What is the potential for overdiagnosis of heparin-induced thrombocytopenia?

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Heparin-induced thrombocytopenia (HIT) is caused by platelet-activating antibodies that recognize platelet factor 4//heparin (PF4/H) complexes. According to the "iceberg model," only a subset of anti-PF4/heparin antibodies of IgG class evincing strong platelet-activating properties cause clinical HIT. Since many centers rely predominantly on an anti-PF4/polyanion enzyme-immunoassay (EIA) to diagnose HIT, we estimated the potential for overdiagnosis when only this single test is available. We examined a database of 100 patients in whom the probability of HIT had been estimated using a clinical scoring system (4Ts), and where patients underwent systematic testing for HIT antibodies using three assays: the platelet serotonin release assay (SRA), an "in-house" EIA that detects IgG anti-PF4/heparin antibodies (EIA-IgG), and a commercial EIA that detects anti-PF4/polyanion antibodies of all three immunoglobulin classes (EIA-GTI). Whereas 16 of 100 patients fulfilled a "classic" definition of HIT (intermediate/high probability plus strong platelet-activating anti-PF4/heparin IgG antibodies), an additional 16 patients fulfilled a "liberal" definition in which any investigated patient (irrespective of the pretest probability) who had a positive EIA-GTI was considered to have HIT. The clinical features of these 16 additional patients—including generally weak antibodies and low risk for thrombosis—suggest underlying non-HIT explanations for thrombocytopenia. Patients with a positive SRA generally corresponded to those with intermediate or high pretest probability of HIT who also had strong EIA-GTI reactivity (>1.20 OD units). We conclude there is the potential to overdiagnose HIT by ~100% if any positive EIA is considered to "confirm" the diagnosis of HIT irrespective of the clinical scenario. Am. J. Hematol. 82:1037–1043, 2007. © 2007 Wiley-Liss, Inc.

Introduction

HIT is a clinicopathologic syndrome that is characterized by thrombocytopenia and/or thrombosis and the presence of platelet-activating antibodies that recognize multi-molecular complexes of platelet factor 4 (PF4) bound to heparin or certain other polyanions [1]. There are several diagnostic tests used to detect the pathologic antibodies, including platelet activation assays (e.g., serotonin release assay [SRA]; heparin-induced platelet activation [HIPA] test) and enzyme-immunoassays (EIAs) that detect antibodies reactive against PF4 bound to heparin or other polyanions, such as polyvinyl sulfonate [2–5]. Available commercial EIAs detect anti-PF4/polyanion antibodies of all three immunoglobulin class, whereas in-house assays can be performed that detect only IgG class antibodies [6–8].

Data from prospective studies support an "iceberg model" of HIT [9], wherein only a subset of "strong" platelet-activating anti-PF4/heparin antibodies of IgG class are likely to cause HIT [7–10]. In contrast, many patients form nonactivating antibodies of IgM or IgA class, or non- or only weakly platelet-activating antibodies of IgG class, and do not develop HIT [7,8]. This is particularly evident in postoperative settings where the frequency of subclinical seroconversion among patients receiving unfractionated heparin (UFH) can range from about 25% (postorthopedic surgery) [7,11] to over 50% (postcardiac or postvascular surgery) [10,12–14]. Since thrombocytopenia is a common problem among hospitalized patients with diverse alternative explanations besides HIT (e.g., postoperative hemodilution, sepsis, multi-organ system failure, etc.), there is a potential for overdiagnosis of HIT, especially if reliance is placed exclusively upon a relatively nonspecific assay such as the EIA.

Previously, we have analyzed archived blood samples collected from prospective studies and demonstrated that only about one-third of samples with detectable anti-PF4/heparin antibodies contain platelet-activating antibodies [7,10]. Similarly, a recent study of referred blood samples for diagnostic testing for HIT found only half of the blood samples that tested positive in the EIA also tested positive for platelet-activating antibodies [8]. These two studies suggest potential for substantial overdiagnosis of HIT, particularly if non-HIT causes of thrombocytopenia prompt diagnostic testing for HIT, and if positive EIAs are considered to "confirm" the diagnosis of HIT without taking into account the pretest probability of HIT, the magnitude of the positive EIA result, or the presence of platelet-activating antibodies.

Recently, we have established a database of 100 consecutive patients on whom we performed systematic assessments for HIT consisting of estimations of the clinical likelihood for HIT using a pretest scoring system, and laboratory testing for HIT antibodies using both the SRA and

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TABLE I. Definitions of HIT Used in this Study

Definition	EIA-GTI OD units	EIA-IgG, OD units	SRA, % release	4T Pretest category
Classic[a]	≥0.40	≥0.45	≥50	Intermediate or high
Liberal	≥0.40	Not considered	Not considered	Low, intermediate, or high
Modified conservative	≥1.20	Not considered	Not considered	Intermediate or high

[a]For the "classic" definition of HIT, any positive PF4-dependent EIA was considered acceptable in supporting the presence of anti-PF4/heparin antibodies in the context of a strong-positive SRA result (>50% serotonin release).

TABLE II. Baseline Characteristics

	Classic ($N = 16$)	Additional liberal ($N = 16$)	HIT test (EIA-GTI) negative ($N = 68$)
Demographics			
Age, median (min–max)	70 (43–85)	66 (48–87)	64 (28–89)
Male, *n* (%)	9 (66.2%)	12 (75.0%)	38 (61.8%)
4T's pretest probability score, *n* (%)			
Low	0 (0%)	11 (68.8%)	53 (77.9%)
Intermediate	8 (50%)	5 (31.2%)	15 (22.1%)
High	8 (50%)	0 (0%)	0 (0%)
HIT test results, *n* (%)			
SRA positive	16 (100%)	1 (6.3%)	0 (0%)
EIA-GTI positive	16 (100%)	16 (100%)	0 (0%)
EIA-IgG positive	16 (100%)	14 (87.5%)	1 (1.5%)
Platelet count nadir, $\times 10^9$/L, median (IQR)	62 (48, 74)	65 (53, 90)	70 (44, 103)

SRA positive: ≥50% serotonin release; EIA-GTI positive: ≥0.40 OD units; EIA-IgG positive: ≥0.45 OD units; The median (IQR) platelet count nadir for the 84 patients comprised of 16 additional liberal and 68 EIA-GTI negative patients was 67 (44, 94) $\times 10^9$/L.

two EIAs [15]. Using the information in this database, we determined the potential for overdiagnosis of HIT by comparing subsets of patients meeting two contrasting definitions of HIT: (1) a "classic" (or "conservative") definition, i.e., intermediate or high pretest probability plus the presence of strong platelet-activating anti-PF4/polyanion antibodies, or (2) those meeting only a "liberal" definition of HIT, i.e., low, intermediate, or high pretest probability plus a positive anti-PF4/polyanion EIA (Table I). We also evaluated a strategy to mitigate against HIT overdiagnosis using a third "modified conservative" definition of HIT.

Results

Sixteen (16%) of 100 patients met the clinical and serologic criteria for the classic definition of HIT (Table II). Of these 16 patients, 8 patients had a high pretest probability and 8 patients had an intermediate pretest probability. All patients tested strongly positive for HIT antibodies by the SRA (>50% release) and had positive testing for anti-PF4/heparin antibodies of IgG class. All 16 patients also tested positive in the EIA-GTI.

In contrast, 32 patients were identified using the liberal definition of HIT. This included the 16 patients identified using the classic definition and an additional 16 patients. Thus, twice as many patients (32 vs. 16) would have been "diagnosed" as HIT using the liberal definition. Of these 16 additional patients, 11 patients had a low pretest probability, and 5 patients had an intermediate pretest probability. Table II compares these two groups of patients as well as the remaining 68 patients who met neither definition of HIT. The classic HIT cases all had intermediate to high clinical pretest probabilities, and positive tests for HIT antibodies using both platelet activation assays and EIA-based assays. In contrast, the additional 16 cases identified using the liberal definition only had low to intermediate clinical pretest probabilities, and generally tested positive for HIT antibodies only using EIA-based assays. (The single patient from this latter group who had a positive SRA result had no known heparin exposure and presented with a de novo autoimmune disorder that included the presence of antiphospholipid antibodies [15]). Of the patients who did not meet any of the three definitions of HIT (Table I), all had either a low or intermediate clinical pretest probability, and only one patient tested very weakly positive for anti-PF4/heparin antibodies in only one assay (EIA-IgG, 0.518 OD units).

The magnitude of the positive HIT testing is shown in Fig. 1. Although all patients meeting the classic and the liberal definitions of HIT were similarly considered to have positive EIA-GTI assay results by fulfilling the manufacturer's recommended absorbance value cut-off of 0.40 OD units, the median absorbance value of 2.39 (IQR, 2.09–2.70) in the classic HIT patients was significantly higher than the median absorbance value of 0.89 (IQR 0.54–1.13) observed in the additional liberal HIT patients ($P < 0.0001$). Likewise, EIA-IgG testing demonstrated significantly stronger results in the classic HIT patients, with a median absorbance value of 2.23 (IQR 1.89–2.65), as compared with a median absorbance value of 0.64 (IQR, 0.41–1.05) in the additional liberal HIT cases ($P < 0.0001$).

Patient outcomes are shown in Table III. The patients meeting the classic definition of HIT differed from the other comparison groups in terms of frequency of thrombosis, bleeding, and in-hospital death. Thrombotic events occurred more frequently in patients meeting the classic definition of HIT. Eleven (68.8%) of the 16 classic HIT patients developed one or more thromboses. In contrast, only 1 (6.3%) of the additional 16 patients meeting the liberal definition, and 5 (7.4%) of the 68 patients who met neither definition of HIT, had one or more thrombotic events. Most thrombotic events occurred between days 5 and 10 after heparin exposure as shown in the thrombosis-free survival curve (Fig. 2A). Thrombosis-free survival from time

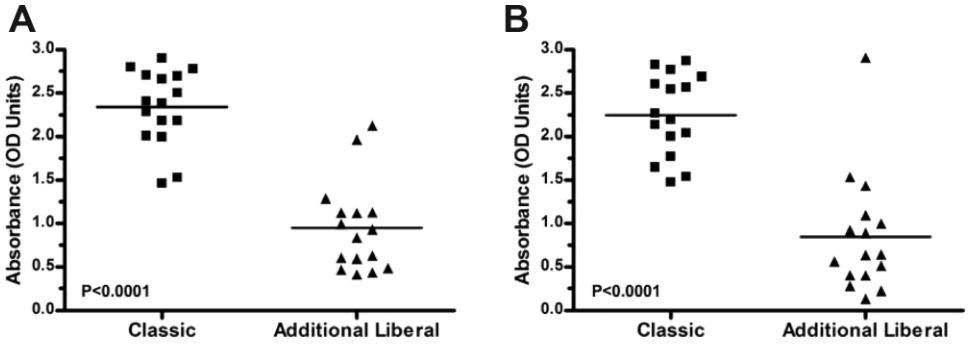

Figure 1. Comparison of the magnitude of EIA results between classic and additional liberal HIT patients. EIA-GTI (A) and EIA-IgG (B) absorbance values of individual patients are shown. Median absorbance value is indicated by the horizontal bar.

TABLE III. Outcomes

	Classic ($N = 16$)	Additional liberal ($N = 16$)	HIT Test negative ($N = 68$)
Thrombosis, *n* (%)	11 (68.8%)	1 (6.3%)	5 (7.4%)
Alternative anticoagulant given, *n* (%)	16 (100%)	8 (50%)	34 (50%)
Bleeding, *n* (%)	0 (0%)	3 (18.8%)	11 (16.2%)
Death, *n* (%)	1 (6.3%)	3 (18.8%)	18 (26.5%)

of heparin exposure was similar in the additional liberal and non-HIT patients, and was significantly higher than in the patients who met the classic definition of HIT ($P < 0.001$). Of the 17 patients who had thrombotic events, 12 patients had one or more thromboses that were clinically apparent prior to the assessment by a hematologist and prior to the initiation of an alternative nonheparin agent (if given). Thrombotic events subsequent to the time of physician assessment for HIT were similarly infrequent in all three patient groups (Fig. 2B; $P = 0.2685$). Two, none, and three events occurred following formal hematology service consultation in the classic, additional liberal, and non-HIT groups, respectively. Both of the events occurring after the time of physician assessment in the classic HIT patients were asymptomatic deep venous thromboses discovered on screening compression ultrasonography, and may well have already been present at the time of hematology assessment. In each of the five occurrences of thrombosis following formal hematology consultation, alternative anticoagulation had already been initiated either at prophylactic doses (two cases) or at therapeutic doses (three cases).

The patients meeting the classic definition of HIT also had fewer bleeding complications. There were no bleeding events (0%) among the 16 patients meeting the classic HIT definition. All of these patients (100%) were given an alternative anticoagulant in the management of their suspected HIT, of which 88% had anticoagulation at full therapeutic doses. By comparison, half of the patients (50%) in both the additional liberal and non-HIT groups were given alternative anticoagulants, of which 69% received prophylactic doses (primarily, low-dose danaparoid). Those receiving prophylactic doses of anticoagulation were similar to patients in whom all anticoagulation was discontinued with regards to the frequency of bleeding complications (17.2% vs. 14.8%) and in-hospital mortality (20.7% vs. 18.5%). These rates were lower than the subset of patients in whom the heparin was continued (20% bleeding complications, 60% in-hospital mortality).

In-hospital mortality was observed in a total of 22 patients in our 100-patient study population. None of the events was considered to be a thrombotic death. Only one (6.3%) patient from the classic HIT group expired during the admission, due to complications of an underlying malignancy (rather than because of HIT) on day 101 following the initial assessment for HIT. In contrast, in-hospital deaths occurred in 3 (18.8%) of the additional liberal and 18 (26.5%) of the HIT test negative patients. All three of the additional liberal patients died from causes other than thrombosis (namely, from sepsis or multiorgan failure). Of the in-hospital deaths among the 18 HIT test negative patients, 2 had nonfatal thrombotic complications, both of whom had an active malignancy (lung and pancreatic carcinomas). A Kaplan–Meier curve showing probability of survival based on in-hospital mortality is shown in Fig. 3. There was no statistically significant difference in survival probability between the three subgroups ($P = 0.2045$), but there was a trend towards lower mortality among the 16 classic HIT patients (6.3%) when compared with the remaining 84 patients (25%) who either tested negative for HIT or who met only the liberal definition of HIT ($P = 0.069$).

In a post-hoc analysis, our database was evaluated for patients meeting a modified conservative definition of HIT. Like the liberal definition of HIT, the modified conservative definition utilized just one laboratory test, the commercially-available EIA-GTI (the results in the SRA and EIA-IgG were not considered). However, unlike the liberal definition, the magnitude of the EIA-GTI result was taken into account, with a higher positive threshold of 1.20 OD units, as well as an intermediate-to-high clinical pretest probability of HIT (using the 4T's scoring system), in order to meet this modified conservative definition of HIT (Table I). Of our 100 patients, 16 patients met the modified conservative definition of HIT. These 16 patients comprised an identical subset of patients identified using the classic conservative definition (Table IV). This indicates that by accepting a higher EIA cut-off threshold of about 1.20 OD units, and by utilizing the pretest probability score, the definition of HIT would overlap considerably with one that utilized the platelet activation assay (SRA).

Discussion

It is important for physicians to avoid overdiagnosis of HIT because inappropriate discontinuation of heparin and initiation of alternative nonheparin anticoagulants for patients without HIT is expensive and could be associated with risk of bleeding without effective reversing agents. In this study, we demonstrate a significant potential for the

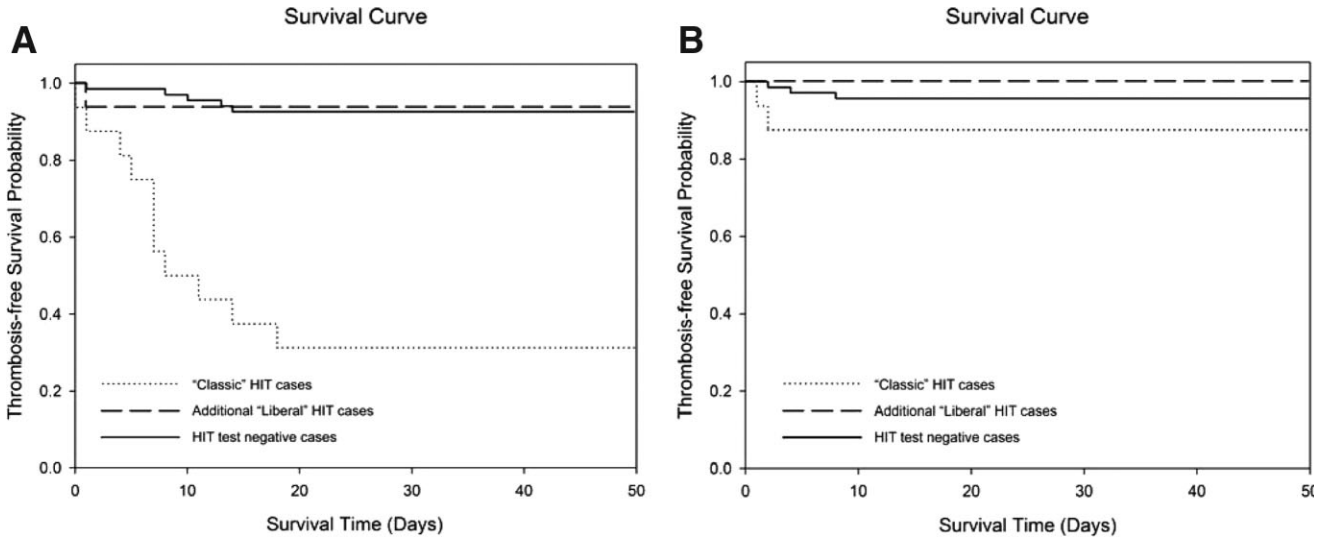

Figure 2. Time-to-event (Kaplan–Meier) analysis of thrombotic event rates among three groups of patients: classic HIT (n = 16), liberal definition of HIT (n = 16), and negative testing for HIT antibodies (n = 68). Thrombotic events included new, progressive, or recurrent thrombosis, thrombotic death, and thrombotic limb amputation. Events are documented in relation to the time of initiation of heparin (A) and from the time of hematologic assessment (B) with a maximum of one event per patient. The three curves differed significantly in Fig. 2A ($P < 0.0001$), whereas they did not in Fig. 2B ($P = 0.2685$). When the classic HIT patients were compared with the combined remaining two groups (HIT test-negative and liberal HIT patients), the differences remained significant for Fig. 2A ($P < 0.0001$) and continued to show no significant difference for Fig. 2B ($P = 0.2383$).

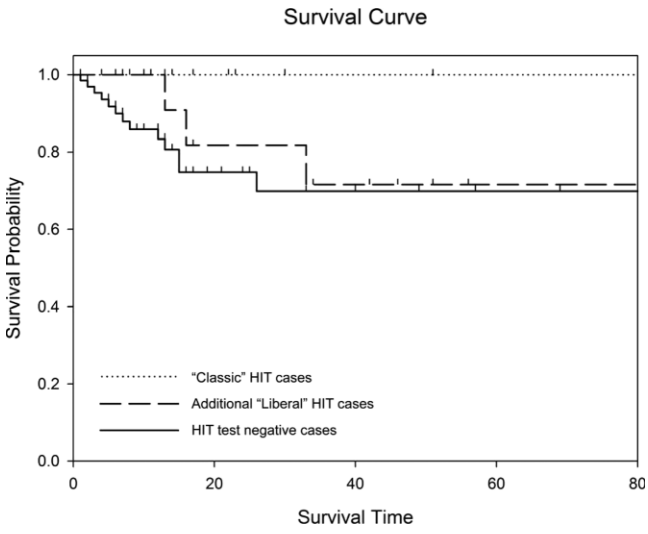

Figure 3. Kaplan–Meier analysis of overall survival among three groups of patients: classic HIT (n = 16), liberal definition of HIT (n = 16), and negative testing for HIT antibodies (n = 68). Although the three groups did not differ significantly (P = 0.2045), the classic HIT patients showed a trend towards lower mortality compared with the combined group of HIT test-negative and additional liberal HIT cases (P = 0.069).

overdiagnosis of HIT by comparing three subsets of patients: those who meet criteria consistent with the classic paradigm of HIT as a disorder caused by strong platelet-activating IgG antibodies that recognize PF4/heparin (or PF4/polyanion), those who meet less stringent criteria (anti-PF4/polyanion antibodies of any immunoglobulin class, with or without platelet-activating properties), and those who are not considered to have HIT by any criteria. The 16 patients who met the strictest classic criteria for HIT had intermediate to high clinical pretest probability scores as well as positive tests for HIT antibodies using both functional and EIA-based assays. In contrast, the use of a more liberal definition of HIT that required only a positive result on an EIA-GTI (commercial) assay irrespective of a pretest assessment, identified an additional 16 patients (32 patients total). These additional patients displayed little resemblance to patients meeting the classic definition, and instead were much more comparable to the (antibody-negative) non-HIT patients, both in thrombotic event-risk, bleeding risk, and in all-cause mortality.

The frequency of thrombosis was low in both the additional liberal and non-HIT patients (6.3 and 7.4%, respectively), and was significantly higher in the classic HIT patients (68.8%, $P < 0.0001$). The majority of these thrombotic events occurred prior to assessment by a hematologist and prior to the initiation of definitive therapy for HIT. This reflects the frequent occurrence of HIT-associated thrombotic events beginning prior to or at the same time as the onset of the HIT-associated platelet count fall [16]. Thrombotic events occurring after the time of assessment in the classic HIT patients was uncommon and was similar to that of the other two groups (12.6%). The dichotomy between the classic HIT patients and the other two comparison groups was also evident when bleeding events were assessed. Despite a much greater use of alternative anticoagulants, there were no bleeding complications observed among the classically defined HIT patients, whereas bleeding complications occurred with a similarly higher frequency in the additional liberal and non-HIT patients (18.8 and 16.2%, respectively). These polar thrombosis- and bleeding-risk profiles highlight the importance of careful patient selection for the initiation of alternative anticoagulants.

Finally, as mentioned, death from any cause during hospitalization was numerically higher in the additional liberal

TABLE IV. Comparison Patients Meeting Modified Conservative Definition of HIT

	Modified conservative positive ($N = 16$)	Modified conservative negative ($N = 84$)
Demographics		
Age, median (min–max)	70 (43–85)	68 (28–89)
Male, *n* (%)	9 (66.2%)	53 (63.1%)
4T's pretest probability score, *n* (%)		
Low	0 (0%)	64 (76.2%)
Intermediate	8 (50%)	20 (23.8%)
High	8 (50%)	0 (0%)
HIT test results		
SRA positive, *n* (%)	16 (100%)	1 (1.2%)
Median SRA release (percent)	91 (IQR = 68.0–98.3)	0.33 (IQR = 0–1.83)
EIA-GTI positive (≥1.20 OD units), *n* (%)	16 (100%)	0 (0%)
Median absorbance (OD units)	2.39 (IQR = 2.09–2.70)	0.22 (IQR = 0.16–0.33)
EIA-IgG positive (≥0.45 OD units), *n* (%)	16 (100%)	12 (14.3%)
Median absorbance (OD units)	2.23 (IQR = 1.89–2.65)	0.19 (IQR = 0.13–0.28)

Patients meeting the modified conservative definition of HIT must have an intermediate or high pretest probability of HIT using the 4T's score, combined with an EIA-GTI result meeting a raised cut-off threshold of 1.20 OD units. SRA and EIA-IgG results are not considered in the modified conservative definition.

and non-HIT patients (18.8 and 26.5% respectively) as compared with the classic HIT patients (6.3%). Indeed, a somewhat counterintuitive finding was that the mortality in the combined non-HIT and additional liberal groups tended to be somewhat greater than in the patients with classic HIT ($P = 0.069$; Fig. 3). This finding contradicts the common view of HIT as a highly lethal condition, and likely reflects the availability of effective treatments for this condition, as well as the high morbidity and mortality of several of the non-HIT disorders that cause thrombocytopenia (e.g., septicemia, multi-organ system failure of multiple causes, advanced malignancy, etc.).

We have demonstrated that the clinical characteristics of the additional liberal patients resemble that of non-HIT patients. Thus, if the pretest probability is not considered and any positive EIA is automatically assumed to be HIT—as with our liberal definition—overdiagnosis will be quite common. This risk of overdiagnosis of HIT may be as great as 100% as shown in our cohort of patients. In other words, if the only criterion for the diagnosis of HIT is a positive result on an EIA-based assay, up to half of the patients may be falsely labeled as having HIT. Similarly, as we have shown previously, the use of a clinical pretest probability assessment alone is not sufficiently accurate in the diagnosis of HIT. Although the 4T's scoring system has a consistently high negative predictive value, it has a variable positive predictive value for HIT that is likely dependent on the experience of the user and on the clinical setting [15]. In the ideal setting, HIT diagnosis would be made using a strategy of maximal diagnostic accuracy similar to that described in our classic definition. This would include assessment of pretest probability (e.g., using a scoring system like the 4Ts) combined with the use of several laboratory tests to detect HIT antibodies, including at least one functional assay to detect heparin-dependent platelet-activating antibodies. Practically, however, this may not be feasible, as many institutions do not perform multiple assays for HIT, and few employ functional assays like the SRA. Even institutions that do perform these functional tests often have lengthy turn-around times for results.

Although both the classic and additional liberal subsets of patients exhibited positive results with the EIA-GTI assay, the magnitude of the HIT antibody detection in patients meeting the classic definition was significantly greater (Fig. 1). Recently, several groups have suggested that absolute optical density values on EIA-based HIT assays can be interpreted in a semi-quantitative manner after observing a correlation between higher OD values with greater likelihoods of HIT and with increased thrombotic sequelae [7,17,18]. We detected a similar relationship in our cohort of patients. Consequently, we examined the possibility of exploiting the differences in the magnitude of the positive results in the EIA-GTI assay in an attempt to refine the diagnosis of HIT. Our hypothesis was that one would be able to distinguish a subset of patients that resemble the classic HIT patients from the remainder of the patients by using a higher EIA-GTI OD threshold in combination with a formal pretest probability assessment. Our cohort of patients was reanalyzed for HIT using a threshold of 1.20 OD units on EIA-GTI and 4Ts pretest probability that was intermediate or high, and identified 16 patients with clinical and laboratory profiles that were consistent with HIT. These 16 patients were the same 16 patients meeting the classic definition of HIT. Hence, with a single commercially-available laboratory test, and the use of a clinical scoring system, the diagnostic accuracy for HIT can be elevated to a level similar to that of performing a multitude of tests including the gold-standard SRA.

The threshold of 1.20 OD units in our modified conservative definition, which is exactly three times the manufacturer's recommended cut-off, was derived by examining our data, and by taking into account similar OD values (such as 1.00 OD units) suggested in the literature to increase the diagnostic usefulness of the commercial EIA [17]. Had we used the threshold of 1.00 OD units, we would have classified one additional patient (who had an EIA-GTI of 1.13 OD units) as meeting the serological criteria for HIT; however, on clinicopathologic grounds, this patient seems unlikely to have had this diagnosis, based upon the weak EIA-IgG (0.636 U), a negative SRA (0% release), lack of thrombosis, and presence of a plausible alternative explanation for the thrombocytopenia (pneumonia/sepsis). It should be emphasized that there cannot be any one single "ideal" OD cut-off that is universally applicable. For any single test sample, different laboratories will yield different absolute absorbance values, because optical densitometry values are relative readings that depend on the operating characteristics of both the assay and of each individual

photometer. In this respect, assessing an absorbance value in a PF4-dependent EIA is more like measuring a global coagulation time (e.g., prothrombin or activated partial thromboplastin time) than measuring a serum sodium or potassium level. Interestingly, our cut-off of 1.20 OD units was also more diagnostically useful when applied to our in-house EIA-IgG assay (in combination with an intermediate or high pretest 4T's score), in comparison with the usual cut-off derived (data not shown). Regardless of the precise cut-off chosen, we and others have now clearly demonstrated that stronger EIA test results are associated with a higher probability of having true HIT.

Our findings indicate that a high degree of diagnostic accuracy for HIT may be achieved using EIA-based assays with increased test thresholds in combination with formal clinical pretest assessments. Based on these data and on our previous validation of the 4Ts clinical model [15], if the pretest probability is intermediate or high and an EIA result is quite strong (in the range of at least 1.00 to 1.20 OD units), then HIT would be very likely and the use of an alternative anticoagulant at therapeutic doses would be recommended. If the probability is intermediate and the EIA result is only modestly positive (0.4–1.2 OD units), we would suggest referring the blood sample for testing using a functional (washed platelet activation) assay, such as the SRA. For many of these patients, it seems reasonable to use prophylactic doses of an alternative non-heparin anticoagulant (e.g., danaparoid, fondaparinux) while awaiting the secondary test results. If the pretest probability score for HIT is low, then any further testing is of questionable utility. This proposed strategy for the management of suspected HIT offers increased diagnostic accuracy with a minimum of investigations, but requires prospective evaluation for validation.

Materials and Methods

Patients and study design

We used a database established during the prospective evaluation of a clinical scoring system for HIT, the *4Ts* [15]. The database includes 100 consecutive inpatients referred for the evaluation of thrombocytopenia or suspected HIT at a single tertiary care centre, the Hamilton General Hospital in Hamilton, Ontario, Canada. All patients underwent a clinical assessment which included a formal evaluation of the pretest probability of HIT by two independent investigators using the 4T's clinical model. Blood samples were drawn at the time of initial assessment for laboratory determination of HIT antibodies. The study was approved by the local institutional ethical review board.

Clinical model

The 4Ts clinical model has been described in detail elsewhere [15]. In brief, the clinical model was used to stratify the pretest probability of HIT into high, intermediate, and low categories. The pretest probability category was determined on the basis of the four following criteria: the magnitude of the fall in platelet count, the timing the thrombocytopenia relative to known or suspected exposure to heparin, thrombotic events, and the presence or absence of an alternative diagnosis.

Diagnostic techniques

Laboratory determination of HIT antibodies was conducted using three separate assays on all patients. The tests consisted of a platelet SRA as previously described [2], an EIA that detects IgG anti-PF4/heparin antibodies (EIA-IgG), and a commercial EIA that detects anti-PF4/polyanion antibodies of all three immunoglobulin classes (EIA-GTI) available from Genetic Testing Institute, (GTI-PF4; GTI, Brookfield, WI). A priori, "positive" test result thresholds were defined as follows: serotonin release of $\geq$50%, EIA-IgG $\geq$ 0.45 OD (optical density) units, and EIA-GTI $\geq$ 0.40 OD units. The threshold of $\geq$50% serotonin release was used because test results of this magnitude are strongly associated with thrombocytopenia that bears a temporal relationship suggestive of clinical HIT (odds ratio, 78; $P < 0.001$) [19] and because about 95% of patients identified with clinical HIT in prospective studies have results of this magnitude [10,19,20]. In contrast, clinical HIT is infrequently seen in association with less robust serotonin releases of 20–49.9%. Test thresholds for the EIA assays were based on manufacturer's recommendations (EIA-GTI) or based upon results generated using normal individuals (EIA-IgG) [7].

Definitions of HIT

Two paradigms for the diagnosis of HIT were defined a priori. A "classic" conservative definition of HIT included patients with an intermediate or high pretest probability of HIT (as determined from the 4Ts score) plus the presence of platelet-activating antibodies detected by SRA plus demonstration of anti-PF4/polyanion reactivity based upon at least one of the two EIAs (EIA-GTI or EIA-IgG) testing positive. A more expansive "liberal" definition of HIT included all patients investigated for HIT who had a positive result with the EIA-GTI, irrespective of the 4Ts clinical score. Thus, by this definition, a patient with a low clinical pretest probability of HIT with a weakly positive EIA-GTI, and who may have tested negative by the SRA and EIA-IgG, would be considered to have had HIT. Our aim in evaluating such a definition of HIT was to estimate the risk for overdiagnosis of HIT—a potential hazard that would be particularly relevant for clinicians without easy access to platelet activation assays such as the SRA, who rely solely on a commercial EIA and who consider any positive test result as supportive of a diagnosis of HIT without regard to either the clinical picture or the magnitude of the positive test result.

In a post-hoc analysis, a third definition of HIT was created to assess the possibility of improving the diagnostic accuracy of the EIA-GTI assay. This "modified conservative" definition of HIT included patients with detectable HIT antibodies that surpassed an increased assay threshold of 1.20 OD units on EIA-GTI in combination with an intermediate or high 4Ts pretest probability score. The three definitions of HIT used in this study are summarized in Table I.

Analysis

Bleeding complications were defined as documented (overt) bleeding requiring transfusion of at least 1 unit of packed red blood cells or causing a drop in hemoglobin of at least 1 g/dL.

The Mann-Whitney U-test (two-sided), a nonparametric statistical method, was used to compare both the EIA-GTI and EIA-IgG results between the classic and liberal groups. Exact *P*-values were calculated due to the small sample size. The SAS System for Windows, Release 8.2 was used to perform the analysis and a *P*-value < 0.05 was considered statistically significant. The time to in-hospital death and times to thrombosis are compared using the log-rank test. The probabilities of no event over time are displayed by Kaplan–Meier (KM) curves.

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