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High sensitivity and specificity of a new functional flow cytometry assay for clinically significant heparin-induced thrombocytopenia antibodies

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SUMMARY

Introduction: Heparin-induced thrombocytopenia (HIT) is a life-threatening condition, in which the anticoagulant heparin, platelet factor 4 (PF4), and platelet-activating antibodies form complexes with prothrombotic properties. Laboratory tests to support clinical diagnosis are subdivided into functional, platelet activation assays, which lack standardization, or immunological assays, which have moderate specificity toward HIT.
In this study, clinical performance of HIT*Alert*, a novel *in vitro* diagnostic (IVD) registered platelet activation assay, was tested in a large cohort of HIT-suspected patients and compared with immunological assays.
Methods: From 346 HIT-suspected patients (single center), clinical data including 4T pretest probability results, citrated platelet-poor plasmas, and sera were collected, allowing direct comparison of clinical observations with HIT*Alert* results. HIT*Alert* performance was compared with PF4 IgG ELISA (246 patients, three centers) and PF4 PaGIA (298 patients, single center).
Results: HITAlert showed high sensitivity (88.2%) and specificity (99.1%) when compared with clinical diagnosis. Agreement of HIT*Alert* with PF4 ELISA- and PF4 PaGIA-positive patients is low (52.7 and 23.2%, respectively), while agreement with PF4 IgG ELISA- and PF4 PaGIA-negative patients is very high (98.1 and 99.1%, respectively).
Conclusion: HIT*Alert* performance is excellent when compared with clinical HIT diagnosis, making it a suitable assay for rapid testing of platelet activation due to anticoagulant therapy.

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Heparin-induced thrombocytopenia, functional

INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a life-threatening medical complication of heparin therapy affecting 0.3–3% of patients exposed to unfractionated heparin [1–3]. HIT is caused by the interaction of platelet-activating antibodies of IgG class with large complexes of platelet factor 4 (PF4) bound to heparin on platelets, resulting in platelet activation and subsequent triggering of the coagulation pathway (as reviewed in [4–6]).

Clinically, HIT is defined by a platelet count below 150×10^9/L or a fall in platelet count of >50% after commencement of heparin therapy, and it is associated with a high risk of thrombotic complications [7, 8]. Because reduced platelet counts after heparin therapy could be caused by other factors, also the timing of platelet count fall, manifestation of thrombosis, and the absence of other causes of thrombocytopenia are scored in HIT-suspected patients, resulting in the 4T pretest probability of HIT [9]. Although the 4T pretest probability has high sensitivity and excellent negative predictive value at scores 0–4, its positive predictive value is only 9–17% [5, 9]. Specific diagnosis of HIT is of utmost relevance regarding continuation of heparin therapy or switching to alternative anticoagulants, which are more expensive to administer and monitor. Therefore, clinicians usually combine the outcome of the 4T test with laboratory test results to enhance diagnostic specificity.

Laboratory tests of HIT-suspected patients are divided into two subgroups. Immunologic assays, such as the PF4 ELISA and Particle Gel Immunoassay (PaGIA), detect circulating anti-PF4/heparin antibodies, but are unable to detect the clinically relevant platelet activation [10]. Typically, immunoassays have wide applicability, are rapid and highly sensitive for HIT but lack specificity [10, 11]. This is due to detection of anti-PF4/heparin antibodies that are not directly associated with platelet activation, leading to HIT overdiagnosis (false positives) and unnecessary switching to alternative anticoagulant therapy.

Functional assays, which include the Serotonin Release Assay (SRA [12]), heparin-induced platelet activation assay (HIPA [13]), and aggregation assay, detect platelet activation and are more specific for HIT than immunologic assays [10, 11]. To date, none of these tests has been registered for *in vitro* diagnostic (IVD) use. Unfortunately, these tests are technically demanding and, in the case of the SRA, also require the use of radioactivity [12], thereby limiting their application in the acute diagnostic setting and availability to the clinician at the moment of decision-making [14]. Clearly, there is currently an unmet need for an easy, rapid functional laboratory assay with high sensitivity and specificity that directly helps clinicians in diagnosing HIT.

In this study, the HITAlert was used, which is an IVD-registered, rapid, commercialized version of the highly sensitive and specific flow cytometry (FCM) assay originally described by Tomer [15, 16]. Sensitivity and specificity of this FCM assay, as compared to the SRA, were found to be 95% and 100%, respectively, in 19 patients and 10 healthy controls [15]. HITAlert was used for laboratory analysis of HIT-suspected patients in two European (Braunschweig, Germany and London, UK) and one North American Center (Stanford, USA). In Braunschweig, several clinical parameters of a large cohort of HIT-suspected patients (346 patients) were analyzed, yielding a unique data set. Furthermore, in all three centers described above, immunologic assays (PF4 ELISA and PaGIA) were performed as well as the HIT*Alert*, allowing direct comparison of immunologic assays with the HIT*Alert*.

Our data show that HIT*Alert* has high sensitivity (88.2%) and specificity (99.1%) when compared with final clinical HIT diagnosis in a cohort of 346 patients.

MATERIALS AND METHODS

Patients and clinical tests

In this study, 346 patients with suspected HIT were monitored at Städtisches Klinikum Braunschweig gGmbH (Braunschweig, Germany). 17 of the 346 patients turned out to be HIT positive as defined by the HITAlert Assay.

Because HIT is essentially a clinical diagnosis, we used the 4T pretest to categorize the clinical samples. In this way, patients were divided into a no HIT, low probability, middle, or high probability HIT group.

From each patient, citrated platelet-poor plasmas (PPP) and sera were obtained from citrate-anticoagulated blood (1/10 Vol, 3.8% trisodium-citrate buffer, Greiner Bio-One GmbH, Frickenhausen, Germany) and stored at −80 °C. These samples allowed direct comparison of clinical data with laboratory data, including a functional assay (HIT*Alert* from IQ Products, Groningen, The Netherlands) and immunological assays (PF4 IgG ELISA and PaGIA).

In two other centers (The Haemophilia Reference Centre, London, UK, 47 patients and Stanford University Medical Center, Stanford, CA, USA, 95 patients), citrated PPP and sera were obtained and stored at −80 °C. These samples allowed direct comparison of HIT*Alert* with PF4 IgG ELISA.

HIT*Alert*, a functional laboratory assay

HIT*Alert* was performed according to the manufacturer's specifications. In brief, platelet-rich plasma (PRP) from healthy donors (preferably 0-type) was prepared by collecting venous blood into a 3.8% Citrate Solution Evacuated Tube (Greiner Bio-One) and centrifuged for 5-min at $100 \times \boldsymbol{g}$. Donors were selected for their reactivity with HIPA-positive and HIPA-negative sera in the HIT*Alert* test. Subsequently, PRP was added to patient serum in the presence or absence of therapeutic concentration of unfractionated heparin [15, 16] in a total volume of 50 μL. To determine heparin dependence of platelet activation, a supratherapeutic dosage of heparin [15, 16] was added. After 1-h incubation at room temperature (+20 to +24 °C) on a horizontal orbital shaker, 5 μL of the suspension was transferred to 45 μL staining solution containing fluorescent-labeled antibodies to a platelet activation marker annexin V [15, 16] and a platelet marker, followed by 15-min incubation in the dark. Samples were measured and analyzed on a standard flow cytometer, capable of detecting FITC and R-PE fluorescence (Braunschweig: FACS Calibur, BD, Franklin Lakes, NJ, USA, London: Beckman Coulter UK Ltd, High Wycombe, UK, Stanford: BD FACS Canto II). In FCM analysis, 100% activation implies that all cells positive for the platelet marker are also 100% positive for the platelet activation marker. Representative FCM results are shown in Figure S1.

Interpretation of HIT*Alert* results

Patient sera that showed ≥7.6% platelet activation in the presence of therapeutic heparin were only considered positive if platelet activation was subsequently reduced ≥50% [15, 16] in the presence of supratherapeutic heparin.

PF4 IgG ELISA and PaGIA, immunological laboratory assays

Patient sera were analyzed with PF4 IgG ELISA or by Particle Gel Immuno Assay (PaGIA) according to the manufacturer's instructions. For PF4 IgG ELISA, OD values ≥ 0.40 were assessed positive, according to the manufacturer's instructions.

Statistical analysis

Agreement between HIT*Alert* performance and final clinical diagnosis (no HIT, low or middle probability, definite HIT) was assessed by receiver operating characteristic (ROC) analysis. ROC analysis permitted calculation of area under the curve, sensitivity, specificity, and likelihood ratio of HIT for given HIT*Alert* test results.

One-way ANOVA (nonparametric Kruskal–Wallis test) was used to compare HIT*Alert* results (platelet activation levels) between different clinical groups (no HIT, low, middle, HIT).

Correlation between HIT*Alert* and ELISA data was analyzed, yielding Spearman's ρ. A nonparametric Mann–Whitney *U*-test was used to compare HIT*Alert* results between either ELISA-positive (OD ≥ 0.4) and ELISA-negative groups or PaGIA-positive and PaGIA-negative groups.

All data were analyzed using GRAPHPAD PRISM software, version 5.0 (GraphPad Software Inc., La Jolla, CA, USA). A *P*-value < 0.05 was considered as statistically significant.

RESULTS

Patient characteristics and clinical diagnosis

In Braunschweig, clinical data including age, sex, and 4T scores were collected from 346 HIT-suspected patients. In Table 1, 4T test results are grouped in a low (0–3), middle (4,5), and high (6–8) probability group and compared with final diagnosis for all patients. Table 2 displays 4T test results of those patients diagnosed with no HIT or HIT only. Of 126 patients that received a low score in the 4T test, 119 patients indeed did not develop HIT. On the other hand, only 1 of 17 patients scoring high in the 4T test actually developed HIT. These data show that 4T testing in our patient cohort had a very high negative predictive value (94.4%) but a very poor positive predictive value (5.9%), which is in agreement with previous observations [5, 9].

Laboratory testing of HIT-suspected patients using the HIT*Alert*

Because 4T testing alone has poor positive predictive value, additional laboratory testing of HIT-suspected patients is strongly recommended to enhance diagnosis. In this study, platelet activation was first measured in sera of 346 HIT-suspected patients, using the HIT*Alert*. In Table 2 and Figure 1, HIT*Alert* results are compared with final clinical diagnosis, which was subdivided into no HIT (224 patients), low probability (61 patients), middle probability (42 patients), or HIT (17 patients). Agreement between HIT*Alert* results and clinical diagnosis in 241 patients with diagnosis no HIT or HIT was assessed by ROC analysis. Area under the (ROC) curve was 0.9701 (95% confidence interval 0.9331–1.007, $P < 0.0001$), and the most optimal separation between HIT-positive and HIT-negative patients was established at a cutoff value of 7.6% platelet activation, with 88.2% sensitivity and 99.1% specificity. Furthermore, ROC analysis revealed that the likelihood for developing HIT was 98.8 times higher for patients who had ≥7.6% platelet activation than patients who had <7.6% platelet activation (Figure 1A and Tables 1, 2).

In Figure 1B, final clinical diagnosis (no HIT, low, middle, HIT) was plotted against percentage platelet activation. In patients with final diagnosis HIT, mean platelet activation was significantly higher (27.6 ± 7%, $P < 0.001$) when compared to patients with diagnosis

Table 1. Patient characteristics and clinical diagnosis

	Clinical Diagnosis						
	No HIT	HIT	Subtotal	Low	Middle	Unknown	Total
4T score							
Low (0–3)	119	7	126	24	17	0	167
Middle (4,5)	88	9	97	33	21	0	151
High (6–8)	16	1	17	4	4	0	25
Unknown	1	0	1	0	0	2	3
Total	224	17	241	61	42	2	346

Clinical data of 346 patients (Braunschweig) were, based on 4T pretest probability, divided into four groups: low, middle, high probability, or 4T score unknown. Subsequently, these groups were further subdivided according to final clinical diagnosis (no HIT, HIT, low, middle, or diagnosis unknown). Analysis of 4T test results in those patients with final diagnosis no HIT or HIT showed that 4T testing has high negative predictive value (119/126, 94.4%) but poor positive predictive value (1/17, 5.9%).

Table 2. Laboratory testing of HIT-suspected patients using the HIT*Alert*

Clinical Diagnosis							
	No HIT	HIT	Subtotal	Low	Middle	Unknown	Total
HITAlert							
NEG < 7.6%	222	2	224	58	38	1	321
POS > 7.6%	2	15	17	3	4	1	25
Total	224	17	241	61	42	2	346

HIT*Alert* results of 346 patients (Braunschweig) were divided into two groups: HIT*Alert* negative or HIT*Alert* positive. Subsequently, these two groups were further subdivided according to final clinical diagnosis (no HIT, HIT, low, middle, or unknown diagnosis).
Analysis of HIT*Alert* results in those patients with final diagnosis no HIT or HIT showed that HIT*Alert* has very high specificity (99.1%) and negative predictive value (99.1%) and high sensitivity (88.2%) and positive predictive value (88.2%) when compared to clinical diagnosis.

no HIT (1.6 ± 0.1%), low (5.4 ± 1.6%), or middle (5.2 ± 1.9%).

Comparison of HIT*Alert* with PF4 ELISA and PaGIA

At this moment, HIT*Alert* is the only *in vitro* diagnostics (CE/IVD) registered functional laboratory assay that is available for HIT analysis. On the other hand, several immunologic assays are IVD-registered, including PF4 IgG ELISA and PaGIA. We directly compared HIT*Alert* performance with PF4 IgG ELISA (Three centers, 246 patients) and PaGIA (Braunschweig only, 297 patients).

In Figure 2A, platelet activation data from 246 patients are plotted against corresponding ODs obtained with PF4 ELISA. Correlation analysis yielded a Spearman's ρ of 0.55 (0.46–0.64, $P < 0.001$), showing that HIT*Alert* and ELISA outcomes are positively related to each other.

Subsequently, we subdivided all HIT*Alert* outcomes in an ELISA-positive group (OD ≥ 0.4, according to manufacturer's instructions) and an ELISA-negative (OD < 0.4) group, resulting in the plot displayed in Figure 2B. Mean platelet activation was significantly lower in patients who were ELISA negative (3.50% ± 0.43%) than in patients who were ELISA positive (20.79% ± 2.44%, $P < 0.0001$). Most sera that were ELISA negative were also HIT*Alert* negative (152/155, agreement 98.1%, see also Table 3A). In the group of ELISA-positive sera however, a large proportion of sera were found HIT*Alert* negative (43/91, agreement 52.7%).

In the literature, PF4 ELISA specificity has been debated, because many HIT-negative patients were found ELISA positive when OD ≥ 0.4 was used as cut-off value. To enhance ELISA specificity, a cutoff value of OD ≥ 1.0 was recommended [17]. If patients with OD ≥ 1.0 were considered ELISA positive, agreement between ELISA- and HIT*Alert*-positive patients was increased to 71.2%, while agreement between ELISA- and HIT*Alert*-negative patients was reduced to 92.8% (Table 3B).

In 297 patient sera, HIT*Alert* was also compared with IVD-registered PaGIA assay (Braunschweig). HIT*Alert* data were subdivided into a PaGIA-positive and PaGIA-negative groups, resulting in the plot shown in Figure 3. Mean platelet activation was significantly lower in PaGIA-negative sera (1.98% ± 0.29%) than PaGIA-positive sera (10.26% ± 2.18%, $P < 0.0001$). The majority of PaGIA-negative sera were also HIT*Alert* negative (213/215, agreement 99.1%). However, only 19/82 of PaGIA-positive sera were also HIT*Alert* positive (agreement 23.2%, see also Table 4).

DISCUSSION

In the present study, a unique data set was collected of HIT-suspected patients, including clinical data (346 patients) and data from functional (HIT*Alert*, 346 patients) and immunological laboratory assays (PF4 IgG ELISA, 151 patients and PaGIA, 297 patients). In these patients, 4T pretest probability had high negative predictive value (94.4%) but poor positive predictive value (5.9%), while HIT*Alert* testing had very

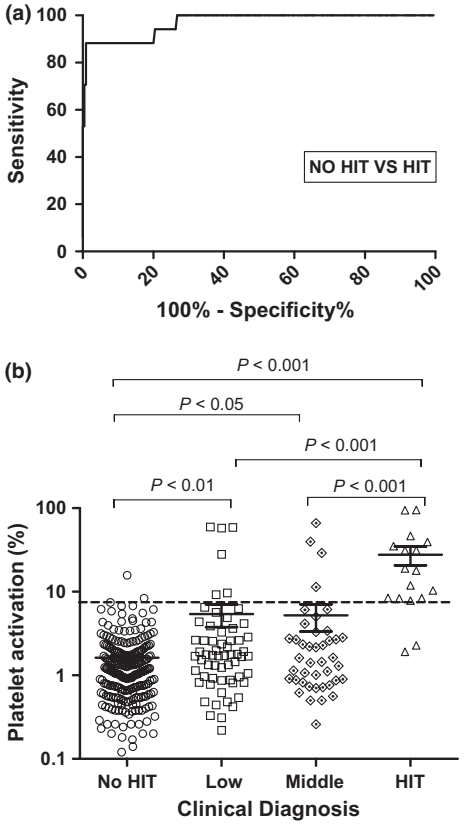

Figure 1. Laboratory testing of HIT-suspected patients using the HIT*Alert*. Agreement between HIT*Alert* results and final clinical diagnosis (HIT, no HIT) was assessed by receiver operating characteristic (ROC) analysis, yielding an area under the curve of 0.9701 (95% CI: 0.9331–1.007, $P < 0.0001$) and cutoff value ≥7.6% platelet activation with a likelihood ratio of 98.82 (a). Final clinical diagnosis (no HIT, low probability, middle probability and HIT) was plotted against HIT*Alert* results (% platelet activation). Mean percentage platelet activation was significantly higher in patients with final diagnosis HIT (27.6 ± 7%, $P < 0.001$) than in patients with diagnosis no HIT (1.6 ± 0.1%), low (5.4 ± 1.6%), or middle (5.2 ± 1.9%) probability (b).

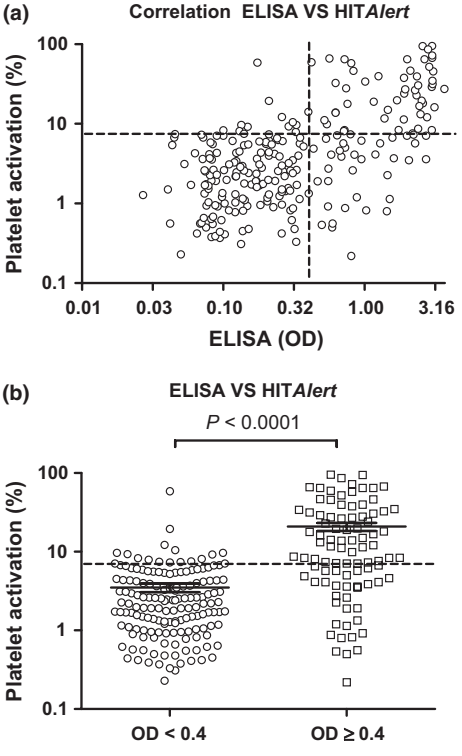

Figure 2. Comparison of HIT*Alert* with PF4 IgG ELISA performance. HIT*Alert* results (% platelet activation) were plotted against PF4 IgG ELISA results (OD). Using correlation analysis, Spearman's ρ = 0.554 (95% CI: 0.458–0.637, $P < 0.0001$) was calculated, indicating a positive relation between percentage platelet activation and ELISA OD's (a). Subsequently, HIT*Alert* results (% platelet activation) were plotted against ELISA results (subdivided into positive, OD ≥ 0.4 and negative, OD < 0.4). Mean percentage platelet activation was significantly higher in patients with positive ELISA results (20.79 ± 2.44, $P < 0.001$) than in patients with negative ELISA results (3.50 ± 0.43). Dashed line represents HIT*Alert* cutoff value, 7.6% platelet activation (b).

high negative predictive value (99.1%) and high positive predictive value (88.2%) toward final clinical diagnosis. These results show that HIT*Alert* is a functional laboratory assay with very high predictive power toward final diagnosis in a large cohort of HIT-suspected patients.

HIT is a serious medical condition, characterized by platelet activation and enhanced risk of thrombotic complications, which are paradoxically caused by administration of the anticoagulant heparin [4–6]. To prevent thrombotic events in HIT-suspected patients, rapid and reliable diagnosis, allowing immediate switch to alternative anticoagulants, is essential [18]. At this moment, functional platelet activation assays are the gold standard, but these assays are technically demanding, lack standardization and therefore do not allow rapid HIT diagnosis. Up till now, HIT*Alert* is the only rapid, standardized functional platelet activation

Table 3. Comparison of HIT*Alert* with PF4 ELISA

	Neg	Pos	Total
(A) PF4 IgG ELISA OD ≥ 0.4			
HITAlert			
NEG < 7.6%	152	43	195
POS ≥ 7.6%	3	48	51
TOTAL	155	91	246
(B) PF4 IgG ELISA OD ≥ 1.0			
HITAlert			
NEG < 7.6%	180	15	195
POS ≥ 7.6%	14	37	51
Total	194	52	246

HIT*Alert* results of 246 patients in three centers were divided into two groups, HIT*Alert* negative or HIT*Alert* positive. Subsequently, these two groups were further subdivided into PF4 IgG ELISA negative (OD < 0.4) or ELISA positive (OD ≥ 0.4). Comparison of HIT*Alert* results with ELISA results showed that HIT*Alert* has very high positive predictive value (94.1%) but moderate negative predictive value (77.9%, A). To increase PF4 IgG ELISA specificity, a cutoff at OD ≥ 1.0 instead of 0.4 has been recommended in the literature. When using this recommended ELISA cutoff value, HIT*Alert* has very good negative predictive value (92.3%), while positive predictive value is moderate in comparison with ELISA results (72.5%, B).

Table 4. Comparison of HIT*Alert* with PaGIA

	PaGIA		
	Neg	Pos	Total
HITAlert			
NEG < 7.6%	213	63	276
POS > 7.6%	2	19	21
Total	215	82	297

HIT*Alert* results of 297 patients (Braunschweig) were divided into two groups, HIT*Alert* negative or HIT*Alert* positive. Subsequently, these two groups were further subdivided into PF4 PaGIA negative or PaGIA positive. Comparison of HIT*Alert* results with PaGIA results showed that HIT*Alert* has very high positive predictive value (90.4%) but moderate negative predictive value (77.2%).

assay available, which has been CE/IVD-registered. Notably, the FCM method on which HIT*Alert* is based shows excellent correlation with the SRA, which is still

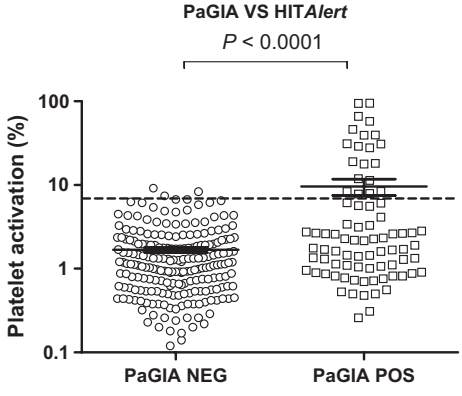

Figure 3. Comparison of HIT*Alert* with PaGIA performance. HIT*Alert* results (% platelet activation) were plotted against PaGIA results (subdivided into positive and negative). Mean percentage platelet activation was significantly higher in patients with positive PaGIA results (10.26 ± 2.18, $P < 0.001$) than in patients with negative PaGIA results (1.98 ± 0.29). Dashed line represents HIT*Alert* cutoff value, 7.6% platelet activation.

considered to be the gold standard concerning laboratory HIT analysis [15, 16].

Several immunological assays, including PaGIA and PF4 ELISAs, are also IVD-registered and are highly standardized in laboratory practice, rapid, and easy to handle. They are however limited to PF4 detection and do not measure (prothrombotic) platelet activation. Furthermore, PF4 ELISAs still require some non-automated pipetting steps, which might limit routine diagnostics on a 24/7 scale. It should be noted, that also 24/7 performance of HIT*Alert* is limited by requirement of a flow cytometer and skilled personnel, but also the ELISA is often only performed during regular hours.

Requirement of donor (O-type) platelets, which are preferred for functional HIT laboratory assays, also limits widespread applicability of functional assays. Furthermore, HIT*Alert* is performed on PRP instead of washed platelets, which might limit sensitivity according to the literature [4]. In this study, we did not investigate whether PRP usage leads to a reduction in HIT*Alert* sensitivity.

Two patients diagnosed with HIT were negative in the HIT*Alert*. One of these patients scored low in 4T testing, was weakly positive in PF4 IgG ELISA (OD

0.55) and PaGIA, but tested negative in the functional assays HIPA, aggregation assay, and HIT*Alert* (1.9% platelet activation). Taken together, all functional assays tested here and 4T test results indicated that this patient was HIT negative, while PF4-based immunological assays indicated HIT positivity. For this patient, switching to alternative coagulants while performing additional functional testing with the SRA assay would be highly recommended.

The second HIT-positive patient scored middle on 4T testing and was PaGIA and ELISA (OD 1.45) positive. This patient was clearly negative in HIT*Alert* testing (2.3% platelet activation), while no additional functional tests were performed. With positive immunoassays, a negative functional assay and a low scoring on the 4T tests, HIT may be unlikely, but this should be confirmed by an additional functional test. The high OD in the ELISA however suggests a high titer of nonactivating antibodies. Also for this patient, switching to alternative coagulants is recommended, while performing the HIT*Alert* again preferably together with an additional functional test such as HIPA or SRA.

Antiphospholipid syndrome (APS) is a disorder with clinical characteristics similar to HIT. In APS patients, many antiphospholipid antibodies are able to form a complex with β2 glycoprotein I (β2GPI) and PF4 with platelet-activating properties [19]. Especially in those patients in which APS is associated with systemic lupus erythematosus (SLE), risk of thrombocytopenia and thrombosis is increased [20]. In laboratory analysis of HIT-suspected patients, APS patients might test positive on PF4 ELISA and PaGIA and they might even test positive in functional (platelet activation) assays, although there is some debate about the latter [21]. A potential laboratory strategy to separate HIT and APS would be specific APS diagnosis using anticardiolipin and anti β2GPI ELISAs, which test negative in HIT-suspected patients.

In the Braunschweig patient cohort, three patients with high platelet activation were assessed HIT*Alert* negative, because there was no reduction in platelet activation in the presence of supratherapeutic heparin. Furthermore, the platelets were already activated in patient sera in the absence of therapeutic heparin. All three patients had 4T score 4–5 and received the final clinical diagnosis low. In one of the patients, PaGIA was positive and ELISA was weakly positive (OD 0.421), while in the other two, PaGIA and ELISA were negative. While lack of heparin dependence rules out HIT in these patients, APS testing could be relevant, especially in the patient with positive ELISA and PaGIA results.

Except for in patients with APS (in the presence of SLE), heparin-independent platelet activation, thrombocytopenia, and thrombotic events have also been described in patients with preceding (perioperative) inflammatory events. Although this clinical situation is rare, it has been observed in postsurgery patients, especially in those who underwent total knee arthroplasty (total knee replacement) [22–24].

Taken together, this study showed good performance of the HIT*Alert* toward HIT diagnosis. Whether applying this functional test upfront makes immunoassays redundant is a question that depends on local logistic and diagnostic structures (e.g., availability of FCM and fresh platelets). The data presented here suggest that such an approach is possible. HIT*Alert* is reliable, easy to perform, rapid, and standardized (IVD-registered) for clinical practice. These properties make HIT*Alert* very suitable for rapid testing of platelet activation due to heparin therapy but also for testing platelet-activating properties of alternative coagulants. Future studies should further unravel HIT*Alert* performance in patient groups with heparin-independent platelet activation events, including APS patients and patients undergoing orthopedic surgery.

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CONFLICT OF INTERESTS

All authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

HSG: study design, wrote and revised the manuscript. MPK, NL and WE: performed research and data analysis (Braunschweig). SS: performed research and data analysis (Stanford). JW and JHNS: study design. HK: Functional HIT testing (HIPA). DAG and GWM: study design, performed research, and data analysis (London), revised manuscript. JLZ: study design, data analysis (Stanford), revised manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Representative HIT*Alert* results.