Evaluating Diagnostic Algorithms for Heparin-Induced Thrombocytopenia using Two Combined Automated Rapid Immunoassays

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Abstract

Heparin-induced thrombocytopenia (HIT) is an autoimmune disorder caused by antibodies against platelet factor 4 (PF4) and heparin complexes. Rapid immunoassays (IAs) for detection of these antibodies mark a milestone in HIT diagnosis, despite a higher false-positive rate compared with functional platelet-activation assays. However, combining different rapid IAs may help to improve their diagnostic specificity. Here, we compared the individual performance of the latex immunoturbidimetric assay (LIA; HemosIL HIT-Ab [PF4-H]; sensitivity 91.7%, specificity 68.4%) and chemiluminescence immunoassay (CLIA; HemosIL AcuStarHIT-Ab [PF4-H]; sensitivity 92.4%, specificity 85.8%) with their combined performance using two unique diagnostic algorithms in a single prospective cohort of suspected HIT patients. Using the simultaneous algorithm adapted from Warkentin et al, the combined LIA–CLIA had a sensitivity of 99.0% and specificity of 64.3%. The sequential algorithm adapted from Rittener-Ruff et al was applied in two theoretical scenarios to reflect real-world circumstances in diagnostic laboratories where access to clinical information is limited: (1) assuming all patients had an intermediate 4Ts score and (2) assuming all patients had a high 4Ts score. This algorithm correctly predicted HIT in 94.5% (high 4Ts) and 96.0% (intermediate 4Ts) and excluded HIT in 82.6% (high 4Ts) and 80.1% (intermediate 4Ts) of patients in either scenario, respectively. Although both combined algorithms improved diagnostic performance of individual IAs, the simultaneous algorithm showed fewer false predictions (7.9%) than the sequential algorithm (intermediate 4Ts: 37.6% and high 4Ts: 41.5%) and proved more practical as it does not rely on physician evaluations. Our findings highlight the importance of accounting for clinician and interlaboratory variability when evaluating diagnostic tests for HIT.

Keywords

- heparin-induced thrombocytopenia
- platelet factor 4
- thrombosis
- antibodies
- laboratory diagnostics

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Heparin-induced thrombocytopenia (HIT) is an immune-mediated adverse drug reaction caused by antibodies against complexes formed between the self-protein platelet factor 4 (PF4) and the anticoagulant heparin (PF4/heparin).[1,2] These antibodies bind to neoepitopes exposed on PF4/heparin to form large immune complexes that can bind and crosslink platelet FcγRIIA receptors, triggering platelet activation.[3–5] Platelet activation stimulates signaling pathways responsible for secreting proinflammatory cytokines, procoagulant microparticles,[6] and thrombin generation,[7] increasing risk of venous or arterial thrombosis.[8,9] Prompt diagnosis is crucial to avoid substantial morbidity and mortality, as HIT patients face a 5 to 10% daily increased risk of thrombotic events.[10,11] HIT is a clinicopathological disorder diagnosed using a combination of clinical evaluations and laboratory testing. Physician evaluations are first performed to determine patients' initial likelihood of having HIT based on specific clinical criteria. For instance, the 4Ts pretest clinical scoring system is used to determine if patients require laboratory testing based on a low (0–3), intermediate (4–5), or high (6–8) 4Ts score.[12] Two main types of laboratory diagnostic tests are subsequently used: immunoassays (IAs) to detect the presence of anti-PF4/heparin antibodies and functional platelet-activation assays that measure the ability of anti-PF4/heparin antibodies to activate platelets. IAs can be broadly classified into enzyme immunoassays (EIA) and rapid IAs, which include the latex immunoturbidimetric assay (LIA)[13] and chemiluminescence immunoassay (CLIA).[14]

Despite the convenience of IAs, functional platelet-activation tests, including the ^{14}C-serotonin release assay (SRA) and heparin-induced platelet aggregation assays (HIPA), are often used to confirm positive results due to their high sensitivities and specificities for HIT.[15,16] In situations where platelet-activation assays are inaccessible, HIT overcall is exacerbated by physicians who must rely on clinical evaluations alone, which can show large variability in individual assessments.[12] Previously, we have shown that HIT is clinically suspected at high rates (86.5% of referred patients are false positive or true negative),[17] demonstrating why laboratory testing is necessary to minimize overdiagnosis and overtreatment. Most IAs are excellent for "ruling-out" HIT, yet their reduced specificity remains a source of concern.[18] Rapid IAs have emerged as potential solutions to these challenges.[19] In contrast to traditional HIT diagnostic assays, automated rapid IAs offer 20- to 30-minute turnaround times and can be performed on-demand. Rapid IAs are reported to have high sensitivities and specificities for HIT antibodies in various studies entailing retrospective cohorts of prescreened suspected HIT patients, when both are used independently.[20,21] Recently, the performance of these assays when used in combination have also been explored due to their complementary nature, as the LIA and CLIA are based on different approaches to antibody detection (LIA: competitive inhibition of HIT-like monoclonal antibody binding to PF4/heparin-coated particles; CLIA, detection of patient antibodies bound directly to PF4/heparin complexes coated on particles).[14,22] However, further prospective evaluations of these assays are required to determine their diagnostic performance in real-world clinical scenarios.

Here, we evaluate the Immucor IgG/A/M PF4-enhanced EIA (IgG/A/M EIA) and compare the independent and combined performance of the LIA and CLIA using a single prospective cohort of suspected HIT patients from across Canada to determine their diagnostic performance. To further validate the combined performance of rapid IAs for HIT diagnosis (LIA–CLIA), we employed two statistical approaches developed in previous retrospective studies: (1) the simultaneous algorithm adapted from Warkentin et al[14] and (2) the sequential algorithm adapted from Rittener-Ruff et al.[22] We aim to provide an assessment of these diagnostic assays in a setting that reflects the reality for most HIT reference laboratories, where access to patients' clinical information is limited.

Materials and Methods

Study Population

Samples used for this study were obtained from patients whose medical requisitions were received by the McMaster Platelet Immunology Laboratory from various referring centers based on a suspicion of HIT. We obtained 1,144 consecutive patient samples between December 2018 and August 2020 and evaluated the operating characteristics of the Immucor IgG/A/M-EIA, LIA, and CLIA using this prospective cohort of patients. These assays were evaluated based on their ability to predict "SRA-positivity" (i.e., the presence of platelet-activating anti-PF4/heparin antibodies) rather than a clinical diagnosis of HIT because access to patient clinical records was limited. Patients were included or excluded from this study based on access to sample (e.g., plasma availability, insufficient sample quantity) at the time of testing.

Detection of Anti-PF4/heparin Antibodies by Immunoassays, Rapid Immunoassays, and Platelet-Activation Assays

The PF4-enhanced EIA detecting IgG/A/M anti-PF4/heparin antibodies was performed as previously described (LIFECODES PF4 Enhanced assay; Immucor GTI Diagnostics, Waukesha, WI).[23] Optical densities (ODs) were measured using a BioTek 800TS microplate reader at 405 nm to determine the presence of anti-PF4/heparin antibodies in patient sera (positive $OD_{405nm} \geq 0.4$).[23] All samples included in this study were subsequently tested for platelet activation in the standard SRA in the presence of therapeutic (0.1–0.3 U/mL) and high (100 U/mL) doses of unfractionated heparin (Pfizer, New York, NY), as previously described.[24] This assay was performed with an anti-human CD32 Fc receptor-blocking monoclonal antibody (IV.3) to confirm FcγRIIa involvement in platelet activation. Patients were classified as SRA-positive if $\geq 20\%$ ^{14}C-serotonin release at therapeutic heparin concentrations was observed along with substantial inhibition of ^{14}C-serotonin release in the presence of high heparin and IV.3 concentrations.

Both the LIA (HemosIL HIT-Ab[PF4-H]) and CLIA (HemosIL AcuStarHIT-Ab[PF4-H]) were performed as previously described according to manufacturer's instructions.[14] Briefly, the CLIA detects antibodies in patient samples using PF4/polyvinyl sulfate (PVS)-coated magnetic particles. Following

incubation, patient samples are magnetically separated, washed, and detected using an isoluminol-labeled anti-human IgG antibody. After addition of a chemiluminescent reagent, detection of the captured anti-PF4/heparin antibodies is performed using the ACL AcuStar hemostasis testing system (Werfen [Instrumentation Laboratory], Bedford, MA) by measuring the level of emitted light, which is proportional to the HIT antibody concentration (U/mL) in each sample. In contrast, the LIA quantifies HIT antibodies based on the ability of citrated patient plasma to inhibit agglutination of KKO-coated latex particles, a mouse monoclonal antibody that mimics HIT antibodies.[25] A competitive agglutination reaction between KKO and HIT antibodies occurs in the presence of PF4/PVS complexes. HIT antibody concentrations are then determined by measuring the reduction in light transmissions due to agglutination using the ACL TOP 500 CTS testing system (Werfen [Instrumentation Laboratory], Bedford, MA). For the LIA and CLIA, results ≥ 1.0 U/mL were considered positive as outlined by manufacturer's instructions.

Operating Characteristics for Anti-PF4 Enzyme Immunoassays and Rapid Immunoassays

The same prospective cohort of 1,144 patient samples were also tested to determine the operating characteristics of the Immucor IgG/A/M-EIA, LIA, and CLIA based on either a positive or negative functional SRA result. The operating characteristics of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio for a positive test result (LR+), likelihood ratio for a negative test result (LR−), and accuracy were evaluated with 95% confidence intervals (CI).

Evaluation of the Combined Performance of Rapid Immunoassays

The combined performance of LIA and CLIA ($n = 996$) was determined by two previously evaluated test algorithms: (1) the simultaneous algorithm adapted from Warkentin et al[14] and (2) the sequential algorithm adapted from Rittener-Ruff et al.[22] Results were considered positive in the simultaneous algorithm if a patient tested positive ($\geq$1.0 U/mL) in either the LIA and/or CLIA.[14] The sequential algorithm involved several steps to determine HIT status. First, patients were either ruled out from having HIT in the presence of a low 4Ts score (0–3) or an intermediate 4Ts score (4–5) with a LIA result $<$ 0.73 U/mL; otherwise HIT was predicted in the presence of an intermediate or high (6–8) 4Ts and a LIA result $>$6.0 U/mL. When the LIA result fell within the "inconclusive" range, namely between the upper (6.0 U/mL) and lower (0.73 U/mL) thresholds, a secondary evaluation was then performed using the CLIA. HIT was predicted when CLIA results were $>$3.0 U/mL and excluded when results were $<$0.13 U/mL. In patients with an intermediate CLIA result (0.13–3.0 U/mL), HIT was predicted in patients with a high 4Ts score and was inconclusive for patients with an intermediate 4Ts score.

The remaining undetermined cases were resolved using a tertiary functional platelet-activation assay with higher specificity. Here, we used the standard SRA as the reference assay to determine when HIT is correctly "excluded" or "predicted." As such, we presented two theoretical scenarios where (1) all patients had an intermediate (4–5) 4Ts score and (2) where all patients had a high (6–8) 4Ts score, to reduce bias and variation in our data while assuming 4Ts scores for our calculations. As such, pre- and post-test probabilities could not be generated using our available data. Rather than depending on clinicians' 4Ts evaluations, this adapted method reflected the situation within diagnostic laboratories that have limited access to patient information. We also excluded a low 4Ts score (0–3) assumption from our analysis, following the observation of a limited NPV of 0.998 for low-risk patients as reported by Cuker et al.[12] A more detailed breakdown of this algorithm can be found in ►**Figs. 1** and **2**.

Statistical Analysis

All figures were generated using GraphPad Prism version 9.1.2 for Mac OS (GraphPad Software, Inc.). The operating characteristics of each assay, including sensitivity, specificity, PPV, NPV, and accuracy were expressed as percentages calculated using Microsoft Excel version 15.37. GraphPad Prism version 9.1.2 for Mac OS was used to calculate 95% CIs (Wilson/Brown method). All other analyses (including positive stratum-specific likelihood ratios; SSLR+) were performed using R version 4.3.0 (2023-04-21). Positive and negative cutoffs for the LIA and CLIA inclusion/exclusion criteria were obtained by receiver operating characteristic analysis to determine results that yielded 100% PPV and 100% NPV as performed by Rittener-Ruff et al.[22]

Results

Prospective Cohort of Suspected Heparin-Induced Thrombocytopenia Patients

Clinical information was unavailable for the 1,144 referred patients suspected of HIT, other than an assumed intermediate (4–5) or high (6–8) 4Ts score; therefore, the official diagnostic status of these patients is unknown. As such, the SRA was used as a surrogate for diagnostic confirmation due to its high sensitivity and specificity for HIT. Based on these results, we classified 229/1,144 (20.0%) of samples as "HIT-positive" (SRA-positive) and 915/1,144 (80.0%) as "HIT-negative" (SRA-negative).

Operating Characteristics of Anti-PF4 Immunoassays and Rapid Immunoassays for Heparin-Induced Thrombocytopenia Diagnosis

Among 1,144 referred samples, 914/1,144 (79.9%) were tested in the commercial IgG/A/M EIA, 1,012/1,144 (88.5%) in the LIA, and 1,128/1,144 (98.6%) in the CLIA. We identified 556/914 (60.8%) patients as EIA-positive ($OD_{405nm} \geq 0.4$), 443/1,012 (43.8%) as LIA-positive ($\geq$1.0 U/mL), and 336/1,128 (29.8%) as CLIA-positive ($\geq$1.0 U/mL). We then determined the operating characteristics of each assay based on the ability to predict SRA positivity in our cohort as summarized in ►**Table 1**.

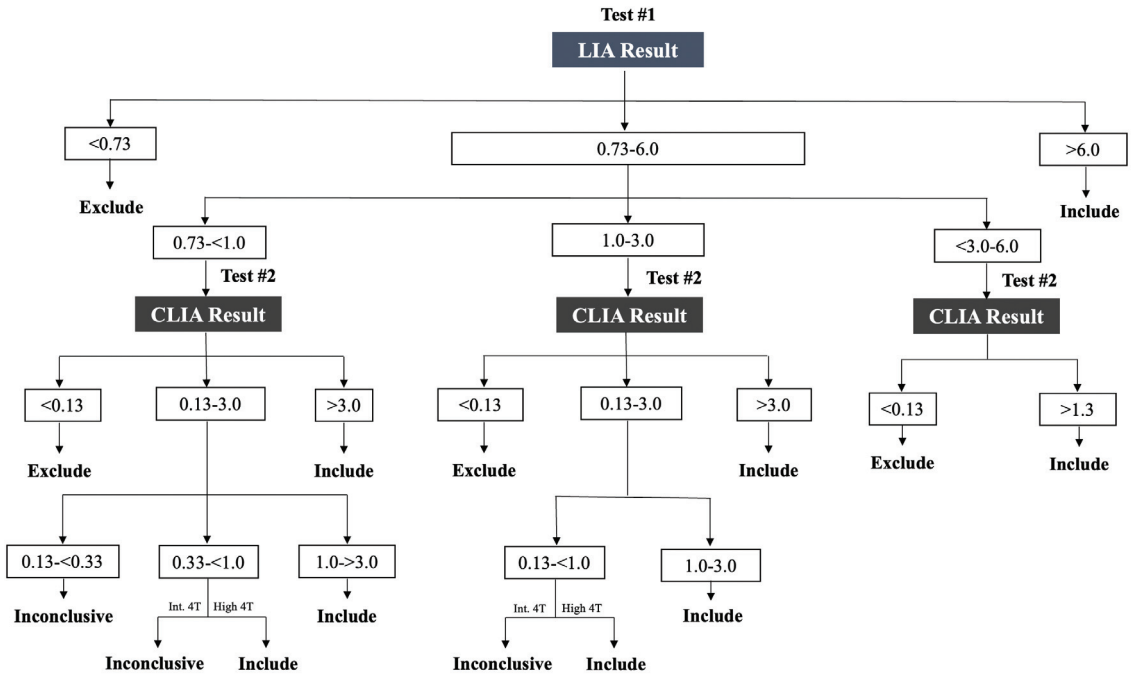

Fig. 1 Analytic approach adopted from Rittener-Ruff et al[22] performing the LIA as the first test. HIT was excluded in the presence of a low 4Ts score (0–3) or a LIA result <0.73 U/mL. HIT was predicted with a LIA result >3.0 U/mL. The CLIA was performed for the remaining cases between the upper (6.0 U/mL) and lower (0.73 U/mL) thresholds in the "inconclusive" range. HIT was predicted when CLIA results were >3.0 U/mL or if patients had a high 4Ts score and excluded when results were <0.13 U/mL. In patients with an intermediate CLIA result (0.13–3.0 U/mL), HIT was predicted in patients with a high 4Ts score and was inconclusive for patients with an intermediate 4Ts score. Any remaining inconclusive cases are resolved using a more sensitive and specific tertiary functional platelet-activation assay, such as the standard SRA. CLIA, chemiluminescence immunoassay; HIT, heparin-induced thrombocytopenia; LIA, latex immunoturbidimetric assay; SRA, ^{14}C-serotonin release assay.

LIA \ CLIA	<0.13 (LR 0.0)		0.13-<0.33 (LR 0.0)		0.33-<1.0 (LR 2.13)		1.0-3.0 (LR 15.62)		>3.0 (LR ∞)	
<0.73 (LR 0.0)	Total (n=375)		Total (n=47)		Total (n=35)		Total (n=18)		Total (n=13)	
	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−
	1	374	0	47	0	35	0	18	5	6
0.73-<1.0 (LR 0.98)	Total (n=47)		Total (n=1)		Total (n=8)		Total (n=12)		Total (n=6)	
	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−
	0	47	0	1	1	7	5	7	4	2
1.0-<3.0 (LR 2.74)	Total (n=110)		Total (n=17)		Total (n=23)		Total (n=22)		Total (n=24)	
	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−
	2	108	2	15	3	20	8	14	13	11
3.0-6.0 (LR 9.12)	Total (n=20)		Total (n=5)		Total (n=10)		Total (n=7)		Total (n=45)	
	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−
	0	20	0	5	4	6	1	6	36	9
>6.0 (LR ∞)	Total (n=11)		Total (n=3)		Total (n=9)		Total (n=2)		Total (n=126)	
	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−	SRA+	SRA−
	1	10	0	3	0	9	0	2	115	11

Fig. 2 Sequential analytic algorithm adopted from Rittener-Ruff et al.[22] Color coding represents categories of sequential LIA–CLIA algorithm results. Dark green = HIT predicted (>6.0 LIA), light green = inconclusive LIA results (0.73–6.0) but HIT is predicted due to a secondary CLIA test result >3.0, red = HIT is excluded (based on first test LIA <0.73), and gray = results within the inconclusive LIA (0.73–6.0) and CLIA (0.13–3.0) range. Inconclusive LIA and CLIA results (yellow) were further classified using patients' 4Ts scores. Any remaining inconclusive results were resolved using the SRA. Likelihood ratios for a positive HIT diagnosis are included for each threshold as determined by Rittener-Ruff et al.[22] CLIA, chemiluminescence immunoassay; HIT, heparin-induced thrombocytopenia; LIA, latex immunoturbidimetric assay; LR, likelihood ratio.

Table 1 Operating characteristics of laboratory diagnostic assays used in heparin-induced thrombocytopenia screening based on ^{14}C-serotonin release assay results

	Immucor IgG/A/M EIA ($n = 914$)			LIA ($n = 1{,}012$)			CLIA ($n = 1{,}128$)			LIA–CLIA[a] ($n = 996$)		
Confusion matrix		**SRA+**	**SRA−**		**SRA+**	**SRA−**		**SRA+**	**SRA−**		**SRA+**	**SRA−**
	+	**177**	**379**	+	**188**	**255**	+	**208**	**128**	+	**199**	**284**
	−	**0**	**358**	−	**17**	**552**	−	**17**	**775**	−	**2**	**511**
Sensitivity (95% CI)	100% (97.9–100)			91.7% (87.1–94.8)			92.4% (88.2–95.2)			99.0% (96.5–99.8)		
Specificity (95% CI)	48.6% (44.98–52.2)			68.4% (65.1–71.5)			85.8% (83.4–87.95)			64.3% (60.9–67.5)		
PPV (95% CI)	31.8% (28.1–35.8)			42.4% (37.9–47.1)			61.9% (56.6–66.94)			41.2% (36.9–45.6)		
NPV (95% CI)	100% (98.9–100)			97.0% (95.3–98.1)			97.8% (96.6–98.7)			99.6% (98.6–99.9)		
LR+ (95% CI)	1.95 (1.8–2.1)			2.9 (2.6–3.2)			6.52 (5.5–7.7)			2.91 (2.6–3.2)		
LR− (95% CI)	0.00			0.12 (0.08–0.19)			0.09 (0.06–0.14)			0.02 (0.0–0.06)		
Accuracy (95% CI)	58.5% (55.3–61.8)			73.1% (70.3–75.8)			87.2% (85.1–89.0)			72.4% (69.6–75.1)		

Abbreviations: CI, confidence interval; CLIA, chemiluminescence immunoassay; LIA, latex immunoturbidimetric assay; LR −, negative likelihood ratio; LR +, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.
[a]Combined LIA–CLIA simultaneous algorithm = positive if CLIA and/or LIA are ≥ 1.0 U/mL.

Operating Characteristics of Combined Latex Immunoturbidimetric Assay and Chemiluminescence Immunoassay Testing for HIT Diagnosis

Using the simultaneous algorithm previously proposed by our group,[14] we then evaluated the combined LIA–CLIA diagnostic performance of suspected HIT patients whose samples had been tested by both automated rapid IA systems ($n = 996$; ►**Table 1**). This approach considered patients positive if either the LIA or CLIA gave a positive result (≥1.0 U/mL). We identified 248/996 (24.9%) patients as LIA–CLIA positive and 748/996 (75.1%) patients as LIA–CLIA negative. The performance characteristics were then evaluated based on patients' SRA results (►**Table 1**). We then performed a semiquantitative analysis based on the 6-point scale developed by Warkentin et al,[14,22] which uses patient SRA results to determine the positive predictivity of the combined LIA–CLIA within a specific stratum (►**Table 2**). Using this predictive analysis, patients are assigned a score for both their LIA and CLIA results representing negative (0), weakly positive (1), moderately positive (2), and strongly positive (3) (►**Table 3**), which are then totaled to yield a final score ranging from 0 to 6. We found higher LIA–CLIA scores correlated with an increased likelihood of testing positive in the SRA. Of the 513 patients with a combined LIA–CLIA score of 0, only 2/531 (0.39%) patients tested SRA positive, yielding a LR− of 0.02 (0.00–0.05). Conversely, among those with a high combined LIA–CLIA scores of 5 and 6, 59/61 (91.8%) and 51/53 (96.2%) had positive SRA results, respectively. Thus, the SSLR+ for these patients with scores of either 5 or 6 was 116.7 (33.23–409.7) and 100.9 (28.61–355.5), respectively. Collapsing these two scores into a single category of strongly positive

Table 2 Evaluation of combined latex immunoturbidimetric assay–chemiluminescence immunoassay scores using a semiquantitative 6-point scale ($n = 996$)

LIA–CLIA score	SRA positive ($n = 201$)	SRA negative ($n = 795$)	Proportion with SRA positive ($n = 201$)	Stratum-specific likelihood ratio (95% CI)
0	2	511	2/513 (0.39%)	LR − = 0.02 (0.00–0.05)
1	18	192	18/210 (8.6%)	SSLR+ = 0.4 (0.24–0.58)
2	16	58	16/74 (21.6%)	SSLR+ = 1.1 (0.65–1.84)
3	31	23	31/54 (57.4%)	SSLR+ = 5.3 (3.20–8.89)
4	24	7	24/31 (77.4%)	SSLR+ = 13.6 (6.07–30.3)
5	59	2	59/61 (91.8%)	SSLR+ = 116.7 (33.2–409.7)
6	51	2	51/53 (96.2%)	SSLR+ = 100.9 (28.6–355.5)

Abbreviations: CI, confidence interval; CLIA, chemiluminescence immunoassay; LIA, latex immunoturbidimetric assay; LR, likelihood ratio; SRA, ^{14}C-serotonin release assay; SSLR, stratum-specific likelihood ratio.
Notes: The semiquantitative 6-point scale analysis was adopted from Warkentin et al.[14] Briefly, weak positive result (1.00–4.99 U/mL) = 1 point; moderate positive result (5.00–15.99 U/mL) = 2 points; strong positive result (>16.00 U/mL) = 3 points. Totaling points from both CLIA/LIA, a final score could range from 0 to 6 points.

Table 3 Semiquantitative six-point scoring system of latex immunoturbidimetric assay and chemiluminescence immunoassay test results

LIA–CLIA results		Score
Negative	0.00–0.99 U/mL	0
Weak	1.00–4.99 U/mL	1
Moderate	5.00–15.99 U/mL	2
Strong	≥16.00 U/mL	3

Abbreviations: CLIA, chemiluminescence immunoassay; IA, immunoassay; LIA, latex immunoturbidimetric assay.
Notes: A semiquantitative six-point scoring system developed by Warkentin et al[14,22] was used to further evaluate the ability of integrated LIA–CLIA results to predict SRA positivity.[14] Briefly, a score of 1, 2, or 3 was given to each sample's LIA and CLIA results based on it being weakly, moderately, or strongly positive. The sample would receive a 0 in the case of a negative result. Each sample would receive a score of 0 to 6 based on the combined results of both automated rapid IAs.

LIA–CLIA scores (110/114; 96.5% SRA positive), the SSLR+ is 108.8 (89.1–132.8).

We further evaluated the combined LIA–CLIA methods proposed by Rittener-Ruff et al,[22] here referred to as the sequential algorithm. Although the original method relies on patients' 4Ts scores, access to 4Ts scores is limited at many diagnostic referral centres (including ours). Therefore, the sequential algorithm was applied in two theoretical scenarios where: (1) all patients had an intermediate 4Ts score (4–5) and (2) all patients had a high 4Ts score (6–8), whereas patients with a low 4Ts score would be automatically excluded. When assuming high 4Ts scores, many results fell within the lowest category for HIT indication (LIA < 0.73 U/mL, 509/996 [51.1%]), rendering them inconclusive according to the proposed methodology.[22] To mitigate the issue of uncertain data, these patients were then excluded for HIT (i.e., categorized as negative) regardless of 4Ts score. When first assuming all patients had an intermediate (4–5) 4Ts score, the sequential algorithm correctly excluded HIT in 657/795 (82.6%) patients, correctly predicted HIT in 190/201 (94.5%) patients, leaving 26/996 (2.6%) patients inconclusive (►**Table 4**). When reevaluating this algorithm while assuming all patients had a high (6–8) 4Ts score, HIT was correctly excluded in 637/795 (80.1%) patients, correctly predicted in 193/201 (96.0%) patients, and left 21/996 (2.2%) patients inconclusive (►**Table 4**). All inconclusive cases in both intermediate and high 4Ts score cohorts were resolved based on SRA results (►**Table 4**); however, no clinical information for these patients was available to confirm diagnosis.

Discussion

It is well reported that anti-PF4/heparin EIAs have high sensitivities (>95%)[26] but reduced specificities (as low as 50%)[18] for detecting platelet-activating HIT antibodies.[17,26] As such, anti-PF4 EIAs are most frequently used to rule out the presence of HIT antibodies followed by tests to confirm their functional platelet-activation ability.[15–17,26] However, functional platelet-activation assays present significant challenges, as they are technically demanding, have long turnaround times, and are only performed at a limited number of reference laboratories.[15,17,27] Although automated rapid anti-PF4 IAs offer a potential solution to these challenges, further practical evaluations of these assays are required. Here, we determined the performance characteristics of the Immucor IgG/A/M anti-PF4 EIA as well as the LIA and CLIA rapid IAs, both individually and in combination, in a single prospective patient cohort of clinically suspected HIT patients referred from across Canada. We also evaluated two different statistical algorithms for interpreting results of the combined LIA–CLIA, previously developed by Warkentin et al[14] and Rittener-Ruff et al,[22] for HIT diagnosis. Our study, involving a larger prospective patient cohort than previously reported, 14 aimed to more accurately reflect real-world conditions by applying combined LIA–CLIA diagnostic algorithms in a standard HIT diagnostic referral center that receives samples from various hospitals without access to patient clinical information or physician evaluations (such as 4Ts scores).

Table 4 Evaluation of sequential algorithm under assumed intermediate or high 4Ts scores for patients tested in both latex immunoturbidimetric assay and chemiluminescence immunoassay ($n = 996$)

4Ts Score	HIT excluded			HIT predicted			Inconclusive	
	SRA+	SRA−	Total correct	SRA+	SRA−	Total correct	SRA+	SRA−
Intermediate	8/665 (1.20%)	657/665 (98.8%)	657/795 (82.6%)	190/305 (62.3%)	115/305 (37.6%)	190/201 (94.5%)	3/996 (0.30%)	23/996 (2.31%)
High	8/645 (1.24%)	637/645 (98.8%)	637/795 (80.1%)	193/330 (58.5%)	137/330 (41.5%)	193/201 (96.0%)	0/996 (0.0%)	21/996 (2.12%)

Abbreviations: CLIA, chemiluminescence immunoassay; IA, immunoassay; HIT, heparin-induced thrombocytopenia; LIA, latex immunoturbidimetric assay; SRA, ^{14}C-serotonin release assay.
Note: Analysis adopted from Rittener-Ruff et al.[22] Patients with a low (0–3) 4Ts score are automatically excluded under this method. LIA testing combined with "assumed" high (6–8) or intermediate (4–5) 4Ts scores was then used to predict or exclude HIT as an initial test, followed by CLIA testing where LIA results were inconclusive. Following CLIA testing, final results were again used to predict or exclude HIT, employing assumed 4Ts scores to inform final predictions when CLIA results were inconclusive. Patients who remain inconclusive despite 4Ts score, LIA, and CLIA must undergo further testing using functional platelet-activation assays. Post-test probability could not be calculated for this study due to limited access to clinical information.

We observed an improvement in diagnostic performance when interpreting LIA–CLIA results using the Warkentin et al[14] simultaneous algorithm when compared with other assays evaluated in this study. The combined LIA–CLIA demonstrated a higher specificity (64.3%) compared with the Immucor IgG/A/M anti-PF4 EIA (48.6%) and improved overall individual LIA and CLIA performances, further supporting the use of both instruments for HIT diagnosis. The simultaneous algorithm also maintained a high sensitivity (99.0%), which is necessary to prevent fatal outcomes in patients who would otherwise be falsely identified as negative.[10] Previously, Rittener-Ruff et al[22] reported the simultaneous algorithm had a reduced ability to diagnose HIT, which could be due to the sole reliance on the 6-point semiquantitative analysis, rather than the originally proposed algorithm. Here, our results align with our previous findings[14,22] that demonstrated a high sensitivity and specificity of the combined LIA–CLIA. We did observe a slight decrease in LIA–CLIA specificity (64.3%) compared with a previous reports (79.5%)[14] for HIT. However, reduced specificity reported in this study compared with the latter for these automated rapid IAs could be caused by our utilization of an entirely prospective patient cohort.[20,21,28,29] We also found that a strong LIA–CLIA score (5 or 6 points) was able to predict the presence of platelet-activating antibodies in 96.5% of SRA-positive patients (compared with 95.5%[14]). Interestingly, 4/114 (3.5%) of patients within this group were SRA-negative and could possibly represent putative SRA-negative HIT cases and should be tested in the PF4-enhanced SRA in future work.

When using the sequential algorithm to assess the samples, we also achieved a high degree of accuracy in pinpointing HIT-positive patients, as determined by SRA results, compared with the Immucor IgG/A/M EIA and individual rapid IA testing. This algorithm correctly predicted HIT in 94.5% of cases when assuming an intermediate 4Ts score or 96.0% of cases when assuming a high 4Ts score. However, we observed a few notable differences when applying this algorithm to our data. Our analysis resulted in a slightly smaller proportion of patients correctly excluded for HIT (intermediate 4Ts: 82.6%; high 4Ts: 80.1%) compared with the previous study (89.9%).[22] We also found 8/201 (4.0%) SRA-positive patients were incorrectly excluded for HIT, despite having a high 4Ts score, compared with Rittener-Ruff et al[22] who reported 100% of HIT patients correctly identified by this method in their cohort. Based on our results, we could not determine the true number of final inconclusive cases because we could not confirm the diagnostic status of suspected patients, compared with 3.4% "gray-zone" patients remaining in the original study.[22] Differences in our findings may also arise from study limitations, including interlaboratory variation, assumptions made regarding patients' 4Ts scores, and the theoretical application of sequential testing. Nonetheless, we believe this approach may face challenges in achieving uniform implementation across varied diagnostic testing centers. This is primarily due to the reliance on pre- and post-test probability scores, which inherently introduces a degree of clinician variability in assigning 4Ts scores. Furthermore, clinical information is not often available to technologists/technicians at most referral centres, which may significantly hinder the effectiveness and consistency of this algorithm if applied across different diagnostic laboratories.

Conclusion

The work presented here further demonstrates how prospective studies utilizing different anti-PF4/heparin assays can more accurately evaluate the everyday performance of HIT diagnostic tests. Unlike previous work, our study accounts for physician and laboratory variations in HIT diagnosis compared with previous evaluations that used well-defined retrospective patient cohorts. Excluding clinical information from analysis can also help evaluate the sole ability of the combined LIA–CLIA to identify HIT patients. Our group previously reported that most patients clinically suspected of HIT are negative following laboratory confirmation (86.5%; false positive or true negative), demonstrating how HIT is overcalled at the clinical evaluation stage.[17] Similarly, it is recognized that pretest probability scores, such as the 4Ts score, can greatly influence diagnostic performance.[17] We found that combining the LIA and CLIA showed a high NPV, suggesting it would be ideal for excluding HIT in a similar method as anti-PF4 EIAs with the additional benefit of on-demand testing and rapid turnaround times. However, due to the low specificity we recommend that functional platelet-activation assays (such as the standard SRA) remain the confirmatory diagnostic tests for HIT. Nevertheless, our findings support the combined use of LIA and CLIA following the simultaneous algorithm where both instruments are available with strict clinical guidelines. However, the potential for increased costs as well as variable access to both instruments remain key limitations of combining both the LIA and CLIA for HIT diagnosis. Future studies should perform prospective evaluations of these assays to further validate their diagnostic performance across various laboratory settings that reflect real-world situations.

Authors Contributions

A.B. and Y.Z. analyzed and interpreted the data, performed statistical analysis, and wrote the manuscript. M.C. performed statistical analysis and wrote the manuscript. J.C. M collected patient samples, performed experiments, and provided technical assistance. T.E.W. and D.M.A. interpreted data and wrote the manuscript. I.N. designed the research, analyzed and interpreted data, and wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflict of Interest

I.N. and T.E.W. have received research funding from Instrumentation Laboratory (Werfen). T.E.W. has received lecture honoraria from Instrumentation Laboratory

(Werfen), and royalties from Informa (Taylor & Francis) and UptoDate (Wolters Kluwer); has provided consulting services to Arbor Biotechnologies, Paradigm Pharmaceuticals, and Veralox Therapeutics; has provided expert witness testimony relating to HIT and non-HIT thrombocytopenic and coagulopathic disorders.

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