Clinical and laboratory tests for the diagnosis of heparin-induced thrombocytopenia

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Summary

A rapid diagnostic work-up is required in patients with suspected heparin-induced thrombocytopenia (HIT). However, diagnosis of HIT is challenging due to a number of practical issues and methodological limitations. Many laboratory tests and a few clinical scoring systems are available but the individual characteristics and the diagnostic accuracy of these are hard to appraise. The 4Ts score is a well evaluated clinical assessment tool with the potential to rule out HIT in many patients. Still, it requires experience and is subject to a relevant inter-observer variability. Immunoassays such as enzyme-linked immunosorbent assays or recently developed rapid assays are able to exclude HIT in a number of patients. But, accuracy of immunoassays differs depending on type of assay, threshold, antibody specificity and even manufacturer. Due to a comparatively low positive predictive value, HIT cannot be confirmed by immunoassays alone. In addition, only some of them are immediately accessible, particularly in small laboratories. While functional assays such as the serotonin release assay (SRA) and the heparin-induced platelet activation assay (HIPA) are considered as gold standard for diagnosis of HIT, they require a highly specialised laboratory. In addition, some of them are not adequately evaluated. In clinical practice, we recommend an integrated diagnostic approach combining not only clinical assessment (the 4Ts score) but immunoassays and functional assays as well. We propose a clear diagnostic algorithm supporting clinical decision-making. Furthermore, we provide an overview of all current laboratory techniques for HIT and discuss diagnostic pathways and strategies to reduce diagnostic errors, and future perspectives.

Keywords

Heparin/adverse effects, immunoassay/methods, thrombocytopenia/chemically induced, thrombocytopenia/diagnosis

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Introduction

Diagnostic work-up of patients with suspected heparin-induced thrombocytopenia (HIT) is hampered by major practical issues and a number of methodological limitations. Not infrequently, suspicion is raised during weekends when haematology consultants and elaborated laboratory services are not available. Thus, surgical registrars or intensive care unit consultants who are inexperienced with such patients may face major clinical decisions at times when there is little support. Most accurate diagnostic tests are functional assays, which are time-consuming, expensive and require a high level of laboratory expertise (1–3). Even in the best-case scenario, results of these gold-standard tests will take at least two days and will only be available from Monday to Friday (4–6). However, the clinical decision regarding whether or not heparin should be stopped and treatment with an alternative anticoagulant started, must be made within a few hours (7–10). Delaying this decision may be life-threatening in patients with HIT (11), while treatment with alternative anticoagulants in non-HIT patients are associated with major risks (12–14). Some clinical scoring systems and a number of immunoassays (▶Table 1) are currently available to help physicians select the most appropriate course of action. However, the diagnostic accuracy varies across these tests and all are associated with limitations (15). Given the large number of publications describing heterogeneous study designs and reporting imprecise and varying results, it is hard to appraise the diagnostic characteristics of individual tests.

In the present article, we will review the currently available clinical and laboratory tests, summarise their diagnostic accuracy data and discuss practical issues. We will also elaborate on test variations and discuss strategies to reduce over-diagnosis.

Diagnostic pathways

While estimating the value of diagnostic tests, it is helpful to appreciate the pathways in which they are used. Thus, we describe typical scenarios requiring a diagnosis of HIT that physicians may find themselves in, which will generally be informed by previous training and the technical infrastructure of the hospital. In

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Table 1: Available immunoassays for the diagnosis of HIT (adapted from 64)).

Type of assay	Available antibody specificities	Measurement scale	Practical issues	Manufacturers
Enzyme-linked immunosorbent assay (ELISA)	Polyspecific IgG specific	Optical density; Low, intermediate and high threshold*	Requires specialised laboratory, determination in batches, daily determination rarely possible	• Genetic testing institute [GTI] Diagnostics, Waukesha, WI, USA (GTI-PF4; HAT; PF4-Enhanced; GTI-IgG) • Hyphen-BioMed, Neuville-Sur-Oise, France (Zymutest HIA IgGAM; Zymutest HIA IgG) • Diagnostica Stago, Asnières-sur-Seine, France (Asserachrom HPIA) • Gen-Probe-Waukesha, Waukesha, WI, USA (Gen-Probe PF4)# • Technoclone GmbH, Vienna, Austria (Technozym)
Particle gel immunoassay (PaGIA)	Polyspecific	Visual assessment of agglutination; Quantification using titration studies°	Determination in standard laboratories possible, 24-hour service, observer-dependent	• Diamed, Cressier sur Morat, Switzerland (ID-H/PF4 PaGIA)
Particle immunofiltration assay	Polyspecific	Visual assessment	Observer-dependent	• Akers Biosciences Inc, Thorofare, NJ, USA (Health-TEST)
Lateral flow immunoassay	IgG specific	Visual or automated assessment°	Determination in standard laboratories possible, 24-hour service	• Diagnostica Stago, Asnières-sur-Seine, France (STic EXPERT HIT) • Milenia Biotec, Giessen, Germany (Milenia Quick-Line HIT)
Chemiluminescent immunoassay	Polyspecific IgG specific	Detection of emitted light; Low, intermediate and high threshold†	Automated determination possible, 24-hour service, expensive	• Instrumentation Laboratory, Bedford, MA, USA (HemosIL AcuStar HIT-Ab; HemosIL AcuStar HIT-IgG)
Latex agglutination assay	Polyspecific	Inhibition of agglutination	Automatized determination possible, 24-hour service, expensive	• Instrumentation Laboratory, Bedford, MA, USA (HemosIL HIT-Ab)

* low threshold: below or equal to OD 0.7, intermediate threshold: between OD 0.8 and 1.4, high threshold: above OD 1.4; ° positive/negative; † low threshold: below 1.0 U/ml, intermediate threshold: between 1.0 and 2.8 U/ml, high threshold: above 2.8 U/ml; # technically identical with GTI assay.

virtually all situations, physicians must make an initial clinical decision while waiting for the results of the functional assay, and the following scenarios may arise. First, the associated laboratory does not provide access to a rapid immunoassay, because enzyme-linked immunosorbent assay [ELISA] is conducted once or twice a week only. In this setting, the initial decision is solely made on the basis of the estimated clinical probability using one of the validated scoring tools. The accuracy of the decision critically depends on the characteristics and appropriate execution of the clinical test. The decision may be revised when the immunoassay test result arrives several days later. However, most authors and recent guidelines recommend *against* conducting an immunoassay in patients with a low risk score (9, 16, 17). In the second scenario, an immunoassay is available Monday to Friday and a functional assay once a week. As above, physicians must decide on the outcome of the clinical tool but decisions can be revised quickly. This strategy puts equal weight on the clinical scoring system as well as laboratory test results. In the third case, an immunoassay is conducted via a 24-hour (h) service and the results of a functional assay will be reported at least once a week. In this preferable situation, physicians can consider clinical characteristics as well as results of immunoassays, and decisions will be modified accordingly within a few days. However, physicians may be tempted to replace the fairly time-consuming task of gathering all information for clinical risk assessment with a laboratory test only (e.g. a rapid immunoassay). In all the above-mentioned scenarios, patient care can relevantly be improved with the help of the local haematology consultancy service which may reduce the number of false-classified 4Ts scorings and improves interpretation of laboratory results. Furthermore, it may save costs by reducing unnecessary testing and treatment with alternative anticoagulants.

Assessing the pretest probability: clinical scoring tools

As illustrated above, standardised assessment of the clinical probability of HIT is an essential step in the work-up of patients with suspected HIT. If conducted correctly, the probability of HIT can be estimated *before* determination of a laboratory test. Several clinical assessment tools have been developed, the outputs of which not only affect the interpretation of any laboratory test result but may in some instances represent the only diagnostic test to guide therapeutic decisions (see ▶Figure 1).

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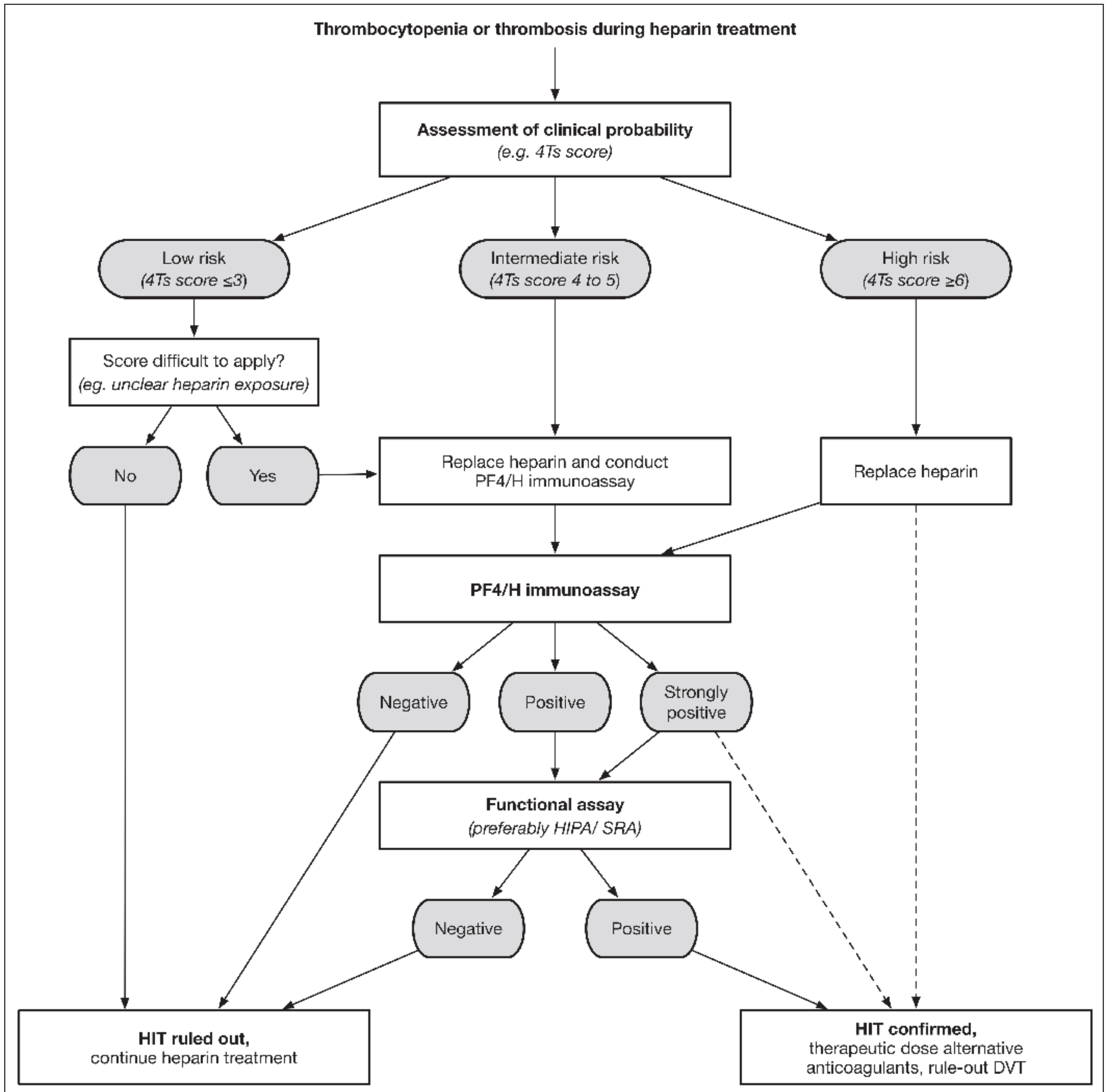

Figure 1: Suggested diagnostic algorithm for diagnosis of HIT (adapted from (8)). The algorithm must be adapted according to the individual setting, taking the availability of laboratory tests such as functional assays into account. Of note, using this algorithm some HIT patients with a low risk 4Ts scoring will be missed, particularly in cases with inadequately determination of the 4Ts score. Thus, several authors suggested conducting an immunoassay in all patients with suspected HIT (24, 33, 34). However, this approach needs careful interpretation of immunoassay test results to avoid over-treatment.

The 4Ts score

The most extensively studied assessment tool, the 4Ts score, incorporates four typical clinical features of HIT: i) thrombocytopenia, ii) characteristic timing of thrombocytopenia, iii) presence of thrombosis or other clinical sequelae, and iv) the absence of other causes of thrombocytopenia (8). The pretest probability is estimated to be low (0 to 3 points), intermediate (4 or 5 points), or high with 6 to 8 points (18, 19). A number of evaluation studies assessed the diagnostic accuracy of the 4Ts score (18, 20–30) and a meta-analysis suggested a high negative predictive value (99.8%; 95% confidence interval [CI]: 97–100%) (19). This result was not

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influenced by the type of performer (laboratory or treating physician), the prevalence, or the clinical setting as studied in sensitivity analyses. According to this meta-analysis, the probability of HIT can be estimated to be 0.2 % (95 % CI: 0.0 to 3.0 %) in low risk 4Ts scorings, 14 % (95 % CI: 9 to 22 %) in intermediate risk scorings and 64 % (95 % CI: 40 to 82 %) in high risk scorings. These results are clearly unsatisfactory for the purpose of confirming HIT. While the use of the 4Ts score as a screening test in the diagnostic pathways has been suggested, some methodological issues have been raised, in particular with regard to determination in clinical practice (31, 32). Most importantly, application of diagnostic accuracy measures to clinical practice was questioned because assessment of the 4Ts score was done by experts instead of referring physicians in most of the diagnostic accuracy studies (31). Indeed, a very recent, well-designed prospective management study considering these issues reported a much more limited sensitivity of the 4Ts score than estimated in the above mentioned meta-analysis (sensitivity 81.3 %; 95 % CI: 67.7, 94.8; specificity 63.8 %; 95 % CI: 59.6–68.0 %) and a limited agreement between physicians and expert observers (Cohens kappa 0.43; 95 % CI: 0.29–0.57) (33). Thus, some authors conclude that a low probability 4Ts score (≤3 points) alone is insufficient to exclude HIT in clinical practice (24, 34). In clinical practice, calculation of the 4Ts score might be challenging due to missing platelet counts, unclear heparin applications, and uncertainty if other reasons are sufficient causes of thrombocytopenia. ►Figure 2 illustrates an example of a 35-year old female patient who was not diagnosed with HIT because of a miscalculated 4Ts score.

The HEP score

The HIT expert probability (HEP) score is another clinical assessment tool which incorporates more clinical features than the 4Ts score (magnitude of platelet count fall, timing of platelet count fall, nadir platelet count, thrombosis, skin necrosis, acute systemic reaction, bleeding and other causes of thrombocytopenia) (29). Each of these features is evaluated using a score ranging from –3 (inconsistent with a HIT diagnosis) to +3 (consistent with a HIT diagnosis). Application of the HEP score resulted in a higher interobserver agreement than the 4Ts score in one evaluation study (29). A cut-off value of 5 was associated with a positive predictive value of 55 % and a negative predictive value of 97 %, showing operating characteristics similar to those observed with the 4Ts score. Nevertheless, the HEP score is more complex and may be more time consuming than the 4Ts. In addition, the number of evaluation studies is much more limited.

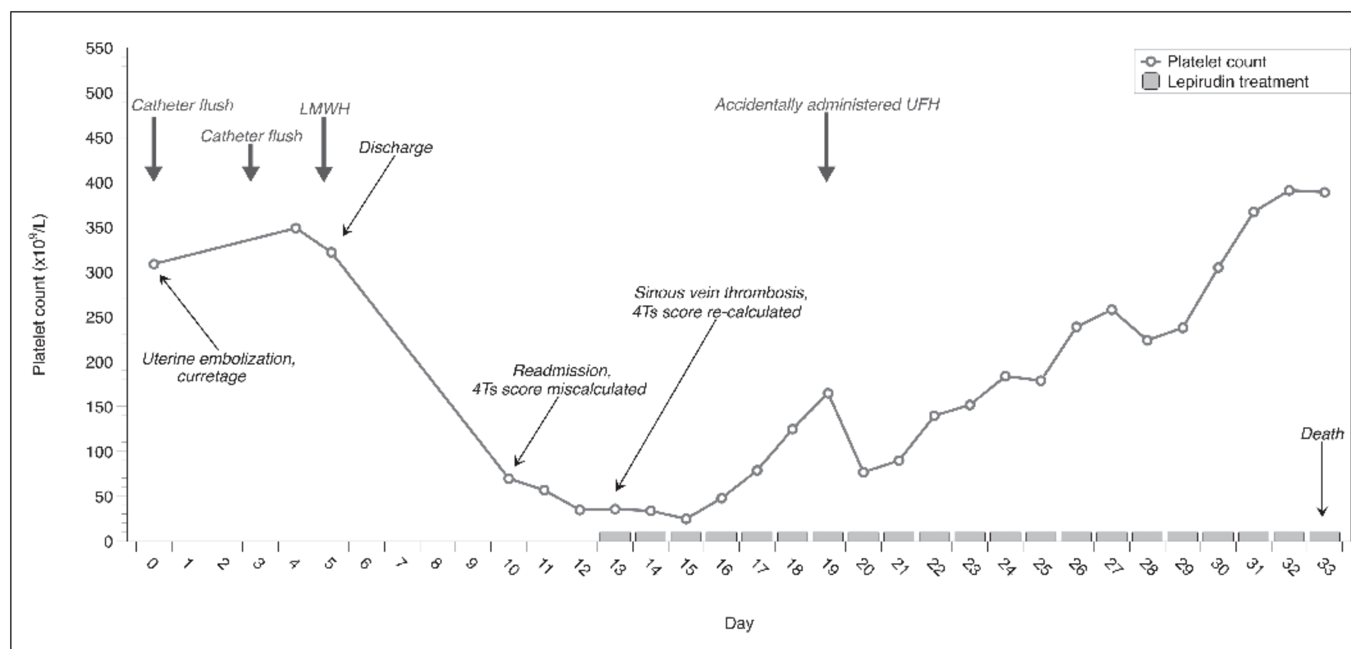

Figure 2: Diagnostic challenges in clinical practice. The 35-year-old female patient was admitted to hospital with vaginal bleeding due to ectopic cervical pregnancy. Uterine embolisation and curettage was performed and the patient was discharged on day 5. The patient presented with fever and abdominal pain five days later (day 10); the platelet count was 70×10^9/l. HIT was wrongly excluded because of a miscalculated 4Ts score (3/8 points; low risk) and no immunoassay test was conducted. On day 13 patient suffered cerebral venous thrombosis and consequent intracranial haemorrhage. The 4T score was re-calculated revealing 6/8 points (high risk). Antibody testing was positive with PaGIA [titre 1:32] as well as with polyspecific GTI-ELISA [OD >3.0]. HIT was confirmed with a functional assay (heparin-induced platelet aggregation test; PAT) demonstrating a strong platelet activation in the presence of 10 IU/ml heparin and no platelet activation in the presence of high-dose heparin. Critical review of the medical records revealed that the 4T score was miscalculated on day 10 because 1) catheter flushes with heparin were not recognised and delayed-onset HIT was not considered (timing = 1 point, correct 2 points), and 2) tubo-ovarian abscess with fever was considered to be a sufficient reason for thrombocytopenia (other reasons = 0 points, correct 1 point). The latter point was corrected on day 13. Despite immediate start with lepirudin and intensive medical support, the patient died on day 33 due to uncontrollable intracranial hypertension.

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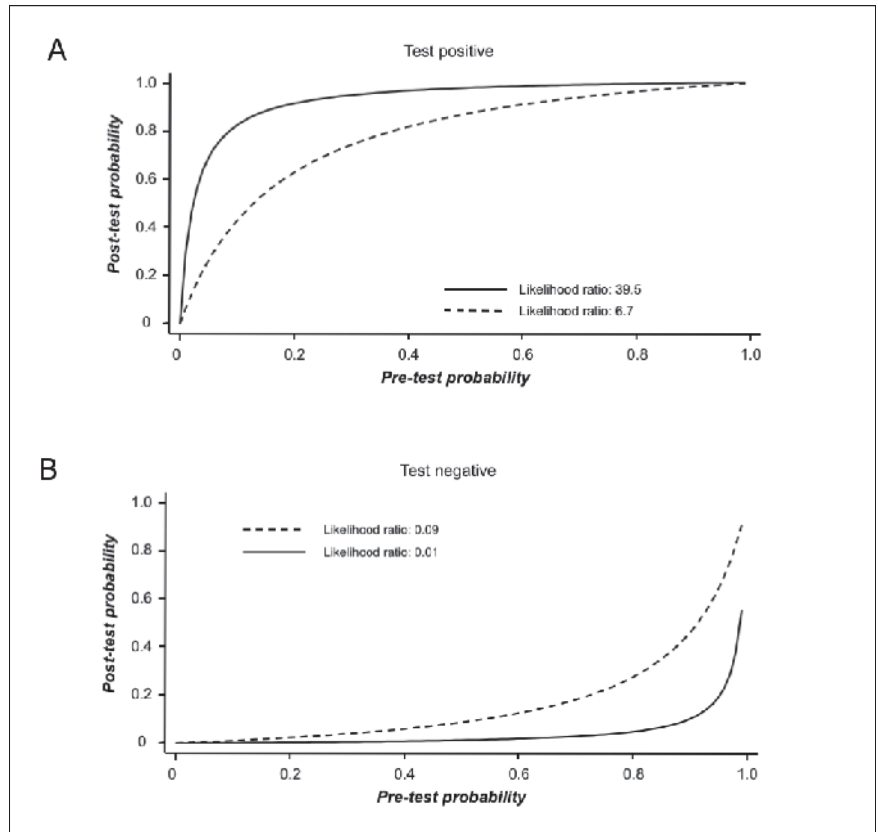

Figure 3: Probability of having HIT with a particular immunoassay test result according to pre-test probability. The probability of having HIT is represented by the post-test probability on the Y-axis, the clinical probability (as measured by a clinical assessment tool) is illustrated by the pre-test probability on the X-axis. Two different immunoassays are shown with curves illustrative of the probability of HIT with a positive and negative immunoassay results as indicated. It is obvious that the probability of having HIT remains low in patients with a low clinical probability despite a positive immunoassay test result. In contrast, the probability of HIT is increasing in patients with a high clinical probability, even with a negative immunoassay test result (applies mainly to assays with a limited sensitivity).

Other scoring systems

Another, simple score to exclude HIT has been suggested by Messmore et al. (35). The system is designed to arrive at low (=0) or possible (=1) probability scores depending on the presence or absence of typical HIT manifestations without knowledge of laboratory test results (except platelet counts). In one evaluation study, it was able to exclude patients without HIT efficiently and it might be more useful for physicians who are not HIT experts. Lillo-Le Louët et al. developed a score to assess the probability of HIT in patients following cardiopulmonary bypass surgery (20). This score incorporates three variables that were predictive for HIT in a retrospective study (a biphasic platelet count profile, an interval of >5 days from CPB to the first day of suspected HIT and a CPB duration of >118 minutes [min]). In an independent study, this score demonstrated a negative predictive value of 78 %, suggesting that it may have inadequate sensitivity to be used as a clinical screening test (36). However, both the Messmore and Lillo-Le Louët scores require more validation in larger prospective studies before firm conclusions regarding their diagnostic accuracy can be drawn.

Immunoassays

Acquired thrombocytopenia is a frequent finding in hospitalised patients treated with heparin. Often, HIT is difficult to exclude or to confirm based on clinical information alone and physicians rely heavily on laboratory tests. Two classes of assays are available: functional (platelet activation) assays and (PF4-dependent) immunoassays. Immunoassays are pivotal in the diagnostic work-up of patients with suspected HIT and rely on detection of antibody binding by ELISA or particle-based immunoassays. However, diagnostic accuracy of immunoassays is quite variable. As an example, ▶Figure 3 illustrates the difference of the probability of having HIT after a positive (or negative test, respectively) between two available assays.

Enzyme-linked immunosorbent assays (ELISAs)

In ELISA, the target antigen (PF4/polyanion complexes) is bound to the solid phase, e.g. microtitre plate wells. Patient serum or plasma is added and an enzyme-labelled secondary antibody is used to detect the amount of anti-PF4/heparin antibodies bound in a semi-quantitative fashion. The intensity of the colour change, measured as optical density (OD), is proportional to the concentration of bound antibodies. The first polyspecific ELISA was developed by Amiral and Greinacher in 1992 (37, 38). Sensitivity was comparable to a heparin-induced platelet activation assay (HIPA) as evaluated in 209 patients with a clinical diagnosis of HIT (38). Since then, several in-house and commercially available assays have been developed and many studies have evaluated their performance characteristics (▶Table 1) (20, 21, 23, 24, 26–28, 39–63). A recent meta-analysis pooled this

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data and calculated the diagnostic accuracy according to different cut-off values: low threshold (OD ≤ 0.7, according to or slightly above the manufacturer's instructions), intermediate threshold (OD 0.8 to 1.4) and high threshold (OD > 1.4). Sensitivity of the polyspecific ELISA was excellent at low threshold (see ▶Table 2) (64) but relevant differences were observed with regard to different thresholds and particular manufacturers. However, specificity was limited for all assays (▶Table 2), restricting their value as a confirmatory test. With regard to ELISA, a significant inter-laboratory variation was observed in a North American proficiency testing programme, in particular with regard to weak positive results (65).

Following *in vitro* observations on the specificity of platelet-activating PF4/H-antibodies, IgG-specific ELISAs were developed and tested in a number of studies (23, 41, 43, 45, 46, 48, 56, 66–72). At low thresholds, sensitivity is again excellent (▶Table 2). However, even though several studies suggested a higher specificity than polyspecific assays (23), this observation was not generalisable in the above-mentioned meta-analysis [64]). Data pooled from all available evaluation studies revealed a specificity of 85.4% for IgG-specific ELISAs (95% CI: 78.2–90.6%), and 86.8% for polyspecific ELISAs (95% CI: 82.0–90.5%). While these ELISA assays can be excellent screening tests, they do have the major drawbacks of being time consuming and requiring a specialised laboratory.

Particle-based immunoassays

Several types of tests have been developed to overcome the drawbacks of ELISA assays: particle gel immunoassays (PaGIA), lateral flow immunoassays, chemiluminescent immunoassays and latex agglutination assays. PaGIA as well as lateral flow immunoassay can be implemented in routine laboratories, conducted 24-h a day and technicians can perform these without specialised training. The polyspecific PaGIA is a particle agglutination assay uses the gel technique of ID-Micro typing with polymer particles coated with PF4/heparin complexes (52). It has been evaluated in a number of studies (21–24, 26, 33, 44, 45, 52, 54, 55, 71, 72, 73, 74).

Table 2: Diagnostic accuracy of immunoassays for diagnosis of HIT.

Type of test	Sensitivity	Specificity	Likelihood ratio	
	(percentages)		Positive (95% CI)	Negative (95% CI)
Polyspecific ELISA				
Low threshold*	96.7 (89.7, 99.0)	86.8 (82.0, 90.5)	7.3 (5.4, 10.0)	0.04 (0.01, 0.12)
Intermediate threshold*	98.4 (90.8, 99.7)	94.9 (90.5, 97.3)	19.3 (10.4, 36.0)	0.02 (0.00, 0.1)
High threshold*	15.0 (14.5, 15.5)	100 (99.3, 100)	73.4 (28.2, 190.9)	0.3 (0.2, 0.5)
IgG-specific ELISA				
Low threshold*	98.3 (95.1, 99.4)	85.4 (78.2, 90.6)	6.7 (4.5, 10.2)	0.02 (0.01, 0.05)
Intermediate threshold*	91.2 (86.2, 94.5)	93.5 (89.1, 96.2)	14.1 (8.1, 24.5)	0.09 (0.05, 0.15)
High threshold*	60.9 (59.7, 62.1)	99.4 (97.6, 100)	97.0 (53.0, 177.6)	0.4 (0.3, 0.5)
PaGIA				
Low threshold°	96.5 (89.8, 98.9)	93.7 (83.1, 97.8)	15.3 (5.5, 42.3)	0.04 (0.01, 0.11)
Intermediate threshold°	98.9	95.9	24.1	0.01
Lateral flow immunoassay	98.4 (85.3, 99.9)	90.3 (84.4, 94.1)	10.1 (6.2, 16.5)	0.02 (0.00, 0.18)
Particle immuno-filtration assay#	0.0	70.1	2.3	0.5
Latex agglutination assay#	100.0	75.6	3.7	0.0
Polyspecific chemiluminescent immunoassay				
Low threshold†	98.9 (92.7, 99.8)	85.6 (79.3, 90.3)	6.9 (4.7, 10.0)	0.01 (0.00, 0.09)
Intermediate threshold†	97.9 (94.6, 100.0)	93.1 (90.4, 95.8)	13.5 (9.5, 18.9)	0.0 (0.0, 0.1)
High threshold†	98.3 (69.5, 99.9)	97.5 (94.4, 98.9)	39.5 (17.5, 89.2)	0.0 (0.0, 0.40)
IgG-specific chemiluminescent immunoassay				
Low threshold†	98.8 (69.2, 100.0)	94.6 (90.7, 96.9)	18.3 (10.6, 31.5)	0.01 (0.00, 0.40)
Intermediate threshold†	78.6 (75.9, 81.2)	98.7 (94.6, 100)	42.3 (20.1, 88.7)	0.2 (0.1, 0.3)
High threshold†	74.2 (71.9, 76.5)	99.1 (95.4, 100)	47.8 (23.2, 98.7)	0.2 (0.1, 0.4)

+ According to results of a recent meta-analysis (73), please note differences between individual manufacturers; * low threshold: below or equal to OD 0.7, intermediate threshold: between OD 0.8 and 1.4, high threshold: above OD 1.4; ° low threshold: positive/negative, intermediate threshold: titre 2 to 3; † low threshold: below 1.0 U/ml, intermediate threshold: between 1.0 and 2.8 U/ml, high threshold: above 2.8 U/ml. #Only one study available.

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Table 3: Commonly used functional assays for diagnosis of HIT.

Type of test	Analytic principle	Endpoint	Platelets used	Confirmation step	Validation
Serotonin release assay (SRA)	Stimulation of platelet serotonin release by patient serum in the presence of heparin	Detection of change in 14C	Washed, 14C-radiolabelled platelets from one selected donor	Suppression with high-dose heparin and inhibition using an FcRIIA blocking antibody	High agreement with clinical HIT (85, 86)
Heparin-induced platelet activation assay (HIPA)	Detection of platelet aggregation induced by patient serum in the presence of heparin	Visual assessment of aggregation in microtitre plates	Washed platelets from four unselected donors	Suppression with high-dose heparin and inhibition using an FcRIIA blocking antibody	High agreement with clinical HIT (38, 87)
Heparin-induced platelet aggregation test (PAT)	Activation of platelets (citrated PRP) in the presence of patient plasma and heparin	Detection of aggregation by aggregometry	PRP of one to four, selected or unselected donors	Suppression with high-dose heparin	Varying agreement with SRA, depending on platelet donor (93), lower sensitivity than SRA/HIPA with clinical criteria (38, 89, 92)
Flow cytometry	Detection of markers for platelet activation (e.g. CD45/GPIIb; platelet microparticles; CD62; annexin V)	Increase of platelet activation markers of donor platelets in presence of heparin	PRP of unselected donors	None	Some agreement with SRA (44, 94-97), requires standardisation and further evaluation
Whole blood impedance aggregometry (Multiplate®)	Activation of whole blood platelets in the presence of patient plasma and heparin	Changes in impedance	Whole blood from one selected donor	Suppression with high-dose heparin	Adequate agreement with SRA in two studies (80, 105), requires confirmation

The sensitivity as well as the specificity of the PaGIA was excellent; the specificity was even higher than ELISA assays with low threshold (▶Table 2, [64]).

The principle of the lateral-flow immunoassay, which is another particle-based immunoassay, is well known from modern pregnancy tests: labelled antibody complexes are retained and become visible during capillary action (71). The diagnostic characteristics have been evaluated in several studies (59, 69–72, 74, 75) from which the data have been pooled and a high sensitivity and reasonable specificity have been confirmed (▶Table 2; [64]).

Nevertheless, PaGIA and lateral flow immunoassays share two disadvantages. First, the results are assessed visually (even though automatic applications exist), which permits variation in interpretation. Second, the results are expressed positively or negatively and titration studies are necessary to determine the anti-PF4/H antibody concentration (24). The particle immunofiltration assay is a different assay, but as yet has not been shown to demonstrate adequate diagnostic accuracy ([64], 76).

A desirable characteristic of tests to be implemented in modern laboratories is that they can be automated allowing them to be run 24 h a day. Two assays have been developed to meet this demand: the chemiluminescent immunoassay (polyspecific HemosIL® AcuStar HIT-Ab and IgG-specific HemosIL® AcuStar HIT-IgG) and the latex agglutination assay (polyspecific HemosIL® HIT-Ab). Both assays can be used with the BIO-FLASH® analyzer (Inova Diagnostics, San Diego, CA, USA) or the ACL TOP coagulometers (Instrumentation Laboratory, Bedford, MA, USA). Magnetic coated particles capture the PF4/heparin antibodies and in case of chemiluminescent immunoassay emitted light is measured (77). The diagnostic accuracy of these assays has been investigated in several large cohorts with favourable results (56, 58, 77-80). At low threshold, sensitivity was very high for both the polyspecific and the IgG-specific tests (▶Table 2) (64). Furthermore, a combination of a high sensitivity with a high specificity was estimated for the polyspecific assay (intermediate threshold) as well as IgG-specific assay (low threshold). Coated latex beads are used instead of magnetic particles with the polyspecific latex agglutination assay. In one evaluation study, sensitivity was found to be excellent, specificity was moderate (79) (▶Table 2).

Diagnostic accuracy measures of rapid immunoassays have also been studied in another recent systematic review and meta-analysis comprising essentially the same primary studies cited above (81). A high sensitivity and specificity (corresponding to a high negative predictive value) was observed for some of the assays as well (PaGIA, lateral flow immunoassay and IgG-specific chemiluminescent immunoassay), suggesting their usefulness in diagnostic algorithms. In addition, implementation of rapid immunoassays is also supported by a study which modelled the cost impact (82).

Functional assays

A subset of PF4/heparin-antibodies is able to activate platelets and cause clinical HIT under certain conditions (8, 83). The presence of platelet-activating antibodies can only be established using

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functional assays. In all tests, patient plasma or serum is incubated with donor platelets which can be prepared in one of two different ways: either as a) washed platelets, or as b) platelet-rich plasma (PRP) or whole blood (1). Washed platelet assays are considered preferable over other PRP or whole blood tests, because remaining plasma/serum may influence the antigen-antibody interaction as well as platelet activation (2, 8, 9, 84, 85). ▶Table 3 summarises the characteristics of the assays most often used.

Washed platelet assays

Both the serotonin release assay (SRA) and heparin induced platelet activation (HIPA) assay utilise washed platelets. Platelet activation is assessed by measurement of the release of ^{14}C-labelled serotonin from test platelets in SRA or by visually determining the formation of platelet aggregates in HIPA (86, 87).

In the HIPA assay, washed platelets from four healthy unselected donors are incubated with patient serum in the presence of buffer or heparin (0.2 IU/ml and 100 IU/ml). Incubation takes place in a round-bottom microtitre plate, with spinning magnetic spheres as a source of shear force. Platelet aggregate formation is determined visually at 5-min intervals; the test is positive if aggregation is observed within 30 min (at 0.2 IU/ml but not at 100 IU/ml heparin) using platelet suspensions from at least two of the four donors.

In the SRA, platelets obtained from a selected donor are pre-incubated with radioactive ^{14}C-serotonin. After washing, platelets are incubated with patient serum and heparin in flat-bottomed microtitre wells in duplicate on a plate shaker. After incubation for 60 min and centrifugation, supernatants of each reaction mixture are collected, and radioactivity is measured. Test results are expressed as percentage of serotonin release (compared to the 100% value obtained by detergent-induced platelet lysis). The test is considered positive if there is >20% release at low heparin concentrations (0.1 to 0.3 IU/ml). However, a number of laboratories use a threshold of >50% serotonin release in order to increase test specificity. The reaction is usually inhibited at supratherapeutic heparin levels (100 IU/ml) (88).

The SRA was initially validated using a set of samples from patients with different degrees of clinical probability of HIT and a very large set of controls obtained from patients with a broad spectrum of clinical characteristics (86, 89). Not only high sensitivity and specificity were observed, but also a clear trend between clinical probability of HIT and the SRA results. These findings were confirmed in a prospective study following up all patients with heparin treatment based on strict clinical criteria (85). Equivalent diagnostic characteristics have been observed in the evaluation of the HIPA test. Initially, Greinacher et al. studied sensitivity in 34 samples, followed by sera from 209 patients (38, 87). Both functional assays are considered the "gold standard" for diagnosing HIT. However, these assays are difficult to perform, require selected healthy platelet donors and are restricted to few reference laboratories. Moreover, the SRA requires the use of the radioisotope, ^{14}C-serotonin, which most laboratories try to avoid due to regulatory and safety issues.

Even though SRA and HIPA are considered as gold standard for the diagnosis of HIT, some cases with incongruous results were observed, e.g. positive tests in combination with negative immunoassays and an atypical clinical presentation (90). These rare cases were generally considered to be "false-positive" SRA results, and indeed some laboratories consider a positive immunoassay to be a quality control check supporting the validity of a "positive" SRA (90). In clinical practice, it is important to always use any laboratory assay in combination with appropriate assessment of the clinical presentations.

Other washed platelet assays that either use ATP release detected by lumiaggregometry, platelet-derived microparticle generation measured by flow cytometry, or proteolysis of FcγRIIa (the receptor through which HIT immune complexes activate platelets) assessed by chemiluminescence have been described, but still require independent validation.

Whole blood assays

Platelet-activating antibodies can be detected using the whole blood impedance analyser (Multiplate®, multiple electrode platelet aggregometry) in the presence of heparin. Blood from a selected donor is collected in hirudin-containing tubes. UFH is then added (0.5 or 100 IU/ml) and the suspension are incubated with patient citrated platelet-poor plasma (PPP) or heat-inactivated serum. Changes in impedance are then recorded over a 15-min period (91). In a multicentre Australian study, this assay, which does not require platelet preparation, demonstrated a sensitivity and specificity of 90.3% and 89.0%, respectively (80, 91).

Other functional assays

A number of other, less elaborate functional assays have been suggested; of these the heparin-induced platelet aggregation test (PAT) and flow cytometry are the most often used. In PAT, platelet aggregometry is performed in the presence of two heparin concentrations using PRP of 1–4, selected or unselected donors (84, 92). However, evaluation studies have revealed varying results, partly explained by the modifications and selection of donors (38, 89, 92, 93). In general, sensitivity was clearly inferior to SRA/HIPA.

Flow cytometry assays have been developed by a number of authors. Serum of patients and platelets from unselected donors are incubated with heparin and different measures of platelet activation are recorded (Annexin V (44, 94, 95), P-selectin (44, 95), and microparticles (96, 97)). Although these assays showed some agreement with the gold standard, standardisation and further evaluation studies are needed.

Strategies to improve the specificity of immunoassays

Several strategies have been developed and introduced to improve the specificity of immunoassays, increase their positive predictive value and limit the number of patients over-treated.

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Determination of PF4/Heparin antibody titres

A number of studies have observed that higher optical density values (in the case of ELISA type assays) are associated with an increased probability of having HIT (43, 98). Higher titres of antibodies have also been correlated with the likelihood of HIT in the case of PaGIA (24) and chemiluminescent immunoassay (58, 80). To confirm these observations, we pooled the data of all available evaluation studies in a recently conducted meta-analysis (64). The cut-off values used in the primary studies were categorised into low, intermediate and high thresholds (corresponding to low, intermediate, and high antibody titres). In line with previous observations, we found a remarkably increased specificity (or positive likelihood ratio) in all immunoassays (poly- and IgG-specific ELISA, PaGIA, poly- and IgG-specific chemiluminescent immunoassay) (64). However, the negative likelihood ratio increased as well, corresponding to a decline in sensitivity. In ►Table 2, we report a summary of the results that might help to define the best threshold.

Application of IgG-specific assays

In vitro data suggest that IgG-specific antibodies account for the vast majority of HIT cases and several studies indeed observed an increased specificity of IgG-specific assays compared to polyspecific tests while sensitivity also remained high (23, 85, 99). We tried to confirm this observation by pooling all available data in the above-mentioned meta-analysis. However, this could be replicated only in part (►Table 2) (64). In addition, sensitivity was somewhat reduced, at least with intermediate and high cut-off values. In clinical practice, we recommend selecting an appropriate combination of antibody specificity and threshold according to the respective likelihood ratios (e.g. polypecific ELISA/ chemiluminescent immunoassay / PaGIA with intermediate threshold or IgG-specific ELISA/ chemiluminescent immunoassay with a low threshold).

Implementation of a high-dose heparin confirmation step

It has been suggested that the specificity of HIT immunoassays could be improved by the implementation of a confirmatory step using supratherapeutic concentrations of heparin. This is because a persistently positive test despite high heparin concentrations can indicate an antibody that reacts against PF4, but not to the PF4/heparin complex. Such antibodies usually do not indicate HIT. While some studies support the use of this step, especially for weakly positive OD values <1.0 units, some of the clinically most relevant high-titre antibodies with strong platelet-activating capacity are not inhibited (100). A recent meta-analysis, however, did not find this strategy helpful (64). Sensitivity was found to be low, at least in a subgroup of samples with a high titre of antibodies (64, 84). Because of this limitation and the corresponding difficulties in interpretation, we recommend against implementing the high-dose heparin confirmation step in routine clinical practice.

Current challenges and future perspectives

While the incidence of HIT in uncomplicated patients can be anticipated to decline due to the increasing use of low-molecular-weight heparins and alternative, non-heparin anticoagulants (101), HIT will remain a particular issue in specific patient populations, which have undergone cardiac surgery or are severely ill patients. Despite the progress in understanding the pathophysiology of HIT, there are still numerous diagnostic issues and treatment challenges.

The clinical dilemma

The management of patients with suspected HIT is associated with two major risks: missing patients with HIT and overtreatment. Physicians rely heavily on immunoassay test results and immunoassays are an essential part of diagnostic pathways as discussed above. However, as few as 10–15% of sera test positive for anti-PF4/heparin antibodies and only up to 50% of these contain clinically relevant, platelet-activating antibodies characteristic of HIT. Therefore, a considerable risk of "overdiagnosis" and subsequent mistreatment of patients without HIT exists (14). These patients are exposed to relevant risks. Therapy with alternative anticoagulants is associated with a high rate of bleeding complications (12), severe anaphylactic reactions (13), higher costs, and requires more management generally than compared to heparin treatment (12, 14). Thus, an important aim of clinical practice and scientific inquiry is to develop and implement diagnostic tests and algorithms that reduce the number of false-positive results.

On the other hand, increasing specificity should not be at the expense of test sensitivity, as missing a diagnosis of HIT is dangerous (64). The risk of severe thromboembolic complications, limb loss and even death is high in untreated HIT patients (11, 102). There is increasing awareness that a low risk 4Ts score does not exclude HIT in all cases (33, 34). In addition, the sensitivity is below 95% in some immunoassays, suggesting that one in 20 HIT patients will be missed as well (64).

Diagnostic algorithms

In order to avoid the above-mentioned risks, the most important challenge in clinical practice is to estimate the probability of an individual patient having HIT. Our considerations above suggest that neither an immunoassay, nor a clinical assessment score alone is able to correctly diagnose HIT. However, combining different diagnostic approaches (clinical and laboratory) can improve diagnostic accuracy and may represent a strategy to solve this clinical dilemma.

Diagnostic algorithms are the most obvious way of combining clinical and laboratory tests for the diagnosis of HIT (24, 33). In ►Figure 1, we illustrate a recently adapted diagnostic algorithm (8). Assessing the clinical probability is suggested for all patients with suspected HIT. Given an appropriate application of the 4Ts score, HIT can be excluded in most patients with a low risk scoring. However, correctly conducting the 4Ts score correctly is

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difficult (31) and determination of an immunoassay is suggested in all cases where there are uncertainties (e.g. unclear heparin exposure, missing platelet numbers).

In contrast, HIT should be considered if an applied 4Ts score is high. In all other cases, determination of a quantitative immunoassay is recommended. However, the diagnostic accuracy varies between different assays and we recommend selecting a test with a high sensitivity as well as a high specificity (64). For example, we recommend choosing an intermediate threshold (cut-off value) in the case of polyspecific ELISA, PaGIA, as well as polyspecific chemiluminescent immunoassay. HIT can be essentially ruled-out if the immunoassay is negative or highly suspected if high titres of antibodies are demonstrated (e.g. OD>=3.0). Even though HIT must be assumed in all other cases with a positive immunoassay, determination of a functional assay is recommended if possible. Depending on the individual setting, a functional assay will be conducted in more cases as well.

There are nevertheless other ways of combining diagnostic tests as well and all have the potential of reducing the number of false-positive and false-negative classifications. For example, a clinical scoring system and an immunoassay can be determined in parallel as suggested by several authors (2, 34, 104), and probabilities can be combined with the use of likelihood ratios and Bayes' theorem (24, 103, 104). However, prospective studies evaluating these tools are still needed.

Conclusion

HIT is a life-threatening situation that requires an immediate diagnostic work-up. Not only missing a patient with HIT can result in catastrophic consequences, but overtreatment also carries a significant risk. The diagnostic work-up is, however, difficult due to a number of practical issues and limitations in the diagnostic accuracy of available assays. The diagnostic pathway should be adjusted to the individual setting using well-defined diagnostic algorithms. The first step should include the assessment of the clinical probability according to a validated scoring system and laboratory investigations should additionally be performed if the probability is intermediate or high. An immunoassay with adequate sensitivity and specificity should be used to avoid over-treatment or failure to recognise HIT. Future efforts to address these challenges should focus on the improvement and clinical evaluation of diagnostic algorithms.

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Conflicts of interest
MN has received research grants or lecture fees from Bayer and CSL Behring. TB declares no conflicts of interest.

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