UZ LEUVEN | LABORATORIUMGENEESKUNDE

Critically Appraised Topic

HIT diagnosis in UZ Leuven

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CLINICAL BOTTOM LINE

Heparin-induced thrombocytopenia type II (HIT) is a frequent and serious complication of heparin treatment which requires initiation of alternative anticoagulation and cessation of heparin as soon as possible. As clinical recognition is often difficult and scoring systems have significant limitations, laboratory tests are an integral part of diagnostic algorithms. The most frequently used test, the immunoassay, is capable of excluding HIT, but the positive predictive value remains low (about 50%, depending on assay used and population tested). By combining quantitative immunoassay (preferably IgG specific) results with 4T scoring (preferably by an experienced scorer), clinicians have at their disposal a post-test probability scheme which allows them to take thought-out treatment decisions for the majority of patients. The high-dose heparin confirmation is only advised for weak positive results as it can give raise to false negatives for strongly reactive samples. The implementation of a functional assay is not considered in UZ Leuven for clinical, organizational/logistic, economic and strategic reasons.
The introduction of on-demand testing (versus average 3 day TAT) can help clinicians to rapidly exclude HIT in a large portion of samples (about 87% for UZ Leuven at the moment), diminishing the unnecessary usage of expensive alternative anticoagulation and patients at unnecessary bleeding (thrombosis) risk. The relative benefits are largely dependent on the treatment strategy and the risk profiles (4T groups) referred for HIT testing.
In UZ Leuven redesigning and rebuilding of the diagnostic platform allows to fit the laboratory of special coagulation (9^{th} floor) in the central laboratory of haematology (7^{th} floor). We suggest to clinicians an evaluation of the polyspecific HemosIL HIT-Ab(PF4-H) (Instrumentation Laboratory). This assay can be executed on an ACL TOP platform, which can be linked to the recently implemented total laboratory automation track system, offering on-demand testing (at the cost of a little specificity as no IgG specific assay is yet available).

CLINICAL/DIAGNOSTIC SCENARIO

Heparin-induced thrombocytopenia type II (HIT-II, named HIT in this paper) is a frequent and serious complication of heparin treatment. Although the pathogenesis still has some unresolved issues [1], the generally accepted concept is the formation of (IgG) antibodies against complexes of platelet factor 4 (PF4) bound to heparin. The multimolecular PF4/heparin-IgG immune complexes activate platelets by binding to their FCγIIa receptor (crosslinking), inducing thrombocytopenia, enhancing thrombin generation and injuring endothelial cells. Once this pathway is triggered, a hypercoagulable state emerges, giving raise to thrombotic complications, associated with high morbidity and mortality [1-4].
The incidence of HIT varies with the duration and type of heparin exposure, the patient population and gender (Table 1). Patients exposed to unfractionated heparin (UFH) show a 10-fold higher likelihood of developing HIT compared to patients receiving low-molecular weight heparin (LMWH). Women have approximately twice the risk of developing HIT as men. [1,4]

Table 1: Incidence of HIT according to Patient Population and Type of Heparin Exposure adapted from CHEST 2012 guidelines[4]

Patient Population (minimum of 4 days exposure)	**Incidence of HIT (%)**
Postoperative Patients	
Heparin, prophylactic or therapeutic dose	1-5
Heparin, flushes	0.1-1
LMWH, prophylactic or therapeutic	0.1-1
Cardiac surgery patients	1-3
Medical patients	
Heparin, prophylactic or therapeutic	0.1-1
LMWH, prophylactic or therapeutic	0.6
Heparin, flushes	<0.1
Patients with cancer	1
Intensive care patients	0.4

Obstetrics patients	<0.1

The most common presentation of HIT is thrombocytopenia, typically 5 to 10 days after the initiation of heparin. Next to the typical-timing HIT, rapid-onset (abrupt platelet fall within 24 h) and delayed-onset (up to 3 weeks after cessation of heparin therapy) types of HIT have been described. Patients who develop rapid-onset HIT already have circulating HIT antibodies, often because of exposure to heparin in the preceding month, occasionally more than 3 months earlier. In up to 25% of patients with HIT, the development of thrombosis precedes the development of thrombocytopenia. Although thrombocytopenia may be severe (though nadir rarely < 20 x 10^9/L), signs of bleeding are rarely encountered. Venous thrombosis is the most common complication of untreated patients presenting with thrombocytopenia (17-57%), followed by arterial thrombotic events (5-10%). Approximately 5-10% of HIT patients die, usually as a result of thrombotic complications. [1-4] The initiation of adequate therapy (cessation of all heparin and initiation of an alternative anticoagulant), for both isolated HIT (not accompanied by thrombosis at diagnosis) and HIT complicated by thrombosis (HITT), comes with a reduction of thrombotic events (grade 1C [4]). The thrombosis rate in patients prior to treatment is estimated at 5% a day [5]. Some studies have reported that about a third of all thrombotic events in HIT patients occurs in the pre-treatment period [5]. Administration of expensive, alternative anticoagulation, on the other hand, comes at an increased risk for bleeding events and a great economic cost.

The diagnosis of HIT is often described as clinico-pathological [1-4]. Both clinical signs (thrombocytopenia /thrombosis/scoring) and evidence for the presence of heparin dependent platelet activating antibodies are necessary. Given the time delay (and limitations) of laboratory results, the increased risk for developing thrombosis if treatment is postponed and the risk for bleeding when alternative anticoagulation is started blindly, clinical assessment often plays an essential role in the initial diagnostic and therapeutic decisions for HIT suspected patients. This can be challenging as the suspected patients, especially the medical patients, can present with numerous other reasons for thrombocytopenia [6,7]. A number of clinical scoring systems have been described to aid physicians. The best studied and prospectively validated system is the 4T score, developed by Warkentin [8] (Figure 1) and further modified by Lo et al [9]. Evidence emerges that this succinct scoring system can be used to rule out HIT [9-11,13,14,16,19,21,23,31,38,39,41,44,45]. A number of problems, however, still remain: first, a significant number of patients with a high score prove not to have HIT, second, the least informative group (intermediate scores) turns out to be the largest group in a great number of studies and third, the inter-rater agreement is only moderate [9-11]. Scoring systems with better performance [12] or for specific subgroups [13] have been described, but prospective validation is still needed and the proposed questionnaire seems rather complex for routine clinical use. It is likely that experience is required for correct clinical estimation of the risk [9].

	2	1	0
Thrombocytopenia	> 50% fall or platelet nadir $20–100 \times 10^9$ L^{-1}	30–50% fall or platelet nadir $10–20 \times 10^9$ L^{-1}	Fall < 30% or platelet nadir < 10×10^9 L^{-1}
Timing of fall in platelet count or other sequelae	Clear onset between days 5–10; or less than one day (if heparin exposure within past 100 days)	Consistent with immunization but not clear (e.g. missing platelet counts) or onset of thrombocytopenia after day 10	Fall in platelet count too early (without recent heparin exposure)
Thrombosis or other sequelae (e.g. skin lesions)	New thrombosis; skin necrosis; postheparin bolus acute systemic reaction	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis not yet proven	None
Other cause for thrombocytopenia not evident	No other cause for fall in platelet count is evident	Possible other cause evident	Definite other cause present

Pretest probability score: 6–8 = high; 4–5 = intermediate; 0–3 = low.

Figure 1: 4T scoring system of HIT according to Warkentin et al. [8]

Laboratory assays to detect these antibodies can be divided in two major categories: immunoassays that detect the presence of antibodies against PF4/polyanion or functional assays detecting evidence of heparin dependent platelet activation. Proposed diagnostic flow charts often start with determining clinical probability, followed by an immunoassay to exclude HIT in a portion of patients. Given the limited value of a positive immunoassay (excellent sensitivity, limited specificity), these are to be confirmed by functional assays. Functional assays, on the other hand, have better specificity and correlate well with clinical HIT but are extremely technically demanding and often require referral of the sample to specialized centres. The generally accepted gold-standard, the serotonin release assay (SRA), is only performed in a few centres, giving raise to long turnaround times (TATs). Consequently most laboratories have decided to use other, faster and less requiring functional assays with less established performance characteristics (flow cytometry (FCM) [14], heparin induced multiple electrode aggregometry (HIMEA) [15,16]) or offer no confirmation at all. This practice could lead to an overdiagnosis of about 100% [17,18] (Figure 2: Iceberg model).

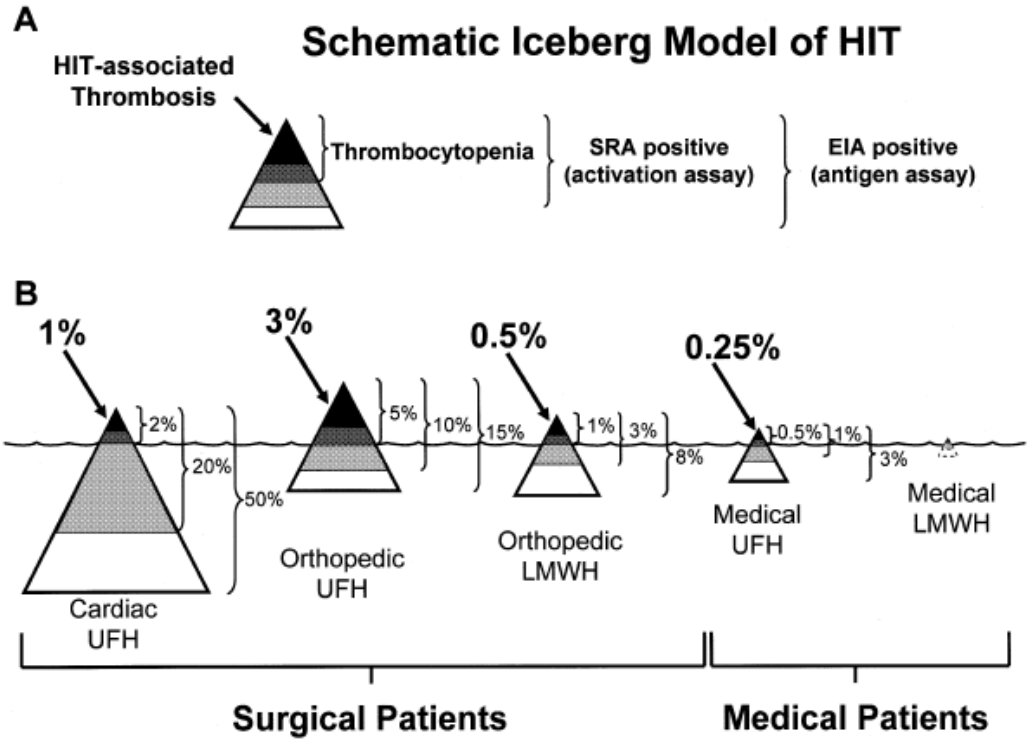

Figure 2: The Iceberg Model of HIT according to Warkentin et al. [8]
Panel A shows the relation between the presence of HIT antibodies detected by an immunoassay or SRA and thrombocytopenia and HIT-associated thrombosis (Iceberg)
Panel B shows the iceberg model depending on type of heparin used and patient population. The surgical incidences might be overestimated as in Europe the use of UFH is largely replaced by LMWH [7].

In UZ Leuven a commercial enzyme immunoassay from GTI Diagnostics (Waukesha, WI, USA) that detects PF4-dependant antibodies of three immunoglobulin classes (IgG/A/M) against PF4/polyvinylsulfonate (PVS) has been used so far. A high-dose heparin confirmation step to increase specificity is routinely performed for positive results, following manufacturers recommendations. Normally, samples are pooled and analysed once a week. This leads to TATs from 1 to 7 days. Supplementary runs can be requested after discussion with a coagulation specialist. Results are reported in a bimodal way: positive or negative, without further written comment. The numeric results (expressed as optical density (OD)) can be routinely reported to external laboratories at their discretion. Standard practice does not include confirming positive samples by a functional assay.

Ongoing rebuilding and redesigning of the diagnostic platform in UZ Leuven will fit the laboratory of special coagulation (9th floor) in the central laboratory of haematology (7th floor). This creates an opportunity to rethink some diagnostic practices prior to moving.

QUESTION(S)

1) *Preferred assay/algorithm for HIT diagnosis (general)?*
2) *Need for functional assay in UZ Leuven?*
3) *Benefits of rapid assay/which assay?*

SEARCH TERMS

1) *MeSH Database (PubMed): MeSH term: "thrombocytopenia + heparin" "thrombocytopenia + heparin + diagnosis "*
2) *UpToDate Online version 2012*
3) *www.google.com: search term: heparin-induced thrombocytopenia*

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APPRAISAL

As clinical signs are non-diagnostic for HIT, especially for patients with numerous co-pathologies [6,7], and consequences of missing pathology are serious, laboratory testing is an integral part of HIT diagnosis. However, a systematic analysis that compares clinical decision with and without laboratory tests has, to our knowledge, not yet been performed. Reports nevertheless show an increased risk for thrombosis if treatment with alternative anticoagulation is not initiated and an increased risk for bleeding if alternative anticoagulation is unnecessarily started [1-4, 48]. The potential of performing useful laboratory tests to optimize decision making seems indisputable.

Assay

The immunoassays, most commonly enzyme linked immunoassays (ELISAs), that test for antibodies (IgG, IgM, IgA) against PF4/heparin (Stago, Hyphen) or PF4/PVS (GTI), are very useful to exclude the diagnosis of HIT as their excellent sensitivity (close to 100%) allows clinicians to confidently rely on negative results (negative predictive value (NPV) close to 100%) [6,7,9,10,13,14,21-24,31,32,36,41,42]. Some other heparin dependent antigens (IL-8 or NAP-2), that induce relevant antibodies, can still be missed using these assays (except possibly with the Hyphen Biomed Zymutest assay). Their occurrence seems very rare (0-6%) [6,7]. The performance characteristics of assays from different manufacturers are comparable (Attachment 3). The GTI assay has been most extensively studied.

The specificity of the immunoassays is limited because not all the detected antibodies are platelet activating. Antibodies against PF4/heparin may also be detected in patients with systemic lupus erythematosus and/or antiphosholipid syndrome (APL) but without HIT [19]. Theoretically, only IgG antibodies are capable of crosslinking FCγIIa receptors on platelets and activating the pathogenic procoagulant cascade. Some reports, however, claim IgM/A antibodies to cause clinical HIT [20]. The great majority of studies, on the contrary, show that only IgG antibodies are important in activating platelets and developing clinical HIT [4,6,7,17,18,21-26]. IgG specific assays have been developed and reported more specific for the diagnosis of HIT [17,21-26]. The value of a positive result nevertheless remains rather mediocre (about 50% positive predictive value (PPV), depending on assay used and population tested). In some studies the sensitivity diminishes a little with the use of these specific assays. This is only a small decrease and appears significant for only one study, in which an in house IgG specific assay was used [6,7,27]. The majority of studies shows no decrease of sensitivity [9,17,21-24,36].

From 2004 on [28], the link between the reaction strength in immunoassays (most often expressed as optical density (OD)) and the likelihood of finding platelet activating antibodies [7,18,23,25,29,31,36,40], clinical signs, like thrombosis [18,23,28,30,33,37,39,41] or probability scores [14,38,39] has been examined. Almost all studies indicate a strong correlation (Attachment 2). Augmenting the OD cut off could further add to specificity. This approach seems limited by a significant loss of sensitivity at higher cut offs [21,36] and the large spread of OD values: a number of patient with demonstrable platelet activating antibodies will have ODs only slightly above the cut off and vice versa [6,7,31,36,39,42]. A large study has shown no single OD cut off reliably distinguishes between the presence of platelet activating antibodies or not [36]. There is, however, a direct and positive relationship between the degree of positivity and the presence of platelet activating antibodies ranging from <5% for ODs 0.40 – 1.00 and 90% for ODs >2.00. In that study about 20% of immunoassay positive samples had OD values between 1.00 and 2.00 with questionable probability for HIT (from 18%-50%). These results were confirmed by another large study [40]. The OD values are not completely interchangeable between laboratories as, even for commercial assays, a number of inter-assay differences may exist (photometers, antibodies, antigens, path

length, reaction time). The correlation has nevertheless been demonstrated using different types of immunoassays (Attachment 2). The use of normalized quantitative values has been proposed to improve inter-laboratory comparability [40].

Another method to increase specificity, the high-dose heparin confirmation step, has been questioned in English literature. This procedure has been reported useless to increase specificity in the post-cardiac surgery setting [35]. Other publications demonstrate a (slightly) increased specificity [32,33,42], but a loss of sensitivity [42] that becomes dramatically for higher OD results [32,33]. In one large study, up to 16% (11/69) of strong reactive sera (OD > 1.0) in a PF4/heparin ELISA with negative confirmation step turned out to contain platelet activating antibodies [32]. Although some hypotheses exist [29,36], the origin and the extent of this observation has not yet been satisfactory clarified. A recent study has demonstrated that the high-dose heparin confirmation nevertheless has independent predictive value for clinical HIT (although the study uses a rather questionable definition of clinical HIT), complementing the information contained in the OD readings [34]. Caution is warranted for high OD results with negative confirmations.

Some functional assays (SRA, heparin induced platelet aggregation (HIPA)) are generally accepted as gold-standard assays for the presence of platelet activating antibodies. Only a few centres perform these technically requiring assays, giving raise to very long TATs and uselessness in urgent clinical settings. Other, easier, functional assays have been proposed as alternative (HIMEA, FCM) [14,15,16,43]. These assays, however, remain technically difficult (and labour-intensive) as compared to immunoassays and will always be impeded by the requirement of human platelets, ideally from known reactive donors. The most user-friendly functional assay seems to be the HIMEA [15,16]. The PPV could be significantly increased by using this assay for confirmation of positive immunoassays [15,16,24]. Recent report [14,15,49] has shown some worrisome technical issues (eg. spontaneous aggregation, false negatives) and the PPV does not seem to completely outclass that of recent described automated immunoassays using an optimal cut off (76% vs. 80%) [24]. Further studies need to determine the exact position of HIMEA/FCM in HIT diagnosis. Perhaps the usage as a first line (and only) test can be examined in future studies? [43]
For UZ Leuven the number of positive samples (one a week, optimistically) seems too small to develop a technically demanding, labour-intensive functional method. Next to the economic and organizational impact (great labour/financial requirements for few tests), the samples would have to be batched, giving raise to extended TATs and little added value: the results will be available only long after patient management decisions have been taken. The introduction of a functional assay (whether in house or by referral of samples) could, on the other hand, identify patients for whom heparin should definitely be avoided in the next 100 days and attract samples of other centres for confirmation in our laboratory, augmenting the number of samples per run. The extent of these benefits depends on unpredictable factors and is not well characterized:
-How often is heparin re-needed within 100 days after HIT diagnosis?
-If the patient is readmitted in another hospital the clinician might still not be aware of the positive HIT status?
-Economic costs of treating a limited number of false positives with alternative anticoagulation versus diagnostic costs/benefits/limitations of confirming with a functional assay?
-How many samples from other centres can be expected and are they prepared to pay an activity based cost per test (which could be high for labour-intensive, technically requiring functional tests)?
-What are the operating characteristics of our in house functional assay (with special regard to platelet donor selections) and how often/how extended tuning with reference centres is needed, what are the costs?
-Percentage of indeterminate results? (2.9% - 9.7% [25,36])

Diagnostic algorithm

The limitations of clinical scoring systems and immunoassays are predominantly the result of their poor PVV for platelet activating antibodies. By combining different literature data/strategies, we aim to optimize the PPV. This has to be done without diminishing the NPV, as the main utility of the test will remain its rule-out capability. Given the rather low prevalence of platelet activating antibodies in suspected samples in general (Attachment 3), small changes in specificity can yield great changes in PPV. The preferred assays therefore seem the IgG specific methods (level 1b). Effort should also be taken to increase pre-test probability (eg. by testing predominantly patients with intermediate/high 4T scores) (level 1b). The high-dose heparin confirmation step should, in our opinion, only be used for weak positive samples to slightly increase specificity (level 2b). [52]

We used operating characteristics from the Warkentin et al. [36] study (for GTI polyspecific assay) to proxy the post-test probability (Bayes theorem [51]) for each 4T risk group at different OD cut offs (Table 2, Figure 3A). As there is significant difference in pre-test probability for the intermediate and high risk groups in the various studies (Attachment 1), for reasons already mentioned above, we estimated pre-test probability for each risk group by pooling all available studies (HIT suspected patients, confirmed by SRA/HIPA,). We are well aware of the limitations of this approach (maybe slightly different operating characteristics of test in our centre, different usage of 4T score and slightly different OD values, pooling of different studies (patient groups, scorers)).

Table 2: Post-test probabilities for combination of 4T scores with assay result

GTI polyspecific Cut off (OD)	**4T score (% pre-test probability)**		
	Low (0.3%; 3/911)	**Intermediate** (12%; 90/750)	**High** (51%; 83/163)

	Negative % [95% CI]	Positive % [95% CI]	Negative % [95% CI]	Positive % [95% CI]	Negative % [95% CI]	Positive % [95% CI]
0.40	0 [0-1]	2 [1-3]	0 [0-2]	48 [41 53]	0 [0-4]	87 [83-90]
1.00	0 [0-1]	6 [3-9]	0 [0-2]	73 [64-81]	2 [1-5]	95 [92-97]
1.40	0 [0-1]	12 [6-24]	1 [0-3]	86 [76-93]	7 [4-11]	97 [95-99]
2.00	0 [0-2]	20 [6-47]	3 [1-4]	92 [80-97]	17 [14-21]	99 [96-100]

This scheme could be further refined by adding the high-dose heparin confirmation step for weak positive results (0.40 – 1.0) in the intermediate 4T group (if the 1.0 cut off is deemed too risky) (level 2b) (Figure 3B). A positive high-dose heparin is an additional argument for the presence of platelet activating antibodies (level 2b). Furthermore, the overall prevalence of HIT in the population of the examined patient should be kept in mind (Table 1). The usage of an IgG specific assay could increase the calculated post-test probabilities even some more (level 1b).

The most problematic population are the weak positives with an intermediate risk. In the pooled analysis about 750/1824 (41%) of samples are obtained from intermediate risk patients, varying from study to study. In the paper of Warkentin et al. [36] about 9% (37/399) of all results had ODs between 0.4 and 1.0. Assuming no covariance (which undoubtedly exist between weak positives and lower risk profiles), the combined chance of having an intermediate risk profile with a weak positive ELISA will be about 4%. In the worst case scenario, all weak positive results will be seen in the intermediate risk category (9% of samples). The in house IgG specific assays tested in this study gave yield to an increased percentage of results in the 0.4 – 1.0 range (18-25%), the probability for SRA positivity remained in the same range (1.4-4.7%).

We have analysed the results of the HIT tests carried out in Leuven over the last 6 months. As no functional confirmatory test is carried out in UZ Leuven the number of samples corresponding to true HIT is unknown. The clinical data concerning the patients were not collected in this study. In UZ Leuven, the different wards have the possibility to request the advice of specialists in the department of Bleeding and Vascular Disorders, who have experience in the diagnosis and treatment of HIT. However, it is not known which proportion of the analyses were carried out following advice of these referents, nor which were the 4T scores of the patients.
A comparison of the proportion of positive HIT tests in our survey (13%) with the proportion of positive HIT tests in several studies (Attachment 3) suggests that many samples were derived from patients with a low 4T score, indicating a potential to further improve the pre-test probability (and hence PPV).

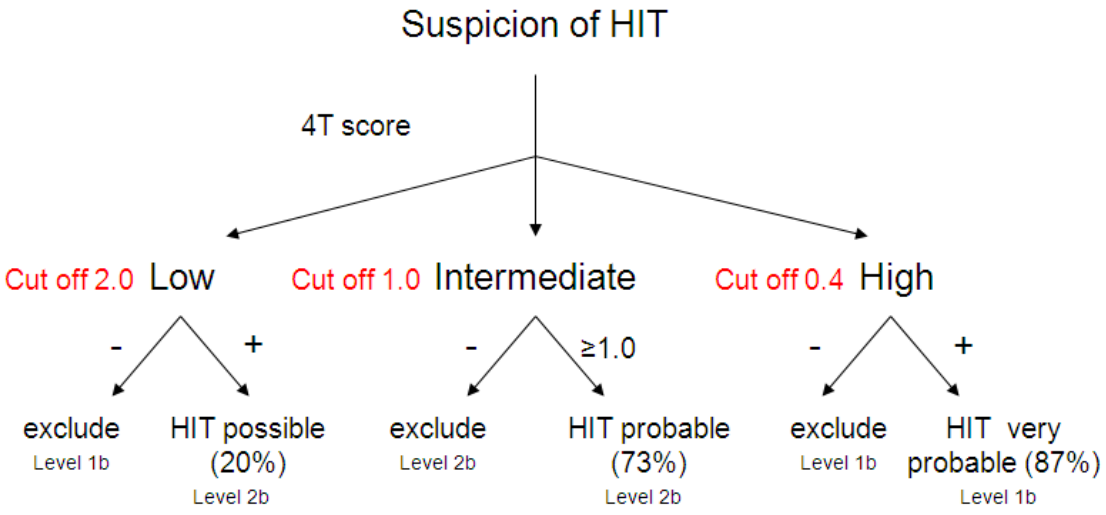

Figure 3A: Proposed algorithm for GTI polyspecific assay using single cut offs

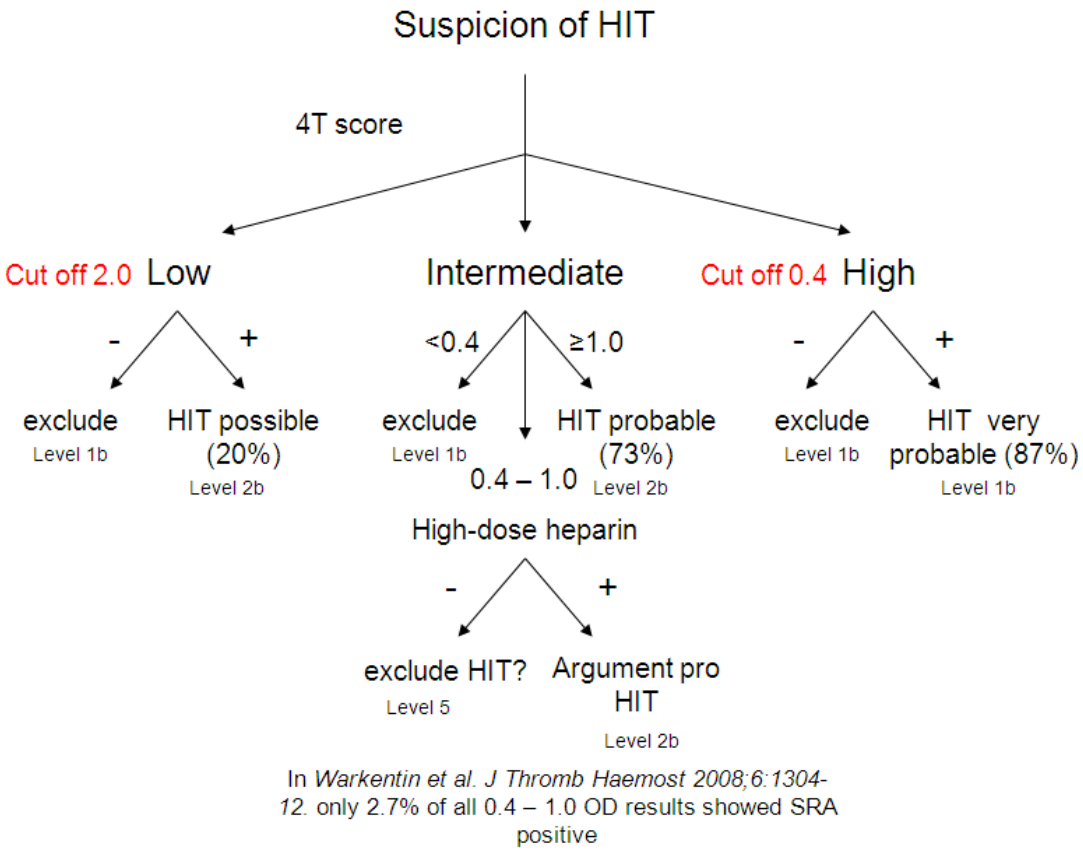

Figure 3B: Proposed algorithm for GTI polyspecific assay using the high-dose heparin confirmation for weak positive intermediate probabilities

Rapid assay

A well designed study that compares patient outcome/treatment decisions for clinical judgement alone, clinical judgement integrated with an immunoassay (batch testing versus immediate result) or clinical judgement integrated with immunoassay, confirmed by functional assay has, to our knowledge, not been performed yet. The benefits can nevertheless be deducted from the importance of accurate and timely diagnosis (risk for thrombosis/bleeding and limited value of clinical scoring systems). In most models [4,48] the TAT for immunoassays is estimated at 24 h. This seems a rather optimistic assumption for our centre and a great inter-laboratory variation exists [50]. Diminishing the TAT from 24 h on seemed to not materially increase cost effectiveness [48]. This, however, depends on the used treatment strategy (switch to alternative anticoagulation awaiting test result or not), the time distribution of the risk for thrombosis (estimated at 5% per day untreated, with about a third of all thrombosis events in the pre-treatment period) and the risk for bleeding events if (unnecessarily) switched to alternative anticoagulation. In most cases the degree of clinical suspicion combined with assay/alternative anticoagulation availability, co-morbidity (baseline bleeding risk) and pharmacokinetic issues (hepatic, renal failure) guides the choice of treatment for each individual patient, so a rather difficult to interpret situation arises [4,16]. For our centre, it can be expected that a decrease in TAT (from average 3 days to on-demand-testing) allows physicians to quick an reliably exclude HIT in a large portion of patients (about 87% of samples). This diminishes unnecessary risks for bleeding and/or thrombosis, depending on treatment strategy. Moreover, rapid and reliable exclusion of HIT gives the clinician the chance to focus on other (sometimes more dangerous) causes of thrombocytopenia. Based on the query results, we tried to estimate the possible benefits of reporting immunoassay results on-demand. The figures in the publication of Bakchoul et al. [21] were used to estimate the number of true HITs (number of positives a year (42) multiplicated by the PPV, 17 HITs a year in our simulation). This German study was chosen because the same assay was used (GTI), the percentage of positive results (possibly reflecting the examined population) was similar to ours (13% vs. 17%) and all results were confirmed by a reference method (HIPA). The benefits of rapid diagnosis largely depend on treatment strategy. If for all intermediate/high risk patients alternative anticoagulation is started, an average 3 days gain in unnecessary expensive medication can be gained for a variable portion of patients. If only the high risk group receives alternative anticoagulation awaiting test result, a lesser gain in medication will be received, but a number of true HIT patients in the intermediate risk group will be at risk for thrombosis for 3 additional days. All figures largely depend on the relative amount of 4T groups, the percentage of true HIT in each 4T group and the decisions by the treating physicians (Table 3,4). The HIT test has a low predictive value for patients at low clinical risk. When implementing a HIT test that could be carried out day and night in the routine laboratory, it may therefore be important to restrict the access to the test to clinicians with proven experience in diagnosis and treatment of HIT. Otherwise, it is plausible that a large number of false positive diagnoses will be made, which would result in many inappropriate treatments and strongly increase the risks and costs.

*Table 3: Pre- and post-test risk distribution for 318 request a year at UZ Leuven assuming initiation of alternative anticoagulation for every patient in intermediate and high risk categories until test result is known and using reported operating characteristics at cut off 0.4 (**A**) or at cut off 1.0 (**B**) [21]*

A

Intermediate + High risk (% of 318)	Increased bleeding risk pre-test (n)	Increased bleeding risk post-test (n)*	Increased thrombosis risk pre-test (n)	Increased thrombosis risk post-test (n)**	Gain/loss (€)***
10	15	2	0	0	- 34 972
30	78	9	0	0	+ 50 010
50	142	16	0	0	+ 136 991
70	204	23	0	0	+ 222 973

B

Intermediate + High risk (% of 318)	Increased bleeding risk pre-test (n)	Increased bleeding risk post-test (n)**	Increased thrombosis risk pre-test (n)	Increased thrombosis risk post-test (n)**	Gain/loss (€)***
10	15	1	0	2	- 33 642
30	78	4	0	2	+ 58 136
50	142	7	0	2	+ 149 914
70	204	10	0	2	+ 241 692

*Table 4: Pre- and post-test risk distribution for 318 request a year at UZ Leuven assuming initiation of alternative anticoagulation for every patient in the high risk categories until test result is known and using reported operating characteristics at cut off 0.4 [21], assuming 15% (**A**) or 50% (**B**) of true HIT cases in the intermediate risk category*

A

Intermediate /High risk (% of 318)	Increased bleeding risk pre-test (n)	Increased bleeding risk post-test (n)*	Increased thrombosis risk pre-test (n)	Increased thrombosis risk post-test (n)**	Gain/loss (€)***
20/10	17	9	2	0	- 23 171
35/15	33	16	2	0	+ 3 716
40/20	49	19	2	0	+ 30 603
20/40	113	16	2	0	+ 138 150

B

Intermediate/ High risk (% of 318)	Increased bleeding risk pre-test (n)	Increased bleeding risk post-test (n)*	Increased thrombosis risk pre-test (n)	Increased thrombosis risk post-test (n)**	Gain/loss (€)***
20/10	23	9	9	0	- 13 993
35/15	39	16	9	0	+ 12 894
40/20	55	19	9	0	+ 39 780
20/40	119	16	9	0	+ 147 328

**Major bleeding risk for patient with HITT treated with argatroban = 8%, we assume the same risk for patients unnecessary treated with this anticoagulant compared to 2.2% risk in the control arm of the study [4]*
***Thrombosis rate of HIT patients not treated with argatroban = 22.4% compared to 6.9% for patients treated with argatroban [4]*
****The gain/loss for immediate exclusion of HIT is calculated as the difference between 3 days of unnecessary alternative anticoagulation cost (700 [48] (used) - 1000 $ [4]a day (argatroban)) spared minus the cost of heparin treatment otherwise given (43 $ full course [48]) minus the assay cost (234 $ a test [48], calculated for all 318 patients).*
This calculation has some major shortcomings: 1) the assay cost is taken rather high (activity based cost per test approximately 66 € for the currently used GTI assay, 113 € for the ACL Acustar assay, 100 € for an ACL TOP assay (lower if in batch)); 2) cheaper alternative anticoagulation is often initiated (fondaparinux (Arixtra®)); 3) the larger picture of reimbursement issues (75 € charged to patient per test, medication forfaits, changes in pathology mix) and costs attributed to thrombosis/bleeding are not included; 4) urgent (TAT 6 h) HIT tests are already carried out during normal working days with the GTI kit in UZ Leuven, but exclusively on demand of the referent internists specialised in bleeding and thrombosis disorders and when the clinical situation is very suggestive of HIT. The development of a novel assay available day and night would thus not result in all cases in a gain of 3 day of treatment; 5) in some clinical situations very suggestive of HIT it may seem difficult not to stop the alternative anticoagulation treatment even if the HIT test is negative

Options for rapid assays

The best described rapid assay is the PaGIA (Biorad, Munich, Germany) that detects IgG, A and M specific to heparin/PF4 complexes (ID-heparin/PF4 PaGIA). In this assay, red-dyed high-density polymer particles coated with complexes of PF4/heparin serve as the solid phase. When antiheparin-PF4 antibodies are present in the plasma, the particles cross-link and remain at the top of the gel chamber (after centrifugation). In case of no significant level of antiheparin/PF4 antibody, all particles sink to the bottom after centrifugation. A number of publications have proposed this assay (whether or not in combination with the 4T score) as a reliable method to rapidly exclude HIT. Some studies have shown this assay to be less sensitive than conventional immunoassays and hence less useful as ruling-out test [19,22,29,43]. Some concern has also risen about the inter-lot variability and the potential of faulty ID-heparin/PF4-polymer lots [46]. Studies that correlate antibody titer (as determined by PaGIA) with functional assay or clinical results indicate a link. The correlation is less well described than link between OD in ELISAs en platelet activating antibodies. Another disadvantage is that only polyspecific testing kits are available. From a logistic perspective the introduction of this assay seems most interesting if the gel cartridges are already in use for other tests (eg. immuno-haematology). In our centre, the immunohaematology lab, were most of these gels are used, is part of the Blood Transfusion Centre and is separated both physically and logistically from the central laboratory.

Another fully automated on demand testing system, HemosIL HIT-Ab(PF4-H) (Instrumentation Laboratory) on an ACL TOP platform, has been compared to a conventional immunoassay (Asserachrom HPIA IgG/A/M) by Davidson et al. [47]. The test principle is latex particle enhanced immunoturbidimetry. A mouse monoclonal antiheparin-PF4 antibody, coated onto latex particles mimics human HIT antibodies. In the presence of PF4/PVS complex and patient sample a competitive agglutination reaction occurs. 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. This assay showed comparable results to the conventional immunoassay (87.7% overall agreement). Evaluation against reference standard methods has yet to be performed and an IgG specific assay is not yet available.

The HemosIL Acustar HIT-IgG(PF4-H) and HIT-Ab(PF4-H) are two chemiluminiscent two-step immunoassays consisting of magnetic particles coated with PF4 complexed to PVS which capture the antiheparin-PF4 antibodies from the sample. After incubation, a magnetic separation, and a wash step, a tracer consisting of an isoluminol-labelled anti-human igG antibody or a mixture of three isoluminol-labelled antibodies (IgG/A/M) is added and may bind with the captured antiheparin-PF4 antibodies on the particles. After a second incubation, magnetic separation and washing step, reagents that trigger the luminescent reaction are added. Emitted light is measured by the Acustar optical system as relative light unit (RLU). Result is expressed in arbitrary units (U/mL) and is available in 30 minutes. The RLUs/arbitrary units are directly proportional to the antiheparin-PF4 antibody concentration in the sample. These assays have already shown well comparable with other commercially available assays (GTI), resulting in overall agreement of 96%. A recent pilot study by Legnani et al.[23] has shown excellent sensitivity (100%) for both assays. The specificity was greatly increased by using the IgG specific assay (from 81 to 96%). The IgG assay also reports excellent NPV (100%) and PPV (85%). This could be in part due to the rather high pre-test probability in this study (17% confirmed HIT). This study, however, has a few shortcomings. First, the number of patients tested is rather small, second, no SRA or HIPA was used. The performed platelet aggregation assay might be less sensitive and give raise to some false negatives. Third, not all patients were confirmed/excluded HIT. Intermediate/low probability patients with negative PaGIA were considered negative without further testing (check also: limitations of PaGIA). A recent Belgian poster describing the use of the assay in 104 HIT suspected patients with low number of clinical HITs confirmed the results of Legnani [24].

The Acustar IgG specific assay seems analytically most interesting (IgG specific testing) and is, at the moment, better studied than HemosIL [23,24,47]. The ACL TOP system, on the other hand, can be connected on the recently implemented track system (Inpeco, Lugano, Switzerland), offering really on-demand testing. This probably comes at the expense of some specificity (as compared to IgG specific assays). For both assays the correlation between quantitative results compared to the current GTI polyspecific assay (used in the study of Warkentin et al. [36]) will have to be determined. This will allow to offer the clinicians a post-test probability scheme including clinical score, strength of reactivity (and high-dose heparin confirmation). The use of a commercial, fully automated system could also contribute to standardization and inter-laboratory comparison.

TO DO/ACTIONS

1) Discuss with clinicians:
    a. on-demand testing at the cost of little specificity versus more specific but later testing
    b. comment on the low threshold for testing (query results)
    c. comment on the post-test probability scheme/algorithm
2) Plan an evaluation/validation project

ATTACHMENTS

Attachment 1: 4T risk group distribution in different studies

Reference	HIT definition	Low			Intermediate			High			Remarks
		HIT	Assay+	Total	HIT	Assay+	Total	HIT	Assay+	Total	
Morel-Kopp 2010 [16]	SRA	2	/	75	2	/	15	5	/	7	
Pouplard 2007 [10]	SRA	0	/	74	14	/	129	8	/	10	
Bakchoul 2009 [21]	HIPA	0	/	316	9	/	130	26	/	54	
Lo 2006, [9] Hamilton	SRA and GTI+	0 1*	11	64	8	13	28	8	8	8	-Interrater Kappa 0.86 (2 raters) -prospective *Patient did not have history of heparin exposure and had APL
Lo 2006, [9] Greifswald	HIPA and in house EIA+	0	4	55	11	19	139	9	15	42	-Rated by requester -prospective -More LMWH use (Europe) (less HIT)
Lillo-Le Louët 2004 [13]	SRA, clinical	0	/	20	24	/	53	11	/	11	Retrospective
Kim 2011 [41]	Chong score	/	/	48	/	/	33	/	/	11	Retrospective
Denys 2008 [14]	FCM	0	4	31	4	34	62	6	8	9	Retrospective, PaGIA
Ruf 2011 [31]	SRA	0	27	36			42			5	Retrospective : only patients with SRA and GTI results available
Legnani 2010 [23]	/	/	/	35	/	/	49	/	/	18	
Weiss 2008 [39]	/	/	3/1*	66	/	13/4*	59	/	10/7*	19	Retrospective by 1 person, GTI *OD 1.0
Tawfik 2011 [11]	SRA	0	1	16	2	2	26	3	4	8	PaGIA, fair interrater agreement Rated by hematologist
Bryant 2008 [44]	SRA	0	6	142	5	13	92	4	5	12	PaGIA Not all PaGIA positives initiated DTI. Rated before serologic testing by hematologist
Crowther 2010 [45]	SRA	0	/	39	1	/	9	1	/	1	Very low HIT prevalence in critically ill despite frequent thrombocytopenia Retrospective by 1 person
Gruel 2008 [19]	SRA	0	/	74	14	/	129	8	/	10	
Janatpour 2007 [38]	Chong score	5	/	36	34	/	48	21	/	21	Retrospective by 2 persons

Attachment 2: Quantitative studies

Reference	Patients	HIT definition	Assay	Results
Greinacher 2010 [40]	935 blood donors (no heparin) 609 trauma, 336 medical and 262 cardiac surgery patients before heparin 2821 suspected	HIPA	In house IgG	97.5 percentiles via quantile regression: significant differences without age/gender influence Donors: 0.21 (females slightly higher) Trauma: 0.35 Medical: 0.48 Cardiac surgery: 0.74 (possibly recent heparin exposure) Correlation HIPA+ <0.5 (10/2000); 0.5 -<1.0 (57/463); 1.0-<1.4 (82/199); 1.4-<2.0 (138/181); >2.0 (5/5) Shift in comparison with Warkentin 2008: very high correlation (>0.9) after using 'normalized' OD grades
Whitlatch 2008 [33]	115 EIA+, retrospectively	ACCP (clinical guidelines)	GTI	244/1194 EIA+ (20%), 115 patients (98 confirm+, 17 confirm -) C+ more likely clinical HIT (72% vs 18%) 3/17 confirm- clinical HIT with high OD (1.1/2.2/3.3) OD higher in clinical HIT+/confirm+ than in HIT?/HIT-/C+, not than C- because 3 high OD outliers and OD predictive of thrombosis
Schenk 2007 [25]	113 patients on MCS blood drawn 5-7 days postop	HIPA	GTI In house IgG	9.7% HIPA indeterminate IgG increases specificity Significant higher OD for HIPA+ Correlation of OD with HIPA+ (logistic regression)
Weiss 2008 [39]	144 suspected		GTI	Significant different OD between low (0.148), IM (0.223) and high (0.470) ODs Wide range Risk of thrombosis increases with OD
Janatpour 2007 [38]	105 suspected	Chong score	GTI	Significant different OD between low (0.223), IM (0.448) and high (0.704) ODs
Althaus 2010 [32]	1000 consecutive suspected	HIPA	IgG specific in house	663 OD<0.5; 217 OD 0.5-1.0; 120 OD>1.0
Bakchoul 2011 [42]	459 suspected	HIPA + intermediate /High	GTI IgG Zymutest	ROC analysis: optimal trade off at 0.62 OD for GTI and 0.69 for Zymutest Median OD 2.87 vs 1.03 for GTI (cfr HIPA) and 2.68 vs 1.23 Zymutest Wide spread of ODs
Ruf 2011 [31]	83 with SRA results available and EIA + or indeterminate	SRA	GTI	Wide variation for OD's with SRA+ 1/10 SRA+, OD<1.0 7/73 SRA-, OD>1.0 Median OD SRA+ 1.912 (0.491-2.894) vs 0.52 SRA- (0.125-1.997)
Baroletti 2012 [30]	318 EIA+ and 50% decrease or <150 x10e9/L		Asserachrom	Doubling of the 30 days thrombosis odds for every 1 unit increase in OD Cancer significant odds (1.91)
Altuntas 2008 [37]	94 EIA+, inhibition		GTI	Significant higher OD in patients with thrombosis (n=48) (1.34+/-0.89 vs. 0.96+/-0.75)

	>50% and 50% decrease in platelets after heparin			OD > 1.27 in isolated group significant higher chance for thrombosis (ROC) No significant differences in inhibition Females 2.8 fold risk
Zwicker 2004 [28]	63 ELISA+ patients (15 with thrombosis at diagnosis)		GTI	Significant higher OD in patients with thrombosis (1.41+/-0.87 vs. 0.80+/-0.46) (27 thrombosis events, 23 patients: 2 with arterial and venous; 2 with HITT and recurrent thrombosis) Threshold 1.0 OD most striking differences Isolated HIT: 6 fold increase in thrombosis risk (30 day) for OD >1.0 (only 1/48 isolated HITs treated with DTI)
Pouplard 2010 [22]	Suspected HIT (101) Non-consecutive	SRA 20%	Zymutest Zymutest IgG	Non HIT: Absorbance median 0.48; range [0.08-2.2] HIT:Absobance median 1.9; range [0.5-2.7]
Kim 2011 [41]	Suspected HIT	Chong score	GTI	Thrombosis: OD median 0.52; range [0.11-2.81] vs. 0.22 [0.02-3.24]
Denys 2008 [14]	Suspected HIT 102 consecutive	FCM	Asserachrom PaGIA	Correlation of OD with 4T groups
Bakchoul 2009 [21]	500 suspected HIT consecutive	HIPA + intermediate /high 4T	GTI Modified GTI (IgG specific) (in house)	Significant higher OD in HIT ROC via OD: AUC identical for polyspecific and IgG specific, best trade-off at 0.65
Warketin 2008 [36]	Study A 417 suspected, 12 excluded Study B 1611 suspected 58 excluded	SRA 50%	 GTI In house IgG In house IgG	-2.9% and 3.6% indeterminate SRAs (excluded) -Study A: <0.40 (0/304) (0/279)* 0.40-<1.00 (1/37) (1/72)* 1.00-<1.40 (2/11) (2/9)* 1.40-<2.00 (5/10) (8/15)* ≥2.00 (33/37) (30/30)* *IgG specific -Study B: <0.40 (1/920) 0.40-<1.00 (18/382) 1.00-<1.40 (16/54) 1.40-<2.00 (45/78) ≥2.00 (109/119) -Logistic regression shows OR 6.36 for every 0.5 OD (GTI) (slight trend to lesser predictivity in postcardiothoracic surgery setting); 11.36 for IgG (study A); 6.39 for every 1.0 OD (study B) -Reference values with heparin receiving controls: <1.66 and <2.90 for UFH non HIT patients for postorthopedic and postcardiac surgery respectively (95% non HIT); lower for LMWH (<0.80) (GTI)
Lo 2006 [9]	100 consecutive	SRA + intermediate /high	GTI In house IgG	Median absorbance in HIT 2.39 (GTI)/2.23 (IgG) significantly higher than in other assay + (non HIT) 0.89/0.64 Cutoff 1.20: 100% sensitive and specific GTI polyspecific (SRA+ and Intermediate/High) Clinical features (thrombosis/bleeding) of assay+- non HIT resemble non HIT-assay-
Greinacher 2007 [7]	1582 (1650) consecutive	HIPA,	In house polyspecific In house IgG	OD overlap
Juhl 2006 [6]	736 (755) consecutive	HIPA,	In house polyspecific In house IgG	OD overlap
Warkentin 2005 [17]	448 samples of prospective	Clinical	GTI In house IgG	Clinical HIT and SRA+ :significant higher OD
Legnani 2010 [23]	102 suspected HIT	Platelet aggregation assay	Acustar Acustar IgG	1-2 U/mL: only 1 out of 4 HIT >2: 16/16 HIT Thrombosis HIT patients(n=7): significant higher U/mL

Attachment 3: Operating characteristics in different studies

Reference	Patients	HIT definition	Assay cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Remarks
Janatpour 2007 [38]	105 suspected (retrospective on basis of DTI initiation)	Chong score	GTI 0.40 1.0	 69 38	 75 85	 78 77	 65 52	
Althaus 2010 [32]	1000 consecutive suspected	HIPA	IgG specific in house 0.5 1.0 0.5 + inhibition 0.5-1.0 + inhibition and all >1.0	 99 83 83 96	 72 94 91 89	 24 58 45 44		663 OD<0.5 217 OD 0.5-1.0 120 OD>1.0 82 HIPA positive Inhibition with 100 U hep (+/-GTI)
Bakchoul 2011 [42]	459 suspected	HIPA + intermediate /High	GTI IgG 0.40 Zymutest 0.5	100 (35/35) 100(94*) (35/35)	88(90*) (374/424) 89(93*) (378/424)	41(45*) (35/85) 43(52*) (35/81)	100 (374/374) 100(99.5*) (378/378)	* High-dose heparin inhibition confirmation
Ruf 2011 [31]	83 with SRA results available and EIA + or indeterminate	SRA	GTI 0.40 1.0	100 (10/10) 80 (8/10)	19 (14/73) 85 (62/73)	14 (10/69) 42 (8/19)	100 (14/14) 97 (62/64)	
Pouplard 2010 [22]	Suspected HIT (101) Non-consecutive [Controls (101; 50 APS)]	SRA 20%	GTI 0.40 Zymutest 0.5 Zymutest IgG 0.5	100 (40/40) 98 (39/40) 98 (39/40)	50.8 (31/61) 77 (47/61) 90 (55/61)	57 (40/70) 74 (39/53) 87 (39/45)	100 (31/31) 98 (47/48) 98 (55/56)	
Lillo-Le Louët 2004 [13]	Suspected HIT (84) CPB retrospective	SRA Clinical	Asserachrom 0.5	100 (35/35)	73 (36/49)	73 (35/48)	100 (36/36)	
Kim 2011 [41]	Suspected HIT 92, Cardiothor	Chong score	GTI 0.40	94 (15/16)	96.6 (28/29)	/	/	
Denys 2008 [14]	Suspected HIT 102 consecutive retrospective	FCM	Asserachrom (kit specific percentage of observed reference) PaGIA	100 (10/10) 100 (10/10)	64 (53/83) 61 (56/92)	25 (10/40) 22 (10/46)	100 (53/53) 100 (56/56)	
Pouplard 2007 [10]	Suspected HIT 213 consecutive	SRA 20%	GTI 0.40	100 (22/22)	82 (156/191)	39 (22/57)	100 (156/156)	

	patients prospective		PaGIA	95 (21/22)	92 (175/191)	57 (21/37)	99 (175/176)	
Bakchoul 2009 [21]	500 suspected HIT consecutive prospective	HIPA + intermediate /high 4T	GTI 0.40 Modified GTI (IgG specific) (in house) 0.40 0.65 1.0 ID-H/PF4 PaGIA	100 (35/35) 100 (35/35) 97 (34/35) 91 (32/35) 94 (33/35)	81 (376/465) 89 (414/465) 92 (430/465) 95 (441/465) 88 (408/465)	28 (35/124) 41 (35/86) 49 (34/69) 57 (32/56) 37 (33/90)	100 (376/376) 100 (414/414) 99.7 (430/431) 99.3 (441/444) 99.5 (408/410)	
Warketin 2008 [36]	Study A 417 suspected, 12 excluded Study B 1611 suspected 58 excluded	SRA 50%	GTI 0.40 1.0 1.40 2.0 In house IgG 0.45 In house IgG 0.45	100 (41/41) 98 (40/41) 93 (38/41) 81 (33/41) 100 (41/41) 99.5 (188/189)	85 (304/358) 95 (340/358) 98 (349/358) 99 (354/358) 84 (299/358) 73 (1001/1364)	43 (41/96) 69 (40/58) 98 (38/47) 89 (33/37) 41 (41/100) 34 (188/551)	100 (304/304) 99.7 (340/341) 99.2 (349/352) 98 (354/362) 100 (299/299) 99.9 (1001/1002)	2.9% and 3.6% indeterminate SRAs (excluded)
Morel-Kopp 2012 [17]	181 PaGIA, GTI-IgG or Asserachrom GAM positives	SRA 50% SRA retest	HIMEA	93 (65/70) 94 (73/78)	89 (99/111) 96 (99/103)	84 (65/77) 95 (73/77)	95 (99/104) 95 (99/104)	Low incidence of APL, heparin independent activation
Morel-Kopp 2010 [16]	97 consecutive HIT (107 samples) 47.4% surgical	SRA 20% for ELISA +	Zymutest IgG 0.5 HIMEA for ELISA+	 100 (9/9)	 67 (4/6)	60 (9/15) 82 (9/11)	 100 (4/4)	Different platelet donors Possibly greater sensitivity for HIMEA (and less for SRA)
Lo 2006 [9]	100 consecutive	SRA + intermediate/high	GTI 0.40	100 (16/16)	81 (68/84)	50 (16/32)	100 (68/68)	Clinical features (thrombosis/bleeding) of assay+ non HIT resemble non HIT assay-

			In house IgG 0.45	100 (16/16)	82 (69/84)	52 (16/31)	100 (69/69)	
Greinacher 2007 [7]	1582 (1650) consecutive, prospective, 52% medical	HIPA, indeterminate excluded 19 polyspecific +, subgroups **not** determined	In house polyspecific 0.50 In house IgG	99 (95/96) 98 (92/94)	93 (1386/1486) 95 (1393/1469)	49 (95/195) 55 (92/168)	99.9 (1386/1387) 99.9 (1393/1395)	IgM/A minor relevance 1 IgM pos/Hipa+: clinically non HIT 1 Ig neg Hipa+: Clinical HIT (NAP?) No thrombosis in IgM/A (+/- 10% pos)
Juhl 2006 [6]	736 (755) consecutive	HIPA, indeterminate excluded (3 GAM **indetermined** , 1 HIPA pos)	In house polyspecific 0.50 In house IgG	94 (50/53) 87 (46/53)	93 (632/683) 96 (653/681)	48 (50/105) 62 (46/74)	99.5 (632/635) 98.9 (653/660)	IgMA only(26.7%) IgM/A: often other cause of thrombocytopenia or thrombosis 3 HIPA+, GAM -: IL/NAP?; 3/4 HIPA IgG neg (IgA/M pos): IL/NAP IgG?
Warkentin 2005 [17]	448 samples of prospective UFH/LMWH as profylaxis after hip replacement trails	Clinical 50% fall 5 days No other cause Raise after heparin stop	SRA 20% GTI 0.40 1.0 In house IgG	100 (14/14) 100 (14/14) 93 (13/14) 100 (14/14)	96 (418/434) 82 (352/429) 92 (398/434)	47 (14/30) 15 (14/91) 28 (14/50)	100 (418/418) 100 (352/352) 100 (398/398)	Specificity greater for LMWH treated patients
Legnani 2010 [23]	102 suspected HIT 33 non HIT (14 LMWH and 19 healthy)	Platelet aggregation assay; negative PaGIA and low/intermediate 4T not tested HIT= PaGIA+ and PA+ or PaGIA-, PA+ and 4T high	PaGIA (indeterminate as positive) Acustar 1.0 U/mL Acustar IgG 1.0 U/mL	94 (16/17) 100 (17/17) 100 (17/17)	86 (72/84) 81 (69/85) 96 (82/85)	80 (16/20) 52 (17/33) 85 (17/20)	99 (72/73) 100 (69/69) 100 (82/82)	Also pos in 1 healthy control and 1 control LMWH
Douxfils 2012 [24]	104 suspected	Clinical	Acustar 1.0 9.41 Acustar IgG 1.0 2.89	100 (9/9) *100* (9/9) 100 (9/9) *100* *(9/9)*	88 (84/95) *98* *(93/95)* 95 (90/95) *97* *(92/95)*	45 (9/20) *82* *(9/11)* 64 (9/14) *75* *(9/12)*	100 (84/84) *100* *(93/93)* 100 (90/90) *100* *(92/92)*	