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Evaluation of Latex Immunoturbidimetric Assay Thresholds and HIT in Cardiothoracic Surgery

Jessica Hernandez, PharmD, BCCP[1,2,3], Hetal Patel, PharmD[2,4],
Phil Biddlecome, PharmD, MBA, BCCCP[2,4], Megan Kildea, PharmD[2,4],
Ruti Dwivedi, PharmD, BCCP[2,4], Shashank Sridhara, PharmD, CPh[2,4],
Scott Silvestry, MD[5], Nicholas Cavarocchi, MD[6], John L. Francis, PhD[7],
and Davide Ventura, PharmD, BCCCP[3,4]

Abstract

Background: Heparin-induced thrombocytopenia (HIT) is a common differential diagnosis in cardiothoracic surgery. The latex immunoturbidimetric assay (LIA) is an enhanced immunoassay that has recently been introduced for the detection of total HIT immunoglobulin and retains a higher specificity of 95% compared to the enzyme-linked immunosorbent assay.

Objectives: To investigate if a semiquantitative relationship exists between increasing LIA levels beyond the current positivity threshold and its correlation to positive serotonin release assay results in cardiothoracic surgery.

Methods: This was a multicenter, observational cohort of cardiothoracic surgery patients initiated on anticoagulation with heparin-based products. To conduct sensitivity and specificity analysis of LIA values, HIT positive was defined as a LIA value ≥1 unit/mL and HIT negative was defined as a LIA level <1 unit/mL. A receiver operating characteristic (ROC) analysis was utilized to evaluate the predictive performance of the LIA.

Results: At manufactures' cutoffs of ≥1.0 unit/mL, LIA sensitivity and specificity was 93.8% and 22%, respectively, yielding a false positive rate of 78%. At a higher cutoff of 4.5 units/mL, LIA sensitivity and specificity was 75% and 71%, respectively, yielding a false positive rate of 29% and an area under the ROC curve of 0.75 ($P = .01$; 95% confidence interval: 0.621-0.889). Bivalirudin was initiated in 84.6% of false positive LIA results.

Conclusion: This study suggests that the diagnostic accuracy of the LIA can be optimized by increasing the LIA positivity threshold. Proposing a higher LIA cutoff, may mitigate unwarranted anticoagulation and bleeding outcomes.

Keywords

heparin-induced thrombocytopenia, immunology, cardiac surgery, bioassay

Introduction

Thrombocytopenia is commonly seen in critically ill patients with an incidence varying between 35% and 45%. Cardiothoracic surgical patients (CTS) and those requiring mechanical circulatory support are particularly susceptible. The prolific use of heparin for circuit patency in cardiopulmonary bypass (CPB) and in extracorporeal membrane oxygenation (ECMO) places heparin-induced thrombocytopenia (HIT) as a common differential diagnosis. Artificial surfaces used in CPB and ECMO circuits are responsible for a pronounced

[1] Ascension Via Christi St. Francis, Wichita, KS, USA
[2] Department of Pharmacy, AdventHealth Orlando, Orlando, FL, USA
[3] College of Pharmacy, University of Kansas, Lawrence, KS, USA
[4] College of Pharmacy, University of Florida, Gainesville, FL, USA
[5] AdventHealth Transplant Institute, Orlando, FL, USA
[6] Department of Critical Care Medicine, AdventHealth Medical Group, Orlando, FL, USA
[7] AdventHealth Hemostasis and Thrombosis Laboratory, Orlando, USA

Corresponding Author:
Davide Ventura, AdventHealth Orlando, 601 E Rollins St, Orlando, FL 32803, 201-693-8242, USA.
Email: davide.ventura@adventhealth.com

elevation in platelet factor 4 (PF4) release generating a substrate for developing HIT with an incidence of 0.1% to 4% in CTS.[1,2] Alternative causes for postoperative thrombocytopenia ranging from hemodilution and consumptive processes to prothrombotic pathologies make the clinical diagnoses for HIT challenging.[2–6]

Existing diagnostic tools include clinical risk scores along with confirmatory functional and immunoassays each with respective limitations.[7] Screening tools, such as the 4Ts score, provide a formidable negative predictive value in the general population but have not been reproduced in CTS. The positive predictive value of the 4Ts score is poor with a 9% to 17% predictive value.[8,9] The enzyme-linked immunosorbent assay (ELISA), a commonly used immunoassay, yields a high sensitivity (98%-99%) for detecting HIT antibodies but limited in specificity, thus yielding a high risk of false positives.[10,11] Functional assays, such as the serotonin release assay (SRA), are considered a gold standard test for HIT diagnosis with a combined sensitivity and specificity of 90% to 100%. However, the SRA is technically demanding and therefore limited to select laboratories resulting in prolonged turnaround times.[7]

Given the limitations of existing immunoassays, novel laboratory tests such as the latex immunoturbidimetric assay (LIA) have been developed. The LIA is the first fully automated latex-enhanced immunoassay that has recently been introduced for the detection of total anti-PF4/heparin immunoglobulins via competitive inhibition, and therefore, decreased aggregation of latex nanoparticles coated with a HIT-mimicking monoclonal antibodies. As compared to ELISA, this on demand test can be performed in approximately 20 minutes. A LIA value ≥1 unit/mL confers a positive test.[12,13] The specificity and positive predictive value of the LIA was noted to be superior with a 90% agreement to an SRA as compared to the ELISA at 75% while maintaining an equivalent negative predictive value.[13,14] The LIA is also suggestive of having a higher sensitivity than the 4Ts score in predicting the likelihood of HIT. As a result of varied differential diagnoses and limitations of established assays, HIT is often over diagnosed and exposes patients to unnecessary bleeding risk with irreversible nonheparin anticoagulants. Understanding how LIA's enhanced diagnostic characteristics perform in a high-risk CTS cohort is vital in mitigating hemostatic complications. Unlike the ELISA, it is important to note that to date, studies which have formally validated or established a true semiquantitative correlation between LIA values and SRA positivity were not conducted specifically in a cardiothoracic surgical population, and instead used a heterogenous demographic.[12,15,16] The
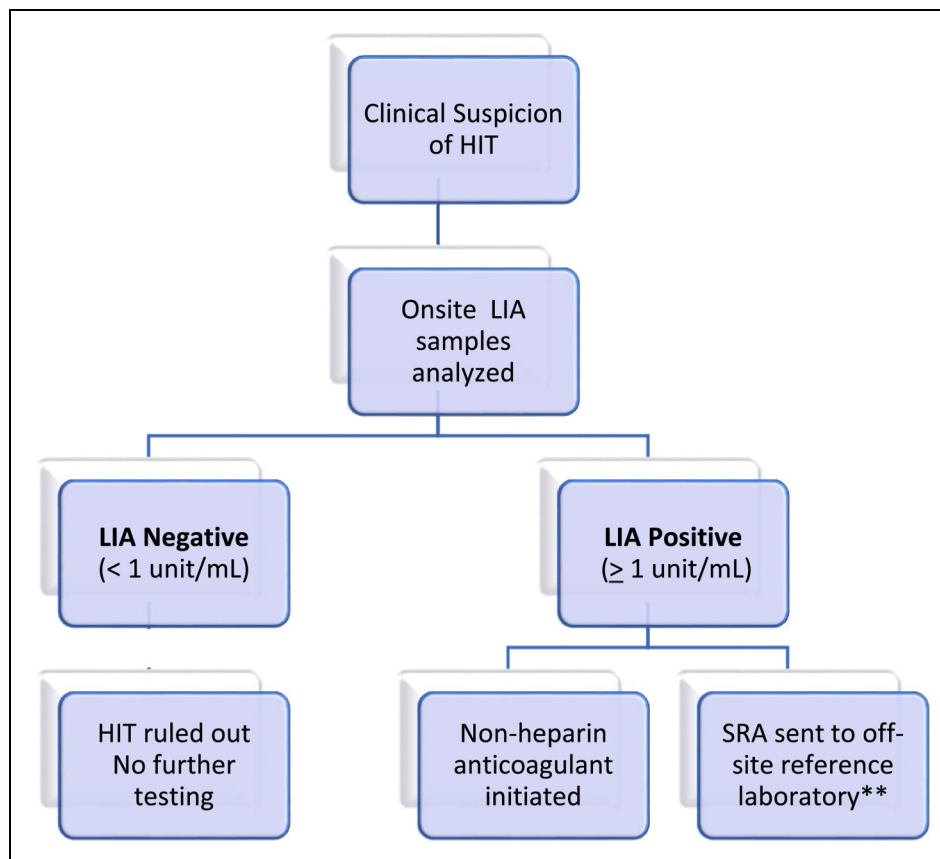

Figure 1. Institutional HIT diagnostic and treatment pathway. **National Reference Laboratory—ARUP Laboratories: 500 Chipeta Way Salt Lake City, UT, USA. Abbreviations: HIT, heparin-induced thrombocytopenia; LIA, latex immunoturbidimetric assay; SRA, serotonin release assay.

Table 1. Patient Demographics.

	Population (N = 100)
Mean age, years	60
Sex, male, (%)	73
Comorbidities, (%)	
CKD	18
VTE	9
Stroke	7
Coagulopathy	3
Indication of CT surgery, (%)	
ECMO cannulation	26
Non-ECMO indication	74
Type of heparin, UFH, (%)	99
Patients on ECMO, (%)	34
4Ts score, (%)	
Low	40
Intermediate	60
High	0
LIA ≥ 1.0, (%)	80
Prevalence of SRA positivity, (%)	16

Abbreviations: CKD, chronic kidney disease; CT, cardiothoracic; ECMO, extracorporeal membrane oxygenation; LIA, latex immunoturbidimetric assay; SRA, serotonin release assay; UFH, unfractionated heparin; VTE, venous thrombotic embolism.

Table 2. Patient Demographics Based on SRA Result.

	SRA negative (n = 84)	SRA positive (n = 16)	P-value
Mean age, years	59.7	60.3	<.001
Sex, male, n (%)	62 (73.8)	11 (68.8)	.676
ECMO, n (%)	30 (35.7)	4 (25)	.407
Comorbidities, n (%)			
CKD	17 (20.2)	1 (6.3)	.182
VTE	8 (9.5)	1 (6.3)	.676
Stroke	4 (4.8)	3 (18.8)	.044
Coagulopathy	3 (3.6)	0	.443
4Ts score, n (%)			
Low	37 (44)	3 (18.8)	
Intermediate	47 (56)	13 (81.3)	
High	0	0	.01
LIA ≥ 1.0	65 (77.4)	15 (93.8)	.1826

Abbreviations: CKD, chronic kidney disease; ECMO, extracorporeal membrane oxygenation; LIA, latex immunoturbidimetric assay; SRA, serotonin release assay; VTE, venous thrombotic embolism.

assumption that specificity will increase as LIA values rise beyond the currently established positivity threshold (≥ 1 unit/mL) without compromising sensitivity is unknown. Moreover, due to the increased risk of HIT in post-CTS patients, it is important to establish and evaluate the validity of LIA thresholds in this cohort. The goal of this study, therefore, was to identify if a semiquantitative relationship exists between LIA values and SRA positivity specifically in CTS patients.

Methods

This was a multicenter, observational, retrospective, cohort study performed in CTS patients at a quaternary medical center from 2018 to 2020. Approval was obtained from the Institutional Review Board at AdventHealth-Orlando (IRB-1685388). Inclusion criteria consisted of adults ≥18 years of age, CTS, initial anticoagulation with unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH), and collection of combined LIA and SRA tests. Upon chart review, 4Ts scores were calculated to evaluate the clinical probability of HIT.

The primary objective was to investigate if a semiquantitative relationship exists between increasing LIA values beyond the current positivity threshold and its correlation to positive SRA results in the diagnosis of HIT in CTS patients. Patients were divided into 2 cohorts, SRA positive and SRA negative. Typically, SRA samples were only analyzed in those patients with a positive LIA (Figure 1). Within each cohort, sensitivity and specificity analysis of LIA thresholds were conducted. LIA positivity for HIT was defined as a value ≥1 unit/mL

and HIT negative was defined as a value <1 unit/mL. Secondary endpoints included the use of alternative anticoagulation limited to bivalirudin and fondaparinux, duration of bivalirudin or fondaparinux, false positive LIA results in SRA negative patients, calculated 4Ts score correlation with LIA values, and prevalence of bleeding. Bleeding was predefined as any documentation of overt bleeding requiring surgical intervention upon chart review, drop in hemoglobin level of ≥2 g/dL, or red blood cell transfusion requirements.

The primary endpoint, defined as the semiquantitative relationship between LIA values and SRA positivity, was analyzed using an area under the receiver operating curve (AUROC) analysis. Sensitivity and specificity for each LIA value ≥1 unit/mL were calculated. Secondary endpoints were analyzed using chi-square or Fischer's exact test for categorical variables and Wilcoxon rank-sum tests for continuous variables. All analyses were performed using SPSS software.

Results

Of the 200 patients reviewed, 100 patients met inclusion criteria. Patients included were predominantly male (73%), with a mean age of 60 years. Indications for CTS included ECMO runs in 34 patients with the remaining indications being non-ECMO in nature including coronary artery bypass graft, valve replacements, lung, and heart transplant. One patient was initially anticoagulated with LMWH and the remaining 99 patients received UFH. LIA values ≥ 1 unit/mL were present in 80% of the patients and prevalence of SRA positivity was 16% (Table 1).

Our study cohort consisted of 84 SRA negative and 16 SRA positive patients. Baseline characteristics were equally distributed between SRA negative and SRA positive patients except for age and 4Ts scores (Table 2). Of the 16 patients identified with a positive SRA test, 15 had LIA values ≥ 1.0 unit/mL (true

positive) and 1 had an LIA value < 1 unit/mL (false negative). In the 84 patients who had a negative SRA result, 65 had an LIA ≥ 1.0 units/mL (false positive) and the remaining 19 had an LIA < 1.0 unit/mL (true negative).

Sensitivity and specificity of the LIA at various cutoffs are shown in Figure 2. The aggregated data for all CTS patients show that the current cutoff for LIA positivity of 1 unit/mL is highly sensitive. However, it lacks specificity and results in elevated false positivity rates. Alternatively, an LIA value of 4.5 units/mL tempers sensitivity but improves specificity from 22% to 71% (Table 3). Using an LIA threshold of 4.5 units/mL resulted in an AUROC predictive diagnosis for HIT of 0.75 (95% confidence interval [CI] 0.621-0.889; $P = .01$).

Secondary endpoints are shown in Table 4. Alternative anticoagulation was initiated in 79.8% and 93.8% of SRA negative and positive patients, respectively. Median days on alternative anticoagulation were 5 days in SRA negative and 9.5 days in SRA positive patients ($P = .007$). Overall, there were a total of 65 patients with LIA false positive results in which 55

were initiated on bivalirudin (84.6%). Clinically significant bleeding was more common in SRA negative patients ($P = .0004$). Correlation of 4Ts with LIA values are shown in Table 5.

Stratum-specific likelihood ratios (SSLR) were calculated to determine the odds of a true HIT diagnosis. The SSLR results separated by LIA value tertiles indicate an increasing probability of diagnosing true HIT as LIA values rise. Incorporating data from the main AUROC analysis, an LIA threshold of 4.5 units/mL or higher increases the likelihood of having true HIT from 1.21 (with an LIA value of ≥ 1 unit/mL) to 2.6 (Table 6).

The likelihood ratio served to further analyze the 4Ts score pretest probability of developing HIT against the post-test probability of LIA. The LIA threshold of 5 units/mL or greater amplifies the odds of SRA positivity whereas the LIA value stratum of 1 to 4.9 units/mL seems to display similar predictive probabilities as the 4Ts score (Table 7).

Discussion

Existing diagnostic tools for HIT have notable limitations and are poorly studied in the CTS patient population. The 4Ts score lacks robust positive predictive probability, ELISA has diminished specificity as low as 50% contributing to high false positives, and the SRA is technically demanding with a delayed turnaround time.[17] Despite the field's advancements in understanding the interplay between HIT pathogenesis and modifications of conventional assays, the detrimental impacts of HIT as a hypercoagulable disorder continue to demand tests with increased accuracy and speed. The LIA offers an
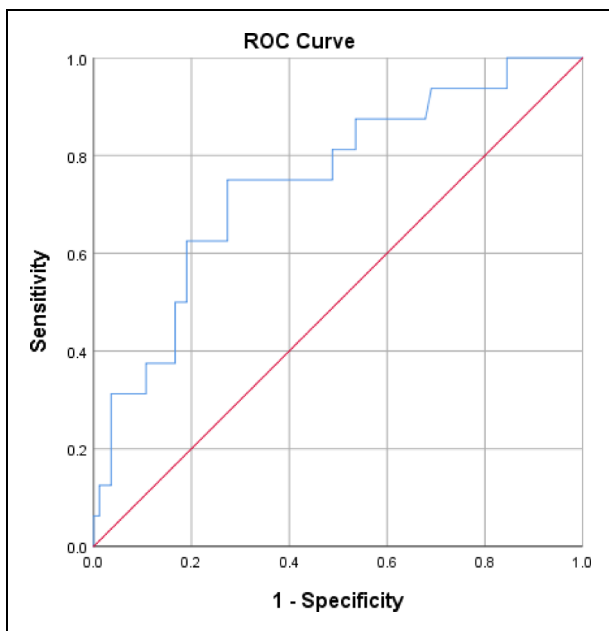

Figure 2. LIA AUROC at various LIA cutoffs. Abbreviations: AUROC, area under the receiver operating curve; LIA, latex immunoturbidimetric assay.

Table 3. Sensitivity and Specificity Analysis.

ROC curve characteristics	LIA traditional cutoff: 1 unit/mL	LIA proposed cutoff: 4.5 units/mL
Sensitivity	93.8%	75%
Specificity	22%	73%
False positivity rate	78%	26%

Abbreviations: LIA, latex immunoturbidimetric assay; ROC, receiver operating characteristic.

Table 4. Secondary Endpoints.

Variable	SRA negative (n = 84)	SRA positive (n = 16)	P-value
Alternative anticoagulation, n (%)	67 (79.8)	15 (93.8)	.2912
Days on alternative anticoagulation, median	5	9.5	.007
False positive LIA results in SRA Negative patients, n (%)	65	-	-
Bleeding events, n (%)	36	0	.0004

Abbreviations: LIA, latex immunoturbidimetric assay; SRA, serotonin release assay.

Table 5. 4Ts Score Correlation With LIA Values.

4Ts score	LIA ≥1 unit/mL n = 80 (%)	LIA <1 unit/mL n = 20 (%)	P-value
Low	34 (42.5)	6 (30)	.093
Intermediate	46 (57.5)	14 (70)	<.001
High	0	0	n/a

Abbreviations: LIA, latex immunoturbidimetric assay; n/a, not applicable.

Table 6a. Stratum Specific Likelihood Ratios (SSLR) based Degree of Positivity

	Stratum Specific Likelihood Ratios (SSLR)		
LIA Values (units/ml)	1 to 4.9	5 to 15	≥ 16
Stratum	Weak Positivity	Moderate Positivity	High Positivity
HIT Positive - n of total positive (%)	5 (10%)	6 (26%)	4 (57%)
HIT Negative - n of total negative (%)	45 (90%)	17 (74%)	3 (43%)
Total n of patients in stratum	50	23	7
SSLR	0.58	1.86	3.125

**HIT Positive defined as SRA positive ***HIT Negative defined as SRA negative

Table 6b. Stratum Specific Likelihood Ratios (SSLR) of Established versus Proposed Likelihood Ratios

	Stratum Specific Likelihood Ratios (SSLR)	
LIA Values (units/ml)	≥ 1	≥ 4.5
Stratum	Established Positive Threshold	Proposed Positive Threshold
HIT Positive - n of total positive	15 (19%)	12 (33%)
HIT Negative - n of total negative	65 (81%)	24 (67%)
Total n of patients in stratum	80	36
SSLR	1.21	2.6

**HIT Positive defined as SRA positive ***HIT Negative defined as SRA negative

Table 7.

Post-test probability for HIT (SRA positive status) with LIA

		Post-test probability of HIT based on LIA values (units/mL)		
4T score	Pretest probability of HIT	Stratum 1-4.9	Stratum 5-15	Stratum > 16
Low	1.9%	1.1%	3.5%	5.7%
Intermediate	6.7%	4.0%	11.8%	18.3%
High	36.6%	-	-	-

Abbreviations: HIT, heparin-induced thrombocytopenia; LIA, latex immunoturbidimetric assay; SRA, serotonin release assay.

attempt to address both these attributes providing rapid sensitivity and specificity of 97.4% and 94%, respectively, at a LIA cutoff of ≥ 1 unit/mL when compared to both IgG and polyspecific ELISA. Though the probability of HIT positive was also observed to increase with rising LIA thresholds, this seminal work by Warkentin et al included a mixed cohort of medical and surgical patients.[12]

To date, no studies have formally validated or established a true semiquantitative correlation between LIA values and SRA positivity exclusively in cardiothoracic surgery. The expanding use of mechanical circulatory support, elevated hemostatic instability, and scarce cardiothoracic demographic representation in HIT diagnostic validation studies prompted us to investigate if the LIA's performance translated specifically to cardiothoracic patients. We sought to determine if a semiquantitative correlation between rising LIA values and SRA positivity exists and if a more suitable LIA threshold for positivity is feasible.

Historical precedent for increasing thresholds of positivity to optimize diagnostics has been researched in ELISA literature as well. To improve specificity, several studies sought to investigate the relationship of higher optical density (OD) thresholds without compromising sensitivity. Warkentin et al[15] were able to observe a relationship between rising ELISA OD thresholds and the odds of a positive SRA. In this study, the odds of a positive SRA increased 6.39 times for every increase of 0.5 OD units ($P < .0001$). Raschke et al, identified an ELISA OD of 0.8 units/mL to be the most discriminant single cutoff by ELISA for the diagnosis of HIT rather than 0.4 units/mL and reduced false positive rates from 31% to 6%. These datasets and the predictive values, however, were not exclusive to cardiothoracic patients.[17] In response to these clinical challenges and paucity in data, Kataria et al investigated the application of ELISA OD thresholds and the SRA results in patients on ECMO. Outcomes from this retrospective study showed that a higher ELISA OD threshold of 1 unit/mL resulted in an improved specificity (89%) while preserving negative predictive value.[16]

Overall, our study in cardiothoracic patients demonstrates that the current LIA cutoff for positivity of ≥ 1 unit/mL in CTS patients is highly sensitive at 93.8%, similar to findings from Warkentin et al.[12] However, specificity is lacking yielding an elevated false positivity rate of 78% in our cohort. By proposing a new LIA cutoff for positivity of 4.5 units/mL, specificity increased from 22% to 71%, therefore, decreasing false positivity rates by 63%. In aggregate, the AUROC of the LIA at the new proposed cutoff of 4.5 units/mL was 0.75 (95% CI 0.621-0.889; $P = .01$) indicating an overall good performance of this screening test for the evaluation of HIT in CTS patients.

SSLR offer an additional perspective into the performance characteristics of the LIA. The SSLR serves to measure the odds a patient has a true positive result within prespecified weak, moderate, and high positivity LIA thresholds. In other words, it allows providers to put into context what the odds of true HIT may be based on the LIA value. Though limited by our total sample size, the SSLR data offers a signal for semiquantitative relationship between rising LIA levels and SRA positivity. A LIA threshold of 4.5 units/mL or higher augments the likelihood of diagnosing true HIT almost 2-fold compared to the established threshold of 1 unit/mL. Additionally, SSLR data was used to describe post-test probabilities of the LIA as compared to the 4Ts score. The 4Ts score is the most widely endorsed clinical assessment tool for HIT. Though alternative scores using CT surgical-specific parameters have been proposed, predictive performance in CTS have been inconsistent. Warkentin et al[12] and Linkins et al[18] have proposed 4Ts score pretest probabilities listed in Table 7. Using Bayesian analysis, we observed that weak positive LIA thresholds between 1 and 4.9 units/mL display a similar pretest and post-test probability performance relative to the 4Ts score. Post-test probabilities of true HIT diagnoses are enhanced at moderate to high positivity LIA thresholds despite low 4Ts scores. Though a low 4Ts score is noted to have a high negative predictive value, moderate to high LIA levels enhanced the probability of detecting true HIT approximately 3-fold despite a low 4Ts score.

This study provides data to support a higher LIA threshold of 4.5 units/mL in CTS patients for the evaluation of HIT. Application of this new LIA value cutoff would observe a decrease in false positive rates from 65 to 24 patients. As a result, unwarranted initiation of inappropriate anticoagulation would also decrease by 61.8%. However, it is important to note that sensitivity is not preserved by increasing the LIA threshold beyond 1 unit/mL.

Aligning with previous ELISA OD threshold studies, the implications of our results suggest that elevating the LIA threshold for positivity may enhance specificity. Though hypothesis generating, the clinical applications of our observations are several fold. Improved specificity along with decreased false positives has the potential to minimize exposure to irreversible and unwarranted anticoagulation, bleeding sequelae, hospital length of stay, and overall healthcare costs. It is noteworthy, however, to highlight the dilutional effect on sensitivity with a proposed increase in LIA threshold to 4.5 units/mL and should be further evaluated in larger studies.

Identifying HIT as a cause of thrombocytopenia in CTS patients, though essential, often leads to overdiagnosis. In fact, early onset thrombocytopenia alone is sometimes a trigger to initiate alternative, nonheparin-based anticoagulants empirically despite the low probability of true HIT associated with early-onset thrombocytopenia in CTS.[19] The findings of this study differ from previously published reports regarding HIT diagnostics. Specifically, this analysis focused exclusively on LIA's performance characteristics in cardiothoracic patients of which 34% were on ECMO suggestive of a particularly critically ill cohort. The risk of thrombosis and bleeding is a delicate balance in the critically ill surgical patients. Therefore, enhancing LIA's capacity to detect true HIT to reduce overdiagnosis and mitigate the use of alternative, irreversible anticoagulants in a high-risk bleeding cohort is of utmost importance.

Our study is limited by its retrospective design and small sample size. Additional limitations include the inability to evaluate the application of LIA thresholds in patients on mechanical circulatory support, confounding variables contributing to bleeding events, and the absence of collection of venous thromboembolism events for the diagnosis of HIT. Finally, it is worth noting that the results in Table 5 are counterintuitive. Based on the 4Ts high negative predictive value, one would assume that the low 4Ts score cohort would have more negative LIA values (< 1 unit/mL), for instance.[20] This counterintuitive outcome may be due to the retrospective nature of this trial. Specifically, given that 4Ts scoring was not always prospectively conducted at our institution, components of the 4Ts score may not have been documented or captured. Furthermore, isolated thrombocytopenia was frequently a trigger to send for LIA testing. These factors combined may have created the observed imbalance. In fact, retrospective 4Ts scoring is a cited limitation as noted by Crowther et al.[21] This group noted discrepant scoring between real-time scoring as compared to central adjudication. Crowther et al further concluded that the validity of low 4Ts score to definitively rule out HIT in critically ill patients may not be sufficient. These observations parallel, at least in part, to our study's observations.

Conclusion

This study is the first to evaluate the semiquantitative relationship between increasing LIA values beyond the current positivity threshold and its correlation to positive SRA results in CTS patients. Our results suggest that a higher LIA cutoff of 4.5 units/mL may improve specificity, therefore, decreasing false positives by 63%. The implications of this study have the potential to minimize bleeding events and better guide the use of irreversible anticoagulants.

Glossary of Abbreviations

CPB	cardiopulmonary bypass
CTS	cardiothoracic surgical patients
ECMO	extracorporeal membrane oxygenation
ELISA	enzyme-linked immunosorbent assay
HIT	heparin-induced thrombocytopenia
LIA	latex immunoturbidimetric assay
LMWH	low molecular weight heparin
OD	optical density
PF4	platelet factor 4
ROC	receiver operating curve
SRA	serotonin release assay
SSLR	stratum-specific likelihood ratio

Authors' Note

All authors contributed to concept and design, analysis and/or interpretation of data; critical writing or revising the intellectual content; and final approval of the version to be published.

ORCID iD

Davide Ventura [ID] https://orcid.org/0000-0002-2378-2792

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