Platelet Count Monitoring and Laboratory Testing for Heparin-Induced Thrombocytopenia

Recommendations of the College of American Pathologists

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• ***Objective.*—Heparin-induced thrombocytopenia (HIT) is an antibody-mediated adverse drug reaction that paradoxically is associated with a brief but dramatically increased risk for thrombosis (transient acquired thrombophilia). The objective of this article is to provide practical recommendations for platelet count monitoring in patients receiving heparin, as well as for selection of laboratory assays to detect pathogenic HIT antibodies.**

***Study Selection.*—Relevant literature that focused on frequency and timing of HIT in various clinical settings and that dealt with laboratory testing for HIT antibodies was critically appraised.**

***Data Extraction and Synthesis.*—The author prepared a preliminary manuscript including recommendations that was presented to participants at the College of American Pathologists Conference XXXVI: Diagnostic Issues in Thrombophilia (November 10, 2001). Support of at least 70% of conference participants was required for recommendations to be adopted.**

***Conclusions.*—The risk of immune HIT varies depending on the type of heparin (unfractionated heparin greater than low-molecular-weight heparin) and patient population (surgical greater than medical). Thus, the intensity of platelet count monitoring should be stratified depending on the clinical situation. Platelet count monitoring should focus on the period of highest risk (usually days 5 to 10 after starting heparin) and should use an appropriate platelet count baseline (generally, the highest platelet count beginning 4 days after start of heparin). However, earlier platelet count monitoring is appropriate if the patient received heparin within the past 100 days, as already circulating HIT antibodies can cause rapid-onset HIT with heparin reexposure. Although both antigen and (washed platelet) activation assays are very sensitive for detecting clinically significant HIT antibodies, activation assays have greater diagnostic specificity for clinical HIT.**

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Heparin-induced thrombocytopenia (HIT) is a paradoxical anticoagulant-induced prothrombotic disorder characterized by thrombocytopenia. It is appropriate to consider HIT as an acquired (immune-mediated) thrombophilia, as it is caused by heparin-dependent, platelet-activating immunoglobulin (Ig) G, and because it is associated with marked in vivo thrombin generation[1,2] (hypercoagulable state) that is strongly associated with venous and arterial thrombosis.[3] It is useful to consider HIT as a clinicopathologic syndrome, that is, both clinical and pathologic criteria should be present to ensure a reliable diagnosis[4] (Table 1[5–10]).

PATHOPHYSIOLOGY

Heparin—a highly sulfated glycosaminoglycan composed of alternating substituted D-glucosamine residues linked 1→4 to either L-iduronic acid or D-glucuronic acid—can form multimolecular complexes with a positively charged, tetrameric glycoprotein member of the C-X-C chemokine subfamily known as platelet factor 4 (PF4).[11,12] Located within platelet α-granules, PF4 can be released from platelets because of weak, nonimmunologically mediated platelet activation caused by heparin.[13] For unknown reasons, some patients generate antibodies that recognize PF4 when it is complexed to heparin. Although IgG, IgM, and even IgA anti-PF4 antibodies are frequently generated, pathogenicity has been best established for HIT antibodies of the IgG class,[3,9,10] particularly when present in high titers[14] and/or when showing strong platelet-activating properties.[9,15] Progressive formation of in situ immune complexes composed of HIT-IgG–PF4–heparin on the platelet surface leads to clustering of the platelet FcγIIa receptors and results in strong platelet activation.[16] Indeed, in vivo activation of platelets[17] by HIT-IgG antibodies is believed to be the major explanation for thrombocytopenia in patients with HIT.

However, other events besides platelet activation contribute to thrombosis in HIT. First, platelet activation induced by HIT-IgG causes profound platelet membrane changes, including the formation of procoagulant platelet-derived microparticles, leading to accelerated thrombin generation.[18,19] Second, PF4 released from platelets binds

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Table 1. Heparin-Induced Thrombocytopenia: A Clinicopathologic Syndrome*

Clinical	Pathologic
Thrombocytopenia† with or without 1 or more of the following:	Activation assay
A. Venous thrombosis	Washed platelet assay
Deep venous thrombosis	Serotonin release assay
Coumarin-induced venous limb gangrene	Heparin-induced platelet activation test
Pulmonary embolism	Using citrated platelet-rich plasma
Cerebral venous thrombosis	Platelet aggregation test
Adrenal hemorrhagic infarction‡	Antigen assay
B. Arterial thrombosis	PF4/polyvinylsulfonate-EIA¶
Lower limb artery thrombosis	PF4/heparin-EIA¶
Cerebrovascular accident	In-house PF4-dependent-EIA that detects HIT-IgG#
Myocardial infarction	Fluid-phase EIA
Other	Particle gel immunoassay
C. Skin lesions (at heparin injection sites)§	
Skin necrosis	
Erythematous plaques	
D. Acute systemic reaction post-intravenous heparin bolus‖	
E. Hypofibrinogenemia secondary to decompensated DIC	

* Only selected laboratory tests for heparin-induced thrombocytopenia (HIT) are listed. DIC indicates disseminated intravascular coagulation; EIA, enzyme immunoassay; and PF4, platelet factor 4.

† Thrombocytopenia broadly defined as any clearly pathologic platelet count fall; a platelet count fall of greater than 50% that occurs between postoperative days 4 and 14 is suspicious for HIT even if the platelet count nadir remains greater than 150×10^9/L[5]; rarely, HIT-associated thrombosis occurs with less marked declines in the platelet count, especially in postoperative patients.[6]

‡ Adrenal infarction has been associated with adrenal vein thrombosis; bilateral adrenal infarction can cause acute or chronic adrenal insufficiency.[5]

§ About three quarters of patients with heparin-induced skin lesions do not develop a platelet fall to less than 150×10^9/L; heparin-induced skin lesions and thrombocytopenia have been associated with arterial thrombosis.[7]

‖ One or more of the following, beginning 5 to 30 minutes after intravenous heparin bolus[5]: chills, rigors, fever, flushing, tachycardia, hypertension, tachypnea, dyspnea, chest pain, cardiopulmonary arrest, nausea, vomiting, diarrhea, headache, or transient global amnesia.

¶ Commercial assay that detects IgM, IgA, and IgG HIT antibodies.[8]

In-house EIAs that detect only IgG HIT antibodies may have acceptably high sensitivity, with greater specificity for clinical HIT, than do assays that detect HIT antibodies of 3 classes (IgA, IgM, and IgG).[9,10]

to heparin-like glycosaminoglycans located on the endothelial cell surface; endothelium injured by HIT antibodies expresses tissue factor.[20,21] There is also recent evidence that HIT antibodies can cause monocytes to synthesize tissue factor.[22,23] Finally, PF4 released from activated platelets binds to heparin and neutralizes its anticoagulant properties. All these events conspire to generate a procoagulant state,[24] as shown by markedly elevated levels of thrombin-antithrombin complexes in patients with HIT.[1,2] Once initiated, the hypercoagulable state can persist for many days, and rarely for several weeks, despite cessation of heparin.

CLINICAL PICTURE

Timing of Thrombocytopenia

The typical presentation of HIT is a platelet count decrease that begins 5 to 10 days after starting heparin (day heparin starts equals day 0).[25] In about 30% of patients, HIT is recognized when there is a rapid fall in the platelet count, starting within minutes or hours of heparin administration. Recent heparin use, generally within the past 100 days, explains this profile, that is, rapid-onset HIT is caused by heparin administered to a patient with already circulating HIT antibodies, rather than new formation of HIT antibodies.[25] Heparin-induced thrombocytopenia antibodies are generally transient and become undetectable at a median of 50 to 85 days following the occurrence of HIT, depending on the assay performed.[25] The rapid formation of pathogenic IgG antibodies as early as 5 days after starting heparin, as well as the transience of these antibodies, appears to explain the temporal profile of HIT. Rarely, thrombocytopenia and thrombosis attributable to HIT can begin several days after discontinuation of heparin (delayed-onset heparin-induced thrombocytopenia and thrombosis[26]); these patients appear to have high titers of platelet-activating IgG antibodies, which clinically resembles a transient autoimmune prothrombotic syndrome.[26]

Severity of Thrombocytopenia

In large studies, the median platelet count nadir has been reported as approximately $55–60 \times 10^9$/L.[5,27–29] Only about 10% of patients have platelet counts less than 20×10^9/L, which is in marked contrast with typical drug-induced immune thrombocytopenia caused by quinine, quinidine, sulfa antibiotics, and others, in which the platelet count nadir almost invariably is less than 20×10^9/L.[5,30] Also, in about 10% of patients with HIT, the platelet count nadir never falls to less than 150×10^9/L,[5] often because the HIT complicates a postoperative course that would otherwise be characterized by postoperative thrombocytosis,[3,31] or occasionally because the patient has a preexisting chronic thrombocytosis. However, in these patients, there usually is a substantial fall in the platelet count attributable to HIT of 50% or more.

Thrombosis

Heparin-induced thrombocytopenia is strongly associated with venous and arterial thrombosis (odds ratio, 37;

Table 2. Sensitivity and Specificity of Selected Activation and Antigen Assays for Detecting Clinically Significant Heparin-Induced Thrombocytopenia Antibodies

Diagnostic Assay	Sensitivity, %	Specificity,* %	
		Early Platelet fall	Late Platelet fall
Platelet [^{14}C] serotonin release assay	90–98†	>95	80–97‡
Heparin-induced platelet aggregation assay	90–98†	>95‡	80–97‡
Platelet aggregation test using citrated platelet-rich plasma	35–85	90§	82§
Platelet factor 4/heparin enzyme immunoassay	>90	>95	50–93
Combination of sensitive activation and antigen assay	100‖	>95‖	80–97‖

* "Early" refers to a fall in the platelet count that begins within the first 4 days of starting heparin; "late" refers to a fall that begins on day 5 or later. The data range for the late platelet count fall indicates cardiac patients receiving unfractionated heparin and orthopedic patients receiving low-molecular-weight heparin, respectively.[8,9]

† Assumes use of certain quality control maneuvers, including use of weak positive control sera and selected and/or multiple platelet donors. Also, in about 5% of heat-inactivated serum, heparin-independent platelet activation is observed. If a new serum aliquot is heat-inactivated, and the test repeated, an interpretable result is achieved in at least half the cases. However, about 30% to 40% of samples (~2% overall) give a repeated "indeterminate" result and the activation assay is nondiagnostic.

‡ Assumes that the heparin-induced platelet aggregation test and serotonin release assay have similar sensitivity and specificity profiles; other platelet activation endpoints that may also give acceptable results using washed platelets include detection of platelet-derived microparticles by flow cytometry.[36]

§ Assumes that a 90% specificity in early thrombocytopenia attributable to non–heparin-induced thrombocytopenia (HIT) disorders (eg, nonspecific platelet activation related to acute inflammatory proteins) declines to an 82% specificity in late thrombocytopenia,[37] which may be attributable to subclinical HIT antibody seroconversion.

‖ Clinicopathologic definition assumes that at least 1 sensitive test must be positive for diagnosis of HIT; specificity of the activation assay is indicated.

95% CI, 5–1600; $P < .001$).[3,5] Approximately half of all patients with HIT are recognized because of new thrombosis that occurs soon after the onset of thrombocytopenia.[27] In the remaining patients, who are recognized because of thrombocytopenia alone (isolated HIT), about 40% to 50% will develop a thrombotic event if treated conservatively (discontinuation of heparin with or without substitution with warfarin).[27,29] Thus, the overall risk of HIT-associated thrombosis is about 75%, ranging from a low of 50% in patients with mild thrombocytopenia (platelet nadir, $>100 \times 10^9$/L), to a high of 90% in patients with more severe thrombocytopenia (platelet nadir, $<30 \times 10^9$/L).[5]

Venous thrombosis is most common, particularly lower limb deep venous thrombosis and pulmonary embolism.[5,27–29] Upper limb deep venous thrombosis is strongly associated with the use of a central line.[32] Arterial thrombosis is also relatively common and occurs most often in lower limb arteries, followed by stroke and myocardial infarction (ie, the opposite of the usual target distribution for arterial disease).[5] The arterial thrombi are often platelet- and leukocyte-rich, and are known as "white clots." Limb loss can be caused by arterial thromboembolism involving large arteries or by microvascular thrombosis; the latter syndrome usually is characterized by progression of deep venous thrombosis to acral necrosis in a patient who develops a supratherapeutic international normalized ratio during treatment with warfarin or other oral anticoagulants, that is, coumarin-induced venous limb gangrene.[1,33]

Other Sequelae

Bleeding is uncommon in HIT; even the occasional patient with severe thrombocytopenia typically does not have petechiae.[5] The uncommon, but characteristic, complication of adrenal hemorrhagic necrosis is believed to result from adrenal vein thrombosis that leads to hemorrhagic necrosis of the adrenal glands. Necrotic or erythematous skin lesions at heparin injection sites occur in 10% to 20% of patients who form HIT antibodies during subcutaneous heparin administration.[6] Only a minority of these patients develop thrombocytopenia, however. Acute systemic reactions that begin 5 to 30 minutes after intravenous heparin bolus can be the first clue that a patient has HIT.[5] Although virtually all patients with HIT can be regarded as having disseminated intravascular coagulation based on laboratory markers of in vivo thrombin generation, in only 5% to 10% of patients is the disseminated intravascular coagulation decompensated, that is, associated with hypofibrinogenemia or prolongation of the international normalized ratio.[5]

Frequency

An unusual aspect of HIT is its variable frequency, depending on the type of heparin and the type of patient population.[9] Unfractionated heparin (UFH) is more likely to cause both HIT antibody formation and clinical HIT, compared with low-molecular-weight heparin (LMWH).[3,9,34] In addition, surgical patients appear to be more likely to develop HIT than medical patients.[34] About 15% of orthopedic surgery patients who receive UFH for 2 weeks develop HIT antibodies; one third of these patients (5% overall) develop clinical HIT (defined as a 50% or greater fall in the platelet count).[9] In contrast, although 50% of cardiac surgery patients form HIT antibodies following heart surgery, only about 5% of these (2%–3% overall) develop clinical HIT.[9,35] The frequency of both HIT antibody formation and clinical HIT is believed to be lower in medical patients receiving heparin,[34] but this patient group has not been studied as extensively.

TEST METHODS

Table 2 lists selected functional (platelet activation) assays and PF4-dependent antigen assays that have been evaluated for detecting clinically significant HIT antibodies. Further details regarding assay methods are provided elsewhere.[8,36–38] There are several advantages and disadvantages of these assays, and since no single assay has 100% sensitivity and specificity, reference laboratories should be able to perform more than 1 assay.

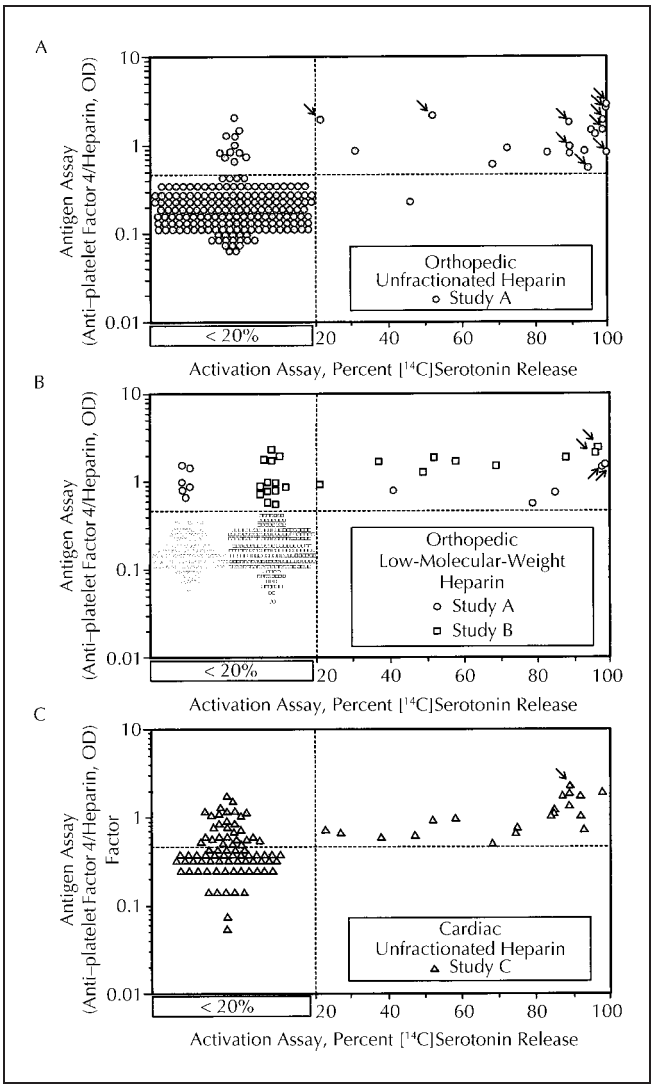

Figure 1. *Comparison of activation and antigen assays for heparin-induced thrombocytopenia–immunoglobulin G (HIT-IgG) antibodies in patients who have undergone cardiac and orthopedic surgery. Quantitative results of activation and antigen tests for HIT-IgG are shown for 3 clinical treatment settings: orthopedic unfractionated heparin (UFH) (A), orthopedic low-molecular-weight heparin (LMWH) (B), and cardiac UFH (C). Results are shown for all 744 patients. All antigen assay data are given quantitatively. For the activation assay results, samples that gave <20% serotonin release are shown without reference to the actual quantitative result (see box designated <20%). Arrows indicate the data points corresponding to the 15 patients with HIT identified in these prospective studies (≥50% fall in the platelet count from the postoperative peak platelet count). The results indicate that whereas the antigen assay is more sensitive for detecting clinically insignificant HIT-IgG, both the antigen and activation assays have equally high sensitivity for detecting clinical HIT. Furthermore, the activation assay has the highest specificity for clinical HIT. Reprinted with permission from* Blood.[9] *Copyright 2000, WB Saunders.*

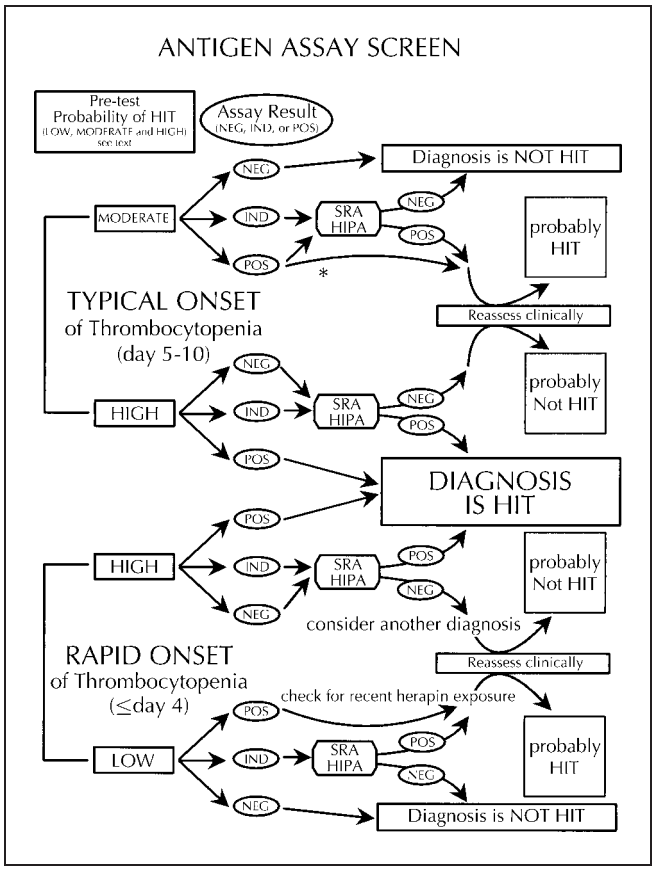

Figure 2. *Diagnostic algorithm if using an antigen assay to screen for heparin-induced thrombocytopenia (HIT). For patients with "typical onset" of thrombocytopenia, the pretest probability of HIT is usually judged to be moderate or high, depending primarily on whether another plausible explanation for thrombocytopenia is apparent, and whether new thrombosis has occurred. For patients with rapid onset of thrombocytopenia, the pretest probability of HIT is usually judged to be low or high, depending primarily on whether there has been recent exposure to heparin, and whether another plausible explanation for thrombocytopenia is apparent. The asterisk indicates that a platelet activation assay could be useful in a patient with only a moderate pretest probability for HIT and a positive platelet factor 4 (PF4)-dependent enzyme immunoassay, as a positive activation assay has a higher specificity for clinical HIT. NEG indicates negative test result; IND, indeterminate test result (eg, result for PF4-dependent enzyme immunoassay weakly positive, eg, <1.0 optical density [OD] units); POS, clear positive test result (>1.0 OD units); SRA, serotonin release assay (activation test); HIPA, heparin-induced platelet activation assay. Reprinted with permission from Warkentin and Greinacher.[8(p257)] Copyright 2001, Marcel Dekker Inc.*

Washed Platelet Activation Assays

Washed platelet activation assays, such as the platelet [14C]serotonin release assay[8,9,39,40] and the heparin-induced platelet activation test,[8,41,42] are used by a few reference laboratories. A major drawback is their technically demanding nature; for example, Eichler et al[43] reported results of a workshop that showed considerable variability among laboratories in reporting results using the platelet activation (heparin-induced platelet activation) test, compared with the antigen assay. Attention to methodologic detail is crucial; for example, use of apyrase in a wash step is necessary to maintain platelet reactivity to HIT antibodies.[8,44]

A fundamental problem is that these assays use a nonspecific endpoint (platelet activation), and thus care must be taken to ascertain that a given platelet activation response is actually caused by HIT antibodies, rather than residual thrombin or immune complexes.[8,38] This can be accomplished by requiring the following reaction criteria to detect HIT antibodies: (1) significant platelet activation at a heparin concentration of 0.1 to 0.3 U/mL that is generally greater than that observed at 0 U/mL heparin; (2) absence of significant platelet activation at a high heparin

concentration (100 U/mL); and (3) platelet activation inhibited by a monoclonal antibody that blocks the platelet Fc receptor.

Prior to performing the assay, residual thrombin in the serum or plasma sample must be heat-inactivated (56°C for 30 minutes). In about 5% of samples tested, the heat-inactivated serum gives an ''indeterminate'' result, that is, the serum causes platelet activation at all heparin concentrations tested. By obtaining another untreated serum aliquot and performing heat-inactivation, the second aliquot may then give a clear positive or negative result (suggesting that heat-aggregated IgG immune complexes may have been formed using the first serum sample). Unfortunately, almost half of these samples (about 2% overall) give persistent indeterminate results, perhaps because of circulating immune complexes; in these cases, an antigen assay must be used for investigation of HIT antibodies.[8,38]

There is considerable variability among HIT sera in their ability to effect platelet activation, as well as in platelets obtained from various normal donors to be activated by HIT sera.[40] Fortunately, these differences in reactivity are hierarchical, that is, HIT sera and platelet donors can be ranked from most to least reactive, with individual serum/platelet reactions following a predictable pattern. This hierarchy has important quality control implications, for example, optimally reactive platelet donors should be used whenever possible, and both weak and strong positive control sera should be used to ensure that the platelets used in any activation assay are capable of responding even to a weak HIT serum.[40] Some laboratories test against a panel of 4 different platelet donors, which may increase diagnostic sensitivity.[41]

Platelet Aggregation Test

The platelet aggregation test (PAT) using citrated platelet-rich plasma and standard platelet aggregometry was among the first assays used to detect HIT antibodies.[45] One method[37] found the sensitivity and specificity to each to be about 80% (using the most reactive platelets and in comparison with nonthrombocytopenic patients receiving heparin). Two studies that directly compared the PAT with either the serotonin release assay[46] or the heparin-induced platelet activation test[42] found the sensitivity of the PAT to be only 33% to 46% of that of the washed platelet assays. A major problem with the PAT is that only a limited number of test conditions and patient/control samples can be tested at any one time. This restriction limits the number of maneuvers that can be used to ensure high test sensitivity and specificity. It is also possible that platelets suspended in citrated platelet-rich plasma will react nonspecifically to heparin in the presence of fibrinogen and other acute phase reactants that may be present in the plasma of an ill patient, suggesting the potential for false-positive diagnosis and lower test specificity.[47] For these reasons, platelet aggregation assays have been superseded in recent years by other assays, particularly the antigen assays. If the PAT is used as an initial test for HIT, a positive test generally supports the diagnosis of HIT, and further testing is usually not required. However, given the lower sensitivity of the PAT (Table 2), a negative test does not exclude HIT in a patient with a moderate or high pretest probability for HIT. In these situations, further testing with the antigen assay or washed platelet activation assay (or both) should be performed. Some laboratories perform the PAT when there is a weak positive antigen assay result so as to help determine the clinical significance of the positive antigen assay.

Platelet Factor 4–Dependent Antigen Assays

Two PF4-dependent antigen assays have been developed that are commercially available for detecting HIT antibodies.[8,38,48,49] One assay, known as the Asserachrom (Stago, Asnières, France), detects antibodies reactive with PF4-heparin complexes. A more recent test, known as the GTI-PF4 (GTI, Brookfield, Wis), detects antibodies reactive with PF4 bound to polyvinylsulfonate. Both tests use goat anti-IgG/IgA/IgM to detect HIT antibodies. However, although there is anecdotal evidence that IgM and IgA HIT antibodies might occasionally cause HIT,[50] prospective studies have shown that assays that detect only HIT-IgG antibodies have very high sensitivity for diagnosing clinical HIT.[9,10] Thus, a drawback of the antigen assays is that they are more likely to detect clinically insignificant HIT antibodies, compared with sensitive washed platelet activation assays. This finding was indeed true even when an activation assay (serotonin release assay) was compared with an in-house antigen assay that detected only IgG antibodies reactive with PF4-heparin (Figure 1).[9]

Newman and colleagues[51] developed a fluid-phase antigen assay that avoids problems of protein (antigen) denaturation inherent in solid-phase assays. This assay may give a lower rate of false-positive reactions and also is useful for assessing in vitro cross-reactivity of HIT-IgG against LMWH and heparinoids. Vun and colleagues[52] found that binding of HIT-IgG to PF4-heparin adsorbed onto positively charged nylon membranes could also permit an antigen assay that avoided using PF4-heparin bound to plastic. There is emerging consensus[9,10,51,52] that antigen assays that detect only HIT antibodies of the IgG class are suitable to confirm a clinical diagnosis of HIT; at present, however, these assays are not commercially available.

Particle Gel Immunoassay (ID-H/PF4 Test)

The particle gel immunoassay (ID-H/PF4 test) has adopted the ID microcolumn system used for red cell serology by using special red-dyed, high-density polystyrene particles coated with PF4-heparin complexes that serve as a solid phase. Patient serum and PF4-heparin–coated microbeads are added to the incubation chamber of the microcolumn card, and after a 5-minute incubation, the card is centrifuged. A strong positive result is indicated by the agglutinated microbeads remaining at the top of the column.[53] The assay is technically easy, rapid, and can be automated. A preliminary study[54] suggested that this assay may have sensitivity intermediate between activation and commercial antigen assays, with specificity resembling that of the activation assays. This assay therefore offers promise as a rapid, routine assay for HIT.

TEST APPLICATIONS

Heparin-induced thrombocytopenia assays are performed most often to investigate patients who develop thrombocytopenia, with or without thrombosis, that begins during, or soon after, heparin treatment. For such patients, both the washed platelet activation assays and antigen assays have similar high sensitivity for detecting clinically significant HIT antibodies (Table 2). Diagnostic specificity is greater for washed platelet activation assays, however, particularly in patients following cardiac sur-

Table 3. Pseudo–Heparin-Induced thrombocytopenia (HIT) Disorders*	
Pseudo-HIT Disorder	**Comment**
Adenocarcinoma	Thrombocytopenia may complicate cancer-associated DIC/thrombosis, especially after *stopping* heparin; warfarin-associated venous limb gangrene has been reported in these patients[59]
Pulmonary embolism	Platelet activation may be secondary to clot-bound thrombin
Diabetic ketoacidosis	Hyperaggregable platelets predispose to thrombocytopenia and thrombosis
Antiphospholipid antibody syndrome	Pathogenesis of thrombocytopenia is obscure, but could involve platelet-activating antibodies in some instances
Thrombolytic therapy	Platelet activation by thrombin bound to fibrin degradation products
Septicemia-associated DIC/purpura fulminans	Symmetric peripheral gangrene secondary to DIC with depletion of protein C has been reported
Infective endocarditis	Infection-associated thrombocytopenia; ischemic events secondary to septic emboli
Paroxysmal nocturnal hemoglobinuria	Platelets susceptible to complement-mediated damage
Posttransfusion purpura	Although timing of thrombocytopenia resembles HIT (ie, 5–10 d after surgery requiring blood products), patients with posttransfusion purpura develop bleeding rather than thrombosis

* Pseudo–heparin-induced thrombocytopenia (HIT) disorders are defined as those that clinically resemble HIT (generally, thrombocytopenia complicated by thrombosis), but with negative testing for HIT antibodies using both activation and antigen assays. For some pseudo-HIT disorders, heparin treatment can result in platelet count recovery (eg, cancer-associated and pulmonary embolism–associated disseminated intravascular coagulation (DIC).

gery, where as many as 50% of patients develop clinically insignificant HIT antibodies detectable by antigen assays.[9]

Heparin-induced thrombocytopenia assays are also performed for research purposes, such as to determine the frequency of HIT antibody seroconversion in various clinical settings during treatment with various preparations of heparin.[3,9,35,55,56] In these situations, the antigen assay is more sensitive for detecting antibodies, and thus for determining differences in frequency of HIT antibodies in different clinical settings. Serologic studies have also shown that many patients develop PF4–heparin-reactive antibodies of the IgA and/or IgM classes that do not cause thrombocytopenia (T.E.W., unpublished data, October 2000).

Reference laboratories should be able to perform at least 1 type of sensitive activation and antigen assay, for several reasons. First, approximately 2% of patient sera repeatedly give indeterminate results by activation assay, and so an antigen test must be performed for diagnosis.[38] Second, there may be rare patients with clinically suspected HIT who test negative for antibodies in antigen assays, but positive in activation assays. There is evidence that such patients may have antibodies against other members of the chemokine family other than PF4, such as interleukin-8 or neutrophil-activating peptide 2.[57] Finally, for a patient who only has a low pretest probability for HIT, a (weak) positive antigen test may not be sufficiently persuasive to give a convincing clinicopathologic diagnosis. Indeed, if a sensitive activation assay tested negative in such a patient, it would suggest the patient actually did not have HIT.[8]

Figure 2 suggests an algorithm for diagnostic testing that assumes an antigen assay is used as a screening test for HIT.[8] In a patient with low or moderate pretest probability for HIT, the sensitivity of an antigen assay is sufficiently high that a negative test essentially rules out the diagnosis of HIT. However, the algorithm suggests that in patients with a weakly positive antigen assay result (indeterminate) or a positive antigen assay in a patient with only a low or moderate pretest probability of HIT, an activation assay may be useful to confirm or refute the diagnosis. However, this practice often will require the serum to be referred to a reference laboratory.

CRITERIA FOR DIAGNOSIS

Heparin-induced thrombocytopenia should be viewed as a clinicopathologic syndrome, that is, the diagnosis should be made only if clinical abnormalities are seen and HIT antibodies are detected (see Table 1). A corollary is that negative testing for HIT antibodies using 2 sensitive assays (preferably, 1 activation and 1 antigen assay) essentially rules out HIT, even if the clinical picture otherwise is suggestive of HIT. Indeed, there are a number of ''pseudo-HIT'' disorders[58,59] that can resemble HIT clinically, but in which HIT antibodies are not detectable and another explanation for thrombocytopenia and thrombosis is apparent (Table 3).

Furthermore, there is evidence that the magnitude of a positive result for HIT antibodies may be diagnostically helpful. For example, Figure 1 shows that a cutoff of 20% serotonin release (the historical definition of a positive test result[39]) was highly sensitive for clinical HIT. However, all but 1 serum gave greater than 50% serotonin release (ie, 14/15 = 93% sensitivity for 50% serotonin release threshold defining a positive test), and all but 2 sera gave greater than 80% serotonin release (ie, 13/15 = 87% sensitivity for 80% serotonin release cutoff). Thus, a general rule suggested by this study is that serum from most patients with clinical HIT will elicit a strong platelet activation response. This finding implies that physicians should require an even more convincing clinical profile for HIT in a patient whose activation assay gives only a weak result, for example, serotonin release from 20% to 50%.

Similarly, there is evidence that the magnitude of a positive antigen assay may be diagnostically useful (Figure 1).[9] For example, although the diagnostic cutoff for a positive HIT result was an optical density (OD) reading greater than 0.45, for 12 (80%) of 15 patients with clinical HIT, the OD was greater than 1.0. Thus, from a clinicopathologic viewpoint, a convincing clinical profile would be

Table 4. Recommendations: Platelet Count Monitoring for Early Detection of Heparin-Induced Thrombocytopenia (HIT)*

1. Patients at highest risk for HIT (postoperative patients receiving prophylactic or therapeutic-dose unfractionated heparin): minimum monitoring during heparin therapy, every second day from day 4 to day 10.†[3,9,34,35,56] *Level 1*

   Patients at intermediate risk for HIT (medical/obstetrical patients receiving prophylactic- or therapeutic-dose unfractionated heparin, postoperative patients receiving prophylactic-dose low-molecular-weight heparin, or patients receiving intravascular catheter ''flushes'' with unfractionated heparin): minimum monitoring during heparin therapy, 2 or 3 times from day 4 to day 10,† when practical.‡[3,9,34,35,55] *Level 1*

   Patients at low risk for HIT (medical/obstetrical patients receiving prophylactic- or therapeutic-dose low-molecular-weight heparin, medical patients receiving only intravascular catheter ''flushes'' with unfractionated heparin): routine monitoring is not recommended.§[34,55] *Level 2*
2. The crucial time period for monitoring ''typical-onset'' HIT is between days 4 and 10† after starting heparin, where the highest platelet count from day 4 (inclusive) onward represents the ''baseline.''[3,5,25] *Level 1*
3. For a patient recently exposed to heparin (within the past 100 d), a repeat platelet count obtained within 24 h following reinitiation of heparin is recommended to identify patients with rapid-onset HIT due to already circulating HIT antibodies.[5,25,26] *Level 1*
4. A platelet count should be measured promptly and compared with recent values in a patient who develops thrombosis during or soon after heparin therapy, or in a patient who develops an unusual clinical event in association with heparin therapy (eg, heparin-induced skin lesions, acute systemic reaction post–intravenous heparin bolus).[5,25,26,58] *Level 2*
5. A platelet count fall of 50% or greater from baseline can indicate HIT, even if the platelet count nadir remains above 150×10^9/L; occasionally, platelet count declines of even lesser magnitude attributable to HIT can be associated with thrombotic events.[5,6,31] *Level 1*

* Definition of levels of evidence: Level 1, 1 or more well-designed prospective studies or 2 or more well-designed retrospective studies; Level 2, retrospective studies or multiple anecdotal studies that reach consensus; and Level 3, isolated anecdotal studies and/or the consensus of expert practitioners.

† First day of heparin use is day 0; platelet count monitoring should be extended beyond day 10 if the platelet count begins to fall unexpectedly during the day 4 to day 10 period, or if heparin therapy is interrupted and restarted because of an intervening surgical or procedural intervention.

‡ Platelet count monitoring may not be practical when low-molecular-weight heparin is given to outpatients.

§ Monitoring as per the ''intermediate'' risk group is appropriate if 1 or more doses of unfractionated heparin were given prior to initiating therapy with low-molecular-weight heparin.[35]

helpful to suggest a diagnosis of HIT if the assay was only weakly positive ($0.45 < OD < 1.0$).

A blood sample obtained during thrombocytopenia is recommended for use in diagnostic testing. Although some investigators have reported that the sensitivity of certain assays may be somewhat higher using a blood sample obtained 1 or 2 days after discontinuation of heparin,[60] this does not appear to be the situation for sensitive washed platelet activation or antigen assays. However, it is important to test ''acute'' sera, since HIT antibodies are transient and usually become undetectable within just a few weeks following an episode of HIT.[25,60]

Table 5. Recommendations for Laboratory Testing for Heparin-Induced Thrombocytopenia (HIT) Antibodies*

1. Heparin-Induced Thrombocytopenia antibody testing is recommended for patients in whom there is clinical suspicion of HIT, based on the temporal features of the thrombocytopenia, or based on the occurrence of new thrombosis during, or soon after, heparin treatment.[3,8,38,58] *Level 1*
2. Acute serum or plasma should be used for testing whenever possible, since HIT antibodies are transient, and may not be detectable even a few weeks after clinical HIT.[25,60] *Level 1*
3. An antigen assay is an appropriate screening test for most laboratories that can perform enzyme immunoassays; however, confirmatory testing using a sensitive washed platelet activation assay (eg, platelet serotonin release assay, heparin-induced platelet activation [HIPA] test) may be appropriate if antigen assay results are weakly positive (indeterminate) or positive testing occurs in a patient with low pretest probability for HIT. In these situations, a negative activation assay suggests the patient likely did not have HIT.[8–10,38,42,48,49,55] *Level 1*
4. Washed platelet activation assays (eg, platelet serotonin release assay, HIPA test) have high sensitivity and specificity for clinical HIT; however, these assays are technically demanding and are most appropriate for reference laboratories.[8–10,38,42,48,49,55] *Level 1*
5. If the platelet aggregation test (PAT) using citrated platelet-rich plasma is used as an initial test for HIT, a positive test generally supports the diagnosis of HIT, and further testing is usually not required. However, given the lower sensitivity of the PAT, a negative test does not exclude HIT in a patient with a moderate or high pretest probability for HIT. In these situations, further testing with the antigen assay, the washed platelet activation assay, or both should be performed.[37,42,46] *Level 1*

* Levels of evidence are explained in the first footnote to Table 4.

SPECIFIC RECOMMENDATIONS

Platelet Count Monitoring

The frequency of platelet count monitoring should take into account the risk for HIT, which depends on the type of heparin used and the patient population under consideration (Table 4). The highest risk for HIT (2%–5%) is in postoperative patients receiving prophylactic doses of UFH (10 000–15 000 U/day) for at least 5 days, particularly postoperative orthopedic, cardiac, and vascular surgical patients. Thus, platelet count monitoring should involve at a minimum testing at 2-day intervals, beginning on postoperative day 4 and continuing until postoperative day 10, or even later if a suspicious platelet count decline begins within the day 5 to day 10 period. In contrast, medical and obstetrical patients receiving prophylactic or therapeutic doses of LMWH have a low risk of HIT (probably less than 0.2%), and many physicians would not perform routine platelet count monitoring. For patients receiving LMWH following major orthopedic surgery, there is an intermediate risk of HIT, approximately 0.5% in one study.[9] For these patients, it seems reasonable to obtain 1 or 2 platelet counts between days 5 and 10, although such testing may not be practicable if patients are discharged early to home with continued outpatient administration of LMWH. Extended prophylaxis with LMWH is not a reason to perform platelet count monitoring, as the risk of HIT declines after the typical 5- to 10-day at-risk period.[5]

Laboratory Testing

Table 5 lists recommendations regarding laboratory testing for HIT.

Treatment

Recommendations regarding treatment of HIT are beyond the scope of this article and are described elsewhere.[24,61] However, with respect to treatment implications of platelet count monitoring to permit early detection of HIT, it is important to note that there is no evidence that early cessation of heparin because of thrombocytopenia attributable to HIT avoids thrombotic complications.[27,29] Indeed, a retrospective study by Wallis and colleagues[29] found a trend toward a *higher* rate of thrombosis among patients in whom heparin was stopped within 48 hours of onset of thrombocytopenia, compared with later cessation of heparin (45% vs 34%; $P = .26$). Since the 30-day thrombotic event rate may be as high as 40% to 50%,[27,29] it is currently recommended that if isolated HIT is suspected based on platelet count monitoring, anticoagulation with an alternative antithrombotic agent should be given (danaparoid, lepirudin, argatroban),[24,61] usually in therapeutic doses.[62] Furthermore, as many of the thrombi occur in the first few days after heparin administration is stopped (approximately 5%–10% per day event rate over the first 1–2 days post–heparin cessation[2,27,63]), awaiting results of laboratory testing is not an acceptable reason to withhold anticoagulant therapy in a patient with a high pretest probability for HIT.[24]

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