Heparin-Induced Thrombocytopenia: A Clinicopathologic Syndrome

Theodore E. Warkentin
Department of Pathology and Molecular Medicine, and the Department of Medicine, McMaster University; and the Hamilton Regional Laboratory Medicine Program, Hamilton Health Sciences Corporation, Hamilton, Ontario, CANADA

Introduction

The American vascular surgeons, Weismann and Tobin in 1958 and Roberts and colleagues in 1964 were the first to describe unusual thrombi affecting the lower limb arteries in some patients treated with heparin for 7 to 14 days. It was not until the mid-1970s however that Silver and colleagues identified the key elements of the heparin-induced thrombocytopenia (HIT) syndrome, namely thrombocytopenia associated with thrombosis beginning several days after starting heparin. Further, in vitro studies performed by these investigators suggested the presence of a heparin-dependent, platelet-aggregating factor in serum thought to be immunoglobulin G (IgG).

In 1992, Amiral and colleagues identified the antigen target of HIT antibodies. The unusual nature of the HIT antigen, a multimolecular complex of heparin and platelet factor 4 (PF4), together with the remarkable, perhaps unparalleled, platelet- and endothelium-activating properties of the pathogenic HIT antibodies are central to the thrombotic events that occur in these patients. From its obscure beginnings, HIT is now accepted as a common and distinct immunohematologic syndrome with broad implications ranging from public health prevention issues to fundamental questions of pathogenesis, which are leading to new insights into the molecular pathogenesis of thrombosis.

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Thrombocytopenia is common in hospitalized patients. Thrombosis too is not an unusual occurrence in patients receiving heparin. Thus, many physicians were sceptical about the existence of a paradoxic, immune-mediated hypercoagulable situation triggered by heparin, especially as heparin-dependent, platelet-activating antibodies were not always demonstrable in patients with putative heparin-induced thrombocytopenia (HIT). However, improvements in the laboratory detection of HIT antibodies as well as greater appreciation of the diverse clinical consequences of HIT have shown that the definition for HIT should now include both clinical and laboratory criteria. In other words, HIT is a clinicopathologic syndrome.

Viewed from this perspective, HIT should be considered in the differential diagnosis when a patient receiving heparin develops an unexpected clinical event—most often, thrombocytopenia with or without thrombosis. The diagnosis should, however, be confirmed whenever possible by demonstrating the presence of the pathogenic HIT antibodies. Clinicians are familiar with other clinicopathologic syndromes, such as the antiphospholipid antibody (lupus anticoagulant) syndrome, where clinical features, such as thrombosis and miscarriages, and laboratory abnormalities, such as non-specific inhibitor or antiphospholipid antibodies, are both required for diagnosis.

Improvements in laboratory methods to detect HIT antibodies and the wider application of these diagnostic tests have broadened the clinical features now known to be associated with the HIT syndrome (Table 1). Prospective seroconversion and platelet count studies have given a more accurate picture of the frequency of HIT among various patient populations receiving heparin. A clinicopathologic framework for HIT means that alternative diagnoses must be considered when HIT antibodies cannot be identified using reliable assays, even in a patient who might otherwise appear to have HIT. Indeed, there are several disorders that clinically resemble HIT, but that are not caused by HIT antibodies (‘pseudo-HIT’).  

Terminology and Definition

Various designations have been used for this disorder: heparin-induced thrombocytopenia, immune (or type II) heparin-induced thrombocytopenia, and heparin-associated thrombocytopenia. It was recently recommended that the term ‘heparin-induced thrombocytopenia (HIT)’ be used, as it indicates the central role of heparin in initiating this syndrome (heparin-induced) and is also the simplest and most widely-used name. In contrast, for other patients who develop thrombocytopenia during heparin treatment, the term ‘non-immune heparin-associated thrombocytopenia’ was recommended, as it clearly indicates the absence of an immune pathogenesis (i.e., negative test for HIT antibodies) and does not imply whether heparin has contributed or not to the thrombocytopenia (heparin-‘associated’).  

Although there is evidence that heparin may contribute to an early, mild, and transient thrombocytopenia, perhaps via direct, non-immune platelet-activating effects, in practice, it is difficult to distinguish this possibility from concurrent clinical
factors that cause platelet count declines. As stated, HIT should be defined on the basis of one or more clinical events together with laboratory demonstration of the pathologic HIT antibodies. The most common clinical event is thrombocytopenia, although associated thrombosis occurs in approximately 33% to 50% of patients.22-25 Usually, the platelet count falls below 150 x 10⁹/L, however, for 10% to 15% of patients recognized as having HIT, the platelet count nadir remains above 150 x 10⁹/L (Fig. 1).26

Sorologic assessment in large clinical trials has shown that a definition of thrombocytopenia applicable to postoperative patients, and relevant to the diagnosis of HIT, is a greater than 50% platelet count fall from the postoperative peak that occurs from days 5 to 14 after starting heparin.16,30-32 Up to 50% of postoperative patients who develop HIT have a relatively high platelet count (greater than 300 x 10⁹/L) on postoperative days 5 to 7 immediately preceding the onset of HIT. Thus, these patients can develop a greater than 50% platelet count fall caused by HIT antibodies, even though the platelet count nadir remains above 150 x 10⁹/L.24 These patients too appear to be at an increased risk for thrombosis.17,24 Occasionally, patients develop thrombotic or other unusual events (e.g., heparin-induced skin lesions, adrenal hemorrhagic infarction, in association with the formation of HIT antibodies, even though the platelet count falls only trivially.28-30 However, viewed from a clinicopathologic perspective, these patients should be considered to have the HIT syndrome.

### Laboratory Testing

Two types of assays are available to detect HIT antibodies: activation or functional assays and antigen assays.23,30,32 Although the concordance between these assays is high, they do not parallel each other precisely. In this regard, the situation closely parallels the antiphospholipid antibody syndrome, where functional (‘lupus anticoagulant’) and antigen (‘anticardiolipin’) assays give similar, but not identical, information.

Activation assays utilizing washed platelets, such as the serotonin release assay,11,12 have been validated in blinded assessment as being strongly associated with thrombocytopenia in patients receiving heparin.23 Figure 2 shows a general scheme for these assays. Specificity for HIT antibodies is increased by various maneuvers that prove that platelet activation was caused by IgG antibodies (e.g., inhibition by platelet FcγIA receptor blockade) and is inhibited by high concentrations of heparin.11,13 Sensitivity is increased by selecting platelet donors whose platelets are readily activated by HIT sera and by using weak positive control HIT sera to ascertain that the platelets remained sensitive to activation by HIT sera following preparation and handling.12 An important disadvantage of washed platelet assays is that they are technically-demanding and not easily performed.33 Other assay-specific disadvantages include the requirement for radiolabels (serotonin release assay) and the use of a subjective visual endpoint (heparin-induced platelet activation assay).

Various in-house and commercial antigen assays for HIT, based on enzyme-linked immunosorbent assay (ELISA) detection of HIT antibodies that recognize surface-bound platelet/factor 4 heparin complexes are now available.5,6,10,14,15,33 These assays are more readily performed by routine laboratories familiar with ELISA techniques. Recently, an assay has become commercially available that uses PF4 bound to polyvinyl sulfonate to
bind HIT antibodies. A fluid-phase PF4/heparin ELISA is also available that may be somewhat more sensitive for HIT antibodies than the traditional ELISA, as well as permitting better assessment of in vitro cross-reactivity among different low molecular weight heparins and heparinoids. The following conclusions are suggested by studies that have compared various antigen and activation assays for HIT. First, washed platelet assays are more sensitive than assays using platelet-rich plasma to detect HIT antibodies. Next, washed platelet assays and antigen assays have similar sensitivity for clinical HIT, whereas antigen assays may have greater sensitivity for subclinical HIT antibody seroconversion. A corollary to this statement is that activation assays have greater positive predictive value for clinical HIT. Finally, both activation and antigen assays occasionally give false-negative results, although the frequency of this phenomenon in experienced laboratories likely is less than 5%. Thus, reference laboratories should be able to perform both activation and antigen assays for HIT.

In about 5% of samples tested, activation assays yield ‘indeterminate’ results, for example, heat-inactivated serum causes heparin-independent platelet activation, most likely because of circulating immune complexes unrelated to HIT. For these patients, an antigen assay is required for diagnosis of HIT. It is also possible, although unproven, that IgM and/or IgA HIT antibodies might cause HIT in the absence of platelet-activating IgG HIT antibodies. This is another possible explanation for false-negative activation assay results.

Conversely, for at least 5% to 10% of patients with clinically-suspected HIT, the PF4/heparin-ELISA gives a negative result, whereas the activation assay shows heparin-dependent, platelet-activating IgG antibodies. There is conjecture that this may be caused by pathogenic antibodies directed against ‘minor’ PF4-like chemokine antigens, such as interleukin-8 or neutrophil-activating peptide-2.

Frequency

Only a minority of patients who form HIT antibodies during heparin treatment develop clinical HIT. In other words, most patients who form HIT antibodies develop seroconversion without thrombocytopenia or thrombosis. The relationship between HIT antibody formation, thrombocytopenia, and thrombosis can be depicted as an ‘iceberg’ (Fig. 3). This model implies that the risk of HIT-associated thrombosis occurs predominantly in patients who also develop thrombocytopenia.
Figure 2. Schematic overview of washed platelet assays. Certain technical aspects of the assay are important for the high sensitivity and specificity of these assays for HIT-IgG antibody-induced platelet activation, including inclusion of apyrase during the platelet wash step (prevents later refractoriness to adenosine diphosphate [ADP]-induced potentiation of platelet activation), composition of the final resuspension buffer (physiologic calcium concentrations), and various maneuvers to exclude platelet activation by other agonists (e.g., an Fc receptor-blocking monoclonal antibody inhibits HIT-IgG-mediated platelet activation, but not activation caused by residual thrombin; high heparin concentrations inhibit HIT-IgG-mediated platelet activation, but not activation caused by circulating immune complexes). At least four different platelet activation endpoints have been used, including adenosine triphosphate (ATP), radioactive \(^{14}\text{C}\)-serotonin, platelet-derived microparticles, and platelet aggregation. Abbreviations: ACD, acid-citrate-dextrose; FcR, Fc receptor; Mab, monoclonal antibody; TTP, thrombotic thrombocytopenic purpura.
This relationship is supported by controlled clinical studies indicating that HIT antibody formation in the absence of thrombocytopenia has no higher risk for thrombosis than seen in control patients. In intriguingly, however, both the frequency of HIT antibody formation, as well as the relative risk of thrombocytopenia in patients who form HIT antibodies, vary considerably among different types of patient populations treated with heparin. For example, postoperative orthopedic patients treated with unfractionated heparin (UFH) have about a 5% risk of HIT, with at least half of these affected patients developing HIT-associated thrombosis. In contrast, although the frequency of HIT antibody formation is considerably higher in postoperative cardiac surgical patients receiving heparin (50% vs 15% by PF4/heparin-ELISA), fewer cardiac surgical patients appear to develop HIT.

Besides the type of patient population receiving heparin, there is also evidence that the immunogenic potential of heparin varies among different heparin preparations. For example, meta-analysis of five studies that have compared UFH derived from porcine intestinal mucosa shows that bovine lung UFH is significantly more likely to cause HIT. But, even porcine UFH is far more likely to cause HIT than low molecular weight heparin (LMWH), at least when given to postoperative orthopedic patients. In these orthopedic patients—a population that appears to have the highest risk for HIT—there is about a seven-fold greater risk of HIT using porcine UFH than LMWH (~5% vs ~0.75%). The reduced risk for developing HIT is an important advantage of LMWH, particularly in patient populations at high relative risks for developing this complication.

Timing of Heparin-Induced Thrombocytopenia: ‘Typical’ and ‘Rapid’ Onset of HIT

HIT typically begins 5 to 10 days after starting heparin. This relatively narrow ‘window period’ for HIT suggests that a point immunization could occur in most patients. This point may involve an interaction between a transient increase in circulating PF4, for example, the release of PF4 resulting from perioperative platelet activation, and the start of heparin treatment. An important aspect to interpreting the various clinical presentation profiles of HIT is the recent observation that HIT antibodies are...
remarkably transient and are usually undetectable 100 days after an episode of acute HIT.45

Thrombocytopenia can occur quickly, even within minutes or hours, in some heparin-treated patients shown to have HIT antibodies. It is widely appreciated that this rapid-onset of HIT occurs in patients who have previously received heparin. Although this phenomenon has been ascribed to an anamnestic immune response,46 recent data suggest that residual circulating HIT antibodies resulting from a recent heparin exposure (usually, within the past 100 days) represents a more plausible explanation for rapid-onset HIT than a rapid regeneration of HIT antibodies.45

This is not a trivial distinction. If there is a minimum time to formation of clinically-significant levels of HIT antibodies, such as 5 days,45 whether this results from an anamnestic response or not, then it may be reasonable to consider a brief course of heparin in certain situations (e.g., for cardiac or vascular surgery), even for patients who have a prior episode of serologically-confirmed, life-threatening HIT. In considering this treatment option, it is important to consider whether suitable alternate anticoagulation is available. Although there are alternative agents available for routine postoperative anticoagulation, such as danaparoid and lepirudin, there is less experience using these agents for cardiopulmonary bypass. Further, the anticoagulant actions of these agents cannot be neutralized, and intraoperative monitoring is not widely available. Before giving heparin in an elective situation to a patient with a previous history of HIT, however, it is important to prove, using a sensitive and reliable assay, that circulating HIT antibodies are no longer detectable. Finally, since HIT antibodies could recur again in several days, heparin use should be restricted to the operative procedure itself, and alternative postoperative anticoagulation should be given (e.g., danaparoid, lepirudin, warfarin).45,47,48

Clinical Features

Figure 1 shows that the median platelet count in patients with HIT is about 60 x 10^9/L. Severe thrombocytopenia, e.g., a platelet count nadir less than 20 x 10^9/L, occurs in less than 10% of patients. Intriguingly, petechiae and other spontaneous hemorrhage is rare, even in patients with very severe thrombocytopenia complicating HIT.17

HIT is strongly associated with thrombosis (Table 2). An association between HIT and thrombosis is possible even when mild thrombocytopenia occurs, for example, in patients whose platelet count falls by more than 50% in the postoperative period, but in whom the platelet count nadir remains above 150 x 10^9/L.17

Table 1 summarizes the various clinical sequelae that have been linked to the HIT syndrome.13 Both prospective and retrospective studies22-27,49 show that venous thrombosis is the most common complication of HIT. Indeed, pulmonary embolism is more common than all arterial thrombotic events combined and may represent one of the most common causes of death in the HIT syndrome.

Underlying clinical factors play a major role in the clinical expression of HIT. For example, medical patients who develop HIT are approximately equally likely to develop arterial or venous thrombosis; in contrast, surgical patients who develop HIT show a marked predominance for venous thrombotic events.25 Recent intra-arterial punctures50 or current or recent use of a central venous catheter51 strongly influence the localization of an arterial or venous thrombosis, respectively, in HIT patients.

Deep Venous Thrombosis

Deep venous thrombosis (DVT) occurs in about 50% of patients diagnosed with HIT.22,23 Other common venous thromboembolic

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**Table 2. Association of HIT and Thrombosis**

<table>
<thead>
<tr>
<th>Thrombotic Event</th>
<th>Patients with HIT (n=9)</th>
<th>Controls (n=656)</th>
<th>Odds Ratio (95% C.I.)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any venous or arterial thrombosis</td>
<td>8</td>
<td>117</td>
<td>36.9 (4.8-1638)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arterial thrombosis</td>
<td>1</td>
<td>2</td>
<td>40.9 (0.6-831)</td>
<td>0.04</td>
</tr>
<tr>
<td>Any venous thromboembolic event</td>
<td>7</td>
<td>115</td>
<td>16.5 (3.1-163)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distal DVT without proximal extension</td>
<td>2</td>
<td>84</td>
<td>1.9 (0.3-10.4)</td>
<td>0.74</td>
</tr>
<tr>
<td>Bilateral DVT (distal or proximal)*</td>
<td>3</td>
<td>18</td>
<td>17.7 (2.6-89.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Proximal DVT</td>
<td>5</td>
<td>29</td>
<td>27.0 (5.4-141)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilateral proximal DVT</td>
<td>2</td>
<td>4</td>
<td>46.6 (3.5-380)</td>
<td>0.002</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>2</td>
<td>2</td>
<td>93.4 (5.7-1374)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

HIT is defined as a platelet count fall to below 150 x 10^9/L in this Table. The data show a stronger association (by odds ratio) between progressively more severe venous thrombosis and HIT.

* At least one proximal DVT was observed in each of the three HIT patients with bilateral DVT; in contrast, 12 of 18 bilateral DVTs in the non-HIT patients involved distal veins only.
events include pulmonary embolism (25%), bilateral lower limb DVT (10%), and upper limb DVT (5%). A clinical trial has shown that venous thromboembolism is strongly associated with HIT. Moreover, the strength of the association is greater in proportion to the severity of the thrombosis (Table 2). Thus, clinicians should keep in mind that the more unusual the thrombosis, the more likely there is an underlying explanation such as HIT.

Venous thromboembolism is the most common thrombotic outcome in HIT. Therefore, in the author’s opinion, whenever possible, patients with clinically-suspected HIT should have objective imaging studies of the lower extremities performed to rule out the presence of subclinical HIT. The reason is that there is a high frequency of subsequent thrombosis (~50%–including sudden death from pulmonary embolism (~5%–despite discontinuing heparin, or substituting heparin with warfarin, in patients with serologically-confirmed HIT. Data from a prospective treatment study also show a high initial frequency of new thrombotic events (~5–10% per day in the first one to two days) despite stopping heparin because of clinically-suspected, and subsequently serologically-confirmed, HIT.

Venous Limb Gangrene
Venous limb gangrene is a recently-recognized iatrogenic complication of HIT that can be caused by coumarin anticoagulation. The syndrome is characterized by the progression of otherwise typical DVT to venous limb gangrene during warfarin or phenprocoumon treatment. Patients with venous limb gangrene characteristically have a supratherapeutic INR, typically greater than 4.0. Laboratory studies suggest that the pathogenesis is a profound disturbance in procoagulant/anticoagulant balance, which appears to be caused by rapid, acquired protein C deficiency during initiation of coumarin anticoagulation, but prior to sufficient reduction in functional prothrombin concentrations so as to effect anticoagulation. Thus, in these patients, persisting thrombin generation as shown by markedly increased thrombin-antithrombin levels, coincided with marked impairment in natural anticoagulant control of thrombin generation, as shown by marked reduction in protein C activity. Although the syndrome has occurred in patients treated with warfarin alone and warfarin combined with anconid, it appears to be safe to give coumarins when thrombin generation is adequately controlled with a drug such as danaparoid or lepirudin. Indeed, many HIT patients will require several months of oral anticoagulant therapy to treat HIT-associated thrombosis.

Cerebral Vein Thrombosis and Adrenal Hemorrhagic Necrosis
Cerebral vein thrombosis and adrenal hemorrhagic necrosis are two uncommon, but well-described complications of HIT caused by thrombosis in the cerebral dural sinuses and adrenal veins, respectively. Clinicians must be aware of these characteristic complications of HIT, as early diagnosis and urgent therapy can be life-saving.

Arterial Thrombosis
Arterial thrombosis most commonly involves the distal aorta and major limb arteries. Thus, limb amputation is a not an uncommon consequence of HIT. Occasionally, patients develop paralysis secondary to spinal cord or plexus infarction. Thrombotic stroke and myocardial infarction (MI) are other sequelae of HIT. Intriguingly, the relative risk of arterial thrombotic events (limb artery occlusion > stroke > MI) is reversed from that seen in the general population (MI > stroke > limb artery occlusion).

Heparin-Induced Skin Lesions
Heparin-induced skin lesions occur at heparin injection sites and range from painful erythematous papules and plaques to extensive dermal necrosis necessitating skin grafting. Only about 10% to 20% of patients who develop HIT antibodies during subcutaneous heparin treatment develop skin manifestations of HIT. Only about 25% of patients who develop heparin-induced skin lesions develop thrombocytopenia, although this subgroup is at an increased risk for thrombotic events, especially arterial thrombosis. HIT antibodies can be readily detected in patients with skin lesions, even if thrombocytopenia does not occur. There is also evidence that the occurrence of ‘classic’ coumarin-induced skin necrosis, typically involving central locations such as the female breast, abdomen, and thigh, is also increased in patients who receive oral anticoagulants to treat HIT.

Acute Systemic Reactions
Acute systemic reactions refer to various symptoms and signs that occur between 5 minutes and 30 minutes after an intravenous heparin bolus in a patient with circulating HIT antibodies. These reactions are characterized by an abrupt, sometimes transient, fall in the platelet count. Delayed recognition of HIT, potentially with tragic consequences, can occur if these events are incorrectly attributed to pulmonary embolism or septicemia.

Pseudo-HIT
This author’s clinicopathologic view of the HIT syndrome means that a patient who develops unusual thrombosis and thrombocytopenia during or shortly after heparin treatment, but who has reproducibly negative tests for HIT antibodies using both activation and antigen assays, must have an alternative diagnosis to explain the unusual clinical events. Indeed, there are occasional patients whose clinical course strongly resembles HIT, but in whom HIT antibodies cannot be demonstrated. I have used the term ‘pseudo-HIT’ to describe some of these patients.

One intriguing example of this phenomenon is that of adenocarcinoma-associated venous limb gangrene. These patients typically present with symptomatic DVT together with malignancy-associated disseminated intravascular coagulation (DIC). These patients appear to be at-risk for developing venous limb gangrene during warfarin treatment, particularly if a supratherapeutic INR is attained. Thus, this syndrome closely resembles that described for HIT patients. I believe that insights gained from elucidating the pathogenesis of HIT can lead to a...
greater understanding of the unusual thrombotic events that occur in other clinical settings.

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