New Approaches to the Diagnosis of Heparin-Induced Thrombocytopenia

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Heparin-induced thrombocytopenia (HIT) is a clinicopathologic syndrome that is most reliably diagnosed when a patient with a clinical scenario that is consistent with heparin-induced immunization is shown to have antplatelet factor 4/heparin, platelet-activating IgG antibodies. A Bayesian diagnostic approach is discussed, wherein the physician estimates the pretest probability of HIT (eg, the timing and severity of thrombocytopenia in relation to heparin treatment and associated thrombosis) and determines the posttest probability using the results of HIT antibody testing. By this approach, the magnitude of a positive test result determines its likelihood ratio in influencing the posttest probability of HIT.

Abbreviations: DVT = deep-vein thrombosis; ELA = enzyme-linked assay; HIT = heparin-induced thrombocytopenia; LMWH = low-molecular-weight heparin; LR = likelihood ratio; PE = pulmonary embolism; PF4 = platelet factor 4; SRA = serotonin release assay; UFH = unfractionated heparin

Heparin-induced thrombocytopenia (HIT) is an immune-mediated disorder caused by IgG antibodies that bind to platelet factor 4 (PF4). The PF4 becomes immunogenic when it binds to heparin. Multimolecular complexes of heparin, PF4, and IgG form on platelet surfaces, and the occupancy of the platelet Fc receptors by HIT-IgG results in platelet activation. Heparin binds PF4 in relation to the chain length and degree of sulfation, perhaps explaining why unfractionated heparin (UFH) is more likely to cause HIT than low-molecular-weight heparin (LMWH). Platelet activation in HIT is associated with the activation of coagulation, as shown by increased levels of markers of in vivo thrombin generation (eg, thrombin-antithrombin complexes). Once these events are triggered, the prothrombotic risk remains for days to weeks, even after stopping heparin therapy.

HIT MYTHS

Certain myths exist regarding HIT. One is that this complication can be diagnosed on clinical grounds alone. While it is true that HIT can be diagnosed in some patients with near certainty based on their characteristic presentation, exclusive reliance on clinical features alone can result in erroneous conclusions. For example, Figure 1 compares two similar clinical scenarios, namely, thrombocytopenia and pulmonary embolism (PE) occurring during UFH prophylaxis following major surgery. However, only one patient (Fig 1, bottom, B) tested positive for HIT antibodies. The other patient (Fig 1, top, A), who tested negative for HIT antibodies using two different assays for HIT antibodies, demonstrated clinical and platelet count recovery when the heparin dose was increased to overcome heparin resistance. This patient’s thrombocytopenia was explained by PE, which can be associated with thrombocytopenia, perhaps because clot-bound thrombin within the thromboemboli activate platelets directly within the high-flow pulmonary circulation. Thus, PE is one of the causes of pseudo-HIT, a term that is used to describe a clinical situation that strongly mimics HIT on clinical grounds, but in which HIT antibodies are not detected.

A second myth is that a positive test result for HIT antibodies automatically means that a thrombocytopenic patient has a diagnosis of HIT. However, this is not necessarily the case. Nonpathogenic PF4/heparin-reactive antibodies are a relatively common occurrence in patients who have received heparin within the past days or weeks. Thus, if a patient treated with heparin develops bacteremia and clinical sepsis, this patient could test positive for HIT antibodies, but the sepsis would have caused the platelet count to fall. Figure 2 illustrates such a clinical dilemma. The patient has features that
strongly support the presence of sepsis (e.g., fever, hypotension, and positive blood culture results) but also has features suggesting HIT (e.g., thrombocytopenia and proximal deep-vein thrombosis [DVT]). This patient tested positive for HIT antibodies by two different assays. However, the patient's subsequent clinical course revealed platelet count recovery while receiving UFH in a therapeutic dose (arguing against the presence of HIT) but also PE on postoperative day 16 (which is consistent with the presence of HIT).

The importance of both clinical and laboratory features in the diagnosis of HIT means that HIT should be considered a "clinico-pathologic syndrome" (Table 1), whereby the diagnosis is made most confidently when the patient has an episode of
thrombocytopenia that cannot otherwise be readily explained (the “clinical” part), together with the presence of anti-PF4-heparin, platelet-activating antibodies that usually give strong positive reactions using sensitive assays (the “pathologic” part).17-19

**CLINICAL FEATURES OF HIT**

Thrombocytopenia, defined as a fall of ≥ 50% in the platelet count, is the most common clinical manifestation of HIT and occurs in > 95% of patients.20 Typically, the thrombocytopenia is of moderate in severity (median platelet count nadir [ie, the lowest value], approximately 50 to 60 x 10⁹ platelets/L). For 90% of patients, the nadir ranges between 15 and 150 x 10⁹ platelets/L.1

The timing of the onset of thrombocytopenia shows three characteristic. The most common (70%) is typical-onset HIT, in which the platelet count begins to fall 5 to 16 days after starting heparin therapy (first day of heparin = day 0).21,22 This characteristic delay reflects the usual short interval for heparin to initiate an humoral immune response. In about 25 to 30% of patients, HIT is recognized because the platelet count falls abruptly within 24 h of starting heparin therapy.21,22 This is termed rapid-onset HIT. This syndrome results from a recent immunizing exposure to heparin, usually within the past few weeks. The platelet count falls quickly because the patient already has circulating HIT antibodies when the heparin is readministered. Rarely, HIT is only recognized by a fall in the platelet count that begins several days after heparin therapy has been stopped (called delayed-onset HIT).23-25 This last syndrome is often clinically severe, as it is associated with high-titer, platelet-activating HIT antibodies that do not require ongoing heparin administration to exert their pathogenic effect.25

Many patients with HIT develop thrombosis, even when heparin had been administered initially only for antithrombotic prophylaxis.26,27 Prospective and case-controlled studies have suggested28-30 that the risk (ie, odds ratio) of thrombosis in patients with HIT is about 20 to 40, which corresponds to an absolute risk of thrombosis of 35 to 75% in many clinical settings. The most common thrombotic event is venous thromboembolism (ie, DVT and PE), especially in postoperative patients. Sometimes, unusual clinical events, such as gradually progressive neurologic dysfunction (ie, cerebral venous thrombosis) or adrenal hemorrhage (ie, adrenal vein thrombosis), can be linked ultimately to venous thrombosis.30 Arterial thrombosis also frequently complicates HIT and manifests most often as an acutely ischemic lower limb due to occlusion of large limb arteries or the distal aorta.30 (Limb ischemia can also result from microvascular thrombosis in the setting of DVT, particularly in patients who develop severe protein C depletion during warfarin anticoagulation therapy during acute HIT [see also the article by Barholomew in this Supplement].) Stroke and myocardial infarction are two other arterial thrombotic complications of HIT.

About 10 to 20% of patients who develop HIT while receiving subcutaneous injections of heparin manifest skin lesions at the heparin injection sites, ranging from painful erythematous plaques to skin necrosis.30,31 Sometimes, patients are recognized as having HIT when they develop acute systemic reactions within 5 to 30 min of the administration of an IV heparin bolus.30,31 These systemic reactions include fever or chills, hypertension, tachycardia, dyspnea, chest pain or tightness, and flushing. They are associated with abrupt declines in the platelet count resulting from receiving the heparin bolus and presumably reflect the biological consequences of sudden generalized platelet activation (and, possibly, leukocyte activation).31

Examination of the peripheral blood film in patients with HIT usually reveals only a reduced platelet count. However, a minority of patients can have disseminated intravascular coagulation, with RBC fragments (ie, schistocytes) or even nucleated RBCs (ie, normoblasts).1,20
Table 1—RIT: A Clinicopathologic Syndrome

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Pathologic</th>
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</thead>
<tbody>
<tr>
<td>Thrombocytopenia (≥50%)*</td>
<td>Anti-PF4/heparin, platelet-activating IgG antibodies (≥50%)*</td>
</tr>
<tr>
<td>Thrombosis (35–75%)</td>
<td>Washed platelet activation assays</td>
</tr>
<tr>
<td>Venous (e.g., DVT, PE, venous limb gangrene), arterial involvement (e.g., cerebral vein thrombosis)</td>
<td>SRA</td>
</tr>
<tr>
<td>Arterial thrombosis (e.g., artery &gt; stroke &gt; myocardial infarction &gt; other sites)</td>
<td>Heparin-induced platelet activation assay</td>
</tr>
</tbody>
</table>
| Microvascular thrombosis | Platelet-derived microparticles
| Microparticles | PF4-dependent ELISA (solubilized)
| PF4-dependent fluid phase immunoassay | PF4-polyvinyl sulfonate
| PF4-polyvinyl sulfonate | PF4-polyvinyl sulfonate
| PF4-dependent fluid phase immunoassay | PF4-polyvinyl sulfonate
| PF4-polyvinyl sulfonate | PF4-polyvinyl sulfonate
| Platelet aggregation studies using citrated platelet-rich plasma† | Platelet aggregation studies using citrated platelet-rich plasma† |

*Thrombocytopenia broadly defined as a proportional platelet count fall (usually > 90%). In a few patients, maximal platelet count falls are associated with thrombosis, skin lesions, or other clinical sequelae in association with formation of HIT antibodies.

†Usually associated with continuous therapy variant of continuous-infused heparin.

‡Reported to be associated with arterial venous thrombosis with subsequent arterial hemorrhage.

§Occurs in 10 to 20% of patients who develop HIT while receiving subcutaneous heparin injections (e.g., enoxaparin plaques and vein necrosis).

¶Occurs in 25% of HIT patients who receive an IV heparin bolus at a time when they have formed HIT antibodies.

††Indicates prolongation of international normalized ratio anticoagulogram, or bleeding attributable to HIT.

§§In a few patients, HIT-like/antiplatelet antibodies could be defined by antibodies directed against chemotherapeutics other than PF4 (e.g., interferon-α or neutrophil activating peptide-2).

∗∗Detected by flow cytometry.

†††These tests are performed infrequently due to limited sensitivity and specificity for HIT.

Heparin use and thrombocytopenia are common events in hospitalized patients. Consequently, the combination does not necessarily indicate HIT. Thus, the clinician must evaluate the patient for other potential explanations for the thrombocytopenia, such as perioperative hemorrhage, sepsis, multiorgan dysfunction syndrome, immune thrombocytopenia caused by other drugs (e.g., quinine, procainamide, rifampin, vancomycin, and sulfonamides), and posttransfusion purpura, to list a few. Very severe thrombocytopenia (platelet count, <15 × 10^9 platelets/L) is usually not caused by HIT. Thus, in a patient whose platelet count has fallen abruptly to 10 × 10^9 platelets/L after angioplasty in which both heparin and a platelet glycoprotein IIb/IIIa antagonist were given, the explanation is most likely acute thrombocytopenia caused by the glycoprotein IIb/IIIa receptor antagonist (see also the articles by Aster and Matthai in this Supplement).

Laboratory Features of HIT

Figure 3 summarizes the following two classes of assays that are used to detect HIT antibodies: platelet activation assays and anti-PF4/heparin (antigen) assays.

Washed Platelet Activation Assay

The activation of platelets suspended in a sodium citrate-anticoagulant was first used in the 1970s for the diagnosis of HIT. Unfortunately, this assay has relatively poor sensitivity for detecting HIT antibodies. In 1986, Sheridan et al reported the use of washed platelets and the release of radiolabeled serotonin for detecting HIT antibodies, which was called the platelet serotonin release assay (SRA). By special methods (i.e., by “washing” the platelets in the presence of the adventitious diphosphate scavenger aspartate, and resuspending the platelets into a calcium-containing and magnesium-containing buffer), the platelets become sensitive to activation by HIT serum or plasma. Further, performing the assay in microwells permits the simultaneous examination of numerous reaction conditions, thus maximizing diagnostic specificity. The typical flow cytometry profile of HIT is one of maximal platelet activation at pharmacologic heparin concentrations (i.e., 0.1 to 0.3 IU/mL), which is inhibited by the addition of a platelet Fc receptor-blocking monoclonal antibody. In addition, high heparin levels (i.e., 10 to 100 IU/mL) inhibit platelet activation by HIT antibodies. This method has been modified to include platelet activation and points other than serotonin release, such as the direct visualization of platelet aggregation (which is widely performed in Europe) or the detection of platelet-derived microparticles by flow cytometry. The major limitation of this assay is its technically demanding nature and therefore its use is limited to a few reference laboratories.
Solid-Phase PF4/Heparin Enzyme Immunoassay

In these antigen assays, PF4 and polyanion are bound in optimal stoichiometric concentration onto a microtiter plate, with enzyme immunoassay (EIA) technology used to detect the binding of patient antibodies to the PF4/polyanion complex. Two PF4 EIAs are commercially available, both of which detect the antibodies of the three major Ig classes (IgE, IgG, IgM, and IgA) against PF4 bound either to heparin (Assenachrom; Stago, France) or polyvinyl sulfonate (GTI; Brookfield, WI). The former assay utilizes recombinant PF4, whereas the latter obtains PF4 from outdated platelets. One manufacturer (GTI) recommends a "confirmatory" step, assessing whether adding high concentrations of heparin inhibits the reaction. To what extent this increases diagnostic specificity has not been reported. Research laboratories that perform "in-house" PF4/heparin EIAs have the option to detect antibodies of just the IgG class, which increases specificity for clinical HIT by avoiding the detection of nonpathogenic IgA and IgM antibodies.

Miscellaneous assays include the particle gel immunoassay (commercially available in Canada and Europe) and the fluid-phase PF4/heparin EIA (used mostly for research applications). The major advantage of the particle gel immunoassay is its rapid turnaround time (15 min), but its operating characteristics regarding the detection of HIT antibodies and the diagnosis of HIT remain to be defined. The fluid-phase EIA is especially useful for detecting in vitro cross-reactivity of HIT antibodies against various heparins and heparinoids.

Iceberg Model of HIT

Schematic Iceberg

The interrelationship of HIT antibodies, as detected using a washed platelet activation assay (e.g., SRA) or the antigen assay (anti-PF4/heparin EIA), with clinical HIT (thrombocytopenia) and HIT-associated thrombosis, can be conceptualized as an "iceberg" (Fig 4, top, A). Several features of HIT are illustrated. First, HIT antibodies are most readily detected using the antigen assay. Second, a subset of antibodies detected by the antigen assay will have platelet-activating properties, and hence will be detected in a washed platelet activation assay. Third,
the results of both types of assays are positive in patients who develop HIT.\textsuperscript{73} Fourth, thrombosis occurs in patients who develop thrombocytopenia, not (usually) in patients who develop HIT antibodies in the absence of a major platelet count fall.\textsuperscript{1,8}

The observations point to the following two corollaries with clinical implications: (1) whereas both types of HIT assays are sensitive for detecting clinically important HIT antibodies, the washed platelet activation assay has greater diagnostic specificity;\textsuperscript{65} and (2) it is more useful to screen for HIT using serial platelet count monitoring rather than serial HIT antibody testing, as platelet count declines exhibit a greater correlation with clinically important events (thrombosis) than HIT antibody formation per se. HIT antibody testing alone would detect many clinically insignificant antibodies and is not recommended.\textsuperscript{65}

Multiple Iceberg Model of HIT

Figure 4, bottom, B, illustrates that the risk of HIT antibody seroconversion, as well as of the development of clinical HIT (i.e., thrombocytopenia with or without thrombosis), varies in different clinical settings (i.e., there are distinct differences in iceberg "size" and "incidence"). Indeed, two major variables influencing the risk of HIT (besides the duration of heparin anticoagulation therapy) are (1) the type of heparin being used (e.g., bovine lung UFH > porcine intestine UFH > porcine intestine LMWH), and (2) the type of patient population in which heparin therapy is being used (e.g., postoperative > medical > obstetric).\textsuperscript{6,7} In practical terms, this means that the frequency of HIT ranges from a low of near-zero (e.g., pregnant women or medical patients receiving LMWH) to about 5\% (e.g., postoperative orthopedic surgery patients given UFH prophylaxis). For unknown reasons, postoperative cardiac surgery patients have a lower frequency of HIT than do patients after orthopedic surgery (2 to 3\% vs 5\%, respectively), even though the former population is more likely to form HIT antibodies (50\% vs 15\%, respectively) [by PF4 EIA].\textsuperscript{19}

Magnitude of a Positive HIT Antibody Test Result

Operating Characteristics of HIT Assays

Clinicians often consider the results of HIT antibody testing as being positive or negative. However,
such a strict dichotomous view may ignore diagnostic information. This is because a strongly positive test result indicates a much greater likelihood that the patient actually has clinical HIT.\textsuperscript{15,19} Figure 5 presents serologic results that were obtained during prospective studies\textsuperscript{15} of several hundred patients to whom antithrombin prophylaxis with LMWH was given after orthopedic surgery. Although 33 patients developed PF4-reactive IgG antibodies, only 4 patients developed clinical HIT. Notably, these 4 patients tested strongly positive in the antigen assay (absorbance, > 1.0 U) and also exhibited the greatest amount of platelet activation in the SRA (> 90% serotonin release).

Figure 6, top, A, presents the results of the PF4 EIA and SRA for a group of 100 patients who were studied prospectively after cardiac surgery.\textsuperscript{15} Half of these patients (50%) developed a positive result to the PF4 EIA, and 20% formed HIT antibodies that were detectable by the SRA. However, only one of these patients (open triangle in Fig 6) developed clinical HIT. (The unusual clinical course of this patient is discussed later.) To be able to determine the operating characteristics of the HIT assays in this patient population, Figure 6, top, A, also shows the results from 16 consecutive patients with HIT who were tested following cardiac surgery in the same laboratory (open circles).\textsuperscript{15,16} It is apparent that most cardiac surgery patients with HIT have strong platelet activation assay results (serotonin release, > 80%) and also test strongly positive in the antigen assay (absorbance, > 1.0 U).

Likelihood Ratios

These data then were used to construct the operating characteristics (ie, sensitivity-specificity tradeoffs) of the PF4 EIA and SRA in post-cardiac surgery patients (Fig 6, bottom, B). These data permit the calculation of the likelihood ratio (LR) of HIT for a given quantitative test result. The LR is defined as the odds that a given level of a diagnostic test result would be expected in a patient with the disorder of interest, as opposed to without it. This is expressed mathematically as the “sensitivity” (ie, the proportion of patients with the disorder of interest who have a positive test result) divided by “1 – specificity” (with specificity is defined as the proportion of patients without the disorder of interest who have a negative test result) at a particular cutoff value between a positive and negative test result. Thus, in post-cardiac surgery patients, an SRA result of 90% reactivity, with a sensitivity of 0.70 (70%) and a specificity of 0.965 (96.5%), yields an LR of 0.70/ (1 - 0.965) ≈ 10. Figure 6, bottom, B, also shows how the LRs are calculated for two representative EIA results.

Bayesian Analysis: Integrating Pretest Probability With the Quantitative Assay Result

The Reverend Thomas Bayes (1702 to 1761) discovered a fundamental principle of logical inference that is relevant to the diagnostic interpretation of laboratory test results. Bayes’ theorem relates the “pretest probability” of a diagnosis to its “posttest probability” (both expressed as odds ratios) in relation to the specific laboratory test result, expressed as the LR (defined above),\textsuperscript{13,14} as follows (in the case of suspected HIT):

\[
\text{Posttest Odds for HIT} = \text{Pretest Odds for HIT} \times \text{LR for Diagnostic Test Result}
\]

Figure 7 illustrates the application of Bayesian analysis to a post-cardiac surgery patient with clinically suspected HIT. Figure 7, top, A, illustrates the clinical course of the patient who tested positive during the prospective study of HIT antibody formation following cardiac surgery (open triangle in Fig 6, top, A).\textsuperscript{15} The patient's clinical course suggested a high pretest probability for HIT, indeed typical of delayed-onset HIT.\textsuperscript{20-25} This is because the magnitude and timing of the fall in platelet count (ie, a > 50% fall beginning on day 7) and an associated clinical sequelae (ie, adrenal hemorrhagic necrosis) are characteristic of HIT. The only typical feature was the delayed onset of thrombocytopenia after...
stopping heparin therapy. Thus, if a high pretest probability of HIT is posited (say, 50%), either of the strongly positive test results i.e., serotonin release, 95% (which is associated with an LR of about 15) or PIP4/heparin EIA, 2.25 optical density units (which is associated with an LR of > 20) clinches the diagnosis with an estimated posttest probability of about 99% (solid line in Fig 7, bottom, B). Even if the clinician were unaware of the syndrome of delayed-onset HIT, and thereby considered HIT to
be unlikely in this scenario (say, only a 10% pretest probability is posited), the strong HIT antibody test result would suggest the correct diagnosis, with an estimated posttest probability of almost 70% (dotted line in Fig 7, bottom, B).

CONCLUSION

I have focused on the clinical-laboratory interrelationships that support or refute a diagnosis of HIT. Because thrombocytopenia in heparin-treated patients has many potential explanations besides HIT, and because of the high probability of the subclinical generation of HIT antibodies in many clinical situations, a clinicopathologic approach for diagnosing HIT is recommended. Thus, HIT antibody test results must be interpreted in the appropriate context of the available clinical information. Furthermore, additional diagnostic information is available as a result of considering the magnitude of a given positive test result, rather than simply viewing the assay result in a dichotomous fashion as merely
positive or negative. Indeed, HIT antibody testing ranks among the most clinically useful of the available platelet-antibody investigations.

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