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ORIGINAL ARTICLE

The dynamic range of immunoassays for heparin-induced thrombocytopenia

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Abstract

Background: Following the current guidelines, immunoassays for the diagnosis of heparin-induced thrombocytopenia (HIT) are interpreted dichotomously, with test results categorized as either positive or negative. However, the extent to which test results hold diagnostic significance across the entire dynamic range remains unclear.

Objectives: We utilized data from the prospective towards precise and rapid diagnosis of heparin-induced thrombocytopenia study, comprising 1393 consecutive patients with suspected HIT, to assess the diagnostic significance of 2 heparin/platelet factor 4

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immunoassay test results across their respective dynamic ranges: HemoSil Acustar HIT IgG (chemiluminescence immunoassay [CLIA]) and Lifecodes PF4 immunoglobulin G (enzyme-linked immunosorbent assay [ELISA]).

Methods: HIT diagnosis was determined by a washed platelet heparin-induced platelet activation assay. For each measurement point in the dataset, we computed likelihood ratios (LRs), sensitivities, and specificities. To provide posttest probabilities for individual test results, we calculated interval-specific LRs and integrated them into a web-based calculator.

Results: The prevalence of HIT was 8.5% (n = 119). An LR of ≥10 was first achieved at 0.3% of the dynamic range (0.4 U/mL; CLIA) and then at 16% (0.64 optical density; ELISA). An LR of ≥100 was present at 9.4% (12 U/mL; CLIA) and 75.0% (3.0 optical density; ELISA). The slope of the linear regression line (LR ~ dynamic range) was 9.5 (CLIA) and 0.9 (ELISA).

Conclusion: Despite both immunoassays showing an association between results and diagnostic significance, the strength of the association varies by assay. CLIA has a larger increase per measurement unit. Posttest probabilities for individual patients can be estimated using a web-based calculator: https://pcd-research.shinyapps.io/BayesianCalculator/.

KEYWORDS

heparin/adverse effects, immunoassay/methods, thrombocytopenia/diagnosis, likelihood functions, platelet factor 4, diagnosis, sensitivity and specificity

1 | INTRODUCTION

Intensive research has led to significant advances in the diagnosis of heparin-induced thrombocytopenia (HIT) [1–3]. Despite these advances, a new study reveals a troubling number of misdiagnoses and missed diagnoses in real-world clinical settings [4]. This begs the question: what factors contribute to this problem? The American Society of Hematology guidelines recommend a stepwise approach based on the dichotomous interpretation of 3 types of diagnostic tests [5]: clinical probability, which is assessed by the 4Ts score [6,7]; heparin/platelet factor 4 (H/PF4) antibodies, as determined by one of the approved immunoassays [8]; and platelet-activating antibodies measured with a functional washed platelet assay, specifically the gold standard tests heparin-induced platelet activation assay (HIPA) or serotonin release assay [9,10]. However, this approach is subject to a key problem: as functional tests are not available for the initial diagnosis, the medical decision is based solely on the positivity of the antibodies in addition to the clinical probability, estimated, for example, using the 4Ts score.

One might hypothesize that higher antibody titers increase the likelihood of HIT. As long as manufacturers typically provide quantitative readings, this information is already available at the time of clinical management decisions. This hypothesis is supported not only by analogies with other immunologically mediated diseases but also by a number of previous observations in the field of HIT. These studies looked at different cutoffs and found higher specificity when higher diagnostic thresholds were used [11–14]. However, the extent of this association along the respective dynamic ranges of the anti-H/PF4 immunoassays remains unclear.

In this analysis of the prospective towards precise and rapid diagnosis of heparin-induced thrombocytopenia (TORADI-HIT) study, we aimed to investigate the diagnostic value of 2 commonly used H/PF4 antibody assays across their dynamic ranges and provide guidance to clinicians for the interpretation of quantitative test results. A web-based online calculator ought to be developed to estimate the posttest probability for individual patients.

2 | METHODS

2.1 | Study design, setting, and population

The TORADI-HIT study is a prospective cohort study conducted in 11 tertiary care hospitals, which has been described in detail elsewhere [4,15–17]. Patients with suspected HIT were enrolled according to the following inclusion criteria: (a) suspected HIT (H/PF4 immunoassay ordered OR clinical assessment tool applied OR consultancy service for HIT assessment requested), (b) age ≥ 18 years, and (c) informed consent provided. Residual serum samples were snap-frozen at −80 °C and sent to the laboratory of the Inselspital Bern University Hospital.

Trained study nurses collected clinical data, the 4Ts score, and routine laboratory data following a prespecified protocol. All laboratory tests mentioned in this analysis were conducted at the central laboratory, as described below. HIPA served as the reference standard defining HIT [9,18]. The washed platelet HIPA test is regarded as a reference standard test for HIT by prominent scientific societies, including the American Society of Hematology and the British Committee for Standards in Hematology [5]. The pros (and cons) of this definition were discussed in detail in earlier publications of this study [4,15–17]. The protocol was approved by ethical committees of all study centers (ie, Kantonale Ethikommission Bern). The study was conducted in accordance with the Declaration of Helsinki. For the preparation of the manuscript, we followed the Standards for Reporting of Diagnostic Accuracy guidelines.

2.2 | Determination of laboratory tests

Two common H/PF4 immunoassays, as well as the washed platelet HIPA, were conducted within a week of sample arrival, and the performing laboratory technicians were blinded to clinical information and results from other assays (same aliquot without freezing). HIPA was done following an established protocol [9,18]. A positive test result was defined as platelet activation occurring in a minimum of 2 out of 4 donors within 30 minutes at a low heparin concentration (0.2 IU/mL), while no aggregation was observed at a high dose of 100 U/mL.

The immunoglobulin (Ig)G-specific enzyme-linked immunosorbent assay (ELISA) Lifecodes PF4 (Immucor Medizinische Diagnostik GmbH) was read at 405 nm on a Tecan Infinite F50 plate reader using Tecan Magellan Software version 7.2 (Tecan Group LTD). The assay's dynamic range spans from 0 to 4 optical density (OD), with positivity marked at OD ≥ 0.4. The instructions of the manufacturer were strictly followed [16]. The automated chemiluminescence immunoassay (CLIA) HemoSil Acustar HIT IgG (Instrumentation Laboratory) was executed on a BIO-FLASH Analyzer (Inova Diagnostics). This assay provides results from 0 to 128 U/mL, with positivity defined as a result ≥1.0 U/mL. Again, the manufacturer's instructions were strictly followed [16]. Immunoassays were strictly performed according to manufacturers' instructions and following strict quality controls [16].

2.3 | Statistical analysis

To assess the diagnostic value of the different immunoassays across their dynamic range, we used each unique value measured in the TORADI-HIT dataset as a threshold to calculate the sensitivity, specificity, and positive likelihood ratio (LR+). LRs+ are powerful measures of diagnostic accuracy that can be interpreted as the likelihood that a given test result would be expected in a patient with the target condition compared with the likelihood of the same result in a patient without the target condition [19,20]. An LR+ of ≥10 is considered very high. To normalize the immunoassay scales, the individual values were then scaled to the upper limit of the dynamic range of each assay. Subsequently, 3 plots were generated depicting the LR, sensitivity, and specificity against the scaled values, and a linear regression line was fitted to the calculated LR+. The LR+ is calculated by:

$$\mathrm{LR}+ = \frac{\text{Sensitivity}}{1-\text{Specificity}} \quad (1)$$

Furthermore, interval LRs (ILRs) were computed for the intervals where the test achieved the following specificities: <70.0%, 70.0% to 89.0%, 90.0% to 94.9%, 95.0% to 97.4%, 97.4% to 98.9%, and ≥99% [21]. The intervals were chosen based on clinical considerations, prioritizing specificity; guidelines do not exist in this regard. Corresponding 95% CIs were calculated using the formula by Simel et al. [22]. Similarly, the ILR represents the likelihood of a test result falling within a specific interval in patients with the disease compared with those without the disease. For ease of clinical application, the calculated ILRs were integrated into an online calculator, enabling the computation of posttest probabilities of HIT using Bayes' theorem [23].

3 | RESULTS

3.1 | Study population

Of the 1393 patients enrolled in the TORADI-HIT study, immunoassay results were available for 1318 patients for CLIA, 1378 for ELISA, and 1273 for particle gel immunoassay (PaGIA). The prevalence of HIT, as defined by a positive HIPA, was 8.5% (n = 119). The 4Ts score was low risk in 666 patients (47.8%), intermediate risk in 636 patients (45.6%), and high risk in 91 patients (6.5%). The population was described in detail in previous publications of the TORADI-HIT study [4,15–17].

3.2 | Diagnostic accuracy of immunoassays across the dynamic range

To assess the diagnostic significance of the immunoassays across their dynamic range, we calculated the LR, sensitivity, and specificity for each unique measurement point. The manufacturers' recommended cutoffs were found to be at 0.8% of the dynamic range for CLIA and 12.5% for ELISA. At the respective cutoffs, the LR+ was 15.8 for the CLIA (sensitivity 95.5%, specificity 94.0%) and 9.1 for ELISA (sensitivity 97.4%, specificity 89.3%). Figure 1 shows the LR plotted against the scaled value of the immunoassays (dynamic range), and Figure 2 shows the specificities (Figure 2A) and sensitivities (Figure 2B). An interpretation is given in the legends of the figures.

We then calculated the LR+ for the lowest value measured above the limit of quantification for each assay. For CLIA, LR+ was 3.4 (0.8% of the dynamic range; sensitivity 99.1%, specificity 70.6%). For ELISA, LR+ was 1.0 (1.00% of the dynamic range; sensitivity 100%, specificity 0.7%). An LR of ≥10 was first achieved at 0.3% of the dynamic range (0.4 U/mL; CLIA) and then at 16% (0.64 OD; ELISA). An LR of ≥100 was present at 9.4% (12 U/mL; CLIA) and 75.0% (3.0 OD; ELISA). This

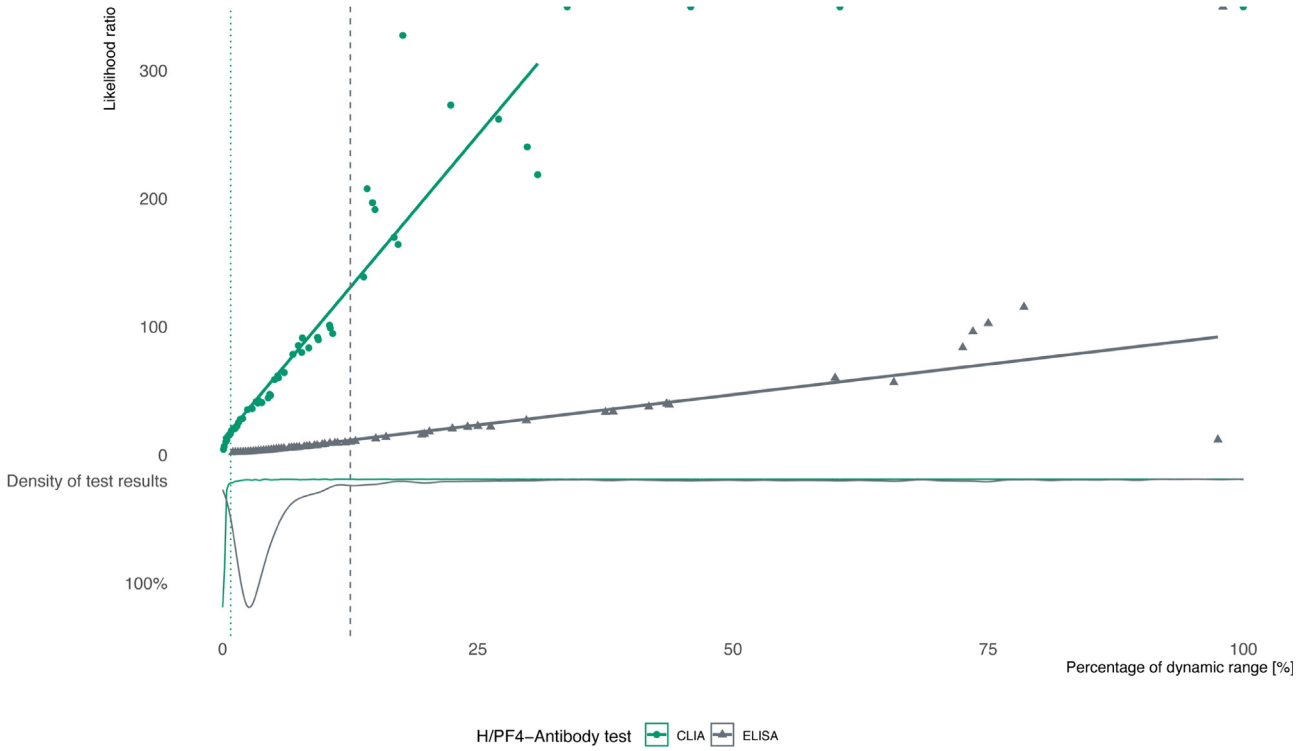

FIGURE 1 Diagnostic significance, expressed as a positive likelihood ratio (LR+), of common heparin/platelet factor 4 immunoassays over the dynamic range of measurements. The x-axis shows the test results scaled to the upper limit of the dynamic range of each assay (100%). LRs+ are powerful measures of diagnostic accuracy that can be interpreted as the likelihood that a given test result would be expected in a patient with the target condition compared with the likelihood of the same result in a patient without the target condition [19]. The density represents the distribution of test results. Dashed vertical lines represent the manufacturers' threshold. The diagnostic significance (LR+) is increasing much more rapidly with the chemiluminescence immunoassay (CLIA) compared with enzyme-linked immunosorbent assay (ELISA) over the range of measurements. Half-points at the y-limit represent infinity LR+, corresponding to 100% specificity. They were not used to calculate the linear regression line. Posttest probabilities for individual patients can be calculated with the web-based calculator: https://pcd-research.shinyapps.io/BayesianCalculator/.

can be interpreted as meaning that the CLIA provides a high degree of diagnostic information, even having lower results than the ELISA.

The slopes of the fitted linear regression lines were 9.5 (95% CI, 8.4, 10.6; intercept = 10.8; 95% CI, −1.0, 22.6), and 0.9 (95% CI, 0.8, 1.1; intercept = −1.3; 95% CI, −5.7, 3.1), respectively (Figure 1).

3.3 | Posttest probabilities according to immunoassay test results

To help physicians in the interpretation of immunoassay test results, we calculated ILRs in the specificity ranges of <70.0%, 70.0% to 89.0%, 90.0% to 94.9%, 95.0% to 97.4%, 97.4% to 98.9%, and ≥99% for the immunoassays and according to the 4Ts score. The intervals were ≤0.09, 0.1 to 0.49, 0.5 to 1.29, 1.30 to 3.09, 3.10 to 6.89, and ≥6.9 U/mL for the CLIA. For the ELISA, the intervals were ≤0.2, 0.21 to 0.59, 0.60 to 0.89, 0.9 to 1.49, 1.50 to 2.39, and ≥2.40 OD. The ILRs are given in the Table. To interpret the results, we implemented a Bayesian calculator. With this web application, one can estimate posttest probabilities of HIT for individual patients (https://pcd-research.shinyapps.io/BayesianCalculator/). The posttest probability is calculated from the pretest probability (prevalence or clinical pretest probability as determined by the 4Ts score) and the LR [23].

4 | DISCUSSION

In this analysis, we used data from the prospective TORADI-HIT study, including 1393 consecutive patients with suspected HIT, to assess the diagnostic significance of H/PF4 immunoassay test results across their dynamic ranges. We were able to demonstrate a strong association between the quantitative test results and the likelihood of HIT. The higher the test result, the higher the likelihood of HIT. However, the strength of this association varies by assay, with CLIA showing a higher increase per measurement unit. This means that, in the case of CLIA, HIT can be ruled in even for results that are relatively small in scale. To calculate posttest probabilities of HIT for individual patients, we developed an easy-to-use Bayesian calculator and implemented it on the website https://pcd-research.shinyapps.io/BayesianCalculator/.

Our study has several strengths. The data were obtained from a large prospective cohort, including patients with "suspected HIT," which closely resembles the target population of the tests. Therefore,

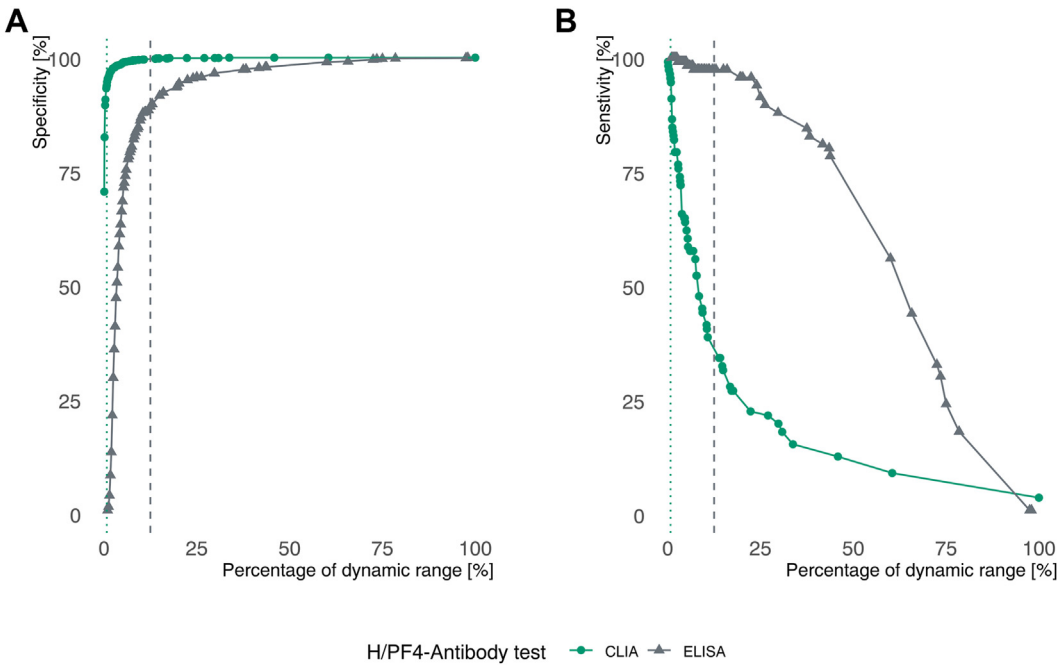

FIGURE 2 Specificity (A) and sensitivity (B) of common heparin/platelet factor 4 immunoassays over the dynamic range of measurements. The x-axis shows the test results scaled to the upper limit of the dynamic range of each assay (100%). Dashed vertical lines represent the manufacturer's cutoff. With chemiluminescence immunoassay (CLIA), specificity increases rapidly and approaches 100%, just above the manufacturer's cutoff (A). For enzyme-linked immunosorbent assay (ELISA), this is only achieved at around 75% of the dynamic range. Heparin-induced thrombocytopenia can, therefore, be ruled in much earlier with CLIA than with ELISA. The sensitivities react in opposite directions, which the manufacturers have compensated for with different thresholds (B). Posttest probabilities for individual patients can be calculated using the web-based calculator: https://pcd-research.shinyapps.io/BayesianCalculator/.

we believe that the results reflect real-world clinical practice. In addition, we included 2 commonly used immunoassays with different analytical techniques, making our results accessible to physicians in a wide range of hospitals. We also provide posttest probabilities of HIT for individual test results using a Bayesian calculator as a web application, making it easily accessible even with a smartphone at the bedside. A potential limitation of our study is that the majority of participants were recruited in Switzerland, which may introduce bias due to similar ethnicity, patient selection, and diagnostic procedures. In addition, certain H/PF4 immunoassays with a quantitative readout, such as HemosIL-H/PF4, were not available at our institution and were, therefore, not included in this study.

Even though this is the first study to comprehensively assess the diagnostic value of H/PF4 immunoassays for the diagnosis of HIT across their dynamic range, our results are consistent with previous publications. In a large systematic review of 49 studies evaluating the diagnostic accuracy of H/PF4 immunoassays (128 tests and 15 199 patients), significant differences were found depending on the cutoff used [8]. The higher the cutoff, the higher the LR. Similar results were obtained in a cross-sectional study of 179 patients with suspected HIT analyzing a polyspecific ELISA, a PaGIA, and a CLIA (HemoSil AcuStar HIT IgG): the higher the cutoff, the higher the LR [11]. In another study, including 114 patients and assessing an ELISA, the positive predictive value was higher with a cutoff of OD 1.0 compared with a cutoff of 0.4 [24]. As part of a quality audit of routine practice, Favaloro et al. [25] analyzed a subset of 1200 specimens by CLIA and serotonin release assay and found increasing LRs using 3 different cutoffs (<0.5 U/mL; 1.0; 2.0). In a retrospective cohort of 341 patients analyzed by CLIA and PaGIA against a composite reference test, different LRs were found for 5 cutoff values [26]. Similar results were reported in 2 earlier studies of the same group [12,27]. These findings are also consistent with observations in other diseases. In particular, in the diagnosis of autoimmune diseases such as rheumatoid arthritis, small vessel vasculitis, and celiac disease, several studies have found a consistent association between elevated antibody titers and an increased likelihood of the disease [28–31].

Our results indicate a strong association between H/PF4 immunoassay test results and the likelihood of HIT. Instead of a qualitative assessment (positive/negative), physicians can use the quantitative value at the bedside to estimate the probability of HIT in individual patients. The higher the result, the higher the likelihood of HIT. To facilitate interpretation, we have implemented a web-based calculator that calculates this posttest probability. Our results also show that immunoassays are not created equal, and different immunoassays have different patterns of reactivity across their dynamic range (Figure 1). In the case of CLIA, HIT can be ruled in even for results that are relatively small in scale. In contrast, with ELISA, even relatively high values are subject to limited specificity, which leaves open the possibility of false-positive results. Our interpretation is that the cutoff values of the manufacturers are not undermined by the results of our study. Rather, the results of the immunoassays can be interpreted differently, namely quantitatively, and thus gain diagnostic significance.

TABLE Diagnostic significance, expressed as a likelihood ratio, of common heparin/platelet factor 4 immunoassays within different intervals of test results.

		Overall	4Ts ≤ 3	4Ts 4-5	4Ts ≥ 6
Intervals	***n***	**LR+**	**LR+**	**LR+**	**LR+**
CLIA (U/mL)					
≤0.099	853	0.01 (0.00; 0.09)	0	0.02 (0.00; 0.16)	0
0.10-0.49	246	0.09 (0.02; 0.35)	0	0.13 (0.03; 0.53)	0
0.50-1.29	62	1.36 (0.63; 2.92)	2.29 (0.34; 15.41)	0.96 (0.30; 3.10)	1.28 (0.27; 5.98)
1.30-3.09	41	4.97 (2.65; 9.34)	7.44 (1.02; 54.54)	3.44 (1.48; 8.00)	2.13 (0.54; 8.36)
3.10-6.89	37	14.05 (7.54; 26.19)	29.72 (8.52; 104.05)	18.10 (7.11; 46.06)	1.59 (0.46; 5.56)
≥6.9	59	59.77 (33.30; 107.29)	297.72 (37.84; 2342.32)	29.61 (15.93; 55.06)	∞
ELISA (optical density)					
≤0.20	905	0.02 (0.01; 0.09)	0	0.02 (0.00; 0.15)	0.04 (0.01; 0.28)
0.21-0.59	255	0.04 (0.01; 0.30)	0	0.07 (0.01; 0.49)	0
0.60-0.89	45	0.50 (0.12; 2.03)	0	0.72 (0.18; 3.01)	0
0.90-1.49	42	4.80 (2.56; 8.98)	11.91 (2.95; 48.12)	4.18 (1.86; 9.39)	1.28 (0.27; 5.98)
1.50-2.39	54	16.82 (10.06; 28.12)	19.85 (4.94; 87.65)	11.33 (5.96; 21.56)	15.3 (2.09; 112.78)
≥2.40	77	57.99 (32.26; 104.24)	89.32 (29.26; 272.67)	42.96 (19.90; 92.74)	29.33 (4.13; 207.95)

LRs+ are powerful measures of diagnostic accuracy that can be interpreted as the likelihood that a given test result would be expected in a patient with the target condition compared with the likelihood of the same result in a patient without the target condition [19]. An LR+ of ≥10 is considered very high. The decreasing LR+ with increasing 4Ts score is explained by different populations: at high clinical probability, the prevalence of heparin-induced thrombocytopenia (HIT) is high, and the immunoassay contributes less to the diagnosis compared with a low prevalence situation. LR+ = 0 indicates that there were no patients with confirmed HIT in this subgroup. LR = ∞ indicates that there were no patients without HIT here. Corresponding posttest probabilities for individual patients can be calculated with the web-based calculator: https://pcd-research.shinyapps.io/BayesianCalculator/.

CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; LR+, positive likelihood ratio.

Although these results are consistent with previous studies, they should be replicated in an independent cohort. With this knowledge, the interpretation of immunoassay test results can be improved. This has the potential to simplify the management of patients with suspected HIT.

5 | CONCLUSIONS

Using data from a large prospective cohort of patients with suspected HIT, we demonstrated a strong association between H/PF4 immunoassay test results and the likelihood of HIT across the full diagnostic range. The higher the result, the higher the likelihood of HIT. However, immunoassays are not equal, and CLIA had the strongest increase in diagnostic significance per unit of measurement. To make this information clinically available, we have developed a web calculator that calculates the posttest probability of HIT for individual patients: https://pcd-research.shinyapps.io/BayesianCalculator/.

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AUTHOR CONTRIBUTIONS

H.N. designed the analysis, wrote the analysis plan, analyzed and interpreted the data, and contributed to the first manuscript draft. S.N. wrote the first draft of the manuscript. C.N. contributed to the analysis plan, formal analysis, and interpretation of the data. J.-D.S., A.G., D.A.T., A.M., W.A.W., A.S., J.A.K.H., B.G., P.V., T.B., and L.G. collected data. M.N. designed and implemented the Towards precise and rapid diagnosis of heparin-induced thrombocytopenia (TORADI-HIT) study, collected data, contributed to the analysis plan and interpretation of data, and wrote the manuscript. All authors contributed to the interpretation of data, reviewed the manuscript critically, and approved the final version of the manuscript.

DECLARATION OF COMPETING INTERESTS

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DATA AVAILABILITY

All data are available from the corresponding author upon reasonable request.

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