Prediction of Heparin Induced Thrombocytopenia (HIT) Using a Combination of 4Ts Score and Screening Immune Assays

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Abstract

Clinical assessment (4Ts) followed by testing for Heparin/platelet factor 4 (HPF4) antibody in intermediate and high risk patients is the standard algorithm of pretest for Heparin induced thrombocytopenia (HIT), and the diagnosis is confirmed by serotonin releasing assay (SRA) in those who have positive antibodies. We conducted a retrospective analysis in a cohort of patients treated in a community hospital who had HIT antibody test by either ELISA or a rapid Particle Immunofiltration Assay (PIFA), regardless of their 4Ts scores. Among 224 patients, 17 had HIT. The PPV for those with a 4 T score $\geq$4 was 10.4%, which misdianosed 3 patients with HIT who tested positive for antibodies. Combining 4 T score $\geq$4 AND positive HIT antibody showed a PPV of 20.3% and a sensitivity of 70.6%, misdiagnosing 5 HIT patients. Using 4Ts $\geq$4 OR positive HIT antibody showed 100% sensitivity and 100% negative predictive value (NPV). The ELISA test had 100% sensitivity and 100% NPV, while the PIFA test missed 2 HIT patients, with sensitivity of 60% and NPV of 96.7%. Our results suggest that SRA testing should be conducted if a patient presents with a 4 T score $\geq$4 OR a positive HIT antibody, and antibody tests should be conducted for every patient suspected of HIT.

Keywords

Introduction

Heparin induced thrombocytopenia (HIT) is a serious immune mediated condition characterized by generation of antibodies against platelet factor 4 (PF4) on exposure to heparin, which upon binding with heparin and platelet factor 4 complex leads to platelet activation and thrombin formation.[1,2] It is a pro-thrombotic state manifested as thrombocytopenia and thrombosis.[3] The gold standard for diagnosis are serotonin release assays (SRA) or heparin induced platelet activation assays (HIPA), which are both technically demanding and performed only in reference laboratories.[4] In most community hospitals, the results are not immediately available for making treatment decisions.[5] Accurate and timely diagnoses for this condition is extremely important to ensure immediate therapy with a non-heparin anticoagulant. Failure to diagnose HIT is associated with a 5-10% daily risk of thrombosis, amputation or death[6]; however, overdiagnosis can lead to inappropriate withdrawal of heparin and administration of alternative anticoagulants with increased cost, fatal bleeding due to exposure of

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thrombocytopenic patients to anticoagulation, and thrombosis from unnecessary suspension of heparin.[7]

The current guidelines for HIT diagnosis suggests the following algorithm for the diagnosis of HIT: All patients should have an initial clinical assessment with "4Ts" score estimation, and for those who have intermediate or high risk 4Ts, a test for the presence of heparin/platelet factor 4 antibodies by ELISA test should be performed. Finally, in those who test positive ELISA, a confirmatory SRA should be administered.[4] Results from a meta-analysis showed robust exclusion of the diagnosis of HIT in the group of patients with low risk "4Ts" scores. However, positive predictive values (PPV) in the intermediate and high-risk groups were suboptimal, which were 0.14 (CI .09-0.22) and 0.64 (CI 0.40-0.82) respectively.[6] The ELISA assay is known to have an excellent negative predictive value of 98 to 99%. However, it has a low positive predictive value owing to the detection of clinically insignificant anti-PF4-Heparin antibodies.[8] All ELISA positive cases are mandated to have confirmation via an SRA.[9]

Although easier to perform than SRA, ELISA tests are not widely available in community hospitals and are usually performed in reference labs.[9] A rapid test kit, Particle Immunofiltration Assay (PIFA), also known as Heparin/Platelet Factor 4 Rapid Assay (H/PF4-RA), provides same day results. Its diagnostic accuracy for predicting SRA positive HIT has not been well studied.

In this retrospective analysis, we identified a cohort of patients who had test results for HIT antibodies and SRA independent of their 4Ts scores and examined the performance of the pretest accuracy of a criterion of dual positivity on 4Ts ≥4, or 4Ts ≥6 and positive HIT antibody test compared to taking the inclusive criterion of 4Ts ≥4 or 4Ts ≥6 OR positive HIT antibody test. Additionally, we compared the positive predictive value with rapid assay H/PF4-RA and the ELISA assay.

Methods

This retrospective study was reviewed and approved by the Institutional Review Board (IRB). A query to the pathology department database was made to generate a list of all consecutive patients who had either HPF4-RA or HPF4-ELISA in addition to an SRA performed for suspected HIT between January 2010 and June 2013. Patients were further eligible if clinical information was available for heparin or low molecular weight heparin (LWMH) exposure and adequate retrospective calculation of "4Ts." Two hundred ninety-four patients were screened. Fourteen patients were excluded due to "indeterminate" SRA results and 56 patients were excluded due to insufficient clinical information for 4 T calculation. After screening was complete, 224 patients were included in the analysis of this study. Electronic medical records were reviewed for platelet counts at various time intervals after heparin exposure, concomitant use of medications, admitting diagnosis, Doppler results, blood/urine cultures, antibiotics use, and clinical course.

Study investigators retrospectively rendered "4Ts" score on each patient based on definition criteria, using all available chart information.[10] Verification of 4Ts calculation was typically performed by at least 1 other investigator until an agreement was reached. Prior exposure was defined as heparin or LMWH use within 30 days or 30-100 days of the onset of thrombocytopenia. Active infection was considered a possible competing cause of thrombocytopenia (score of 1 point) and was diagnosed via a positive blood and/or urine cultures or use of antibiotics. Prior use of myelosuppressive chemotherapy drugs or cardiopulmonary bypass surgery (CABG) within 4 days for thrombocytopenia evaluation was considered another definite cause of thrombocytopenia (score of 2 points).

The HPF4-RA (Akers Bioscience, Inc, Thorofare, NJ) test was performed in the hospital laboratory following the manufacturer's instructions. ELISA and SRA tests were performed at Quest Diagnostic laboratory. The cut off for a positive ELISA assay was OD ≥0.400. A reading of serotonin release of equal to or above 20% at low dose (0.1 IU/ml and/or 0.5 IU/ml) UFH followed by reduction of the percentage release by one half or greater with the high dose (100 IU/ml) was considered positive according to the manufacturer's manual (Test ID: CPT 86022 SRA-Unfractionated, Quest Diagnostics NJ USA).

Statistical Analysis

Sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), and accuracy of "4Ts" scores, HPF4-ELISA, HPF4-RA test, and the combination of 4Ts and antibody tests were calculated. Biostatistics soft wares SPSS version 23 and SAS Macro were used for confidence interval and p-value calculations.

An exact Clopper-Pearson confidence interval was used in these analyses because the case (positive ELISA, SRA, or antibody test) and non-case counts were below 10 in each of the diagnostic test groups. A Fisher's Exact Test for 2-sample independent sensitivities/specificities was conducted to calculate p-values comparing the sensitivity, specificity, PPV, and NPV of each diagnostic test group. Accuracy of the tests are defined as the overall probability that a person is correctly classified and is calculated by adding the sensitivity multiplied by disease prevalence and the specificity multiplied by the complementary proportion of disease prevalence.

Results

Patient Characteristics and Prevalence of HIT

Among the 224 patients in this study cohort, the median age was 73 years old and the range was from 22 to 98 years old. There was 51% male and 49% female patients. In 68 (30%) patients, SRA was ordered simultaneously with HIT antibody assays. One hundred and seven (47%) patients had exposure to both intravenous and subcutaneous heparin, while 112 (50%) had subcutaneous heparin only. Among those who received intravenous heparin, 11 (10.3%) received treatment for deep vein thrombosis (DVT), and at least 49 (45.8%) received

Table 1. Prevalence of HIT on Different Medical Services.

	Total patients	SRA positive	Prevalence (%)
Entire cohort	224	17	7.6
Medical floor	97	9	9.3
Surgical floor	80	1	1.3
Medical ICU	14	4	28.6
Surgical ICU	33	3	9.1
CABG (can be on any floor)	37	1	2.7

treatment for cardiac issues, including 4 who received treatment for both. The duration of heparin use was measured as the interval between heparin use and platelet drop. Ninety-seven patients received heparin for 5-10 days or less than 1 day with prior exposure of less than 30 days; 24 patients received heparin for over 10 days or less than 1 day with prior exposure in the last 30-100 days; 103 patients received heparin for less than 4 days from the start of heparin without no recent exposure. Patients who received subcutaneous heparin all received at prophylactic dose for DVT prevention. Low molecular heparin usage was only documented in 5 (2%) patients, 2 with therapeutic dose, and 3 with prophylactic dose.

Seventeen patients were diagnosed with HIT based on an SRA result, yielding a prevalence of 7.6%. The prevalence was highest among those in the medical ICU (28.6%), followed by the medical floor (9.3%). Thirty-seven patients who underwent cardiac surgery requiring cardiopulmonary bypass pump were suspicious for HIT, and only 1 case was confirmed with HIT (Table 1).

Performance of the "4Ts" Score Calculation

Among 224 patients, 89 (39.7%), 98 (43.8%) and 37 (16.5%) patients were assigned to low, intermediate and high-risk groups respectively by "4Ts" calculation (Table 2). Three HIT patients were diagnosed in the low-risk group, 10 in the intermediate-risk group, and 4 in the high-risk group. The PPVs of 4Ts were 3.4% (95% CI 1.2-9), 10.2% (95% CI 6.9-14.9) and 10.8% (95% CI 4.7-23.2) in the low, intermediate and high-risk groups respectively. The highest specificity was observed among those in the high-risk group. For those who scored at least a 6 in the 4Ts test, the specificity was 84.1% (95% CI 78.4-88.8) and a sensitivity of 23.5% (95% CI 6.8-49.9), as well as a PPV of 10.8% (95% CI 4.7-23.2). By widening the positive group eligibility to 4 T $\geq$4, the sensitivity increased to 82.3% (95% CI 56.6-96.2), but the specificity decreased to 41.6% (95% CI 34.8-48.6), with the PPV remaining at 10.4% (95% CI 8.3-12.9) (Table 2). In measuring the accuracy of the 4Ts, 4 T $\geq$6 still ranked the highest at 78.07% (95% CI 72.13-83.26). Three HIT cases occurred in the low risk group measured by 4 T scores (4Ts 1-3).

Performance of the HIT Antibody Screening Tests

Among 224 patients, 83 (37%) showed positive HIT antibody test, which included either HIT ELISA tests (total 135 patients,

and 54 positive) or HPF-4 RA test (total 89 patients, 29 positive), while none had both tests. The incidence of a positive HIT Ab test in the 4Ts risk categories were: 27% in the low risk group, and 44.9% and 40.5% in the intermediate- and high-risk groups respectively. Only 15 of the 83 patients who had positive HIT antibody test were confirmed with HIT using an SRA, with a PPV of 18.1% (Table 2). Two HIT patients had false negative HIT antibody tests, both of whom had a false negative HPF-4RA test and their 4Ts were 7 and 2 respectively. All 3 patients in 4Ts 0-3 category had a positive Ab test, 2 of whom had an ELISA OD >1, and 1 who tested positive for HPF-4RA.

Further analysis showed that in the 4 T $\geq$6 group with the highest risk of HIT, 15 (40.5%) patients tested positive for the HIT antibody. However, among the 4 SRA + patients in this group, 1 tested negative for HIT antibodies. Therefore, the positive HIT Antibody test and the positive pretest by 4Ts (4Ts $\geq$4 or 4Ts $\geq$6) identified an overlapping but not inclusive group of patients (data not shown).

Enhancement in Diagnostic Accuracy using Combination of "4Ts" Score and HIT Antibody Test

In an attempt to increase the PPV of HIT pretesting, we combined test results from the HIT antibody (HIT Ab) and 4 T scores of 4 or greater. Table 2 shows a diagnostic test group who tested positive for both HIT Ab and 4 T scores 4 or more and a test group who tested positive for HIT Ab or with a 4 T score of 4 or more.

In the group of patients who had 4 T $\geq$4 AND HIT Ab +, PPV increased to 20.3% (95% CI 14.7-27.5), but the NPV of 97% (95% CI 93.8-98.5) was similar to using HIT antibody test alone (98.6%) or using 4Ts score alone (96.7%) (Table 3). It is also notable to mention that this measure did not increase sensitivity, as 5 HIT patients would be missed if this combination criterion were selected. In the group of patients who had 4 T $\geq$4 or HIT Ab +, the sensitivity and NPV went up to 100%, indicating that all HIT patients would be diagnosed. The PPV however remains low at 10.7% (95% CI 9.8-11.6). The combination of 4Ts 1-3 AND HIT Ab negative reciprocally showed a PPV of 0.

Similarly, if the criterion of 4Ts $\geq$6 AND HIT Ab + were used, the specificity, NPV and accuracy of the test would be the highest. However, this cutoff would result in a sensitivity of 17.7% (95% CI 3.8-43.4).

If the criterion of 4 T $\geq$4 AND HIT Ab + were used, 59 patients (26.3% of the entire cohort) patients will need treatment for suspected HIT, while if the criterion of 4 T $\geq$4 OR HIT Ab + were used, 71% (159/224) of the patients would need to be treated waiting for confirmatory SRA result. On the other hand, 4Ts $\geq$6, and 4Ts $\geq$6 AND HIT Ab+ had an accuracy of 78% (95% CI 72.1-83.3) and 88.4% (95%CI 83.5-92.3). The likelihood of HIT was the highest and alternative anticoagulation should certainly be used waiting for SRA confirmation.

Table 2. Sensitivity, Specificity, Positive and Negative Predictive Values of 4TSCORE, HIT Ab test, and the Accuracy (overall probability that a patient is correctly classified) = Sensitivity × Prevalence + Specificity × (1 − Prevalence).

	Total Number N (%)	SRA positive	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)
4Ts 1-3	89 (39.7)	3	17.7 (3.8-43.4)	58.5 (51.4- 65.2)	3.4 (1.2-9)	89.6 (87.1-91.7)	55.36 (48.59-61.98)
4Ts 4-5	98 (43.8)	10	58.8 (2.9-81.6)	57.5 (50.5-64.3)	10.2 (6.9-14.9)	94.4 (90.5-96.8)	57.59 (50.83-64.15)
4Ts ≥6	37 (16.5)	4	23.5 (6.8-49.9)	84.1 (78.4-88.8)	10.8 (4.7-23.2)	93.1 (91.1-94.6)	78.07 (72.13-83.26)
4Ts ≥4	135 (60.3)	14	82.4 (56.6-96.2)	41.6 (34.8-48.6)	10.4 (8.3-12.9)	96.7 (91-98.8)	44.64 (38.01-51.41)
HIT Ab +	83 (37.0)	15	88.2 (63.6-98.5)	67.1 (60-73.5)	18.1 (14.5-22.3)	98.6 (95-99.6)	68.75 (62.24-74.76)
4Ts ≥4 AND HIT Ab +	59 (26.3)	12	70.6 (44-89.7)	77.3 (71-82.8)	20.3 (14.7-27.5)	97.0 (93.8-98.5)	76.79 (70.7-82.15)
4Ts ≥4 OR HIT Ab +	159 (71.0)	17	100 (80.5-100)	31.4 (25.1-38.2)	10.7 (9.8-11.6)	100*	36.61 (30.29-43.28)
4Ts ≥6 AND HIT Ab +	15 (6.7)	3	17.7 (3.8-43.4)	94.2 (90.1-97)	20 (7.2-44.5)	93.3 (91.8-94.6)	88.39 (83.46-92.28)
4Ts ≥6 OR HIT Ab +	105 (46.9)	16	94.1 (71.3-100)	57 (50-63.9)	15.2 (12.9-18)	99.2 994.6-100)	59.82 (53.08-66.30)
4Ts 1-3 AND HIT Ab negative	65 (29.0)	0	0 (0-19.5)	68.6 (61.8-74.9)	0	89.3 (88.4-90.2)	63.39 (56.72-69.71)

The exact Clopper-Pearson confidence interval was used in this situation because there are not over 10 counts for both case and non-cases. In the above table, the test that is the most effective to detecting HIT is the HIT antibody test because the sensitivity, positive and negative predictive value are comparatively higher than any of the 4 T tests alone. If the patient presents with a 4 T score over 6 and tests positive for HIT antibodies, then the accuracy of the test increases from 78.07 (CI: 72.13-83.26) to 88.39 (83.46-92.28).

Comparison of the Diagnostic Accuracy Between the HIT ELISA Test and the HPF-4 RA Rapid Test

In comparing the predictive value and accuracy between HIT ELISA test and the rapid test HPF-4 RA, we found that overall they have similar accuracy of about 68% (Table 3). However, while a positive result in ELISA test had 100% sensitivity and 100% NPV, the HIT rapid test alone had a 60% (95% CI 58.0-78.7) and 96.7% (95% CI 90.7-98.9) sensitivity and NPV respectively. Both tests had low PPV, but the HIT ELISA test was higher than that of the rapid test (22.2% versus 10.3%, p = 0.0223). Overall, HIT tests with an OD ≥1 was associated with the highest PPV (50%, 95% CI 33-67), as well as the highest specificity (92.7%, 95%CI 86.6-96.6) and accuracy (91.1%, 95%CI 85-95.3) (Table 3).

Discussion

Due to the inferior single assay performance either by 4Ts or HIT lab tests, some clinicians in our community hospital would send for HIT antibody test and SRA test simultaneously (30% of the cohort), an SRA test despite a negative HIT Antibody test, or an HIT antibody test in patients who had 4Ts in the low risk groups. This practice, which does not follow the established test algorithm, provided a distinctive study subject composition to allow us to examine the independent contribution to the prediction of HIT from results of 4Ts and HIT antibody tests.

In this cohort, we showed that the pretest PPVs for diagnosis of HIT with 4Ts calculation score (4Ts ≥4 or 4Ts ≥6) were only 10.4% and 10.8% respectively, whereas that of the HIT antibody test was 18.1%. Out of the 17 HIT patients, 3 were in the 4Ts 0-3 low risk group. A dual positivity of the 4Ts score (4Ts ≥4) and positive HIT Ab test increased the PPV moderately to 20.3%, but that criterion would still result in false

negatives in 29.4% (5 patients) of HIT patients. The reason for this finding could be explained by the fact that there were patients who tested positive for HIT Ab and had a 4 T score greater than 4 or 6 with neither testing criteria group capturing the total HIT positive population. A selection criterion of 4 T ≥4 or positive HIT Ab test was able to encompass every patient who had HIT with a sensitivity and NPV of 100%, but with a reduced PPV of 10.7%.

The findings of our study confirmed our initial concern of missing out HIT patients in the low risk category. In our study, 3 cases of HIT appeared to have 4Ts in the low risk group, and all of them had a positive result on an HIT antibody assay. Although those 3 cases only represented 3.4% (3/89) of all patients in the 4Ts 0-3 group, it accounted for an alarming 17.6% of all SRA positive patients. This observation raised concern on the current guidelines, which recommends no further HIT Ab test in the low risk patients.[8] In the original observation[10], no one in the low risk group tested positive for HIT Ab.[10] Conversely, a prospective study by Linkins et al. evaluated the performance of a combination of 4Ts score estimation and the HIT antibody test.[11] They reported an HIT incidence of 1.9% in the low 4Ts score group compared to the 6.7% and 36.6% patients diagnosed of HIT in the intermediate and high-score groups respectively. Therefore, the authors recommended performing HIT antibody testing in patients without full information of previous heparin use or missing counts, even in the low risk group. Similar to the aforementioned study, the incidence of HIT in the low risk groups in our study was 3.3%, which supports the revised guideline recommendations as stated above.[8,11-13] Our result also showed that 4Ts 0-3 and negative HIT Ab had a PPV of 0, revealing a more effective criterion in ruling out HIT than being in the low risk group alone. This result was consistent with the report from Andrews et al in the prospective evaluation of the MICU patients.[14]

Table 3. Comparison of the Accuracy of the HIT Antibody ELISA Test and the HIT Rapid Test.

	Total test Positive	Total number	SRA Positive	Total negative	sensitivity	specificity	PPV	NPV	Accuracy
HIT ELISA test, OD >1	18	135	9	117	75 (42.81-94.51)	92.68 (86.56-96.6)	50 (32.99-67.01)	97.44 (93.44-99.02)	91.11 (84.99-95.32)
HIT ELISA test, OD <1 (0.4-0.99)	36	135	3	99	25 (5.49-57.19)	73.17 (64.43-80.76)	8.33 (3.17-20.18)	90.91 (87.64-93.38)	68.89 (60.36-76.57)
HIT ELISA test	54	135	12	81	100 (73.54-100)	65.85 (56.76-74.16)	22.22 (18.27-26.75)	100	68.89 (60.36-76.57)
HIT Rapid test	29	89	3 (5 total SRA positive)	60	60 (14.66-94.73)	69.05 (58.02-78.69)	10.34 (5.01-20.17)	96.67 (90.67-98.85)	68.54 (57.83-77.97)
HIT Ab test, either OD or Rapid	83	224	17	141	88.24 (63.56-98.54)	67.15 (60.3-73.5)	18.07 (14.53-22.26)	98.58 (94.96-99.61)	68.75 (62.24-74.76)
P value OD+ vs Rapid +	-	-	-	-	<0.0001*	0.6186	0.0223*	0.0332*	0.7359
P value OD≥1 vs Rapid+	-	-	-	-	0.00177*	<0.001*	<0.001*	0.956	<0.0001*

*Marks a statistically significant value at alpha = 0.05.

The exact Clopper-Pearson confidence interval was utilized in this situation as well because the case and non-case counts are not all over 10. To calculate the p-values in comparing the sensitivity, specificity, PPV, and NPV of the tests, a Fisher's Exact Test for 2-sample independent sensitivities. The best test for HIT in Table 3 would be testing positive for those with OD>1, as the overall accuracy of the test is 91.11% (CI: 93.44-99.02, p < 0.0001), which is statistically significantly higher than the rapid HIT test or only testing positive for OD. Although there are some statistically significant p-values comparing the tests, it is important to note that because the prevalence of the disease is below 10% in this sample, the prevalence dependent NPV and PPV are skewed.

The 5 false negative HIT patients found from both the 4 T and HIT Ab test also warrants further attention. Our data showed that patients whose 4Ts ≥4 or 4Ts ≥6 did not necessary test positive for HIT Ab and vice versa. This result highlights another concern on the current recommended algorithm for testing, which recommends no further SRA test if a patient is tested negative for HIT Ab.[8] In our study, the cohorts in the intermediate or high risk by clinical criteria and the cohort who would show positive HIT Ab test were only partially overlapping. Thus, these 2 tests may cast their predictions independently of each other. Using a selection criterion of 4 T ≥4 OR positive HIT Ab test captured every patient who had HIT in this study. Therefore, we suggest that cases suspected for HIT in the intermediate or high-risk group should move on to SRA testing even if an HIT antibody test were negative. Our study illustrated the poor predictive values with objective laboratory tests alone, and there is urgent need to improve diagnostic laboratory screening tests.

A significant fraction of this study cohort was tested for the presence of antibody against Heparin/platelet factor 4 (HPF4) using Particle Immune-Filtration Assay (PIFA). This assay has the advantage of rapid same day result turnover and has a sensitivity of 91.3%, specificity of 98% and overall agreement of 97.2% when tested against an ELISA assay (package insert information). However, in this study, we showed that its sensitivity was 60%, and NPV was 96.7%. This test also has a very low PPV (10%), so in order for it to be more effective that the ELISA assay, it must also have a perfect NPV in addition to its rapid turnover rate. Unfortunately, 2 patients with HIT showed a false negative rapid test, thus demonstrating its inferiority to the ELISA assay. A positive ELISA assay (OD >0.4) had 100% sensitivity and NPV, while the subgroup of OD ≥1 had PPV of 50% and overall accuracy of 91.1% as previously reported.[8] These results suggest that a negative HIT antibody by the PIFA rapid test cannot be used as a sole evidence to rule out HIT.

Our study showed an inferior performance of "4Ts" assessment compared to other published studies.[6,11] The PPV of "4Ts" in the high risk (4Ts ≥6) or combined high and intermediate risk (4Ts ≥4) groups in our study was 10.8% and 10.4% respectively. The PPV was 0.64 (95% CI 0.40-0.82), and 0.22 (95% CI 0.15-0.31) in the respective groups in the meta-analysis.[6] One explanation is the low prevalence rate of HIT (7.6%) in this study, comparing to 4%-42% in other reports.[6] On the other hand, sensitivity and specificities are not affected by the prevalence rate. The sensitivity of our study was 82.4% (95% Ci 56.6-96.2) in the 4 T ≥4 group, while results from a previously published prospective study showed a sensitivity of 97% (95% CI 86.2-99.8).[15] Although the overall specificity for 4Ts ≥4 was only 41.6%, 4Ts ≥6 had a significantly higher specificity of 84% and overall accuracy of 78%, confirming that critical clinical utility of clinical scoring. The inferior performance in sensitivity could be a result of inaccurate 4 T assessment. Because of these conclusions, there are many reasons as to why 4Ts testing is very prone to observer subjectivity.

We examined our data carefully to look for potential pitfalls that can explain the underperformance of 4Ts. (1) In this study, all "4Ts" calculations were performed retrospectively by study personnel incorporating all clinical information, some of which became available a few days after the HIT antibody testing, such as a more accurate assessment of the value and time course of the platelet nadir in addition to venous Doppler results for testing deep vein thrombosis. The chart was also reviewed thoroughly for documentation of prior heparin use. Even so, there could be error in the data collection, particular when some information was not available. This error may occur more frequently in a community hospital setting. (2) The "4Ts" score assigned to each patient was initially agreed upon by 2 investigators, during which another 2 other senior investigators also checked the calculation independently and reached a separate consensus. Judgmental bias could have occurred from this protocol, such as the determination of the start day of platelet decline as well as the day of platelet nadir. (3) We also made a rule that any positive culture or antibiotic use would receive a score of "1," as noted for possible competing cause, which could be controversial.

The subjectivity of 4 T score assignment was well recognized by experts. Based on the information in a randomized study on HIT in critically ill patients, the interpretation of 4 T scores between the real-time scoring by research coordinators compared to retrospective central adjudicators was not entirely consistent.[16] The difference lies in the availability of the information and disagreement in the cause of thrombocytopenia. Linkins et al emphasized that "patients suspected to have HIT may have more than 1 cause of thrombocytopenia, and 4Ts score alone may be insufficient to exclude HIT in more complex patients."[11] Overall, although the 4Ts assignment in our study was preformed retrospectively through verification by multiple investigators, it was still prone to observer subjectivity.

Limitations to this study include the small sample size and retrospective nature of this research in addition to the previously stated observer bias in the retrospective calculation of 4Ts scores. Because of the low prevalence of HIT in the study population, the NPV and PPV were skewed toward the lower end, which may not be generalizable populations. To this extent, it is important to note that this study was imperative to demonstrate the importance of incorporating multiple low-cost tests to ensure maximum effectiveness and efficiency in diagnosing HIT especially within a community hospital setting.

Through these findings, the most prevalent conclusion that is worth emphasizing is that the sensitivity in those who scored at or above a 4 in the 4Ts increased when a positive HIT antibody test result was incorporated. The 100% sensitivity when combining these results indicates the utility of an objective lab test result as a supplement to the subjective 4 T test, which has the potential to ameliorate detrimental false negative outcomes for HIT. Based on these results, we propose the following recommendations for HIT pre-tests, particularly to patients treated in community hospitals where the 4Ts calculation can

be suboptimal due to observer bias and insufficient information. (1) We recommend performing HIT antibody tests on all patients regardless of their 4Ts scores to provide an objective measure of HIT probability. (2) As recommended by the traditional algorithm, patients with 4Ts ≥ 6, or 4Ts ≥ 4 and/or positive HIT Antibody test should receive alternative anticoagulation therapy because of their high probability of HIT. (3) In order to effectively capture all potential positive HIT patients, physicians should consider broadening the criterion for further SRA testing in patients with 4Ts ≥ 4 or positive HIT antibody tests, and should only exclude those who score 1-3 for 4Ts 1-3 AND tested negative for HIT antibodies. (4) While a negative HIT antibody test by an ELISA assay may effectively rule out HIT, a negative HIT antibody test by a PIFA rapid assay should not be used as the sole concluding evidence to rule out HIT; an assessment combining the 4Ts scoring and HIT antibody result should also be performed.

Contributions

Rajat Thawani: Collection of data, data analysis and manuscript writing.

Srikant Nannapaneni: IRB application process, collection of data, data analysis and manuscript writing.

Vivek Kumar: Statistical data analysis, manuscript writing and proof reading.

Phone Oo: Collection of data and proof reading.

Michael Simon: Data collection, referencing and proof reading.

Anna Huang: Statistical data analysis, manuscript writing and proofreading.

Ishan Malhotra: Data collection and proof reading.

Yiqing Xu: Research idea, data interpretation, data collection, principle manuscript writing and proof reading.

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