Received: 11 January 2022 | Accepted: 27 June 2022

DOI: 10.1111/jth.15811

ORIGINAL ARTICLE

Combinations of rapid immunoassays for a speedy diagnosis of heparin-induced thrombocytopenia

Luana Rittener-Ruff | Matteo Marchetti | Elena Matthey-Guirao | Francesco Grandoni | Francisco J. Gomez | Lorenzo Alberio

Division of Haematology and Central Haematology Laboratory, Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Lausanne, Switzerland

Correspondence
Lorenzo Alberio, Division of Haematology, Department of Oncology, Central Haematology Laboratory, Department of Laboratories, Lausanne University Hospital (CHUV) Rue du Bugnon 46, CH – 1011 Lausanne, Switzerland.
Email: lorenzo.alberio@chuv.ch

Funding information
Swiss National Science Foundation

Abstract

Background: Early recognition and treatment of heparin-induced thrombocytopenia (HIT) are key to prevent severe complications.

Objective: To assess the diagnostic performance of rapid immunoassays (IA) in detecting anti-PF4/heparin-antibodies.

Methods: Diagnostic performances of lateral-flow IA (LFIA; STic Expert HIT) and latex IA (LIA; HemosIL HIT-Ab) were analyzed in pilot ($n = 74$) and derivation cohorts ($n = 267$). Two novel algorithms based on the combination of HIT clinical probability with sequentially performed LIA and chemiluminescent IA (CLIA; HemosIL AcuStar-HIT-IgG) were compared with published rapid diagnostic algorithms: the "Lausanne algorithm" sequentially combining CLIA and particle-gel IA (PaGIA) and the "Hamilton algorithm" based on simultaneously performed LIA and CLIA.

Results: LFIA missed 6/30 HIT. The sensitivity and specificity of LIA were 90.9% and 93.5%. The Lausanne algorithm correctly predicted HIT in 19/267 (7.1%), excluded it in 240/267 (89.9%), leaving 8/267 (3%) cases unsolved. The algorithm sequentially combining CLIA and LIA predicted HIT in 19/267 (7.1%) with 1/19 wrong prediction, excluded it in 236/267 (88.4%), leaving 11/267 (4.1%) cases unsolved. The algorithm employing LIA as a first assay predicted HIT in 22/267 (8.2%), excluded it in 235/267 (88%), leaving 9/267 (3.4%) cases unsolved. Finally, the Hamilton algorithm correctly predicted HIT in 10/267 (3.7%), excluded it in 229/267 (85.7%), leaving 28/267 (10.5%) cases unsolved.

Conclusion: LFIA cannot be used to exclude or predict HIT when using frozen plasma. A Bayesian approach sequentially employing two rapid immunoassays for anti-PF4/heparin antibodies is most effective for the accurate diagnosis of HIT. Based on retrospective data, the combination LIA/CLIA is a candidate for a prospective validation.

KEYWORDS
anti-PF4/heparin antibodies, Bayesian inference, diagnostic algorithm, heparin-induced thrombocytopenia, rapid immunoassays

Manuscript Handled by: Andreas Greinacher Final decision: Andreas Greinacher, 27 June 2022

1 | INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is an immune-mediated adverse reaction that occurs in 0.2%–3% of patients treated with unfractionated or low-molecular-weight heparin.[1,2] HIT leads to a severe prothrombotic state due to circulating antibodies directed against platelet factor 4 (PF4) bound to heparin chains, which are able to activate platelets and other cells.[1,3] Thus, the risk for thrombotic complications, both arterial and venous, is enhanced.[2] A speedy diagnosis,[4] avoidance of any heparin, and switching to alternative anticoagulants are key to preventing any further complications from HIT.[1,2]

HIT is a clinicopathological syndrome whose presence can be assessed by combining its clinical pre-test probability, estimated for example by a scoring system such as the 4T, with the magnitude of circulating anti-PF4/heparin antibodies.[5,6] Two different classes of assays are available for HIT testing. The first group consists of functional assays, such as the heparin-induced platelet activation (HIPA) or serotonin-release assay (SRA) that are considered the gold standard for HIT diagnosis.[7] However, they are technically challenging, expensive, restricted to specialized laboratories, and not available for immediate diagnostic work-up.[1,7] The second group encompasses immunoassays (IA), classically enzyme-linked immunosorbent assays (ELISA) that detect anti-PF4/heparin antibodies. ELISA are easy to perform and widely available. However, they are characterized by high sensitivity and low specificity.[8] Therefore, ELISA are helpful in excluding HIT but as only around 50% of anti-PF4/heparin antibodies are platelet activating, HIT is over-diagnosed when only the qualitative ELISA result is considered.[9,10]

In recent years, several rapid IA detecting anti-PF4/heparin antibodies (within 10–30 min) have been developed.[11] These IA are based on different techniques such as, particle-gel IA (PaGIA),[12] chemiluminescent IA (CLIA),[13] lateral flow IA (LFIA),[14] and latex immune-turbidimetric assay (LIA).[15] The most recent American Society of Hematology (ASH) guidelines on HIT indicate that the incorporation of these emerging rapid IA into diagnostic algorithms for HIT is a key research priority.[16] A rapid Bayesian diagnostic algorithm for guiding clinical management decisions has been developed by Marchetti and colleagues at the Division of Hematology and Central Hematology Laboratory of the University Hospital of Lausanne, Switzerland (Figure 1).[17] This algorithm is a combination of the pre-test probability of HIT assessed with the 4T score with the magnitude of first-line, and when required second-line, rapid IA (CLIA and PaGIA, respectively). Briefly, in a first step the combination of 4T score and CLIA excludes (clinical probability low or intermediate, CLIA <0.13 U/ml) or predicts (clinical probability intermediate or high, CLIA >3.0 U/ml) HIT. In a second step, the unsolved cases are further investigated with PaGIA (see Figure 1 for diagnostic combinations). Of note, this Bayesian algorithm excludes HIT in a more stringent way than suggested by the ASH guidelines[16] and is able to predict accurately the result of the gold-standard functional assay HIPA in about 97% of cases with a laboratory turnaround time of less than 1 hour.[4,17]

Essentials

- Integration of rapid immunoassays into diagnostic algorithms for heparin-induced thrombocytopenia (HIT) is a research priority.
- Lateral flow, latex, chemiluminescent and particle gel assays for HIT antibodies were investigated.
- Quantitative results of latex, chemiluminescent and particle gel assays are diagnostically useful.
- The sequential combination of two rapid immunoassays is most effective for HIT recognition.

However, there is still a gray area of about 3% of cases where the "Lausanne algorithm" cannot predict or exclude HIT. Moreover, the second IA of the algorithm (PaGIA) is not available in the United States and needs a technician-dependent optical reading, which is subjective and semi-quantitative.[18] Therefore, we decided to test whether new rapid, automated, non-reader dependent IA, the STic Expert HIT (LFIA)[14] and HemosIL HIT-Ab (LIA),[13] would perform better than the PaGIA and could possibly substitute it in a new version of the Lausanne algorithm.

Based on the results of a pilot study in which we evaluated the diagnostic characteristics of the rapid IA for the detection of clinically relevant anti-PF4/heparin antibodies, we compared the performance of four rapid diagnostic algorithms for HIT in a retrospective derivation cohort: the Lausanne algorithm (Figure 1),[17] two newly developed algorithms based on the 4T score combined with sequential CLIA and LIA or LIA and CLIA, respectively (the second rapid IA being performed in cases not solved by the combination of the 4T score with the first rapid IA, similarly to the concept of the Lausanne algorithm), and the "Hamilton algorithm".[19] The latter has been developed by Warkentin and colleagues at the McMaster University (Hamilton, Canada) and is based on the combination of the simultaneously performed LIA and CLIA. Specifically, the quantitative results of both rapid IA are transformed in a score ranging from 0 to 6 (for each IA: negative = 0 points; weak-positive = 1 point; moderate-positive = 2 points; strong-positive = 3 points), which correlates with the probability of HIT.[19]

2 | METHODS

2.1 | Study design

The study design is shown in Figure 2. Two cohorts of patients investigated for suspected HIT at our institution were tested. First, a retrospective pilot cohort extracted from patients investigated for suspected HIT from January 1, 2017 to August 26, 2019 who had been tested with both IA of the Lausanne algorithm (CLIA

1st step Assess HIT pre-test probability with the 4T score Result (score)	2nd step CLIA HIT-IgG testing in patients with a 4T score ≥2 (and/or with unexplained heparin resistance) Result (U/ml)	Likelihood ratio (95% CI in Tab. 2)	Expected patients (%)	3rd step PaGIA-H/PF4 testing in patients with CLIA HIT-IgG results in the intermediate "grey-zone" (0.13-3.0 U/ml) Result (titer)	4th step Assess the individual post-test probability for HIT Low 4T score P (95% CI)	Int. 4T score P (95% CI)	High 4T score P (95% CI)	5th step Clinical decision (expected patients, %)
Pre-test probability for HIT according to 4T score: 0-3: **Low** (<1%) 4-5: **Int** (10-14%) 6-8: **High** (50-64%) **Proposed approach:** 0-1 : **HIT ruled out** * ≥ 2 : **ad 2nd step**	**<0.13**	LR 0.00	70-80	--	**0%**	**0%** (0-3)	**0%** (0-25)	**≤3% post-test probability for HIT :** HIT ruled out, Continue heparin **(Expected scenario in up to 90% of cases)**
	0.13-<0.33 †	LR 0.60		≤1	**0%**	**0%** (0-2)	**0%** (0-17)	
				2	**0%**	**3%** (0-18)	**24%** (4-70)	
				4	**0%** (0-1)	**29%** (13-53)	**82%** (63-92)	
				8	**4%** (1-12)	**76%** (41-93)	**97%** (88-99)	
				≥16	**100%** (2-100)	**100%** (70-100)	**100%** (96-100)	
	0.33-<1.0 ‡	LR 3.40	10-20	≤1	**0%**	**0%** (0-9)	**0%** (0-53)	**Undetermined post-test probability for HIT:** Perform HIPA functional assay as diagnostic gold-standard. While result pending, manage according to clinical judgment **(Expected scenario in ≤5% of cases)**
				2	**0%**	**14%** (2-55)	**65%** (20-93)	
				4	**0%** (0-1)	**70%** (46-86)	**96%** (91-99)	
				8	**18%** (5-50)	**95%** (80-99)	**100%** (98-100)	
				≥16	**100%** (15-100)	**100%** (93-100)	**100%** (99-100)	
	1.0-3.0 **	LR 63.13		≤1	**0%** (0-2)	**0%** (0-65)	**0%** (0-95)	**≥75% post-test probability for HIT :** HIT ruled in, Stop heparin, Start alternative anticoagulant therapy **(Expected scenario in 5-10% of cases)**
				2	**4%** (1-22)	**76%** (30-96)	**97%** (82-100)	
				4	**35%** (17-59)	**98%** (94-99)	**100%** (99-100)	
				8	**80%** (47-95)	**100%** (99-100)	**100%**	
				≥16	**100%** (75-100)	**100%**	**100%**	
	>3.0 **	LR ∞	5-10	--	**100%** (4-100) ‡‡	**100%** (79-100)	**100%** (98-100)	

Notes: *, If data are complete and in absence of unexplained heparin resistance. †, Optimal cut-off according to ROC analysis. ‡, Recommended cut-off according to manufacturer. **, High cut-off derived from the retrospective and prospective cohorts. ††, perform PaGIA and interpret as if CLIA LR of 0.60. ‡‡, perform PaGIA and interpret as if CLIA LR of 63.13.
Legend: P, Probability; CI, confidence interval.

FIGURE 1 "Lausanne rapid diagnostic algorithm" for heparin-induced thrombocytopenia (HIT) sequentially combining chemiluminescent immunoassay (CLIA) and particle-gel IA (PaGIA).[17] The first step is to assess the pre-test probability for HIT with the 4T score. In case of a 4T score ≥2 or unexplained heparin resistance, automated CLIA HIT-IgG is performed as first-line test. For cases with results situated in the CLIA intermediate "gray zone", PaGIA is performed as second-line test. Finally, for cases that remain unresolved despite a combination of 4T score, CLIA, and PaGIA (HIT undetermined), individualized clinical judgment will define initial management decisions, while awaiting for the results of the functional HIPA assay as diagnostic gold standard. Gray, situations in which HIT cannot be excluded or predicted.

and PaGIA). We selected all patients investigated with HIPA for whom we still had plasma and completed the cohort with samples in which HIT had been excluded by our algorithm.[17] Out of these samples, 74 were analyzed with STic Expert, and because of limited plasma volume, 60 with HemosIL HIT-Ab. Second, a retrospective derivation cohort including 267 patients out of the 321 consecutive ones investigated for suspected HIT from September 1, 2019 until August 9, 2020 with the Lausanne algorithm. For these 267 patients, plasma samples were still available for additional testing with HemosIL HIT-Ab. Demographic data are summarized in Table 1.

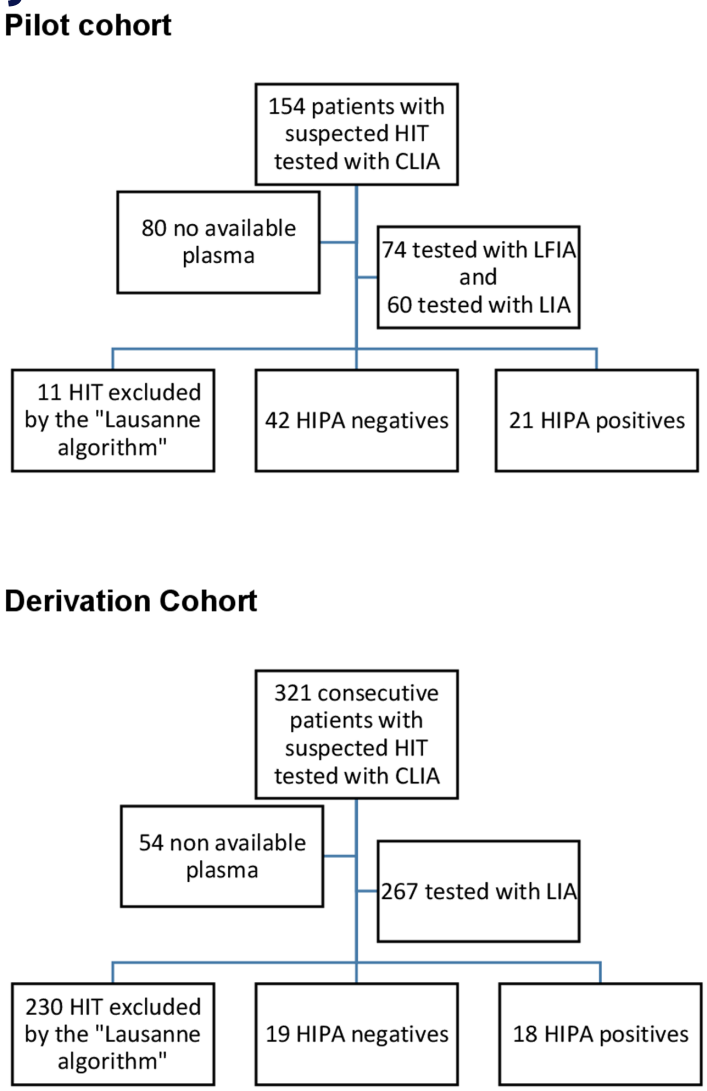
2.2 | Samples collection and storage

Blood was drawn into 3 ml plastic syringes (Monovette, Sarstedt, Numbrecht, Germany) containing 0.3 ml 0.106 mol/L trisodium citrate. Plasma was prepared by double centrifugation at 1500× *g* for 10 min at room temperature. Plasma samples were then frozen for storage in polypropylene tubes at −80°C.

2.3 | Immunoassays for the detection of anti-PF4/heparin antibodies

The Zymutest-HIA-IgG (Hyphen BioMed) is a commercially available immunoglobulin G (IgG)-specific ELISA coated with heparin-protamine complexes in which PF4 is provided by a platelet lysate added to the reaction mixture. Analytical turnaround time (TAT) is around 3 h. The cut-off recommended by the manufacturer is set at approximately 0.3 OD (depending on the daily determination of the control sample).[17]

The HemosIL Acustar HIT-IgG (Instrumentation Laboratory) is an automated CLIA with PF4 bound to polyvinyl-sulfonate particles.[13] Anti-PF4/heparin-antibodies form a complex with PF4/polyvinyl-sulfonate, which is adsorbed on magnetic beads. After separation of the microparticles, an isolumiol-labeled anti-human-IgG-antibody is added. After washing, the AcuStar optical system measures the light emission intensity in relative light units that are directly proportional to the anti-PF4/heparin-IgG-antibody concentration. The cut-off recommended by the manufacturer is ≥1.0 U/ml. The time to results is approximately 30 min.



Notes:

In the pilot cohort, plasma was available for 74 patients, who were tested with LFIA patients (11 HIT excluded by the "Lausanne algorithm", 42 HIPA negative, 21 HIPA positive). Because of limited plasma volumes, LIA was evaluated with only 60 patients (11 HIT excluded by the "Lausanne algorithm", 32 HIPA negative, 17 HIPA positive)

In the derivation cohort, plasma was available for 267 patients.

FIGURE 2 Study design. Retrospective pilot cohort, January 2017 to August 2019 (non-consecutive patients, $n = 154$, 50 heparin-induced platelet activation (HIPA) positive cases). Retrospective derivation cohort, September 2019 to August 2020 (consecutive patients, $n = 321$, 23 HIPA positive cases).

The ID-PaGIA-H/PF4 (Bio-Rad/DiaMed SA) is a manual PaGIA that detects IgG, IgM, and IgA directed against PF4/heparin complexes.[12] Ten microliters of plasma are added into a reaction chamber of the ID-test card, followed by 50 µl of polymer particles (red high density polystyrene beads coated with PF4/heparin complexes). After incubation for 5 min at room temperature, the ID-card is centrifuged for 10 min (85 × *g*, 1030 rpm). Anti-PF4/heparin-antibodies cross-link the red polymer particles, which remain on the top of the gel chamber. In the absence of a significant level of anti-PF4/heparin-antibodies, the particles sink to the bottom of the gel chamber. The result of the card is read by the laboratory operator. In case of an indeterminate or positive result with the undiluted

TABLE 1 Patients' demographics and clinical characteristics

	Retrospective derivation cohort *n* = 267	
	HIPA positive	HIPA negative
Patients, *n* (%)	18 (6.8)	249 (93.2)
Females, *n*/total (%)	9/18 (50)	82/249 (33)
Age, years		
Median	73	69
IQR	70.8–75.2	58.6–77.1
Range (min/max)	40; 83	20; 94
Clinical setting, *n* (%)		
Internal medicine	4 (22.2)	88 (35.4)
ICU	4 (22.2)	42 (16.9)
Surgery	0	24 (9.6)
External institutions	10 (55.6)	95 (38.1)
4T score		
Median	4.5	3
IQR	4–5.7	3–4
Range (min/max)	0; 7	0; 7
CLIA, U/ml		
Median	7.13	0.01
IQR	5.03–26.10	0–0.04
Range (min/max)	0.51; 59.6	0.00; 2.79
LIA, U/ml		
Median	6.35	0.23
IQR	2.98-6.9	0.08-0.41
Range (min/max)	0.74–7.01	0–5.49

Abbreviation: CLIA, chemiluminescent immunoassay; IQR, interquartile range; LIA, latex IA.

sample, the analysis is repeated in serially diluted plasma samples using specific diluent II (Diamed SA). The titer of the positive result with the highest dilution, followed by an indeterminate or negative result in the subsequent dilution, is reported after confirmation by dual control.[18] According to the manufacturer, the official cut-off is a positive result in the undiluted probe. The time to results is approximately 20 min.

STic Expert HIT (Diagnostica Stago SAS) is a LFIA to detect IgG antibodies against PF4/polyanion complexes.[14] This test can be used either on plasma or serum and does not detect antibodies of classes IgA or IgM. Five microliters of the patient sample are pipetted into the port as well as two drops of Sample Buffer. The latest contains a complex of ligand labeled PF4 and a polyanion and forces the sample to migrate through the membrane. Patients' anti-PF4/heparin IgG antibodies, if present, bind to the ligand labeled PF4-polyanion complex and colored gold nanoparticles carrying anti-ligand antibody bind to the ligand labeled PF4/polyanion complex during the migration. When the fluid passes the test line (line T) on the strip, immobilized anti-human IgG antibodies on the nitrocellulose membrane capture the patient's IgG antibodies. The excess of gold particles migrates through the membrane and is captured at the control line (line C). According to the fabricant leaflet, the assay characteristics are 100% sensitivity and 93% specificity. The time to result is <15 min.

In this study, LFIA results were read visually by the first author without knowing the clinical history and classified as such: negative, doubtful positive (+/−), weakly positive (+), positive (++), and strong positive (+++). A doubtful positive result (+/−) was considered to be "positive" as the primary aim of this screening test is to exclude HIT. To have more complete and repeatable data, as already reported by Sachs and colleagues,[14] each test membrane was scanned by CanoScan LIDE210 (Canon) and the density of the test band was measured using ImageJ (National Institutes of Health, Bethesda, Maryland, USA). A ratio was calculated to quantify the density according to the control density. Based on this ratio, a test with a band density greater than 1 was considered positive. The time to results is approximately 10 min.

HemosIL HIT-$Ab_{(PF4\text{-}H)}$ (Instrumentation Laboratory) is an automated, latex-particle-enhanced immuno-turbidimetric assay (LIA) to detect total heparin associated antibodies found in HIT patients.[15] A monoclonal antibody that mimics human HIT antibodies is coated onto latex particles. The particles agglutinate when mixed with complexes of PF4/polyvinylsulfonate, which results in higher absorbance. In the presence of plasma containing functional anti-PF4/heparin antibodies, a competitive agglutination reaction occurs. Thus, no or minimal increase in absorbance by patient sample indicates a positive test result. The degree of agglutination is inversely proportional to the concentration of antibodies in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates. The cut-off recommended by the manufacturer is ≥1.0 U/ml. There is an automatic rerun of the test if the results are above 5.6 U/ml with an additional dilution of ¼ allowing the test range to expand to 16 U/ml. The time to results is approximately 13 min. The test was performed using the ACL TOP 350 coagulometer (Instrumentation Laboratory) and following the manufacturer's recommendations.

2.4 | Functional assay for the detection of platelet-activating anti-PF4/heparin antibodies

The HIPA is recognized along with SRA as one of the two gold-standard tests for the detection of heparin-dependent, platelet-activating antibodies.[7] The re-calcified patient plasma is added to washed reactive platelets from four selected, blood group 0 healthy donors. If functional anti-PF4/heparin-antibodies are present, platelet aggregation is observed at low heparin concentration with at least two donors after 30 min and is suppressed at high concentration. This functional assay was performed blindly in a specialized laboratory at the Institute for Immunology and Transfusion Medicine, University Hospital of Greifswald, Germany.

2.5 | Diagnosis of HIT

According to the Lausanne algorithm,[17] the first step is to assess the pre-test probability for HIT with the 4T score. In case of a 4T score[5,6] ≥2 or (despite a 4T score of 0) unexplained "heparin resistance" [defined biologically by the failure to reach the therapeutic target despite administration of 1.5 fold the usually required dose of unfractionated heparin[20] (i.e., >27 IU/kg body weight per hour) and clinically by the occurrence of venous or arterial thrombosis or extension of thrombosis in a patient receiving unfractionated heparin with therapeutic target range],[21] the automated CLIA HIT-IgG is performed as a first-line test and is expected to solve about 80% of cases. Specifically, HIT is excluded in the presence of a low or intermediate clinical probability and a CLIA result <0.13 U/ml; HIT is predicted in the presence of an intermediate or high clinical probability and a CLIA >3.0 U/ml (of note, HIPA is always performed for confirmation of a predicted HIT). For the remaining 20% of cases with results situated in the CLIA intermediate "gray zone" (0.13–3.0 U/ml),[17] PaGIA testing is performed as a second-line test (see Figure 1 for diagnostic combinations). This additional assay is expected to solve at least 50% of cases that were situated in the CLIA intermediate gray zone (in these cases, HIPA is performed as well). Finally, for the ≤5% of cases that remain unresolved despite a combination of 4T score, CLIA, and PaGIA (HIT undetermined), individualized clinical judgment will define initial management decisions, while awaiting for the results of the functional HIPA assay as a diagnostic gold standard.[17] The frequency of HIT in the pilot cohort is high because we purposely selected cases investigated by HIPA. HIT frequency in the derivation cohort is 22 out of 267 patients (8.2%), as expected.[16,22]

2.6 | Statistics

The statistical analysis was performed using MedCalc (version 15.11.0). Performance characteristics were compared using the receiver-operating characteristic (ROC) curve, which is a graph of sensitivity against 1 − specificity. A perfect test would have sensitivity and specificity both equal to 1. The performance characteristic of a diagnostic assay was quantified by calculating the area under the ROC curve (AUROC). The ideal test would have an AUROC of 1.0, whereas a random guess would have an AUROC of 0.5.

The results of the rapid IA were analyzed in order to determine their ability to predict or exclude HIT by means of ROC analysis. The comparison of IA with a functional gold-standard test permits calculation of likelihood ratios (LR) for result intervals, and identification of cut-off of IA results associated with 100% negative (NPV) and positive (PPV) predictive values for a positive functional assay.[17,22] In conclusion, pre-test probability of HIT was assessed by the 4T score. This value was transformed into a post-test probability by combining it with the LR (95% CI limits) of the sequential quantitative IA result (online calculator: http://www.sample-size.net/post-probability-calculator-test-new) as performed by Marchetti et al.[17]

3 | RESULTS

3.1 | Diagnostic performance of rapid IA in the retrospective pilot cohort

The results of the four IA (CLIA, PaGIA, LFIA, and LIA; Supplementary material, Data set S1) were analyzed based on their ability to predict HIT diagnosis and are summarized in Table S1. CLIA and PaGIA performed as already observed in our previous research.[17,22] Using a band density of 1 as the threshold for a positive test, the sensitivity of the LFIA was 80.0% and its specificity 81.8%. The PPV was 75.0% and the NPV 85.7%. For the LIA, according to the official cut-off, the results showed a sensitivity of 87% and a specificity of 59.5%. PPV and NPV were 57.1% and 88.0%, respectively. Of note, among the four IA, only the PaGIA did not show false negative results, while according to their respective official cut-offs, the LIA produced three negative results out of 23 (13%) HIT positive cases, the CLIA four out of 30 (13%),[17] and the LFIA six out of 30 (20%). While lowering the quantitative threshold for positivity allows identification of a cut-off with a 100% NPV for CLIA (0.13 U/ml[17]) and LIA (0.73 U/ml), this was not possible for FLI.

3.2 | Diagnostic performance of rapid IA in the retrospective derivation cohort

Patients' demographics and clinical characteristics of the derivation cohort are summarized in Table 1. No relevant differences were observed between HIPA positive and negative patients for age, gender, clinical settings, and laboratory results.

Because of its performance in the pilot cohort with 20% false negative results and the impossibility to improve its sensitivity by lowering the threshold for positivity, the LFIA was not further evaluated in the derivation cohort. The detailed performance results of the other IA (CLIA, PaGIA, and LIA) are reported in the Supplementary material (Data set S2).

In the retrospective derivation cohort, CLIA showed a sensitivity of 90.9% and a specificity of 99.2%, with a NPV of 99.2% and a PPV of 90.9%. For the PaGIA, the sensitivity was 100% and the specificity 45.2%, with a NPV of 100% and a PPV of 56.4%, according to the official cut-off. The LIA showed a sensitivity of 90.9% and a specificity of 93.5%; its NPV and PPV were 99.1% and 55.6%, respectively (Table S1).

3.3 | Identification of likelihood ratios for the quantitative results of rapid automated IA

Likelihood ratios (LR) of quantitative interval results were identified by ROC analysis and are displayed in Table 2.

TABLE 2 Likelihood ratios (LR) for a positive HIT diagnostic calculated from the retrospective derivation cohort ($n = 267$)

Immunoassay $N = 267$	Result interval	HIT positive	HIT negative	LR	95% CI	100% PV	95% CI
LIA (U/ml)	0.00–0.73	0	219	0.00	0.00–0.41	Negative	83.9–100
	0.73–1.00	1	12	0.98	0.13–7.15		
	1.00–3.00	4	14	2.74	1.21–9.26		
	3.00–6.00	5	1	9.12	7.17–478.46		
	6.00–8.00	11	0	∞	15.69 to ∞	Positive	98.5–100
CLIA (U/ml)	0.00–0.13	0	222	0.00	0.00–0.41	Negative	83.9–100
	0.13–0.33	0	10	0.00	0.00–9.68		
	0.33–1.00	2	11	2.13	0.50–8.98		
	1.00–3.00	4	3	15.62	3.71–65.21		
	3.00–6.00	15	0	∞	21.75 to ∞	Positive	98.5–100

Abbreviations: CLIA, chemiluminescent immunoassay; CI, confidence interval; HIT, heparin-induced thrombocytopenia; LIA, latex IA; LR, likelihood ratio; *N*, total number of patients investigated in the prospective derivation cohorts; PV, predictive value.

3.4 | Performance of diagnostic algorithms incorporating two rapid IA

We evaluated the diagnostic efficiency of the original Lausanne algorithm (Figure 1, Table S2) and of two newly developed algorithms based on the 4T score sequentially combining CLIA and LIA (instead of PaGIA), as shown in Figures 3 and 4 (Tables S3 and S4, respectively). Finally, we also investigated the diagnostic approach recently proposed by Warkentin and colleagues (Hamilton algorithm),[19] which employs the semi-quantitative results of simultaneously performed LIA and CLIA to calculate a laboratory-based HIT probability score (Table 3; see Notes for scoring system). The diagnostic performances of the four different approaches are summarized in Table 4.

In the derivation cohort, the Lausanne algorithm correctly excluded HIT in 89.9% and correctly predicted HIT in 7.1% of the patients investigated for suspected HIT, leaving 3% of the cases in the gray zone; these cases had to be solved by HIPA (Table 4 and Table S2 for details). These results confirm our previous experience.[17] Of note, 86% of patients with HIT were accurately predicted, there were no false HIT predictions, and we observed 3/267 probably false negative HIPA results (Table 4, Notes). The latter observation is in line with our research[17] and others' experience.[15,23]

A newly developed algorithm based on the predictive values indicated in Table 3, employing the CLIA as the first rapid IA and sequentially using the LIA instead of the PaGIA (Figure 3), correctly excluded HIT in 88.4% of cases, leaving 4.1% of cases in the gray zone, which had to be solved by HIPA; 86% of patients with HIT were accurately predicted. There was 1/267 false HIT prediction and 3/267 probably false negative HIPA results (Tables 4 and S3). A third algorithm sequentially employing LIA as the first and CLIA as the second rapid IA (Figure 4) correctly excluded 88% of cases, leaving 3.4% of cases in the gray zone, which had to be solved by HIPA. 100% of HIT patients were accurately predicted. There were no false HIT predictions and 3/267 probably false negative HIPA results (Tables 4 and S4). In two instances, the mismatch between both algorithm predictions and HIPA cannot be solved by the clinical course (Table 4, Notes, B).

The Hamilton algorithm[19] correctly excluded HIT in 85.8% of cases (score of 0), predicted 45% of patients with HIT (scores of 4 to 6 according to the original publication), leaving 10.5% of cases unsolved (detailed results are presented in Table 3).

4 | DISCUSSION

While unrecognized HIT is linked with high morbidity and mortality, alternative non-heparin anticoagulants are expensive and associated with a high bleeding rate.[1] Consequently, it is essential to have a rapid and accurate diagnostic approach[4] to guide clinical management of patients with suspected HIT and accurately identify those who will need to be switched to non-heparin anticoagulants, such as argatroban or danaparoid.[24–27] We have recently published our original Lausanne algorithm,[17] which is based on the Bayesian use of the 4T score[5] and two sequential rapid IA (CLIA and PaGIA) for anti-PF4/heparin antibodies. This algorithm is able to accurately predict or exclude HIT in more than 95% of patients evaluated for suspected HIT, with a laboratory turnaround time of ≤1 h.[4,17]

We set out to evaluate the diagnostic accuracy of two other rapid IA for detecting anti-PF4/heparin antibodies, the STic Expert (a LFIA)[14] and the HemosIL HIT-Ab$_{(PF4-H)}$ (a LIA),[15] because our algorithm incorporated the PaGIA,[12] a manual, semi-quantitative assay, which may be difficult to interpret.[18] In the meantime, the search for an alternative to PaGIA has become an urgent necessity because of BioRad's abrupt decision to cease production of this IA after the introduction of the new *in vitro* diagnostic medical devices regulation (IVDR).[28] In an initial pilot cohort, selected on purpose with a high number of HIPA-positive samples, we evaluated whether the respective performances of LFIA and LIA were strong enough to be investigated in a subsequent derivation cohort (Table S1).

The main benefit of the LFIA is that it is ready to use, rapid, and visually readable. Published data indicate a good diagnostic

1st step	2nd step		3rd step	4th step			5th step
Assess HIT pre-test probability with the 4T score	**CLIA HIT-IgG testing in patients with a 4T score ≥2 (and/or with unexplained heparin resistance)**		**Hemosil HIT-AB testing in the intermediate "grey-zone" (0.13-3.0 U/ml)**	**Assess the individual post-test probability for HIT**			**Clinical decision** (expected patients, %)
Result (score)	**Result** (U/ml)	**Likelihood ratio** (95% CI in Tab. 2)	**Result** (titer)	**Low 4T score P** (95% CI)	**Int. 4T score P** (95% CI)	**High 4T score P** (95% CI)	
Pre-test probability for HIT according to 4T score: 0-3: **Low** (<1%) 4-5: **Int** (10-14%) 6-8: **High** (50-64%) **Proposed approach:** 0-1 : **HIT ruled out** * ≥ 2 : **ad 2nd step**	**<0.13**	LR 0.00	--	**0%**	**0%** (0-3)	**0%** (0-25)	**≤3% post-test probability for HIT :** HIT ruled out, Continue heparin
	0.13–<0.33 †	LR 0.60	0.00-<0.73	0%	0% (0-3.9)	0% (0-30)	
			0.73-<1.0	0.6% (0.1-4.1)	8.7% (1.3-41.1)	51.1% (12.2-88.4)	
			1.0-<3.0	2% (0.7-5.3)	24.7% (10.6-47.5)	78.1% (56.3-90.8)	
			3.0-6.0	26.1%(4.1-74.3)	85.1% (41.2-97.9)	98.4% (88.4-99.8)	
			>6.0	100%(8.7-100)	100%(60.5-100)	100% (94.4-100)	
	0.33–<1.0 ‡	LR 3.40	0.00-<0.73	0 % (0-1.4)	0% (0-18.5)	0% (0-71.2)	**Undetermined post-test probability for HIT:** Perform HIPA functional assay as diagnostic gold-standard. While result pending, manage according to clinical judgment
			0.73-1<.0	3.2 % (0.4-19.6)	35.1% (6.7-79.8)	85.6% (44-97.7)	
			1.0-<3.0	10.3 % (4-24)	64.9% (40.1-83.7)	95.3% (88-98.2)	
			3.0-6.0	66.7% (19.7-94.2)	97% (79.9-99.6)	99.7% (97.7-100)	
			>6.0	100 % (34.9-100)	100% (89.7-100)	100% (99-100)	
	1.0-3.0	LR 63.13	0.00-<0.73	0% (0-20.7)	0% (0-80)	0% (0-97.8)	
			0.73-<1.0	38.4% (7.6-82)	90.9% (57.1-98.7)	99.1% (93.5-99.9)	
			1.0-<3.0	68.1% (43.5-85.5)	97.2% (92.5-99)	99.7% (99.3-99.9)	
			3.0-6.0	97.4% (82-99.7)	99.8% (98.7-100)	100% (99.9-100)	**≥75% post-test probability for HIT :** HIT ruled in, Stop heparin, Start alternative anticoagulant therapy
			>6.0	100% (90.9-100)	100% (99.4-100)	100% (99.9-100)	
	>3.0	LR ∞	--	**100%** (18.7-100) ‡‡	**100%** (79-100)	**100%** (98-100)	

Notes: *, If data are complete and in absence of unexplained heparin resistance. †, Optimal cut-off according to ROC analysis. ‡, Recommended cut-off according to manufacturer. ††, perform PaGIA and interpret as if CLIA LR of 0.60. ‡‡, perform PaGIA and interpret as if CLIA LR of 63.13.
Legend: P, Probability; CI, confidence interval.

FIGURE 3 Diagnostic algorithm sequentially combining chemiluminescent immunoassay (CLIA) and latex IA (LIA). The first step is to assess the pre-test probability for heparin-induced thrombocytopenia (HIT) with the 4T score. In case of a 4T score ≥2 or unexplained heparin resistance, the automated CLIA HIT-IgG is performed as first-line test. For cases with results situated in the CLIA intermediate "gray zone", Hemosil HIT-AB testing is performed as second-line test. Finally, for cases that remain unresolved despite a combination of 4T score, CLIA, and LIA (HIT undetermined), individualized clinical judgment will define initial management decisions, while awaiting for the results of the functional heparin-induced platelet activation (HIPA) assay as diagnostic gold standard. Gray, situations in which HIT cannot be excluded nor predicted.

performance.[14,29,30] However, we observed that it can sometimes be difficult to evaluate the positivity of the test so that inter-reader reproducibility was variable. To avoid this problem, we tested an automated quantification of the band density[14] (Supplementary material, Data set S1). Nevertheless, even using the density of the band, we could not determine clinically useful cut-offs given the fact that there were false positive results even with negative densities and false negative results at high positive densities. As shown above (Table S1), the LFIA did not have a strong enough performance compared with CLIA and PaGIA, in particular because it missed six out of 30 (20%) HIT positive cases and because its official cut-off cannot be adapted to improve sensitivity, as we previously did with the CLIA.[17] Therefore, we did not include the LFIA in our derivation cohort because this assay would not allow HIT to be accurately and safely excluded. This is in line with published data[7] and the performance observed in the external quality exercises of the ECAT performed between 2016 and 2021: out of 678 analyses the LFIA (STic Expert HIT) generated 71 (10.5%) borderline and 87 (12.8%) false negative results.

The LIA is a fully-automated and rapid immunoassay. Thus, it allows a greater standardization and a reduction of intra- and inter-laboratory variations. With only two false negative results in the retrospective derivation cohort (Table S1), the LIA compared very well with CLIA and PaGIA. Moreover, its official cut-off could be adapted to improve sensitivity (Table 2).

The derivation cohort was used to verify the diagnostic efficiency of the Lausanne algorithm and to evaluate alternative approaches relying on automated IA, such as CLIA and LIA (Figures 3 and 4). We compared the respective performances of four rapid diagnostic algorithms for HIT, based on the sequential (CLIA/PaGIA,[17] CLIA/LIA, LIA/CLIA) or simultaneous (LIA and CLIA, Hamilton algorithm)[19] use of two rapid IA for anti-PF4/heparin antibodies.

Our data summarized in Table 4 confirm that the Lausanne algorithm,[17] combining the CLIA as the initial rapid IA with the PaGIA as a secondary assay for unsolved cases, is very effective in classifying HIT status,[4] with only 3% of cases remaining in the gray zone and no false HIT predictions. Of note, the algorithm depicted in Figure 4, with the LIA as the first test and the CLIA as the second one, also

1st step Assess HIT pre-test probability with the 4T score Result (score)	2nd step Hemosil HIT-AB testing in patients with a 4T score ≥2 (and/or with unexplained heparin resistance) Result (U/ml)	Likelihood ratio (95% CI in Tab. 2)	3rd step CLIA HIT-IgG testing in the intermediate "grey-zone" (0.73-3.0 U/ml) Result (titer)	4th step Assess the individual post-test probability for HIT Low 4T score P (95% CI)	Int. 4T score P (95% CI)	High 4T score P (95% CI)	5th step Clinical decision (expected patients, %)
Pre-test probability for HIT according to 4T score: 0-3: **Low** (<1%) 4-5: **Int** (10-14%) 6-8: **High** (50-64%) **Proposed approach:** 0-1 : **HIT ruled out** * ≥ 2 : **ad 2nd step**	**0.00–<0.73**	LR 0.00	--	**0%**	**0% (0-6.3)**	**0% (0-42.2) ††**	**≤3% post-test probability for HIT :** HIT ruled out, Continue heparin
	0.73–<1.0 †	LR 0.98	<0.13	0% (0-0.2)	0%(0-3)	0% (0-24.8)	
			0.13-<0.33	0.6% (0.2-2.4)	8.8%(2.3-28.4)	51.1%(20.7-81.2)	
			0.33-<1.0	3.3% (1.4-7.6)	35.2%(18.6-56.4)	85.5%(71.3-93.4)	
			1.0-3.0	38.9% (7.7-83)	91%(57-98.7)	99.1%(93.5-99.9)	
			>3.0	100% (18.7-100)	100%(78.5-100)	100% (97.5-100)	
	1.0–<3.0 ‡	LR 3.35	<0.13	0% (0-0.6)	0% (0-9.4)	0% (0-53)	**Undetermined post-test probability for HIT:** Perform HIPA functional assay as diagnostic gold-standard. While result pending, manage according to clinical judgment
			0.13-<0.33	2%(0.5-7.8)	24.7%(7.6-57.5)	78.1%(47.1-93.6)	
			0.33-<1.0	10.4%(4.7-21.6)	65%(43.8-81.5)	95.3%(89.5-98)	
			1.0-3.0	68.3%(22-94.3)	97.2 %(81.9-99.6)	99.7%(98-100)	
			>3.0	100%(43.7-100)	100%(92.5-100)	100%(99.3-100)	
	3.0-6.0	LR 58.57	<0.13	0% (0-10.1)	% (0-64.4)	0% (0-95)	
			0.13-<0.33	26.2%(8.2-59.5)	85.1% (58.8-95.9)	98.3% (93.7-99.6)	
			0.33-<1.0	66.8% (45.9-82.7)	97% (93.2-98.7)	99.7% (99.3-99.9)	
			1.0-3.0	97.4% (83-99.7)	99.8% (98.7-100)	100% (99.9-100)	**≥75% post-test probability for HIT :** HIT ruled in, Stop heparin, Start alternative anticoagulant therapy
			>3.0	100% (93.1-100)	100% (99.5-100)	100% (99.9-100)	
	>6.0	LR ∞	--	**100% (13.7-100)** ‡‡	**100% (72-100)**	**100% (96.5-100)**	

Notes: *, If data are complete and in absence of unexplained heparin resistance. †, Optimal cut-off according to ROC analysis. ‡, Recommended cut-off according to manufacturer. ††, perform PaGIA and interpret as if LIA LR of 0.98. ‡‡, perform PaGIA and interpret as if LIA LR of 58.57.
Legend: P, Probability; CI, confidence interval.

FIGURE 4 Diagnostic algorithm sequentially combining latex immunoassay (LIA) and chemiluminescent IA (CLIA). The first step is to assess the pre-test probability for heparin-induced thrombocytopenia (HIT) with the 4T score. In case of a 4T score ≥2 or unexplained heparin resistance, the automated Hemosil HIT-AB is performed as first-line test. For cases with results situated in the LIA intermediate "gray zone", CLIA HIT-IgG testing is performed as second-line test. Finally, for cases that remain unresolved despite a combination of 4T score, LIA, and CLIA (HIT undetermined), individualized clinical judgment will define initial management decisions, while awaiting for the results of the functional heparin-induced platelet activation (HIPA) assay as diagnostic gold standard. Gray, situations in which HIT cannot be excluded nor predicted.

TABLE 3 Analysis of the prospective derivation cohort ($n = 267$) according to dual LIA and CLIA tests per the six-point scale, the "Hamilton algorithm"[19]

CLIA/LIA score	HIPA positive	HIPA negative	Proportion HIPA +	Stratum-specific likelihood ratio	4T score		
					Low	Intermediate	High
0	0	229	0	0.00	111	111	7
1	1	14	1/15 (6.7%)	0.99	6	8	1
2	1	5	1/6 (16.7%)	2.77	2	2	2
3	6	1	6/7 (85.7%)	83	2	4	1
4	2	0	2/2 (100%)	∞	0	0	2
5	5	0	5/5 (100%)	∞	1	4	0
6	3	0	3/3 (100%)	∞	1	0	2
Total	18	249					

Note: The score, ranging from 0 to 6, is based on the semi-quantitative results of simultaneously performed LIA and CLIA.[19] For each IA:<1.00 U/ml = 0 points.1.00–4.99 U/ml = 1 point.5.00–15.99 U/ml = 2 points.≥16.00 U/ml = 3 points.Abbreviations: CLIA, chemiluminescent immunoassay; HIPA, heparin-induced platelet activation; LIA, latex IA; LR, likelihood ratio for a positive HIPA result; SSLR+, stratum-specific likelihood ratio for a positive result within the stratum indicated.

TABLE 4 Diagnostic performances of four diagnostic algorithms incorporating rapid immunoassays for anti-PF4/heparin antibodies

Diagnostic algorithm	Algorithm's output				
	HIT excluded	HIPA positive predicted	HIT predicted	Undetermined	Mismatches
1st CLIA/2nd PaGIA[17]	**240**/267 (89.9%)	**16**/18 (88.9%)	**19**/22 (86%)	**8**/267 (3%)[a]	**4**[e]
1st CLIA/2nd LIA	**236**/267 (88.4%)	**15**/18 (83.3%)	**19**/22 (86%)	**11**/267 (4.1%)[b]	**6**[f]
1st LIA/2nd CLIA	**235**/267 (88.0%)	**17**/18 (94.4%)	**22**/22 (100%)	**9**/267 (3.4%)[c]	**5**[g]
Both LIA and CLIA[19]	**229**/267 (85.8%)	**10**/18 (55.6%)	**10**/22 (45%)	**28**/267 (10.5%)[d]	**0**

UPN	4T score	CLIA, U/ml	PaGIA, titer	LIA, U/ml	Zymutest-HIA-IgG, OD	ELISA	HIPA	Clinical course
(A) Probably false negative HIPA results:								
19.264	5	2.17	004	5.49	1.364 (P)	Strongly positive	Negative	APLA negative. Clinical evolution compatible with HIT (anticoagulation with agatroban; Plt count D0 50 G/L, D1 44 G/L, D3 96 G/L, D5 157 G/L, D7 247 G/L)
19.295	5	0.36	008	5.87	0.095 (N)	Negative	Negative	APLA negative. Clinical evolution compatible with HIT (anticoagulation with argatroban; Plt count D0 35 G/L, D1 62 G/L, D3 68 G/L, D5 138 G/L, D7 190 G/L) Anti-platelet treatment with Aspirin and clopidogrel
20.151	6	1.66	002	3.32	0.255 (N)	Strongly positive	Negative	APLA negative. Clinical evolution compatible with HIT (anticoagulation with agatroban; Plt count D0 27 G/L, D1 44 G/L, D3 136 G/L, D5 202 G/L, D7 240 G/L)
(B) Unsolved mismatch between HIT prediction (CLIA/LIA and LIA/CLIA algorithms) and HIPA outcome:								
19.246	7	1.20	001	1.00	0.429 (P)	Negative	Negative	APLA negative. Clinical evolution compatible with HIT (anticoagulation with agatroban; Plt count D0 56 G/L, D1 74 G/L, D3 105 G/L, D5 136 G/L, D7 193 G/L)
20.212	7	2.79	001	0.92	0.209 (N)	Negative	Negative	APLA negative. Clinical evolution compatible with HIT (anticoagulation with argatroban; Plt count D0 61 G/L, D1 60 G/L, D3 114 G/L, D5 200 G/L, D7 251 G/L)
(C) Possibly false positive HIPA result:								
20.097	3	0.51	0	1.25	0.734 (P)	Strongly positive	Positive	Good clinico-biological evolution under heparin treatment (Plt count D0 67 G/L, D1 80 G/L, D3 107 G/L, D5 189 G/L, D7 204 G/L; D-dimers D0 2′401 ng/ml, D7 2′312 ng/ml)
(D) False HIT prediction (CLIA/LIA algorithm):								
20.175	4	0.15	004	5.35	0.088 (N)	Negative	Negative	Good clinico-biological evolution under heparin treatment (Plt count D0 89 G/L, D1 96 G/L, D2 91 G/L, D3 95 G/L, D4 194 G/L, D7 133 G/L; D-dimers D0 15′263 ng/ml, D1 13′595 ng/ml)

Note: Zymutest-HIA-IgG OD: Optical densities; decision point at 0.347. Detailed information on the observed mismatches between algorithm prediction and HIPA.

Abbreviations: CLIA, chemiluminescent immunoassay; D, day; HIPA, heparin-induced platelet activation; HIT, heparin-induced thrombocytopenia; LIA, latex IA; N, negative; P, positive; PaGIA, particle-gel IA; Plt, platelet.

[a]1 HIPA positive, 7 HIPA negative (including UPN 19.246 and 20.212).

[b]2 HIPA positive, 1 possibly false positive HIPA (UPN: 20.097), 8 HIPA negative.

[c]1 possibly false positive HIPA (UPN: 20.097), 8 HIPA negative.

[d]8 HIPA positive, 20 HIPA negative.

[e]3 probably false negative HIPA (UPN: 19.264; 19.295; 20.151), 1 possibly false positive HIPA (UPN: 20.097).

[f]3 probably false negative HIPA (UPN: 19.264; 19.295; 20.151), 2 unsolved mismatches (UPN: 19.246; 20.212), 1 false HIT prediction (UPN: 20.175).

[g]3 probably false negative HIPA (UPN: 19.264; 19.295; 20.151), 2 unsolved mismatches (UPN: 19.246; 20.212).

demonstrated an excellent diagnostic performance, identifying all patients with HIT, with no false HIT predictions, and 3.4% of cases in the gray zone. On the other hand, the sequential use of CLIA as the first assay followed by LIA appears to be possibly less satisfactory with one false HIT prediction and 4.1% of the cases in the gray zone. Finally, we evaluated the Hamilton algorithm, a rapid diagnostic approach proposed by Warkentin and colleagues[19] based on the stratified results of both the CLIA and LIA (Table 3). While this approach requires both IA to be performed in all patients evaluated for a suspected HIT, both tests are necessary in only about 25% of cases with the sequential algorithms. Moreover and clinically more relevant, the discrimination achieved by the Hamilton algorithm is not as good as with the sequential IA combinations, as there are more results with an undermined score of 2–3 (4.8%) and only 50% of HIT cases were predicted (Table 4).

Overall, according to our data, a Bayesian diagnostic algorithm based on the combination of the 4T score with two sequential rapid IA for anti-PF4/heparin antibodies is the most effective approach for reaching an accurate classification of patients investigated for suspected HIT.

4.1 | Limitations of the study

First, we evaluated the diagnostic performance of STic Expert HIT (LFIA) retrospectively by testing frozen plasma samples, as done in the original publication.[14] However, since this rapid assay is a screening test primarily designed for testing fresh plasma or serum in emergency conditions, its evaluation with frozen samples may have contributed to the poor performance that we observed. Second, although the derivation cohort consisted of 321 patients consecutively investigated for a suspicion of HIT and confirmed the performances previously observed for PaGIA and CLIA,[17,22] in 54 (16.8%) instances we did not have plasma available. Therefore, our data are based on 267 patients only, should be considered hypothesis-generating, and need to be confirmed in a wider, ideally multi-centric and prospective cohort.

In conclusion, according to our data, the LFIA is not effective enough in detecting anti-PF4/heparin antibodies and cannot be used to exclude or predict HIT, at least when using frozen plasma. We demonstrated that a Bayesian approach employing two sequential rapid IA (CLIA/PaGIA, CLIA/LIA, LIA/CLIA) is very effective for the diagnosis of HIT. Because of the abrupt discontinuation of its manufacture, the PaGIA has to be substituted by the LIA in the Lausanne algorithm. We would recommend incorporating the LIA as the first IA, according to the approach depicted in Figure 4. These data need to be verified in a prospective multi-centric cohort.

AUTHOR CONTRIBUTIONS

LRR (ORCID 0000-0001-6916-997X) performed research, analyzed data, wrote the manuscript. MM (ORCID 0000-0002-7741-0740) analyzed data, edited the manuscript. EMG (ORCID 0000-0003-0395-2384) performed research. FG (ORCID 0000-0003-2208-2070) was in charge of the patients. FJG (ORCID 0000-0002-8816-037X) performed research. LA (ORCID 0000-0001-9686-9920) conceived research, analyzed data, wrote the manuscript. All authors read and approved the submitted version of the manuscript.

ACKNOWLEDGMENTS

This work constitutes the medical master thesis of Luana Rittener-Ruff. Open access funding provided by Université de Lausanne.

We thank the physicians caring for the patients, the technicians of the hemostasis laboratory, PD Thomas Thiele and Prof. Andreas Greinacher for support with the HIPA assay, Prof. Dr. med. Michel Duchosal for support, and Prof. Dr. med. Claudio Sartori for acting as expert of the master thesis of Luana Rittener-Ruff. We thank the companies Axon Lab AG (Baden-Dättwil, Switzerland) and Stago CH S.A. (Glattbrugg, Switzerland) for having kindly supplied the reagents required for the study.

LA is supported by a grant from the Swiss National Science Foundation (SNF 320030-197392).

CONFLICT OF INTEREST

The authors have no potential conflict of interest.

ORCID

Luana Rittener-Ruff https://orcid.org/0000-0001-6916-997X
Matteo Marchetti https://orcid.org/0000-0002-7741-0740
Elena Matthey-Guirao https://orcid.org/0000-0003-0395-2384
Francesco Grandoni https://orcid.org/0000-0003-2208-2070
Francisco J. Gomez https://orcid.org/0000-0002-8816-037X
Lorenzo Alberio https://orcid.org/0000-0001-9686-9920

REFERENCES

1. Arepally GM. Heparin-induced thrombocytopenia. *Blood*. 2017;129(21):2864-2872.
2. Greinacher A. CLINICAL PRACTICE. Heparin-induced thrombocytopenia. *N Engl J Med*. 2015;373(3):252-261.
3. Marchetti M, Zermatten MG, Bertaggia Calderara D, Aliotta A, Alberio L. Heparin-induced thrombocytopenia: a review of new concepts in pathogenesis, diagnosis, and management. *J Clin Med*. 2021;10(4):683.
4. Cuker A, Cines DB. Diagnosig HIT: the need for speed. *Blood*. 2020;135(14):1802-1803.
5. Warkentin TE. Heparin-induced thrombocytopenia: pathogenesis and management. *Br J Haematol*. 2003;121(4):535-555.
6. Cuker A, Gimotty PA, Crowther MA, Warkentin TE. Predictive value of the 4Ts scoring system for heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood*. 2012;120(20):4160-4167.
7. Favaloro EJ, McCaughan G, Mohammed S, et al. HIT or miss? A comprehensive contemporary investigation of laboratory tests for heparin induced thrombocytopenia. *Pathology*. 2018;50(4):426-436.
8. Nagler M, Bachmann LM, ten Cate H, ten Cate-Hoek A. Diagnostic value of immunoassays for heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood*. 2016;127(5):546-557.
9. Alberio L. Heparin-induced thrombocytopenia: some working hypotheses on pathogenesis, diagnostic strategies and treatment. *Curr Opin Hematol*. 2008;15(5):456-464.

10. Warkentin TE. HIT paradigms and paradoxes. *J Thromb Haemost*. 2011;9(Suppl 1):105-117.
11. Sun L, Gimotty PA, Lakshmanan S, Cuker A. Diagnostic accuracy of rapid immunoassays for heparin-induced thrombocytopenia. A systematic review and meta-analysis. *Thromb Haemost*. 2016;115(5):1044-1055.
12. Meyer O, Salama A, Pittet N, Schwind P. Rapid detection of heparin-induced platelet antibodies with particle gel immunoassay (ID-HPF4). *Lancet*. 1999;354(9189):1525-1526.
13. Legnani C, Cini M, Pili C, Boggian O, Frascaro M, Palareti G. Evaluation of a new automated panel of assays for the detection of anti-PF4/heparin antibodies in patients suspected of having heparin-induced thrombocytopenia. *Thromb Haemost*. 2010;104(2):402-409.
14. Sachs UJ, von Hesberg J, Santoso S, Bein G, Bakchoul T. Evaluation of a new nanoparticle-based lateral-flow immunoassay for the exclusion of heparin-induced thrombocytopenia (HIT). *Thromb Haemost*. 2011;106(6):1197-1202.
15. Warkentin TE, Sheppard JI, Linkins LA, Arnold DM, Nazy I. Performance characteristics of an automated latex immunoturbidimetric assay [HemosIL([R]) HIT-ab(PF4-H)] for the diagnosis of immune heparin-induced thrombocytopenia. *Thromb Res*. 2017;153:108-117.
16. Cuker A, Arepally GM, Chong BH, et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: heparin-induced thrombocytopenia. *Blood Adv*. 2018;2(22):3360-3392.
17. Marchetti M, Barelli S, Zermatten MG, et al. Rapid and accurate Bayesian diagnosis of heparin-induced thrombocytopenia. *Blood*. 2020;135(14):1171-1184.
18. Schneiter S, Colucci G, Sulzer I, Barizzi G, Lammle B, Alberio L. Variability of anti-PF4/heparin antibody results obtained by the rapid testing system ID-H/PF4-PaGIA. *J Thromb Haemost*. 2009;7(10):1649-1655.
19. Warkentin TE, Sheppard JI, Smith JW, et al. Combination of two complementary automated rapid assays for diagnosis of heparin-induced thrombocytopenia (HIT). *J Thromb Haemost*. 2020;18(6):1435-1446.
20. Raschke RA, Reilly BM, Guidry JR, Fontana JR, Srinivas S. The weight-based heparin dosing nomogram compared with a "standard care" nomogram. A randomized controlled trial. *Ann Intern Med*. 1993;119(9):874-881.
21. Guermazi S, Znazen R. [Resistance to curative treatment by unfractionned heparin]. *Rev Med Interne*. 2009;30(4):331-334.
22. Nellen V, Sulzer I, Barizzi G, Lammle B, Alberio L. Rapid exclusion or confirmation of heparin-induced thrombocytopenia: a single-center experience with 1,291 patients. *Haematologica*. 2012;97(1):89-97.
23. Warkentin TE. Challenges in detecting clinically relevant heparin-induced thrombocytopenia antibodies. *Hamostaseologie*. 2020;40(4):472-484.
24. Alatri A, Armstrong AE, Greinacher A, et al. Results of a consensus meeting on the use of argatroban in patients with heparin-induced thrombocytopenia requiring antithrombotic therapy - a European perspective. *Thromb Res*. 2012;129(4):426-433.
25. Colucci G, Nagler M, Klaus N, Conte T, Giabbani E, Alberio L. Practical guidelines for argatroban and bivalirudine in patients with heparin-induced thrombocytopenia. *J Transl Sci*. 2015;1(2):37-42.
26. Alberio L, Angelillo-Scherrer A, Asmis L, et al. Recommendations on the use of anticoagulants for the treatment of patients with heparin-induced thrombocytopenia in Switzerland. *Swiss Med Wkly*. 2020;150:w20210.
27. Marchetti M, Barelli S, Gleich T, et al. Managing argatroban in heparin-induced thrombocytopenia: a retrospective analysis of 729 treatment days in 32 patients with confirmed heparin-induced thrombocytopenia. *Br J Haematol*. 2022;197:766-790.
28. Bank PCD, Jacobs LHJ, van den Berg SAA, et al. The end of the laboratory developed test as we know it? Recommendations from a national multidisciplinary taskforce of laboratory specialists on the interpretation of the IVDR and its complications. *Clin Chem Lab Med*. 2020;59:491-497.
29. Leroux D, Hezard N, Lebreton A, et al. Prospective evaluation of a rapid nanoparticle-based lateral flow immunoassay (STic expert([R]) HIT) for the diagnosis of heparin-induced thrombocytopenia. *Br J Haematol*. 2014;166(5):774-782.
30. Berroeta C, Crespin M, Bouabdallah K, et al. Diagnostic performance of a new rapid lateral flow immunoassay in patients suspected of heparin-induced thrombocytopenia and its clinical consequences. *Semin Thromb Hemost*. 2016;42(1):69-74.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rittener-Ruff L, Marchetti M, Matthey-Guirao E, Grandoni F, Gomez FJ, Alberio L. Combinations of rapid immunoassays for a speedy diagnosis of heparin-induced thrombocytopenia. *J Thromb Haemost*. 2022;20:2407-2418. doi: 10.1111/jth.15811