the considerable heterogeneity in availability and type of diagnostic testing among various medical centers, as well as the evolving medical literature on the diagnostic implications of a positive or negative test result (see Chap. 11).

D. Treatment of HIT

Medicolegal issues involving the treatment of HIT are complicated by such issues as treatment paradoxes, use of nonapproved medications, new information about the natural history of isolated HIT, as well as the recent availability of new drugs for prevention and treatment of thrombosis complicating HIT, all of which have influenced the standard of care.

Treatment “Paradoxes”

There are many seemingly counterintuitive treatment paradoxes in the management of HIT (Warkentin, 2001; see Table 1 in Chap. 13). Many of these paradoxes are known to specialists, although a generalist practitioner may not be familiar with them. For example, prophylactic platelet transfusions are considered to be relatively contraindicated in HIT, even when a patient is severely thrombocytopenic. This is because bleeding is rare in HIT, and because platelet transfusions at least theoretically might increase the risk for thrombosis. Thus, a platelet transfusion ordered for a patient with HIT who suffers a subsequent thrombosis might be regarded as prima facie (“at first view”) evidence for malpractice. However, in a complex case of HIT, mitigating factors could include uncertainty about the cause of thrombocytopenia at the time of transfusion, and other factors suggesting a high risk for bleeding. Further, although published anecdotes suggest a link between platelet transfusions and subsequent thrombosis, the growing awareness that the natural history of HIT itself is for thrombosis to occur frequently even in the absence of platelet transfusions suggests that any causal association between a platelet transfusion and a subsequent adverse outcome is speculative. Indeed, this view is reflected in the “weakest” grade (level 2C) assigned this recommendation proscribing platelet transfusions.

Another example of a counterintuitive treatment paradox is the recommendation that LMWH is contraindicated as treatment for HIT (Hirsh et al.,
This recommendation reflects the observation that even though using LMWH will prevent most cases of HIT (Warkentin et al., 1995, 2000, 2003), use of these preparations in a patient who already has established HIT has a high probability of treatment failure (Ranze et al., 2000). Although this recommendation against use of LMWH for treating HIT was given a relatively “high-grade” recommendation by the ACCP Consensus Conference (1C+), an alternate view holds that LMWH therapy might be acceptable provided that in vitro cross-reactivity testing is negative using platelet aggregation assays (Slocum et al., 1996). However, problems with such an approach involve treatment delays pending laboratory testing, as well as the availability of other agents with low (danaparoid) or absent cross-reactivity (argatroban, lepirudin). Nevertheless, this contrary viewpoint does show that expert opinion potentially can differ substantially regarding treatment recommendations.

Nonapproved Medications

Although three drugs are widely regarded as safe and effective for managing HIT, differences exist in their approval status among different countries. For example, although danaparoid is approved in the United States for prevention of deep vein thrombosis following orthopedic surgery, it does not have a specific indication in the United States for prevention or treatment of HIT-associated thrombosis. Nevertheless, its former availability on the U.S. market meant that physicians had the legal right to use the drug for “off-label” treatment of HIT (Preuss and Conour, 1999). According to the U.S. Food and Drug Administration (FDA, 1982), “accepted medical practice often includes drug use that is not reflected in approved labeling.” Danaparoid was withdrawn from the U.S. market by the manufacturer in April 2002 (although it remains available in some other countries [see Chap. 14]). Another example of a drug that has been used “off-label” to treat HIT is bivalirudin, a hirudin derivative approved in the U.S. and Canada for anticoagulation during percutaneous coronary interventions (Francis et al., 2003; see Chap. 17). For theoretical reasons, it seems likely that the pentasaccharide anticoagulant, fondaparinux, will be effective for treating HIT, and it is possible that this agent (now approved in the U.S., Canada, European Union, and elsewhere for antithrombotic prophylaxis in certain orthopedic settings) will see increasing “off-label” use for treating or preventing thrombosis in HIT in the coming years (see also Chaps. 4 and 8).

Argatroban is the only drug currently approved in the United States for treatment and prevention of thrombosis in patients with HIT (lepirudin having been approved in the United States only for treatment of thrombosis complicating HIT). However, protocols do exist for “off-label” treatment of HIT using either danaparoid or lepirudin for isolated HIT; in the case of a
patient with liver impairment, for example, where use of argatroban is relatively contraindicated, the non-approved treatment with danaparoid or lepirudin for a patient with isolated HIT might be safer (see Chaps. 14 and 15). A further consideration is that previous successful experience with a particular agent could favor its use again by a physician who may be reluctant to try a newer drug, with its requisite “learning curve.” In the event that a physician chooses an “off-label” therapy for HIT, she should be prepared to justify the treatment chosen.

An Evolving Standard of Care

Advances in scientific knowledge, of course, can and do have dramatic impact upon the standard of care. This is well illustrated in HIT, in which an emerging consensus regarding diagnosis and treatment began only within the past few years (Warkentin et al., 1998). For example, we are aware of legal cases in which expert opinion held that earlier use of coumarin anticoagulation may have prevented limb loss “within a reasonable degree of medical probability”; ironically, however, subsequent research showed that it actually had been the coumarin anticoagulant that explained the patient’s limb loss, via the syndrome of warfarin-induced venous limb gangrene (Warkentin et al., 1997, 1999). Another example: an expert argued successfully that the patient’s severe episode of venous thromboembolism was not related to HIT, since HIT was only associated with arterial thrombosis. Again, new data emerged in the mid-1990s showing that not only did the spectrum of thrombosis in HIT include venous thrombosis, but that it actually predominated over arterial thrombosis (see Chap. 3).

Currently, an important issue in HIT that is undergoing a major conceptual shift is that of treatment of “isolated HIT”, i.e., HIT recognized on the basis of thrombocytopenia alone. Until recently, it was assumed that prompt cessation of heparin would avoid most complications of HIT. However, since 1996, it has become increasingly evident that patients with serologically confirmed HIT have a high risk for subsequent thrombosis despite stopping heparin, with thrombotic event rates as high as 10% at two-day, 40% at one-week, and 50% at one-month follow-up (Warkentin and Kelton, 1996; Wallis et al., 1999; Warkentin, 2003; see Chaps. 4 and 13). Indeed, the study by Wallis and colleagues (1999) suggests that the highest risk for thrombosis might paradoxically occur in those patients in whom heparin is discontinued relatively soon after onset of thrombocytopenia.

This growing awareness of the unfavorable natural history of isolated HIT has led to new guidelines recommending that physicians consider using alternative anticoagulation to these patients (Hirsh et al., 1998, 2001; Warkentin and Greinacher, 2004; see Chaps. 13–16). Also, in November, 2000, the
U.S. Food and Drug Administration approved a new anticoagulant, argatroban, for the treatment of thrombosis in patients with HIT-associated thrombosis, as well as for the prevention of thrombosis in patients with isolated HIT. Does a new regulatory approval based upon this scientific context now signal a new standard of care? Or should sufficient time elapse for it to become apparent that adoption of this practice has become widespread?

V. SUMMARY

This chapter has summarized some of the medicolegal implications of HIT. The rapid progress of scientific information relating to HIT, as discussed in this book, and the implications of these scientific advances for the evolution of the corresponding standard of care indicate the importance for physicians who use heparin to be familiar with issues of diagnosis and treatment of this adverse effect of heparin.

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I. RISK-BENEFIT ASSESSMENT FOR ANTITHROMBOTIC PROPHYLAXIS

Antithrombotic prophylaxis has become virtually routine in certain perioperative settings, especially for hospital inpatients judged to be at medium or high risk for thrombosis. In low-risk situations, such as minor operations in healthy outpatients, the need for antithrombotic prophylaxis is not uniformly well established. According to the guidelines of 18 surgical societies (AWMF-Leitlinien, 2003):

In case of leg trauma, the grade of measures for thrombosis prophylaxis depends on the severity of the trauma, the degree of immobilization, the duration for healing, and on individual risk factors for thrombosis. For each patient immobilized by trauma, the measures for thrombosis prophylaxis have to be adjusted individually based on a benefit (prophylaxis of thrombosis) to risk (bleeding, HIT) consideration. To date, early mobilization is most effective. In patients with recent trauma and/or lower limb surgery involving immobilization of a joint, pharmacologic thrombosis prophylaxis is recommended, especially in case of additional risk factors for thrombosis.

Prophylaxis against thrombosis thus involves a medical decision involving risk-benefit assessment for an individual patient.
II. CHOICE OF ANTITHROMBOTIC PROPHYLAXIS

The choice of an antithrombotic prophylactic agent leads to the principle of freedom of choice of therapy, meaning that it is primarily the right of the physician to decide on the treatment (Bundesgerichtshof, 1982). This right is limited by the duty to choose the treatment that, benefit being equal, carries the lowest risk for the patient. Choosing a higher-risk agent has to be justified by special circumstances in individual cases or a higher chance of successful treatment (Bundesgerichtshof, 1987). Thus, the doctor violates his or her duty of care if choice of prophylaxis increases patient risk without medical justification.

These considerations have implications for choice of prophylaxis for heparin-induced thrombocytopenia (HIT): If serious side effects, such as HIT, are very rare and associated complications usually not serious, then use of heparin prophylaxis is certainly indicated. (This also applies to the more common entity known as nonimmune heparin-associated thrombocytopenia, which is a benign event without adverse consequences.) Under these circumstances, effective pharmacological prophylaxis using heparin can be justified to decrease the risk to immobilized patients even in an otherwise low-risk category.

However, if the frequency of HIT is greater, even as high as 0.5-5% in certain clinical settings (Greinacher, 1996a; Warkentin et al., 1998) (see Chap. 4), the risk-benefit assessment leads to a different conclusion: prophylaxis with heparin entails significant risk and may no longer be less dangerous than no prophylaxis. Whether there are alternate antithrombotic maneuvers available that are as effective as UFH, but cause HIT less frequently, has to be defined by the medical community.

The decision for or against prophylaxis thus depends on the overall risk/benefit ratio, including the frequency and clinical effect of HIT in a particular patient population, compared with the expected benefit of the heparin in reducing thrombotic events. If, as current opinion suggests, the benefits of a particular heparin preparation outweigh the low risk of HIT, then prophylaxis using heparin may be justified, even in patients at relatively low risk for thrombosis. The relative frequencies of HIT in different clinical settings are discussed in Chap. 4.

It remains debated among medical experts which of the various anticoagulants should be given to an individual patient. Recently this topic has gained increased attention as several new drugs for antithrombotic prophylaxis have become available, e.g., hirudin, danaparoid, and fondaparinux. Current guidelines (AWMF, 2003) do not favor any particular drug or drugs. However, they state that some agents have a very low (or absent) risk of inducing HIT. This does not mean that the new drugs are the medical standard
for antithrombotic prophylaxis. The situation is different, however, if there is a clear indication for one of these new agents, e.g., a patient with a recent history of HIT.

According to the Stufenplanverfahren des Bundesinstituts für Arzneimittel und Medizinprodukte (official measurements to reduce drug-associated risks of the German Federal Institute for Drugs and Medicinal Products), it is necessary to monitor the platelet count in every patient treated with heparin. However, there is no medical consensus about the details of the monitoring. The usual recommendation is one platelet count measurement before commencing the heparin therapy, followed by three measurements a week from day 5 and one weekly count from day 20 (Greinacher, 1996b). However, as new information on the different risks of developing HIT among patient populations emerge from clinical studies, an individualized approach to platelet monitoring taking into account the particular degree of risk may be appropriate (see Chap. 4).

III. THE INFORMED PATIENT

Another important legal aspect is the duty of the physician to inform the patient about the actual risk of thrombosis, including the possible risks and expected benefits of prophylaxis. This information is divided into two parts: procedure-related and therapy-related. One example is the current discussion on pharmacological prophylaxis of thrombosis in outpatients. The Bundesgerichtshof (federal court) (1996a,b,c) is of the opinion that the current discussion in medical science about the danger of thrombosis and the means of pharmacological prophylaxis in outpatients justify a duty to provide information. In these cases, “patient autonomy demands information about possible dangers of treatment and the means available to avoid or alleviate such undesired effects.”

Information about prophylaxis against thrombosis must include hemorrhage; allergic reactions, including HIT and its possible complications; the need for platelet count monitoring; and, for long-term prophylaxis, osteoporosis. Even if the risk of HIT is very low, information about it must be provided. Furthermore, information about certain life-threatening consequences of HIT, such as permanent organ damage and even death, as well as possible countermeasures against these outcomes, must be communicated. Statistical probabilities in mathematical terms are not of major importance: they are, according to the Bundesgerichtshof (1994a), of “minor importance only.” Critical, however, is whether the complications are relatively specific for the treatment intervention in question. Thus, even extremely rare risks need to be mentioned if they are known to be associated specifically with an intervention
and if their occurrence would have noticeable influence on the patient’s life and occupation (Bundesgerichtshof 1994b, 1996a; Oberlandesgericht Hamm, 1995). Consequently, there should be no doubt about the duty to inform about the risks associated with HIT, as these complications are known to be caused by heparin and, therefore, are specific for this intervention. Indeed, the court (Oberlandesgericht Celle) ruled that the physician should have informed the patient, at least briefly, about the risk of HIT (Oberlandesgericht Celle, 2002).

If a doctor recommends using heparin in a low-risk patient outside an approved indication, the patient must also be informed about this. This is because in legal terms, approval of a drug is “like a seal of quality, that independent of the actual quality or safety—can be decisive for the patient’s decision-making in the area where the pharmaceutical law is applicable, so he has to be informed” (Bundesgerichtshof, 1996b).

Physicians are allowed to use drugs to treat diseases for which they have not been approved, provided the medical necessity arises and there is a rationale or precedence for its benefit and reasonable safety in the clinical context (Oberlandesgericht Köln, 1991; Bundessozialgericht, 2002). The regulatory approval of a drug merely creates a state of confidence; that is, the doctor can rely on the fact “that the risk versus benefit ratio is considered favorable in the light of the evidence provided by the manufacturer and the examination of the Bundesinstitut für Arzneimittel und Medizinprodukte” (Weißauer, 1994).

On the other hand, for a doctor using a nonapproved drug or an approved drug outside its approved indication, this state of confidence does not cover him or her in the event of damaging side effects. The physician may then be required to justify the treatment, for example, by showing that the drug has been used with a favorable side effect profile, that reputable specialists recommend its use, and that no alternative treatments are available. This is not an uncommon situation in the management of complications of HIT itself, where an alternative anticoagulant, danaparoid sodium, is often used outside its approved indication (i.e., antithrombotic prophylaxis following orthopedic surgery), or even without any approval in some countries, for the treatment or prevention of thrombosis associated with HIT.

The physician must inform the patient about reasonable precautions in optimizing the safety of a prescribed treatment. This includes educating outpatients about typical signs and symptoms of therapeutic complications. For example, informing patients about the possibility and significance of skin reactions at heparin injection sites as an early manifestation of HIT is appropriate. The main content of the information given to the patient should be documented in writing by the physician, as legal protection in the event of a subsequent adverse event occurring. It is especially prudent to document the
informed consent process if practice outside of usual medical care is contemplated, or if the patient refuses recommended treatment, such as antithrombotic prophylaxis. Incomplete or lacking documentation can lead to a reversal of the burden of proof in favor of the patient if, in the event of a pulmonary embolism or deep vein thrombosis, the doctor has to prove that he had informed the patient.

IV. THE HARMED PATIENT

The mere violation of approved medical practice or failure in the duty to obtain informed consent by themselves do not constitute a punishable offence nor justify claims for compensation. For the physician’s mistake in treating or informing the patient to become punishable, it must be proved that the patient was harmed.

If it can be demonstrated that a patient who was not informed about the risks of HIT would nevertheless have chosen this prophylactic treatment despite its potential risks, then violation of the duty to obtain informed consent becomes irrelevant. This is no longer true if the patient who suffered HIT or any other complication can prove that having known about the risks, he or she would have refused the treatment. In this context, the frequency of HIT among various patient populations could be a factor determining the likelihood that a patient would have given informed consent. For example, a court may determine that a reasonable patient might not have given informed consent to receive unfractionated heparin if the frequency of HIT is shown to be about 5% (e.g., postoperative orthopedic patients), particularly if other therapeutic options exist (e.g., low molecular weight heparin or warfarin).

In case of a treatment error, such as omission to provide antithrombotic prophylaxis, proof is needed that adequate prophylaxis against thrombosis would have prevented the damage. In civil cases of alleged malpractice, the burden of proof required by the plaintiff is relatively low, such as prima facie evidence. In cases of gross negligence, the burden of proof even is reversed: if, for example, there has not been any prophylaxis despite constitutional risk factors and immobilization of a patient, or there was no radiological imaging performed despite suspicion of thrombosis, the physician would have the difficult task to prove that the pulmonary embolism or deep vein thrombosis would have occurred even with prophylaxis.

This required burden of proof in a civil litigation is substantially less than in a criminal case. In criminal law, one of the basic tenets is that the benefit of doubt goes in favor of the accused. This means that negligent treat-
ment, or lack of treatment when indicated, can only be proved to be causative in a criminal case if the “correct” treatment (e.g., prophylaxis against thrombosis) would have prevented death or damage to the patient with a “probability bordering on certainty.” This legal term cannot be defined in precise statistical terms (e.g., 95 or 99%). Rather, by raising a “reasonable doubt,” even a “high” or “very high” probability that an omitted treatment might otherwise have prevented death or disability would not be sufficient to prove the causality of a violation of duty in a criminal court (Bundesgerichtshof, 1988). “Probability bordering on certainty” means the exclusion of reasonable doubts, i.e., doubts which are based upon concrete facts. Given the level of uncertainty in medical knowledge, the required “probability bordering on certainty” in a criminal case (e.g., when the physician is accused to have omitted prophylaxis against thrombosis as a breach of the duty of care) cannot be postulated.

V. COST OF ANTITHROMBOTIC PROPHYLAXIS

Cost is not an argument against indicated prophylaxis. The less expensive but equally effective drug should be used, but social law’s duty to work economically does not legitimize lowering the standard of medical care. Medical duty for care and the duty to work economically are not mutually exclusive, as social law acknowledges the need for approved treatment. However, economic considerations and price have to give way to aspects of effectiveness of a drug and its indication. So, if there is an appropriate indication for prophylaxis for an individual patient’s health, insurance must bear the cost.

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Appendixes
APPENDIX 1. TEN CLINICAL “RULES” FOR DIAGNOSING HIT

Rule 1: A thrombocytopenic patient whose platelet count fall began between days 5 and 10 of heparin treatment (inclusive) should be considered to have HIT unless proved otherwise (first day of heparin use is considered “day 0”).

Rule 2: A rapid fall in the platelet count soon after starting heparin is unlikely to represent HIT unless the patient has received heparin in the recent past, usually within the past 100 days.

Rule 3: A platelet count fall of more than 50% from the postoperative peak between days 5 and 14 after surgery associated with heparin treatment can indicate HIT even if the platelet count remains higher than $150 \times 10^9/L$.

Rule 4: Petechiae and other signs of spontaneous bleeding are not clinical features of HIT, even in patients with very severe thrombocytopenia.

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

Rule 6: Localization of thrombosis in patients with HIT is strongly influenced by independent acute and chronic clinical factors, such as the postoperative state, atherosclerosis, or the location of intravascular catheters in central veins or arteries.

Rule 7: In patients receiving heparin, the more unusual or severe a subsequent thrombotic event, the more likely the thrombosis is caused by HIT.

Rule 8: Venous limb gangrene is characterized by (1) in vivo thrombin generation associated with acute HIT; (2) active deep vein thrombosis in the limb(s) affected by venous gangrene; and (3) a supratherapeutic international normalized ratio (INR) during coumarin anticoagulation. This syndrome can be prevented by: (1) delaying initiation of coumarin anticoagulation during acute HIT until there has been substantial recovery of the platelet count (to at least $100–150 \times 10^9/L$) while receiving an alternative parenteral anticoagulant (e.g., lepirudin, argatroban, danaparoid), and only if the throm-
bosis has clinically improved; (2) initiating coumarin in low, maintenance doses (e.g., 2–5 mg warfarin); (3) ensuring that both parenteral and oral anticoagulant overlap for at least 5 days, with at least the last 2 days in the target therapeutic range; and (4) if applicable, physicians should reverse coumarin anticoagulation with vitamin K in a patient recognized with acute HIT after coumarin therapy has been commenced.

**Rule 9:** Erythematous or necrotizing skin lesions at heparin injection sites should be considered dermal manifestations of the HIT syndrome, irrespective of the platelet count, unless proved otherwise. Patients who develop thrombocytopenia in association with heparin-induced skin lesions are at increased risk for venous and, especially, arterial thrombosis.

**Rule 10:** Any inflammatory, cardiopulmonary, or other unexpected acute event that begins 5–30 min after an intravenous heparin bolus should be considered acute HIT unless proved otherwise. The postbolus platelet count should be measured promptly, and compared with prebolus levels, because the platelet count fall is abrupt and often transient.

*Disclaimer:* The preceding 10 clinical “rules” have been formulated primarily for didactic purposes, and are not intended necessarily to imply any standard of care in relation to their clinical application.
### APPENDIX 2. ESTIMATING THE PRETEST PROBABILITY OF HIT: THE FOUR T'S

Points (0, 1, or 2 for each of 4 categories: maximum possible score = 8)\(^a\)

<table>
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<th>2</th>
<th>1</th>
<th>0</th>
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<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>Nadir, 20–100 (at least 30% fall); or any &gt;50% fall (nadir ≥ 20)</td>
<td>Nadir, 10–19 × 10(^9)/L; or any 30–50% fall; (or &gt;50% fall associated with heart surgery)</td>
<td>Nadir, &lt;10 × 10(^9)/L; or any &lt;30% fall</td>
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<tr>
<td>(acute)</td>
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<tr>
<td><strong>Timing</strong>(^b) of platelet count fall, thrombosis, or other sequelae (first day of heparin course = day 0)</td>
<td>Clear onset between days 5–10; or ≤1 day (if heparin exposure within past 30 days)</td>
<td>Consistent with day 5–10 fall, but not clear (e.g., missing platelet counts); or ≤1 day (heparin exposure within past 31–100 days); or platelet fall after day 10</td>
<td>Platelet count fall ≤4 days without recent heparin exposure</td>
</tr>
<tr>
<td><strong>Thrombosis or other sequelae (e.g., skin lesions, ASR)</strong></td>
<td>New thrombosis; skin necrosis; ASR after iv heparin bolus</td>
<td>Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis (not yet proven); asymptomatic upper-limb DVT</td>
<td>None</td>
</tr>
<tr>
<td><strong>Other cause for thrombocytopenia not evident</strong></td>
<td>No explanation for platelet count fall is evident</td>
<td>Possible other cause is evident</td>
<td>Definite other cause is present</td>
</tr>
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ASR, acute systemic reaction; DVT, deep venous thrombosis.

\(^a\) Pretest probability score: 6–8 = high; 4–5 = intermediate; 0–3 = low.

\(^b\) First day of immunizing heparin exposure considered day 0; the day the platelet count begins to fall is considered the day of onset of thrombocytopenia (it generally takes 1–3 more days until an arbitrary threshold that defines thrombocytopenia is passed). The scoring system shown here has undergone minor modifications from previously published scoring systems (see Chap. 3).
APPENDIX 3. PLATELET COUNT MONITORING FOR HIT

We recommend:

1. Monitoring for typical-onset HIT: stratifying the intensity of platelet count monitoring for HIT based upon its risk
   
   A. Patients at highest risk for HIT (1–5%) (e.g., postoperative patients receiving prophylactic-dose UFH after major surgery): monitoring during heparin therapy, at least every second day from day 4 to day 14.*
   
   Patients receiving therapeutic-dose UFH: platelet count monitoring once daily from day 4 to day 14.*

   B. Patients at intermediate risk for HIT (0.1–1%) (e.g., medical/obstetrical patients receiving prophylactic-dose UFH, or postoperative patients receiving prophylactic-dose LMWH, or postoperative patients receiving intravascular catheter “flushes” with UFH): monitoring during heparin therapy, at least every 2 or 3 days from day 4 to day 14, when practical.

   C. Patients at low risk for HIT (<0.1%) (e.g., medical/obstetrical patients receiving prophylactic- or therapeutic-dose LMWH, or medical patients receiving only intravascular catheter “flushes” with UFH): routine platelet count monitoring is not recommended.

These are draft recommendation (Seventh American College of Chest Physicians Consensus Conference on Antithrombotic Therapy, September 2003). Readers should consult the publication (Warkentin and Greinacher, 2004) to obtain the final recommendations.

2. Monitoring for rapid-onset HIT: for a patient recently exposed to heparin (within the past 100 days), a repeat platelet count within 24 hours following reinitiation of heparin.

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* The crucial time period for monitoring “typical-onset” HIT is between days 4 and 14 (first day of heparin = day zero), where the highest platelet count from day 4 (inclusive) onwards represents the “baseline.” Platelet count monitoring can cease before day 14 when heparin is stopped.

† Once-daily platelet count monitoring recommended as daily blood draws required for aPTT monitoring.

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

§ Monitoring as per “intermediate” risk is appropriate if UFH was given before initiating LMWH.
3. When to suspect HIT:
   A relative (proportional) platelet count fall of 50% or greater that is otherwise clinically unexplained should be considered suspicious for HIT, even if the platelet count nadir remains above $150 \times 10^9$ /L.

   For any patient who develops thrombosis during or within several days after heparin therapy, or who develops an unusual clinical event in association with heparin therapy (e.g., inflammatory or necrotic skin lesions at heparin injection sites, acute systemic reaction post–intravenous heparin therapy), a repeat platelet count should be measured promptly, and compared with recent values.
APPENDIX 4. TREATMENT RECOMMENDATIONS*

Nonimmune Heparin-Induced Thrombocytopenia

Recommendation. Heparin should not be discontinued in patients clinically suspected of having nonimmune heparin-associated thrombocytopenia (grade 2C).

Immune Heparin-Induced Thrombocytopenia

Discontinuation of Heparin for Clinically Suspected HIT

Recommendation. All heparin administration should be discontinued in patients clinically suspected of having (immune) HIT (grade 1C+).

Recommendation. A clearly visible note should be placed above the patient’s bed stating “NO HEPARIN: HIT” (grade 2C).

Recommendation. Heparin can be safely restarted in patients proved not to have HIT antibodies by sensitive activation or antigen assay (grade 2C).

Anticoagulation of the HIT Patient with Thrombosis

Recommendation. Therapeutic-dose anticoagulation with a rapidly acting anticoagulant, e.g., danaparoid (grade 1B), lepirudin (grade 1C+), or argatroban (grade 1C), should be given to a patient with thrombosis complicating acute HIT. Treatment should not be delayed pending laboratory confirmation in a patient strongly suspected of having HIT.

Anticoagulation of the HIT Patient Without Thrombosis

Recommendation. Alternative anticoagulation with an appropriate anticoagulant, such as danaparoid, lepirudin, or argatroban, should be considered in patients with clinically suspected HIT even in the absence of symptomatic thrombosis. Anticoagulation should be continued at least until recovery of the platelet counts to a stable plateau. Patients should undergo imaging studies for lower limb DVT, especially those at highest risk for venous thromboembolism, such as postoperative patients (grade 1C+).

* The grades of recommendation are from Guyatt et al. (2001) and Hirsh et al. (2001) (in Chap. 13) and are described in Chap. 13.
Longer-Term Anticoagulant Management of the HIT Patient with Thrombosis

**Recommendation.** The drug of choice for longer-term anticoagulation of HIT patients is an oral anticoagulant of the coumarin class (e.g., warfarin or phenprocoumon). However, in a patient with acute HIT, oral anticoagulant therapy should be delayed until the patient is adequately anticoagulated with a rapidly acting parenteral anticoagulant, and ideally not until there has been substantial platelet count recovery (at least $\geq 100 \times 10^9/L$). Oral anticoagulants should be started in low maintenance doses (e.g., <5 mg warfarin), with at least 5 days of overlap with the parenteral anticoagulant (including at least 2 days in the target therapeutic range). If applicable, oral or intravenous vitamin K should be given to reverse coumarin anticoagulation in a patient recognized as having acute HIT after coumarin has been commenced (grade 1C).

**Recommendation.** Prothrombin complex concentrates should not be used to reverse coumarin anticoagulation in a patient with acute or recent HIT unless bleeding is otherwise unmanageable (grade 2C).

Reexposure of the HIT Patient to Heparin

*Heparin Reexposure of the Patient with Acute or Recent HIT*

**Recommendation.** Deliberate reexposure to heparin of a patient with acute or recent HIT for diagnostic purposes is not recommended. Rather, the diagnosis should be confirmed by testing acute patient serum or plasma for HIT antibodies using a sensitive activation or antigen assay (grade 1C+).

*Heparin Reexposure of the Patient with a History of Remote HIT*

**Recommendation.** Heparin should not be used for antithrombotic prophylaxis or therapy in a patient with a previous history of HIT, except under special circumstances (e.g., cardiac or vascular surgery) (grade 2C).

Cardiopulmonary Bypass or Vascular Surgery

*Management of the Patient with Acute or Recent HIT*

**Recommendation.** Alternative anticoagulation should be used for heart or vascular surgery in a patient with acute or recent HIT with detectable HIT antibodies. Bivalirudin, lepirudin, or danaparoid are appropriate alternatives for intraoperative anticoagulation, provided that appropriate, rapid-turnaround laboratory monitoring and blood product support to manage potentially severe bleeding complications are available. Another approach is to give heparin together with a potent antiplatelet agent (grade 2C).
Management of the Patient Following Disappearance of HIT Antibodies

Recommendation. In a patient with a previous history of HIT, heart or vascular surgery can be performed using heparin, provided that HIT antibodies are absent (by sensitive assay), and heparin use is restricted to the surgical procedure itself (grade 1C).

HIT During Pregnancy

Recommendation. If available, danaparoid (and possibly daparinux) is preferred for parenteral anticoagulation of pregnant patients with HIT or in those who have a previous history of HIT (grade 2C).

Adjunctive Therapies for HIT

Medical Thrombolysis

Recommendation. Regional or systemic pharmacological thrombolysis should be considered as a treatment adjunct in selected patients with limb-threatening thrombosis or pulmonary embolism with severe cardiovascular compromise (grade 2C).

Surgical Thromboembolectomy

Recommendation. Surgical thromboembolectomy is an appropriate adjunctive treatment for selected patients with limb-threatening large-vessel arterial thromboembolism. Thrombocytopenia is not a contraindication to surgery. An alternative anticoagulant to heparin should be used for intraoperative anticoagulation (grade 1C).

Intravenous Gammaglobulin

Recommendation. ivIgG is a possible adjunctive treatment in selected patients requiring rapid blockade of the Fc receptor–dependent platelet-activating effects of HIT antibodies (e.g., management of patients with cerebral venous thrombosis, severe limb ischemia, or very severe thrombocytopenia) (grade 2C).

Plasmapheresis

Recommendation. Plasmapheresis, using plasma as replacement fluid, may be a useful adjunctive therapy in selected patients with acute HIT and life- or limb-threatening thrombosis who are suspected or proved to have acquired deficiency of one or more natural anticoagulant proteins (grade 2C).

Dextran

Recommendation. Dextran should not be used as primary therapy for acute HIT complicated by thrombosis (grade 1B).
Acetylsalicylic Acid and Dipyridamole

Recommendation. Antiplatelet agents, such as aspirin, may be used as adjuncts to anticoagulant therapy of HIT, particularly in selected patients at high risk for arterial thromboembolism. The possible benefit in preventing arterial thrombosis should be weighed against the potential for increased bleeding (grade 2C).

Platelet Glycoprotein IIb/IIIa Inhibitors

Recommendation. GP IIb/IIIa inhibitors should be considered as experimental treatment in HIT and used with caution if combined with anticoagulant drugs (grade 2C).

Caveats for the Treatment of HIT

Low Molecular Weight Heparin (LMWH)

Recommendation. LMWH should not be used to treat patients with acute HIT (grade 1C).

Oral Anticoagulants (Vitamin K Antagonists)

Recommendation. Oral anticoagulants are contraindicated in patients with acute HIT, unless combined with an agent that reduces thrombin generation (grade 1C+).

Ancrod

Recommendation. Ancrod should not be used to treat patients with HIT (grade 1C).

Platelet Transfusions

Recommendation. Prophylactic platelet transfusions are relatively contraindicated in patients with acute HIT (grade 2C).

Disclaimer. The listed recommendations represent the general views of the editors (as of September 2003) and are intended primarily as an educational guide for physicians who must treat patients with clinically suspected, or serologically proven, HIT. The editors wish to emphasize that these recommendations cannot be applied indiscriminately to all clinical situations, for reasons that can include concomitant clinical factors, diagnostic uncertainty, availability of alternative anticoagulant options, and the availability and turnaround times for HIT antibody and anticoagulant monitoring assays.
# APPENDIX 5. DANAPAROID DOSING SCHEDULES IN HIT PATIENTS

<table>
<thead>
<tr>
<th>Clinical indication</th>
<th>Danaparoid dosing schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous thromboembolism</td>
<td></td>
</tr>
<tr>
<td>Prophylaxis (prior HIT)</td>
<td>750 U sc, b.i.d. or t.i.d.</td>
</tr>
<tr>
<td>Prophylaxis (acute HIT)</td>
<td>Treatment doses (see below) may be appropriate for prophylaxis of acute HIT (see pp. 16, 348–349, 379)</td>
</tr>
<tr>
<td>Venous or arterial thromboembolism:</td>
<td>2250 U iv bolus followed by 400 U/h for 4 h, 300 U/h for 4 h, then 150–200 U/h for ≥5 days, aiming for a plasma anti-Xa level of 0.5–0.8 U/mL</td>
</tr>
<tr>
<td>Treatment (either prior or acute HIT)</td>
<td>Subcutaneous administration schedule: 1500–2250 U sc b.i.d. (given almost 100% bioavailability, 2250 U sc b.i.d. is approximately equal to an iv infusion rate of 200 U/h)</td>
</tr>
<tr>
<td>Embolectomy or other peripheral vascular surgery</td>
<td>Preoperative: 2250 U iv bolus, intraoperative flushes: 750 U in 250 mL saline, using up to 50 mL (see p. 355); postoperative: 750 U sc t.i.d. (low-risk patients) or 150–200 U/h (high-risk patients) beginning at least 6 h after surgery</td>
</tr>
<tr>
<td>Hemodialysis (on alternate days)</td>
<td>3750 U iv before 1st and 2nd dialyses; 3000 U for 3rd dialysis; then 2250 U for subsequent dialyses, aiming for plasma anti-Xa level of &lt;0.3 U/mL predialysis, and 0.5–0.8 U/mL during dialysis (see also Chap. 18).</td>
</tr>
<tr>
<td>Hemofiltration</td>
<td>2250 U iv bolus, followed by 600 U/h for 4 h, then 400 U/h for 4 h, then 200–400 U/h aiming for a plasma anti-Xa level of 0.5–1.0 U/mL (see also Chap. 18).</td>
</tr>
<tr>
<td>Cardiopulmonary bypass surgery (CPB)</td>
<td>125 U/kg iv bolus after thoracotomy; 3 U/mL in priming fluid of apparatus; 7 U/kg/h iv infusion commencing after CPB hookup, and continued until 45 min before expectation of stopping CPB (see also Chap. 19)</td>
</tr>
</tbody>
</table>
### Clinical indication | Danaparoid dosing schedule
---|---
**Cardiac catheterization**<br>Preprocedure: 2250 U iv bolus (3000 U if 75–90 kg and 3750 U if >90 kg)<br><br>**Percutaneous transluminal coronary angioplasty (PTCA) or intra-aortic balloon pump**<br>Preprocedure: bolus as per foregoing;<br>postprocedure: 150–200 U/h for 1–2 days after PTCA (or until removal of balloon pump)<br><br>**Catheter patency**<br>750 U in 50 mL saline, then 5–10 mL per port, or as required<br><br>**Pediatric dosage considerations**<br>Prophylaxis: 10 U/kg sc b.i.d.<br>Treatment: 30 U/kg b.w., iv bolus, then 1.2–2.0 U/kg b.w./h depending upon severity of thrombosis

*Abbreviations*: b.w., body weight; b.i.d., twice daily; iv, intravenous; t.i.d., three times daily.<br>*Compatibility with intravenous solutions*: Danaparoid is compatible for dilution with the following solutions: saline, dextrose, dextrose-saline, Ringer’s, lactated Ringer’s, 10% mannitol.<br>*Preparation of solution for infusion*: One option is to add four ampules containing 3000 U (i.e., 750 anti-Xa U/0.6 mL ampule) of danaparoid to 300 mL of intravenous solution (i.e., a solution that comprises 10 U danaparoid per milliliter of intravenous solution; thus, an infusion rate of 40 mL/h corresponds to a dose of 400 U/h; 20 mL/h to a dose of 200 U/h, and so on.<br>*Adjust iv danaparoid bolus for body weight*: <60 kg, 1500 U; 60–75 kg, 2250 U; 75–90 kg, 3000 U; >90 kg, 3750 U.
APPENDIX 6. DOSING SCHEDULES FOR LEPIRUDIN TREATMENT OF PATIENTS WITH HIT

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bolus&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>IV infusion&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Target aPTT ratio&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIT with isolated thrombocytopenia (dose regimen B in HAT trials)</td>
<td>None&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.10 mg/kg b.w./h&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>HIT and thrombosis (dose regimen A1 in HAT trials)</td>
<td>0.40 mg/kg&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.15 mg/kg b.w./h&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Thrombosis prophylaxis in patients with a history of HIT</td>
<td>15 mg sc b.i.d.&lt;sup&gt;f&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HIT with thrombosis and concomitant thrombolysis (dose regimen A2 in HAT trials)</td>
<td>0.20 mg/kg b.w. iv&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.10 mg/kg b.w./h&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Renal dialysis every alternate day</td>
<td>0.10 mg/kg b.w. iv predialysis</td>
<td>—</td>
<td>2.0–2.5</td>
</tr>
<tr>
<td>Continuous venovenous hemofiltration (CVVH)</td>
<td>0.005 mg/kg b.w./h (initial rate)</td>
<td>—</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>PCI; UA or acute MI without ST elevation</td>
<td>0.40 mg/kg b.w. iv</td>
<td>0.15 mg/kg b.w./h</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Vascular surgery</td>
<td>0.40 mg/kg b.w. iv</td>
<td>0.10 mg/kg/h</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Vascular surgery (intraoperative vessel flushes)</td>
<td>Use up to 250 mL (0.1 mg/mL solution)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Postoperative anticoagulation</td>
<td>0.10 mg/kg b.w./h</td>
<td>—</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>Cardiac surgery using CPB (dose regimen C in HAT trials)</td>
<td>0.25 mg/kg b.w. iv&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.50 mg/min&lt;sup&gt;a,g&lt;/sup&gt;</td>
<td>Monitored by ECT: &gt;2.5 µg/mL before start of CPB, 3.5–4.5 µg/mL during CPB&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Repeat aPTT determinations should be made 4–6 h after any dose adjustment.
Abbreviations: aPTT, activated partial thromboplastin time; b.w., body weight; CPB, cardiopulmonary bypass; ECT, ecarin clotting time; iv, intravenous; MI, myocardial infarction; PCI, percutaneous coronary intervention; UA, unstable angina.

a A maximum body weight of 100 kg should be used for dose calculations.
b Adjust for renal insufficiency.
c The ratio is based on comparison with the normal laboratory mean aPTT. If Actin FS or Neothromtin reagents are used, the aPTT target range is usually 1.5–3.0.
d Used in the HAT-1, HAT-2, and HAT-3 trials.
e This is the author’s recommended starting dose in all HIT patients, unless life- or limb-threatening thrombosis is present.
f Tested in a prospective, randomized trial after orthopedic surgery (Eriksson et al., 1996, 1997).
g Stop 15 min before end of CPB; put 5 mg into CPB after disconnection to avoid clotting of pump.
h The target lepirudin level pre-CPB (>2.5 μg/mL) is lower than the level sought during CPB (3.5–4.5 μg/mL) because of the addition of lepirudin to the pump priming fluid (0.2 mg/kg b.w.).
**APPENDIX 7. DOSING SCHEDULE FOR LEPIRUDIN IN PATIENTS WITH HIT AND RENAL IMPAIRMENT**

<table>
<thead>
<tr>
<th>Creatinine clearance (mL/min)</th>
<th>Serum creatinine, mg/dL (μmol/L)</th>
<th>Adjusted iv infusion rate (% of original dose [see Appendix 6])&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>45–60</td>
<td>1.6–2.0 (141–177)</td>
<td>50</td>
</tr>
<tr>
<td>30–44</td>
<td>2.1–3.0 (178–265)</td>
<td>25</td>
</tr>
<tr>
<td>15–29</td>
<td>3.1–60 (266–530)</td>
<td>10</td>
</tr>
<tr>
<td>&lt;15</td>
<td>&gt;6.0 (&gt;530)</td>
<td>0.005 mg/kg/h b.w. iv (adjusted for aPTT)</td>
</tr>
</tbody>
</table>

Abbreviations: aPTT, activated partial thromboplastin time; b.w., body weight; iv, intravenous.

<sup>a</sup> No initial bolus of lepirudin is given.
APPENDIX 8. DOSING SCHEDULES FOR ARGATROBAN TREATMENT OF PATIENTS WITH HIT (APPROVED INDICATIONS)

<table>
<thead>
<tr>
<th>Clinical use</th>
<th>Bolus(^a)</th>
<th>IV infusion(^a)</th>
<th>Monitoring and adjusting therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis or treatment of thrombosis(^b,c,e)</td>
<td>—</td>
<td>2 µg/kg/min (For hepatically impaired patients, reduce initial dose.(^d))</td>
<td>Dose adjusted (not to exceed 10 µg/kg/min) to achieve steady state aPTT 1.5–3.0 times the baseline value (not to exceed 100 s)(^e,f,g)</td>
</tr>
<tr>
<td>Percutaneous coronary intervention (PCI)(^b,h,i)</td>
<td>350 µg/kg (given over 3–5 min)</td>
<td>25 µg/kg/min</td>
<td>Infusion dose adjusted (15–40 µg/kg/min) to achieve an ACT 300–450 s; additional bolus doses of 150 µg/kg may be given as needed(^j,k)</td>
</tr>
</tbody>
</table>

Abbreviations: HIT, heparin-induced thrombocytopenia; IV, intravenous; aPTT, activated partial thromboplastin time; ACT, activated clotting time; PCI, percutaneous coronary intervention.

\(^a\) Based on patient’s body weight.

\(^b\) Includes patients with active HIT who have isolated thrombocytopenia or associated thrombosis, as well as patients with a documented history of HIT who are no longer thrombocytopenic but require anticoagulation.

\(^c\) Argatroban is approved in the United States as an anticoagulant for prophylaxis or treatment of thrombosis in patients with HIT, and in Canada as an anticoagulant in patients with HIT who in the opinion of their attending physician requires anticoagulation.

\(^d\) For patients with moderate hepatic impairment, an initial dose of 0.5 µg/kg/min is recommended.

\(^e\) The aPTT should be checked at least 2 h after the initiation of argatroban or any dosage change.

\(^f\) For patients in studies ARG-911 and ARG-915, the mean ± SEM dose of argatroban was 1.9 ± 0.1 µg/kg/min.

\(^g\) For transferring a patient to warfarin anticoagulant therapy: After substantial resolution of thrombocytopenia, initiate warfarin therapy using the expected daily dose of warfarin (do not use a loading dose) while maintaining argatroban infusion. At least 5 days of warfarin therapy are required to lower functional prothrombin concentrations to a therapeutic, steady state level. For monitoring the conversion to warfarin during coadministration of argatroban at doses up to 2 µg/kg/min, see text and Fig. 16.7.

\(^h\) Argatroban is approved in the United States as an anticoagulant in patients with or at risk for HIT undergoing PCI. Argatroban has not been evaluated in hepatically impaired patients undergoing PCI. These recommendations do not consider the combination use of argatroban with glycoprotein IIb/IIIa antagonists, wherein lower doses of argatroban (e.g., 250–300 µg/kg bolus followed by infusion of 15 µg/kg/min) have been shown to provide effective anticoagulation with an acceptable bleeding risk (Jang et al., 2003).

\(^i\) Includes percutaneous transluminal coronary angioplasty (balloon angioplasty), stent implantation, and atherectomy; oral aspirin 325 mg should be given 2–24 h prior to PCI.

\(^j\) The ACT should be checked 5–10 min following the initial bolus dose and after any additional bolus dose or change in the infusion rate. In studies ARG-216, ARG-310, and ARG-311, the majority of patients required only one bolus dose during the interventional procedure, and the mean ± SEM dose of argatroban was 23.1 ± 0.7 µg/kg/min.

\(^k\) After the procedure, the sheaths should be removed no sooner than 2 h after discontinuing argatroban and when the ACT is <160 s.
APPENDIX 9. TIMELINES OF AN EPISODE OF HIT

HIT-IgG detectable

HIT-IgG not detectable

Venous
DVT, PE
Venous gangrene
Adrenal infarction
Cerebral sinus thrombosis
Arterial
Lower limb thrombosis
Stroke
MI
Other

Skin lesions at
heparin injection sites
Acute systemic reaction (ASR)
post-heparin bolus

Platelet count x 10^9 (mm^-3)

0 5 d 10 d 2 wk 1 mo 3 mo 6 mo 1 yr 10 yr

Unfractionated Heparin (UFH)

Typical-onset HIT

Delayed-onset HIT

Rapid-onset HIT

can occur if heparin given
and HIT-IgG still present
(usually within 100 days)

Risk of HIT-associated thrombosis

DVT, Deep-vein thrombosis; MI, Myocardial infarction; PE, Pulmonary embolism.

a Associated with coumarin (e.g., warfarin) treatment of DVT complicating HIT.
b Upper limb, mesenteric and renal artery thrombosis can complicate HIT.
c In some patients with rapid-onset HIT, thrombocytopenia may not have complicated the recent heparin exposure.
Index

Abciximab, 443, 485, 492
Abciximab-induced immune thrombocytopenia, 35
ACCP Consensus Conference on Antithrombotic Therapy, 335
Acetylsalicylic acid, 356–357
Acquired antibody-mediated platelet dysfunction, 29–30
Acquired anticoagulant deficiency, 81–83
Acrocyanosis, 321
Activation assays, 273
vs. antigen assays, 293–295
citrate-anticoagulated blood, 285–287
HIT antibodies, 271–287
in vitro cross-reactivity, 302–303
Acute coronary syndrome, 136
anti-PF4-heparin antibodies, 203 lepirudin, 425
Acute myocardial infarction, 444
Acute postinfectious autoimmune thrombocytopenia, 27
Acute thrombocytopenia, 25–42
ADCC, 226
Adenocarcinoma-associated disseminated intravascular coagulation (DIC), 313, 314, 315–318
venous limb gangrene, 315–318
Adenocarcinoma-associated thrombotic endocarditis, 80
Adenosine diphosphate (ADP), 230
Adenosine triphosphate (ATP), 273, 278
ADP, 230
Adrenal hemorrhagic infarction, 80–81
AECA, 254
Africa, 30
AITP, see Autoimmune thrombocytopenic purpura (AITP)
Alpha-methyldopa, 36
Alteplase, 488
American College of Chest Physicians (ACCP) Consensus Conference on Antithrombotic Therapy, 335
Amiodarone, 488
Amiral, Jean, 11–12
Amphotericin B, 488
Ancrod (Arvin), 359
cardiopulmonary bypass, 542–543
Anemia, warm-type autoimmune hemolytic, 28
Angina pectoris, 444
Angiomax, see Bivalirudin (Angiomax)
Annexin V-binding assay, 273

613
Antibivalirudin antibodies, 500

Antibodies
- antibivalirudin, 500
- anticardiolipin, 321
- antichemokine, preexisting, 172
- antiendothelial cell, 254
- antiheparin-PF4, 253
  - acute coronary syndrome, 203
drug-dependent, 41–42
  - immunoglobulin-binding assays, 41
- endothelial cells, HIT, 259–262
- heparin-dependent, pathogenicity, 168–171
- heparin-induced thrombocytopenia (HIT)-associated, beta-1, 3-glucan sulfates, 207–209
- heparin-induced thrombocytopenia (HIT)-IgG, 340
  - IgG, 155
  - PF4-H, platelet activation, 170
  - PF4-H-reactive, 169
- platelet, characterization, 39–42
  - platelet-activating, HIT, 7–8
  - platelet-bound characterization, 40–41
- quinidine-dependent, 42
- Antibody-dependent cellular cytotoxicity (ADCC), 226
- Antibody-independent platelet activation, 152
- Anticardiolipin antibodies, 321
- Antichemokine antibodies, 172
- Anticoagulants
  - acquired deficiency, 81–83
cardiopulmonary bypass, 531–546
  - with danaparoid sodium, 378–379
decision making, 545–546
- HIT, 344
  - oral, 358–359
    - hemodialysis, 521–522
  - U.S. approval, 18
- Antiendothelial cell antibodies (AECA), 254

Antigen assays, 273, 285
  - vs. activation assays, 293–295
  - children, 563–564
cross reactivity, 303
- HIT antibodies, 288–295
- Antigen-presenting cells (APC), 190–191
- Antigens
  - antibody binding, 170
  - fluid-phase, 273
glycoproteins, 26
  - heparin-dependent, HIT, 165–172
  - surface-bound, 273
target, 273
- Antiheparin-PF4 antibodies, 253
  - acute coronary syndrome, 203
- Antiphospholipid antibody syndrome (APLAS), 255, 271, 272, 314, 321–324
  - vs. HIT, 323–324
  - thrombocytopenia, 324
- Antiplatelets, 356–357
- Anti-Xa-inhibiting pentasaccharide, 135–136
- Aortic aneurysms, 37
- Aortic valve replacement, 80
- APC, 190–191
- APLAS, see Antiphospholipid antibody syndrome (APLAS)
- APTT, 401
- Apyrase, 279
- Argatroban-911, 447–448
- Argatroban-915, 448–541
- Argatroban (Novastan), 342–343, 347, 399, 437–467, 581–582
  - breastfeeding, 465–466
cardiopulmonary bypass, 541
  - chemical description, 438
  - chemical structure, 439
  - children, 466, 565
clinical pharmacology, 438–443
clinical use, 444–445
  - conversion to warfarin, 455–460
Index

[Argatroban (Novastan)]
cross-reactivity, 443
distribution, metabolism and excretion, 440–441
dosing and monitoring, 454–460, 455
drug-drug interactions, 442–443
everly, 466
hemodialysis, 520–521
HIT, 445–454
acute anticoagulation, 451–453
cardiopulmonary bypass surgery, 464–465
extracorporeal membrane oxygenation, 464–465
hemodialysis, 463–464
percutaneous coronary intervention, 460–463
prospective studies, 453–454
stroke, 464
isolated HIT, 15–16
vs. lepirudin, 418–419
mechanism of action, 438–439
pharmacokinetic-pharmacodynamic relationship, 441
pregnancy, 465–466
reexposure, 453
reversal, 444
special populations, 441–442
therapy duration, 454–455
Arixtra, see Fondaparinux (Arixtra)
Arterial obstructive disease, peripheral, 444
Arterial thromboembolism, 83–84
danaparoid sodium, 375–376
dosing schedules, 377
prophylaxis, 380
Arvin, 359
cardiopulmonary bypass, 542–543
Aspirin, 485
hemodialysis, 512
Asserachrom, 290, 292
Atherogenesis, 258
ATP, 273, 278
Atropine, 443
Autoantigens, cryptic, 166
Autoimmune disease, 238
Autoimmune hemolytic anemia, warmtype, 28
Autoimmune thrombocytopenia acute postinfectious, 27
drug-induced, 36
idiopathic, 36
Autoimmune thrombocytopenic purpura (AITP), 25–26, 26–30
chronic, 27–28
clinical manifestations, 27–28
diagnosis, 28–29
pathogenesis, 26–27
secondary, 27
therapy, 29
Azathioprine, 29

BAT, 489, 491
Best, Charles H., 1
Beta-1,3-glucan sulfates anticoagulant activity, 207–209
HIT-associated antibodies, 207–209
BetaTG, 171
Beta-thromboglobulin (betaTG), 171
Bivalirudin (Angiomax), 343, 399, 475–501
administration, 485
adverse effects, 488–489
cardiopulmonary bypass, 537–541
chemistry, 476–478
clinical use, 489–496
cost, 500–501
dosage, 481–484
HIT, 19, 496–500
monitoring, 485–486
pharmacodynamics, 479–481
pharmacokinetics, 478–479
pharmacology, 478
reversal, 488
Bivalirudin Angioplasty Trial (BAT), 489, 491
Black population of Africa, 30
Blue toe syndrome, 321
Bone marrow transplantation, 27
HIT, 93
Breastfeeding, 465–466
Burden of proof in malpractice, 576–577
CACHET, 492
Calcium, 152
Cancer, consumptive thrombohemorrhagic disorders, 37
Carbohydrate-based heparin alternatives, 209–210
Carbohydrates, sulfated
HIT, 207
PF4, 198–202
Cardiac catheterization, 377
Cardiac surgery, 134–135
Cardiopulmonary bypass
ancrod (Arvin), 542–543
anticoagulation, 381–382, 531–546
argatroban, 541
HIT, 464–465
bivalirudin (Angiomax), 494, 498–499, 537–541
danaparoid sodium, 532–534
dosing schedules, 377
HIT, 351–354
lepirudin, 425–426
platelet inhibition, 541–542
recombinant hirudin (lepirudin), 534–536
CDR3, 190
Cellular immune response, 189–191
Cerebral venous (dural sinus) thrombosis, 79–80
Chemokine receptor, 257
Children
antigen assays, 563–564
argatroban, 466
danaparoid sodium, 384–385
dosing schedules, 377
EIA, 562–563
[Children]
HIT, 91–93, 353–354, 553–567
clinical presentation, 555–562
frequency, 553–555
laboratory testing, 562–564
pathophysiology, 553
prevention, 566–567
therapy, 564–566
lepirudin, 427–428
low molecular weight heparin (LMWH), 566–567
Chlorpromazine, 488
Chong, Beng, 9
CHOOSE, 499
Chronic autoimmune thrombocytopenic purpura (AITP), 27–28
Circulated platelet-rich plasma (c-PRP) activation assays
HIT antibodies, 285–286
vs. washed platelet assays, 287, 302–303
aggregation assays, disadvantages, 286
CISN, 76, 87–88, 336
Citrate-anticoagulated blood activation assays, 285–287
Citrate anticoagulation, regional, 523
Citrated-anticoagulated blood, 287
Classic coumarin-induced skin necrosis (CISN), 87–88
Clexane, 119
Clinical heparin-induced thrombocytopenia (HIT), 204–205
Clinical practice guidelines, 577
Clinicopathologic syndrome, 108
Commercial low molecular weight heparin (LMWH), 206
Congenital hypercoagulability, 83
Consumptive thrombohemorrhagic disorders, 36–38
clinical disorders, 36–37
diagnosis, 37–38
pathogenesis, 36
Coronary artery bypass surgery, 
  see Cardiopulmonary bypass
Corticosteroids, 32–33
Coumarin, 349
  children, 565–566
  conversion to, 455–460
  multiple digital necrosis, 80
  transitioning to, 406
  venous limb gangrene, 13–14, 75–79, 77
Coumarin-induced skin necrosis (CISN), 76, 87–88, 336
C-PRP, see Circulated platelet-rich plasma (c-PRP)
Crohn’s disease, 27
Cross reactivity antigen assays, 303
Cryptic autoantigens, 166
Cyclic thrombocytopenia, 30
Cytopenia, idiopathic autoimmune, 36
Dalteparin, 119
  adverse effects, 389
  with anticoagulants, 378–379
  availability, 391
  cardiopulmonary bypass, 532–534
  chemistry, 372
  children, 384–385, 564–565
  clinical use, 374–391
  dosing schedules, 377
  hemodialysis, 513–515
HIT
  antibodies, 386–389
  thrombosis, 12
  laboratory monitoring, 385–386
  vs. lepirudin, 419–420
  low-dose, 336
  pharmacodynamics, 373–374
  pharmacology, 373
  pregnancy, 384–385
  prophylaxis, 379
Deep venous thrombosis (DVT), 12
  bivalirudin (Angiomax), 489–490, 490
  danaparoid sodium, 374
  lower limb, 73–75
  upper limb, 75
Dermatan sulfate, 343
  hemodialysis, 522
Desmopressin, 407
Dexamethasone, 485
Dextran, 356
Diabetic ketoacidosis, 314, 318–321, 322
Diazepam, 488
DIC, see Disseminated intravascular coagulation (DIC)
Digoxin, 485
Diltiazem, 443
Diphenhydramine, 443, 485
Dipyridamole, 356–357
Disseminated intravascular coagulation (DIC), 5, 28, 81–83
  adenocarcinoma-associated, 313, 314, 315–318
  venous limb gangrene, 315–318
  argatroban, 465
  clinical manifestations, 82
  consumptive thrombohemorrhagic disorders, 37
  danaparoid sodium, 374
DIT, 33–36
  drugs inducing, 34
  therapy, 34
Dixon, R.H., 5
Dobutamine, 443, 485
Dopamine, 443, 485
Drug-dependent antibodies, 41–42
  immunoglobulin-binding assays, 41
Drug-induced autoimmune thrombocytopenia, 36
Drug-induced immune thrombocytopenia (DIT), 33–36
  drugs inducing, 34
  therapy, 34
Dural sinus thrombosis, 79–80, 80
DVT, see Deep venous thrombosis (DVT)

Ecarin clotting time (ECT), 401–402
ECT, 401–402
EIA, see Enzyme immunoassay (EIA)
ELAM, 252
Elderly, argatroban, 466
Embolectomy, danaparoid sodium, 377
Embolism, pulmonary, 12
Endarterectomy, 495
Endocarditis
adenocarcinoma-associated thrombotic, 80
infective, 314, 327
Endothelial cell injury, immune, 254–255
Endothelial leukocyte adhesion molecule (ELAM), 252
Endothelium, 255–256
antibodies, HIT, 259–262
hemostasis, 252–254
platelet factor 4, 256–258
Enoxaparin, 119
Enzyme immunoassay (EIA), 107, 132, 133, 288
children, 562–563
fluid-phase, 289–291
fluid-phase PF4-heparin, schematic figure, 291
Enzyme-linked immunosorbent assay (ELISA), 186, 401–402
Epinephrine, 485
Epoprostenol, 352
Eptifibatide, 443, 485
Esmolol, 485
Evan’s syndrome, 28
EVOLUTION, 499
Expert witnesses, malpractice, 576
Extracorporeal circuit, 84–85
Extracorporeal membrane oxygenation, 464–465

Factor Xa (FXa), 199
Familial thrombocytopenia, 28
Fcγ1a receptor-mediated platelet activation, 227–232
Fcγ1a receptor-mediated signal transduction, 230–232
FcγR111a, 224–227
Arg/His131 polymorphism, 234–235
HIT, 232–236
plasma-soluble, 233
FcγR111a polymorphism
Arg/His131 variant, 237
autoimmune disease, 238
disease, 236–241
HIT, 238–241
infectious disease, 238
Fc receptor blockade, washed platelet assays, 284
Femoral artery, white clots, 321
Fentanyl, 443
Fibronectin, 152
Flow cytometry, 228–229, 273, 285
Fluid-phase antigen, 273
Fluid-phase enzyme immunoassay (EIA), 289–291
Fluid-phase PF4-heparin-enzyme immunoassay (EIA), 291
Fondaparinux (Arixtra), 17, 135–136, 210, 211–212, 343, 581
Furosemide, 485
FXa, 199

GAG, 179–180, 183, 198, 253
Glucan sulfates
anticoagulant activity, 207–209
HIT-associated antibodies, 207–209
Glycoproteins, 27
antigens, 26
inhibitor inducing immune thrombocytopenia, 35
Glycosaminoglycans (GAG), 179–180, 183, 198, 253

Graft rejection, 255
thrombosis, 84–85
Harenberg, Job, 12
HAS, 489, 491
HAT, 107
  frequency variability, 108
  nonimmune, 154, 338–339
HAT-1, 407, 409–412
  efficacy, 409–412
  meta-analysis, 415–418
  safety, 412
HAT-2, 407, 412–414
  efficacy, 412–414
  meta-analysis, 415–418
  safety, 414
HAT-3, 407, 414–415
  meta-analysis, 417–418
Hemodialysis
  argatroban, 444
  HIT, 463–464
danaparoid sodium, 383–384
dosing schedules, 377
HIT, 509–523
  clinical presentation, 510–511
  management, 511–523
lepirudin, 407, 426–427
without anticoagulant, 512–513
Hemofiltration
danaparoid sodium, 383–384
dosing schedules, 377
lepirudin, 407
Hemoglobinuria, paroxysmal
  nocturnal, 314, 327
Hemolytic uremic syndrome (HUS), 38
Hemostasis, endothelium, 252–254
Heparan sulfate-containing proteoglycans (HSPG), 255–256
Heparin, 255–256, 485
cardiopulmonary bypass, 543–544
causing thrombosis, 1–5
discontinuation, 336
discovery, 1
incidental exposure, 63–64
nonimmune
  platelets, 157–158
  [Heparin]
    PF4, 179–180
    platelet activation
      antibody-independent, 152
      platelet-related prohemorrhagic effects, 153–154
      platelets, 149–152
      repeat use
      HIT, 62–63
      structure, 198–199
Heparin-associated thrombocytopenia (HAT), 107
  frequency variability, 108
  nonimmune, 154, 338–339
Heparin-Associated Thrombocytopenia (HAT)-1, 407, 409–412
  efficacy, 409–412
  meta-analysis, 415–418
  safety, 412
Heparin-Associated Thrombocytopenia (HAT)-2, 407, 412–414
  efficacy, 412–414
  meta-analysis, 415–418
  safety, 414
Heparin-Associated Thrombocytopenia (HAT)-3, 407, 414–415
  meta-analysis, 417–418
Heparin-coated devices, 121–122
Heparin-dependent antibodies, 168–171
Heparin-dependent antigens, 165–172
Heparin-dependent platelet activation endpoints, 276–278
interpretation, 276
test conditions, 275–276
Heparin-induced platelet activation (HIPA) assay, 272, 273, 277, 278, 292, 293–295, 298–299
heparin-induced, 272, 273, 277, 278, 292, 293–295, 298–299
Heparin-induced skin lesions at subcutaneous heparin injection sites, 85
Heparin-induced thrombocytopenia (HIT), 54–70, 262–263, see also
Pseudo-heparin-induced thrombocytopenia (HIT)
animal models, 235–236
antibodies
  activation assays, 271–287
  antigen assays, 288–295
  beta-1,3-glucan sulfates, 207–209
  clinical HIT, 204–205
c-PRP, 285–286
cross-reactivity, 205–209
danaparoid sodium, 386–389
epitopes recognition, 182–189
heparin, 130–131
immune complexes, 202–203
low molecular weight heparin (LMWH), 205–207
PF4-heparin complex, 202–205
test discrepancies, 296
washed platelets, 279–282
antibody-containing immune complexes, 204
antibody seroconversion, population-based studies, 132–136
anticoagulants, 344
vs. APLAS, 323–324
bone marrow transplantation, 93
cardiac surgery, 134–135
cardiopulmonary bypass, 351–354
children, 91–93, 353–354, 553–567
clinical
  HIT antibodies, 204–205
  picture, 53–95
complications, 85–87
  cardiac and neurological, 91
diagnosis, 579–580
doubts, 300
  sensitivity-specificity tradeoffs, 301
endothelial cells antibodies, 259–262
estimating pretest probability, 93–95
FcγR111a, 232–236

Heparin-induced thrombocytopenia (HIT)
FcγR11a polymorphisms, 238–241
frequency, 107–138
incidental UFH flushes, 120–121
medical patients, 112–114, 117
platelet count monitoring, 136–138
pregnancy, 119–120
surgical patients, 115–116, 117
variability, 108, 110
hemodialysis, 509–523
heparin
  discontinuation, 70, 341
  repeat use, 62–63, 350–351
  variable duration, 122
heparin-coated devices, 121–122
heparin-dependent antigens, 165–172
heparin dose-dependence, 123
heparin-platelet interactions, 154–157
history, 1–19
hypercoagulable state, 70
IgG, 259, 261, 340
immune
  frequency, 111–123
  therapy, 339–351
  immune basis, 4–5
  immune vascular injury, 251–263
  induction, 197
  laboratory testing, 9–11, 271–304
  classification, 273
legal aspects, 573–592
medical thrombolysis, 354
molecular immunopathogenesis, 179–192
monocyte Fcgamma receptors, 236
natural history, 71–72
neonates, 91–93
nonimmune, 8–9
type 1 vs. type 2, 9
orthopedic patients, LMWH vs. UFH, 118–119
paradoxical thrombosis, 5–8
<table>
<thead>
<tr>
<th>Term</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin-induced thrombocytopenia (HIT)</td>
<td>149–158, 339–341</td>
</tr>
<tr>
<td>pathogenesis</td>
<td>149–158, 339–341</td>
</tr>
<tr>
<td>platelet activation, dynamic model</td>
<td>232–233</td>
</tr>
<tr>
<td>platelet factor-4-heparin</td>
<td>11–12</td>
</tr>
<tr>
<td>platelet Fc receptor</td>
<td>223–241</td>
</tr>
<tr>
<td>pregnancy</td>
<td>91, 353</td>
</tr>
<tr>
<td>prospective studies</td>
<td>10–11</td>
</tr>
<tr>
<td>risk reduction</td>
<td>17–19</td>
</tr>
<tr>
<td>sequelae</td>
<td>54</td>
</tr>
<tr>
<td>subclinical, antibody seroconversion</td>
<td>137</td>
</tr>
<tr>
<td>sulfated carbohydrates</td>
<td>207</td>
</tr>
<tr>
<td>sulfated polysaccharides</td>
<td>122, 197–213</td>
</tr>
<tr>
<td>test</td>
<td>295–302</td>
</tr>
<tr>
<td>interpretation</td>
<td>295–302</td>
</tr>
<tr>
<td>thrombocytopenia</td>
<td>54–70</td>
</tr>
<tr>
<td>defined</td>
<td>67</td>
</tr>
<tr>
<td>delayed onset</td>
<td>64–65</td>
</tr>
<tr>
<td>diminishing risk</td>
<td>59</td>
</tr>
<tr>
<td>platelet count monitoring</td>
<td>67</td>
</tr>
<tr>
<td>rapid onset</td>
<td>59–60</td>
</tr>
<tr>
<td>severity</td>
<td>66–70</td>
</tr>
<tr>
<td>timing</td>
<td>54–65</td>
</tr>
<tr>
<td>typical onset</td>
<td>54–59</td>
</tr>
<tr>
<td>thromboembolectomy</td>
<td>354–355</td>
</tr>
<tr>
<td>thrombosis</td>
<td>70–85, 83, 128</td>
</tr>
<tr>
<td>anticoagulation</td>
<td>341–348</td>
</tr>
<tr>
<td>frequency</td>
<td>123–132</td>
</tr>
<tr>
<td>heparin resistance</td>
<td>90</td>
</tr>
<tr>
<td>longer-term anticoagulants</td>
<td>349–350</td>
</tr>
<tr>
<td>pathogenesis</td>
<td>72–73</td>
</tr>
<tr>
<td>treatment</td>
<td>12–14</td>
</tr>
<tr>
<td>without hemorrhage</td>
<td>70</td>
</tr>
<tr>
<td>without thrombocytopenia</td>
<td>69–70</td>
</tr>
<tr>
<td>thrombotic and hemorrhagic manifestations</td>
<td>5–7</td>
</tr>
<tr>
<td>thrombotic complications</td>
<td>71–72</td>
</tr>
<tr>
<td>transient antibodies</td>
<td>60–61</td>
</tr>
<tr>
<td>treatment</td>
<td>335–360, 580–583</td>
</tr>
<tr>
<td>paradoxes</td>
<td>336, 580–581</td>
</tr>
<tr>
<td>Heparin-induced thrombocytopenia (HIT)</td>
<td>unrecognized, 109</td>
</tr>
<tr>
<td>vascular surgery</td>
<td>351–354</td>
</tr>
<tr>
<td>venous thrombosis</td>
<td>73–83</td>
</tr>
<tr>
<td>without thrombosis, anticoagulation</td>
<td>348–349</td>
</tr>
<tr>
<td>Heparin-induced thrombocytopena (HIT)-associated thrombosis (HITT), 262</td>
<td></td>
</tr>
<tr>
<td>monocyes</td>
<td>262</td>
</tr>
<tr>
<td>Heparin induced thrombocytopena thrombosis syndrome (HITTS), 71–72</td>
<td></td>
</tr>
<tr>
<td>Heparinization, regional</td>
<td>512</td>
</tr>
<tr>
<td>Heparin mimetics</td>
<td>212</td>
</tr>
<tr>
<td>Heparin-platelet factor 4 (HPF4)</td>
<td>156, 171</td>
</tr>
<tr>
<td>Heparin-platelet interactions</td>
<td>154–157</td>
</tr>
<tr>
<td>Heparin sulfate</td>
<td>181</td>
</tr>
<tr>
<td>HIPA assay, see Heparin-induced platelet activation (HIPA) assay</td>
<td></td>
</tr>
<tr>
<td>Hirudin</td>
<td>152, 397–407, 399</td>
</tr>
<tr>
<td>chemistry</td>
<td>397–398</td>
</tr>
<tr>
<td>pharmacokinetics</td>
<td>398–401</td>
</tr>
<tr>
<td>pharmacology</td>
<td>398</td>
</tr>
<tr>
<td>Hirudo medicinalis</td>
<td>13</td>
</tr>
<tr>
<td>Hirugen</td>
<td>399</td>
</tr>
<tr>
<td>Hirulog Angioplasty Study (HAS), 489, 491</td>
<td></td>
</tr>
<tr>
<td>HIT, see Heparin-induced thrombocytopena (HIT)</td>
<td></td>
</tr>
<tr>
<td>HITTS, 71–72</td>
<td></td>
</tr>
<tr>
<td>HIV, 27</td>
<td></td>
</tr>
<tr>
<td>HPF4, 156, 171</td>
<td></td>
</tr>
<tr>
<td>H/PF4-PaGIA, 292</td>
<td></td>
</tr>
<tr>
<td>HSPG, 255–256</td>
<td></td>
</tr>
<tr>
<td>Human immunodeficiency virus (HIV), 27</td>
<td></td>
</tr>
<tr>
<td>Human umbilical vein (HUVEC), 255, 260</td>
<td></td>
</tr>
<tr>
<td>HUS, 38</td>
<td></td>
</tr>
<tr>
<td>HUVEC, 255, 260</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>443</td>
</tr>
<tr>
<td>Hypercoagulability, congenital, 83</td>
<td></td>
</tr>
</tbody>
</table>
Idiopathic autoimmune cytopenia, 36
Idiopathic immune thrombocytopenia, 27
Idraparinux, 211–212
IgA, 170, 203
IgG, see Immunoglobulin G (IgG)
IgM, 170, 191, 203, 260
IL-8, see Interleukin-8 (IL-8)
IL-10, 181
ILP, 118
Immune complexes, 202–203
Immune endothelial cell injury, 254–255
Immune heparin-induced thrombocytopenia (HIT)
frequency, 111–123
therapy, 339–351
Immune thrombocytopenia
abciximab-induced, 35
drug-induced, 33–36
drugs inducing, 34
therapy, 34
idiopathic, 27
passive, 33
Immune vascular injury
HIT, 251–263
HIT-associated thrombosis, 262
Immunofluorescence assays, 303
Immunoglobulin A (IgA), 170, 203
Immunoglobulin G (IgG), 203, 260, 355
agonists, 227–232
antibodies, 155
HIT, 6–7
plasma, 233–234
posttransfusion purpura (PTP), 32–33
Immunoglobulin M (IgM), 170, 191, 203, 260
Immunoreceptor tyrosine-based activation motif (ITAM), 226
Incidental unfractionated heparin (UFH) flushes, 120–121
Infectious disease, 238
Infective endocarditis, 314, 327
Informed consent, 578

Interferon-gamma-inducible protein (IP-10), 181
Interleukin-8 (IL-8), 171, 172, 181, 184, 187, 232–233
Interleukin-10 (IL-10), 181
Intravenous heparin bolus, 89–90
IP-10, 181
Isolated heparin-induced thrombocytopenia (HIT), 582–583
natural history, 14–15, 128–132
therapeutic-dose anticoagulation, 16
treatment, 14–16
Isolated limb perfusion (ILP), 118
ITAM, 226
Kasabach-Merritt syndrome, 28, 37
Kelton, John, 10
Leech, 13
Legal aspects, 573–592
acute coronary syndrome, 425
allergic reactions, 423–425
antibodies, 422–423
anticoagulation tests, 401–405
aPTT ratios, 400
vs. argatroban, 418–419
cardiopulmonary bypass, 425–426, 534–536
children, 427–428, 565
clinical studies, 407–409
vs. danaparoid sodium, 419–420
dosing, 402, 405–406
hemodialysis, 426–427, 515–516
percutaneous coronary intervention, 425
postmarketing drug monitoring program, 418–420
pregnancy, 427
removal of, 406–407
thrombosis, 13
transitioning to warfarin, 406
vascular surgery, 425–426
Lidocaine, 485
### Index

<table>
<thead>
<tr>
<th>Term</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litigation</td>
<td>573</td>
</tr>
<tr>
<td>Livedo reticularis</td>
<td>88–89, 321</td>
</tr>
<tr>
<td>LMWH, see Low molecular weight heparin (LMWH)</td>
<td></td>
</tr>
<tr>
<td>Low-dose danaparoid</td>
<td>336</td>
</tr>
<tr>
<td>Lower limb deep venous thrombosis (DVT)</td>
<td>73–75</td>
</tr>
<tr>
<td>Low molecular weight heparin (LMWH)</td>
<td>17, 180, 336, 357–358, 580–581, 357–358, 580–581</td>
</tr>
<tr>
<td>antibodies</td>
<td>205–207</td>
</tr>
<tr>
<td>children</td>
<td>566–567</td>
</tr>
<tr>
<td>commercial characteristics</td>
<td>206</td>
</tr>
<tr>
<td>early vs. late onset thrombocytopenia</td>
<td>111</td>
</tr>
<tr>
<td>hemodialysis</td>
<td>512</td>
</tr>
<tr>
<td>vs. UFH, orthopedic patients</td>
<td>118–119</td>
</tr>
<tr>
<td>Luminography</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>278</td>
</tr>
<tr>
<td>ATP release</td>
<td>273</td>
</tr>
<tr>
<td>Lupus anticoagulants</td>
<td>321</td>
</tr>
<tr>
<td>Lupus anticoagulant syndrome</td>
<td>271, 272, 321–324</td>
</tr>
<tr>
<td>Lymphoproliferative disorders</td>
<td>27</td>
</tr>
<tr>
<td>MAIPA assay</td>
<td>39–40, 301</td>
</tr>
<tr>
<td>Malpractice</td>
<td>573</td>
</tr>
<tr>
<td>burden of proof</td>
<td>576–577</td>
</tr>
<tr>
<td>expert witnesses</td>
<td>576</td>
</tr>
<tr>
<td>standard of care</td>
<td>575–576</td>
</tr>
<tr>
<td>McLean, Jay</td>
<td>1</td>
</tr>
<tr>
<td>Medicolegal system</td>
<td>575–577</td>
</tr>
<tr>
<td>Melphalan</td>
<td>118</td>
</tr>
<tr>
<td>Meningococcemia</td>
<td>326</td>
</tr>
<tr>
<td>Methyladpeta</td>
<td>36</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>443</td>
</tr>
<tr>
<td>Meyer, Dominique</td>
<td>11–12</td>
</tr>
<tr>
<td>Microparticles</td>
<td>228–229</td>
</tr>
<tr>
<td>platelet-derived</td>
<td>279</td>
</tr>
<tr>
<td>Midazolam</td>
<td>443</td>
</tr>
<tr>
<td>Monoclonal antibody immobilization of platelet antigens (MAIPA) assay</td>
<td>39–40</td>
</tr>
<tr>
<td>thrombocytopenia</td>
<td>301</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
</tr>
<tr>
<td>Fcy receptors</td>
<td>236</td>
</tr>
<tr>
<td>thrombosis</td>
<td>262</td>
</tr>
<tr>
<td>Morphine</td>
<td>443, 485</td>
</tr>
<tr>
<td>Mustard, Fraser</td>
<td>10</td>
</tr>
<tr>
<td>Myeloproliferative disease</td>
<td>80</td>
</tr>
<tr>
<td>Nadroparin</td>
<td>119</td>
</tr>
<tr>
<td>Nafamostat mesilate, hemodialysis</td>
<td>522</td>
</tr>
<tr>
<td>NAP-2</td>
<td>171, 181, 187</td>
</tr>
<tr>
<td>pre-existing antibodies</td>
<td>172</td>
</tr>
<tr>
<td>Negligence, professional</td>
<td>573</td>
</tr>
<tr>
<td>Neoantigens</td>
<td>166</td>
</tr>
<tr>
<td>Neonates, HIT</td>
<td>91–93</td>
</tr>
<tr>
<td>Neutrophil-activating peptide 2 (NAP-2)</td>
<td>171, 181, 187</td>
</tr>
<tr>
<td>pre-existing antibodies</td>
<td>172</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>252</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>485</td>
</tr>
<tr>
<td>Nonidiosyncratic heparin-induced platelet activation</td>
<td>152–153</td>
</tr>
<tr>
<td>Nonimmune heparin, platelets</td>
<td>157–158</td>
</tr>
<tr>
<td>Nonimmune heparin-associated thrombocytopenia (nonimmune HAT)</td>
<td>154, 338–339</td>
</tr>
<tr>
<td>Nonimmune heparin-induced thrombocytopenia (HIT)</td>
<td>8–9</td>
</tr>
<tr>
<td>type 1 vs. type 2</td>
<td>9</td>
</tr>
<tr>
<td>Nonimmune heparin-platelet interactions</td>
<td>149–158</td>
</tr>
<tr>
<td>Novastan, see Argatroban (Novastan)</td>
<td></td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>211–212</td>
</tr>
<tr>
<td>Onyalai</td>
<td>30</td>
</tr>
<tr>
<td>Oral anticoagulants</td>
<td>358–359</td>
</tr>
<tr>
<td>hemodialysis</td>
<td>521–522</td>
</tr>
<tr>
<td>Orgaran, see Danaparoid sodium (Orgaran)</td>
<td></td>
</tr>
<tr>
<td>PAF</td>
<td>252</td>
</tr>
<tr>
<td>PAI-1</td>
<td>495</td>
</tr>
<tr>
<td>Paradoxic thrombosis</td>
<td>5–8</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>314, 327</td>
</tr>
</tbody>
</table>

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MARCEL DEkker, Inc.
270 Madison Avenue, New York, New York 10016
Particle gel immunoassay (ID-H/PF4 test), 273, 291–293
Passive immune thrombocytopenia, 33
PAT, 273
PBMC, 190
PBP, 181
PCI, see Percutaneous coronary intervention (PCI)
Pentasaccharides, 210–211
anti-Xa-inhibiting, 135–136
Percutaneous coronary intervention (PCI)
argatroban, 444, 460–463
bivalirudin (Angiomax), 490–493, 497–498
lepirudin, 425
Percutaneous transluminal coronary angioplasty (PTCA)
bivalirudin (Angiomax), 494
danaparoid sodium, 377
Peripheral arterial obstructive disease, 444
Peripheral blood mononuclear cells (PBMC), 190
PF4, see Platelet factor 4 (PF4)
PF-4H, see Platelet factor 4-heparin (PF4-H)
PF4-M2, 184
Phenprocoumon, see Coumarin
Phenylephrine, 443
Phlegmasia, 14
Plasma immunoglobulin G (IgG), 233–234
heat inactivation, 279
Plasmapheresis, 355–356
Plasma-soluble FcγR111a, 233
Plasminogen activator inhibitor-1 (PAI-1)
bivalirudin (Angiomax), 495
Platelet-activating antibodies, 7–8
Platelet-activating factor (PAF), 252
Platelet activation
Fcγ1a receptor-mediated, 227–232
heparin-dependent
[Platelet activation]
endpoints, 276–278
interpretation, 276
test conditions, 275–276
interpretation by HIT serum in absence of added heparin, 284–285
PF4-H antibodies, 170
Platelet aggregation assays, 9–10
Platelet aggregation test (PAT), 273
Platelet antibodies, 39–42
Platelet autoantibodies and alloantibodies, 39–40
Platelet basic protein (PBP), 181
Platelet-bound antibodies, 40–41
Platelet count, monitoring, 578–579
frequency, 136–138
Platelet-derived microparticle generation, 279
Platelet dysfunction, acquired antibody-mediated, 29–30
Platelet factor 4 (PF4), 155, 165, 262
antigenicity, 166–168
endothelium, 256–258
heparin, 179–180
polyvinylsulfonate antigen assay, 289
structure, 198
sulfated carbohydrates, 198–202
sulfated polysaccharides, 199–201
in vivo, 201–202
Platelet factor 4-heparin (PF4-H), 201, 223, 271
antibodies, 202–205
platelet activation, 170
reactive, 169
Platelet factor 4-heparin enzyme immunoassay (PF4-H-EIA), 273, 293–295, 298–299
thrombocytopenia, 298–299
Platelet factor 4-M2 (PF4-M2), 184
Platelet Fc receptor, 223–241
Platelet glycoprotein IIb/IIIa inhibitors, 357
Platelet inhibition, cardiopulmonary bypass, 541–542
Platelet microparticle assay, 273
Platelet-rich plasma (PRP), 273, 275, 287
Platelet-rich plasma (PRP) aggregation test, 233–234
Platelets
  circulated platelet-rich plasma (c-PRP), 273
  heparin, 149–152
  nonimmune heparin, 157–158
  transfusions, 336, 359–360, 580
Platelet specific alloantigens, posttransfusion purpura (PTP), 31
Polyanion, 182–186
Polysaccharides, sulfated
  HIT, 122, 197–213
  PF4, 201–202
Porcine mucosal unfractionated heparin (UFH), 117–118
Postoperative orthopedic patients, 56–57
Posttransfusion purpura (PTP), 31–33, 314, 327–328
  clinical picture, 32
  diagnosis, 32
  pathogenesis, 31–32
  therapy, 32–33
Potassium, 485
Pregnancy, 80
  argatroban, 465–466
  bivalirudin (Angiomax), 495
  danaparoid sodium, 384–385
  HIT, 91, 353
    frequency, 119–120
    lepirudin, 427
Primary biliary cirrhosis, 27
Prochlorperazine, 488
Professional negligence, 573
Prostacyclin, 152, 252
  hemodialysis, 522
Prosthetic device, 84–85
Protein, 186–189
Protein C receptor, 253
PRP, see Platelet-rich plasma (PRP)
P-selectin, 252
Pseudo-heparin-induced thrombocytopenia (HIT), 313–330, 580
  HIT, 328–329
    treatment, 328–330
PTCA
  bivalirudin (Angiomax), 494
  danaparoid sodium, 377
PTP, see Posttransfusion purpura (PTP)
Pulmonary embolism, 12, 314, 318, 319, 320
Purpura fulminans, septicemia, 314, 325–327
Quinidine
  antibodies, 42
  drug-induced immune thrombocytopenia (DIT), 34
Quinine, drug-induced immune thrombocytopenia (DIT), 34
Raynaud’s phenomenon, 80
Recombinant hirudin (r-hirudin), see Lepirudin
Regional citrate anticoagulation, hemodialysis, 523
Regional heparinization, hemodialysis, 512
Reteplase, 488
Reviparin (Clexane), 119
Rheumatoid arthritis, 27
r-Hirudin, see Lepirudin
Rhodes, Glen R., 5
Roberts, Brooke, 3–4
Sarcoidosis, 27–28
Scintillation counter, 278
Secondary autoimmune thrombocytopenic purpura (AITP), 27
Septicemia
  consumptive thrombohemorrhagic disorders, 36–37
  purpura fulminans, 314, 325–327
Serotonin release, flow cytometry, 273
thrombocytopenia, 298–299
Serum, heat inactivation, 279
Sheridan, Dave, 10
Silver, Donald, 5, 6
Skin necrosis
classic coumarin-induced, 87–88
coumarin-induced, 87–88
without coumarin therapy, 88
Sodium bicarbonate, 485
Solid-phase PF4-heparin-EIA, 288
Solid-phase red cell adherence assay (SPRCA), 303–304
Splenectomy, AITP, 29
SPRCA, 303–304
SRA, see Serotonin release assay (SRA)
Standard of care
evolving, 582–583
malpractice, 575–576
Streptokinase, 354, 488
Stroke
argatroban, 444, 464
danaparoid sodium, 374
Subclinical heparin-induced thrombocytopenia (HIT), 137
Sulfated carbohydrates
HIT, 207
PF4, 198–202
Sulfated polysaccharides
HIT, 122, 197–213
PF4, 201–202
Surface-bound antigen, 273
Systemic lupus erythematosus, 27, 28, 255
Target antigen, 273
TAT complexes, 78
T-cell receptor (TCR), 189
TCR, 189
TG, 171
Thrombin, 153
inhibition, 399
Thrombin-antithrombin (TAT) complexes, 78
Thrombocytopenia, see also Heparin-associated thrombocytopenia (HAT); Heparin-induced thrombocytopenia (HIT);
Immune thrombocytopenia abciximab-induced immune, 35
acute, 25–42
acute postinfectious autoimmune, 27
APLAS, 324
autoimmune, 27
cyclic, 30
early vs. late onset, 110–111
familial, 28
heparin-induced platelet activation (HIPA) assay, 298–299
HIPA test, 298–299
HIT-IgG, 259
idiopathic immune, 27
laboratory results, 299–302
monoclonal antibody immobilization of platelet antigens (MAIPA) assay, 301
passive immune, 33
PF4-H-EIA, 298–299
rapid vs. typical onset, 297–299
SRA, 298–299
in vitro cross-reactivity, 302–303
activation assays, 302–303
washed platelet assay, 298–299
Thromboembolectomy, 354–355
Thromboembolism, see Arterial thromboembolism
Thromboglobulin (TG), 171
Thrombolytic therapy, 314, 324–325
Thrombomodulin, 258
Thrombosis, 83, 128, 251
frequency, 123–132
heparin resistance, 90
paradoxical, 5–8
treatment, 12–14
<table>
<thead>
<tr>
<th>Index</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombotic thrombocytopenic purpura (TTP), 28, 38</td>
<td></td>
</tr>
<tr>
<td>Thromboxane A2, 227</td>
<td></td>
</tr>
<tr>
<td>Tirofiban, 352, 443, 485</td>
<td></td>
</tr>
<tr>
<td>Tissue plasminogen activator (t-PA), 252, 354</td>
<td></td>
</tr>
<tr>
<td>Tobin, Richard W., 1–2</td>
<td></td>
</tr>
<tr>
<td>Towne, Jonathan, 8</td>
<td></td>
</tr>
<tr>
<td>T-PA, 252, 354</td>
<td></td>
</tr>
<tr>
<td>TTP, 28, 38</td>
<td></td>
</tr>
<tr>
<td>UFH, see Unfractionated heparin (UFH)</td>
<td></td>
</tr>
<tr>
<td>Unfractionated heparin (UFH), 165–166, 179, 352</td>
<td></td>
</tr>
<tr>
<td>early vs. late onset thrombocytopenia, 111</td>
<td></td>
</tr>
<tr>
<td>HIT frequency</td>
<td></td>
</tr>
<tr>
<td>incidental flushes, 120–121</td>
<td></td>
</tr>
<tr>
<td>porcine mucosal, 117–118</td>
<td></td>
</tr>
<tr>
<td>limitations, 197</td>
<td></td>
</tr>
<tr>
<td>Unstable angina</td>
<td></td>
</tr>
<tr>
<td>argatroban, 445</td>
<td></td>
</tr>
<tr>
<td>bivalirudin (Angiomax), 493–494</td>
<td></td>
</tr>
<tr>
<td>Upper limb deep venous thrombosis (DVT), 75</td>
<td></td>
</tr>
<tr>
<td>Urokinase, 354</td>
<td></td>
</tr>
<tr>
<td>Urokinase-type plasminogen activator, 253</td>
<td></td>
</tr>
<tr>
<td>Urticaria, 89</td>
<td></td>
</tr>
<tr>
<td>Vancomycin, 488</td>
<td></td>
</tr>
<tr>
<td>Vascular restenosis, 495</td>
<td></td>
</tr>
<tr>
<td>Vascular surgery</td>
<td></td>
</tr>
<tr>
<td>HIT, 351–354</td>
<td></td>
</tr>
<tr>
<td>lepirudin, 425–426</td>
<td></td>
</tr>
<tr>
<td>Vena cava filters, 336, 360</td>
<td></td>
</tr>
<tr>
<td>Venous thromboembolism, 75</td>
<td></td>
</tr>
<tr>
<td>danaparoid sodium, 374, 375–376</td>
<td></td>
</tr>
<tr>
<td>dosing schedules, 377</td>
<td></td>
</tr>
<tr>
<td>prophylaxis, 380</td>
<td></td>
</tr>
<tr>
<td>Verapamil, 443, 485</td>
<td></td>
</tr>
<tr>
<td>Viral hepatitis, 27</td>
<td></td>
</tr>
<tr>
<td>Vitamin K antagonists, 358–359</td>
<td></td>
</tr>
<tr>
<td>Von Willebrand factor (vWF), 38</td>
<td></td>
</tr>
<tr>
<td>factor VIII, 407</td>
<td></td>
</tr>
<tr>
<td>platelet function, 153</td>
<td></td>
</tr>
<tr>
<td>VWF, 38</td>
<td></td>
</tr>
<tr>
<td>factor VIII, 407</td>
<td></td>
</tr>
<tr>
<td>platelet function, 153</td>
<td></td>
</tr>
<tr>
<td>Warfarin, see Coumarin</td>
<td></td>
</tr>
<tr>
<td>Warm-type autoimmune hemolytic anemia, 28</td>
<td></td>
</tr>
<tr>
<td>Washed platelet assays, 271–272, 273, 293–295</td>
<td></td>
</tr>
<tr>
<td>vs. c-PRP activation assay, 280, 287, 302–303</td>
<td></td>
</tr>
<tr>
<td>disadvantages, 285</td>
<td></td>
</tr>
<tr>
<td>heparin-independent platelet activation, 282</td>
<td></td>
</tr>
</tbody>
</table>