DR BRIGITTE TARDY-PONCET (Orcid ID : 0000-0001-5409-942X)
DR DOMINIQUE LASNE (Orcid ID : 0000-0001-6600-0263)
DR ANDREAS GREINACHER (Orcid ID : 0000-0001-8343-7336)

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Heparin-induced thrombocytopenia: construction of a pre-test diagnostic score derived from the analysis of a prospective multinational database, with internal validation

Brigitte Tardy-Poncet[1], Emmanuel de Maistre[2], Claire Pouplard[3], Emilie Presles[1], Martine Alhenc-Gelas[4], Dominique Lasne[5], Marie-Hélène Horellou[6], Christine Mouton[7], Anne Serre-Sapin[8], Anne Bauters[9], Philippe Nguyen[10], François Mullier[11], Julien Perrin[12], Grégoire Le Gal[13], Pierre-Emmanuel Morange[14], Lélia Grunebaum[14], Agnès Lillo-Le Louet[16], Ismail Elalamy[17], Yves Gruel[3], Andreas Greinacher[18], Thomas Lecompte[19], and Bernard Tardy[1], on behalf of the GFHT-HIT study group

1. CIC 1408, Inserm U1059 SAINBIOSE, F-Crin INNOVTE, Université de Lyon, Saint-Etienne, France (B Tardy-Poncet MD, E Presles M Biostat, Prof B Tardy MD)
2. Hemostasis unit, CHU Dijon, Dijon, France (E de Maistre MD)
3. Division of Hematology - Hemostasis, University Hospital of Tours, Tours, France (Prof C Pouplard Pharm D, Prof Y Gruel MD)
4. Hemostasis unit, Hopital Européen Georges Pompidou, AP-HP, Paris, France (M Alhenc-Gelas Pharm D)

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5. Hemostasis unit, Hôpital Necker, AP-HP, Paris; Université Paris Sud Paris Saclay, Inserm U1176, Le Kremlin-Bicêtre, France (D Lasne Pharm D)
6. Hematology Laboratory Unit, Hôpital Universitaire Paris-centre, Paris, France (MH Horellou MD)
7. Hemostasis unit, CHU Bordeaux, Bordeaux, France (C Mouton MD)
8. Hemostasis unit, CHU Clermont-Ferrand, Clermont-Ferrand, France (A Serre-Sapin Pharm D)
9. Hemostasis unit, CHU Lille, Lille, France (A Bauters Pharm D)
10. Hemostasis unit, CHU Reims, Reims, France (Prof P Nguyen MD)
11. Namur Thrombosis and Hemostasis Center, Hematology Laboratory, Université catholique de Louvain, CHU UCL Namur, Yvoir, Belgium (Prof F Mullier MD)
12. Hemostasis unit, CHU Nancy, Nancy, France (J Perrin Pharm D)
13. Department of Medicine, University of Ottawa, Ottawa Hospital Research Institute, Thrombosis Research Group, Ottawa, Canada (Prof G Le Gal MD)
14. C2VN, Aix Marseille University, INSERM, INRA; Laboratory of Hematology, La Timone Hospital, Assistance Publique des Hôpitaux de Marseille, Marseille, France (Prof P-E Morange MD)
15. Laboratory of Hematology, CHU Strasbourg, Strasbourg, France (L Grunebaum MD)
16. Pharmacovigilance center, Georges Pompidou European Hospital, AP-HP, Paris, France (A Lillo-Le Louet Pharm D)
17. Hematology and Thrombosis Center, Tenon University Hospital, INSERM UMRS 938, Sorbonne University, Paris, France (Prof I Elalamy MD)
18. Institut fuer Immunologie und Transfusionsmedizin, Universitaetsmedizin Greifswald, Greifswald, Germany (Prof A Greinacher MD)
19. Department of Medicine, Geneva University Hospitals, and Geneva Platelet Group (GpG), Faculty of Medicine, University of Geneva, Geneva, Switzerland (Prof T Lecompte MD)
Correspondence to: Prof Bernard Tardy, Centre d'Investigation Clinique, Inserm CIC 1408, CHU Saint Etienne, 42055, France, Phone: 33 477120797, Fax: 33477127820 bernardtardy@yahoo.fr

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Essentials

- Pre-test scores for Heparin Induced Thrombocytopenia (HIT) diagnosis present limitations.
- We prospectively included 2,280 patients with HIT suspecion into derivation and validation cohorts.
- Multivariate analysis revealed eight clinical variables associated with HIT, easily collectable at the time of suspicion, from which a score was derived.
- The diagnostic performances of the score make it a promising candidate for incorporation into a diagnostic algorithm for HIT.

Abstract (235 words)

Background: Diagnosis of heparin-induced thrombocytopenia (HIT) requires pre-test probability assessment and dedicated laboratory assays.

Objective: To develop a pre-test score for HIT.

Design: Observational; analysis of prospectively collected data of hospitalized patients suspected with HIT (ClinicalTrials.gov NCT00748839).

Setting: Thirty-one tertiary hospitals in France, Switzerland, and Belgium.

Patients: Patients tested for HIT antibodies (2,280 evaluable), randomly allocated to derivation and validation cohorts.

Measurements: Independent adjudicators diagnosed HIT based on the prospectively collected data and Serotonin Release Assay results.

Results: HIT was diagnosed in 234 (14.7%) and 99 (14.5%) patients in the two cohorts. Eight features were associated with HIT (in brackets, points assigned for score calculation of the score): unfractionated heparin (1); therapeutic-dose heparin (1); cardiopulmonary bypass (cardiac surgery) (2); major trauma (3); 5- to 21-day interval from anticoagulation initiation to suspicion of HIT (4); ≥ 40% decrease in platelet count over ≤ six days (3); thrombotic event, arterial (3) or venous (3). The C-statistic was 0.79 [95% CI, 0.76-0.82]. In the validation cohort, the area under the receiver operating characteristic curve was 0.77 [95% CI, 0.74-0.80]. Three groups of scores were defined; HIT prevalence reached almost 30% in the high-probability group.

Limitation: The performance of the score may depend on settings and practices.

Conclusion: The objective, easy-to-collect, clinical features of HIT we evidenced were incorporated into a pre-test score, which may guide clinical decisions regarding diagnostic testing and anticoagulation.
Primary Funding Source: French Health Ministry.

Key words: Heparin-induced thrombocytopenia, observational study, pre-test score, heparin, thrombocytopenia

Introduction

Heparin-induced thrombocytopenia (HIT) is a prothrombotic and potentially devastating complication of heparin therapy.[1,2] Its pathogenesis is complex, but an accepted distinctive feature is the occurrence of antibodies directed against complexes of chemokines and polyanionic macromolecules (such as PF4 - heparin complexes) and targeting platelets, which can be strongly activated. Monocytes, neutrophils and endothelial cells are also targeted.[3] It is crucial to diagnose HIT as early but also as reliably as possible to enable appropriate management, while avoiding overdiagnosis with the risks associated with the use of alternative anticoagulation regimens.[4] There are often several potential explanations for thrombocytopenia other than HIT in patients receiving heparin. Laboratory testing for HIT, designed to detect HIT antibodies, also has limitations.[5] Immunoassays (IA) are highly sensitive, but show insufficient specificity.[6] Platelet activation assays such as heparin-induced (washed) platelet activation test (HIPA) and serotonin release assay (SRA) are more specific than immunoassays but are more demanding and time-consuming, and false positive, false negative and undetermined results are possible.[7-9] The results of laboratory testing are combined with a clinical estimate of HIT probability. Scoring systems were developed to help with pre-test probability assessment: 4Ts score, HIT Expert Probability (HEP) score, and post-cardiopulmonary bypass score.[10-13] However, selection of predictors in those scores was based on either personal clinical experience,[10,11] or analyses of retrospectively collected patients' data.[12] Two were prospectively evaluated, but populations comprised fewer than 500 patients[11,13,14-18] and/or the prevalence of HIT was low (less than 10%).[15,19-21] In addition, the accuracy (i.e. sensitivity and specificity) of those scores is difficult to estimate as various laboratory assays were used to diagnose HIT. Currently, the 4Ts score, which facilitates a systematic approach by using four basic relevant items, appears to be the most widely used. However it has some limitations[21-23], such as inter-observer variability (the experience of attending physicians in calculating the score being crucial), and the often challenging appraisal of other possible causes of thrombocytopenia.[5,21-23] A limited agreement (at best 65%) between attending physicians, haematologists and experts for the three categories of the 4Ts score has been reported.[23] Up to one third of patients could be scored differently.[24] Disagreements occurred more frequently regarding the timing of thrombocytopenia and other possible causes.[23,24] Finally some potential reported predictors of HIT such as unfractionated heparin (UFH) rather than low molecular weight heparin (LMWH) and therapeutic doses of heparin therapy rather than

prophylactic doses[25-27] were neither tested nor incorporated in the available scores. Thus, it appeared that there was a need to develop a new pre-test score including accurate predictors with standardized measurement as well as other potential predictors.

The objectives of this study were: (i) to better identify features associated with HIT, and to develop a model through multivariate analysis of data prospectively collected among a large cohort of patients with suspected HIT; (ii) to use this model to construct a score for assessment of the pre-test probability of HIT; and (iii) to internally validate the score.

Patients and methods

Study design

The prospective observational HIT score study (NCT00748839) was conducted in 31 tertiary hospitals (28 in France, two in Switzerland and one in Belgium) under the auspices of the Groupe Français d'études sur l'Hémostase et la Thrombose (GFHT). The study was developed and coordinated by the Centre for Clinical Investigation (CIC 1408) of the Centre Hospitalier Universitaire, Saint-Etienne, France. The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki, Good Clinical Practice, and relevant French legal and regulatory requirements regarding data protection (Commission National Informatique et Liberté no. 908138, French Advisory Committee on the Processing of Information in the Field of Health Research no.08077). The protocol was also approved by Swiss and Belgian ethics committees (CER 11-024; B039201010164). All patients received written information about the study, emphasizing their right to refuse participation or to withdraw at any time. As no experimental interventions were envisaged in the protocol, no written informed consent was required for patient inclusion.

Patients

All patients aged ≥18 years were eligible for inclusion if HIT was suspected on the grounds of newly developed thrombocytopenia or downwards change in platelet count, or venous or arterial thrombosis, during administration of UFH, low molecular weight heparin (LMWH), or fondaparinux, and if an immunoassay for HIT antibodies was performed in the specialized thrombosis units participating in the study: the assay was the one locally implemented (Asserachrom-HPIA, Stago, Asnière-sur-Seine, France; Zymutest HIA IgG/A/M, Hyphen

Biomed, Neuville-sur-Oise, France; GTI's PF4 Enhanced ELISA, Diagnostics Inc., Waukesha, WI, USA). Patients were not included if the physicians responsible for their care: (i) considered there was insufficient clinical data prior to laboratory testing; (ii) found the timing of thrombocytopenia unclear; (iii) detected a definite alternative cause of thrombocytopenia; (iv) could not ensure follow-up of the patient, in particular to assess whether platelet count normalised.

Data collection

Data for each patient were recorded locally by the persons responsible for laboratory testing and pharmacovigilance, and the senior physician in charge of the patient, using the Case Report Form (CRF) (supplemental material) previously used by the GFHT-HIT group.[28] All data (19 items, 103 variables) were centralized in the Centre for Clinical Investigation, Centre Hospitalier Universitaire, Saint-Etienne, France. Data quality was regularly monitored through electronic editing checks to detect implausible, incomplete or inconsistent data. In addition, submitted data were compared with source medical records during periodic site monitoring visits.

Day 0 was the first day of exposure to heparin or fondaparinux, even if there was a short discontinuation (48 hours or less).

Diagnosis of HIT was locally given at the end of the hospital stay of included patients, recorded in the eCRF as either likely or possible, or unlikely HIT. Local investigators relied on a structured analysis on all clinical data, including heparin withdrawal or not, use of alternative non-heparin anticoagulant, and subsequent changes in platelet counts, and ELISA results, but they were blinded to the SRA result performed within the frame of the protocol. Calculation of the 4Ts score was left at the discretion of the investigators.

Plasma collection and laboratory testing

Plasma samples were prepared according to clinical practice for suspected HIT. They were centrally stored at -80°C. SRA was performed as previously described[29] in four GFHT laboratories (without formal cross-validation), the operators being unaware of the patients' clinical data and other laboratory data. The assay was considered positive if a >20% release of serotonin was detected at low heparin concentration but not at high heparin concentration. The assays were batched for study purpose and performed using washed platelets from two to three donors selected for the sensitivity of their platelets to HIT antibodies.

HIT diagnosis

Local diagnosis

Diagnosis of HIT was locally given at the end of the hospital stay of included patients, recorded in the eCRF as either likely or possible, or unlikely HIT. Local investigators relied on a structured analysis on all clinical data, including heparin withdrawal or not, use of alternative non-heparin anticoagulant, and subsequent changes in platelet counts, and ELISA results, but they were blinded to the SRA result performed within the frame of the protocol.

Experts' adjudication

HIT was diagnosed through independent adjudication by two experts among a panel of eight (B.T-P., E.deM., D.L., M-H.H., G.LeG., A.L-LeL., A.G., and T.L. – details at the end of the article), based on the eCRF data: clinical and laboratory data including changes in platelet counts over time, both before and after suspicion, results of laboratory testing for HIT antibodies (ELISA and SRA), and any follow-up data until hospital discharge. When 4Ts scores were locally determined, adjudicators were blinded to them. If the two experts disagreed, a third expert adjudicated the case and the majority vote was adopted. Experts who were also investigators did not adjudicate their own patients. The experts adjudicated all cases locally considered as likely or possible HIT and also adjudicated 30% of cases (selected at random) locally considered as unlikely HIT. The protocol stipulated that for the latter cases, if there were at least one disagreement between the experts and the local investigators, an additional 10% of such cases would be adjudicated. The agreement between the two experts, and between the experts and the SRA results, was calculated (Kappa coefficient).

Statistical analysis

Based on the literature available at the time of the study design, the estimated prevalence of HIT among suspected cases was about 5 to 8%. As the study was designed to take into account a dozen variables, the goal was to recruit a total of 100 to 150 HIT cases, corresponding to a total population sample size of 2,000 patients with suspected HIT. To validate the diagnostic score, 1,000 additional patients (validation cohort) were deemed necessary. Thus a total of 3,000 patients were to be recruited and allocated by randomly splitting the sample into two cohorts: two-thirds of

study participants into the derivation cohort and one-third into the validation cohort. The two cohorts were compared with respect to clinical and laboratory variables.

Development of the model and score construction and internal validation

Analyses were performed on all included patients with available data. Data were processed and analysed using SAS-WINDOWS software version 9.4 (SAS Institute Inc., Cary, NC) and R software, version 3.6.0. Quantitative variables are presented as the number of cases, mean (± standard deviation) or median (interquartile range) as appropriate. Qualitative variables are presented as the number of cases and percentages. The model was developed based on data from the derivation cohort. Clinical variables potentially associated with HIT were evaluated. Each variable was investigated separately as a potential predictive factor, by univariate logistic regression analysis. Any factor that reached the threshold set at $p \leq 0.20$ in the univariate analysis was included in the multivariate analysis. A backward logistic regression method was used, including the variables retained after the univariate analysis. A statistical significance threshold of $p \leq 0.05$ was chosen for selection of the independent explanatory factors. To detect model instability, bootstrapping methods were employed. The Hosmer-Lemeshow goodness-of-fit statistic was used to assess model calibration.

A diagnostic score, herein referred to as 'GFHT-HIT pre-test score', was developed based on regression coefficients from the final multivariate model. Importantly, we disregarded features that are notoriously difficult to capture and/or could be subject to high inter-observer variability[30] such as heparin exposure within past 100 days or presence of other possible causes of thrombocytopenia. The discrimination ability of the score was assessed on the basis of a receiver operating characteristic (ROC) curve and cut-off points were assessed with their respective sensitivity and specificity. Finally, three groups were defined with increasing HIT clinical probability: low, intermediate and high.

In the validation cohort, in addition to the ROC curve, we assessed the predictive accuracy of the score by calculating the prevalence of HIT in three diagnostic probability groups. Operating characteristics were evaluated with standard measures of sensitivity, specificity and predictive values.

Results

Patient characteristics

A total of 2,296 patients were enrolled in 31 tertiary hospitals. Sixteen patients were excluded due to insufficient data or impossibility to follow–up, resulting in a cohort of 2,280 evaluable patients (mean age, 67±15 years; female, 41.5%). Of those, 1,597 were randomly selected for the derivation cohort and 683 for the validation cohort (Figure 1). In the derivation cohort there were 234 cases diagnosed with HIT. As our recruitment goal of 150 cases adjudicated as HIT was reached before the recruitment of the planned total sample size of 3,000 patients, the study was prematurely stopped.

The main characteristics of the patients included in the derivation and validation cohorts are shown in Table 1. There were no statistically significant differences between the cohorts for any item. Many patients in their majority were hospitalized in an intensive care unit (56.8%). Almost half of the patients received UFH, and a therapeutic dose of heparin. Thromboembolism occurred in 12.3% of the patients. Of note, the median of collected platelet counts per patient was eight (Q1-Q3: 5-11) over a median anticoagulant exposure of eight days (Q1-Q3: 5-14). Two experts among the panel independently adjudicated 1,141 cases, comprising all the 616 cases considered by the local investigators as likely or possible HIT and 525 of the 1,664 cases considered as very unlikely HIT by the local investigators as per protocol (Figure 1). Among the adjudicated cases, there were 109 (9.6%) disagreements between the two experts, which needed a third expertise: in 25 cases, one expert ruled out the diagnosis of HIT while the other ruled it in; in the other cases, one expert considered the diagnosis of HIT as likely whereas the other ruled it out. The Kappa coefficient of agreement between the experts was 0.81 [95% CI, 0.78-0.84]. The Kappa coefficient of agreement between the experts' adjudications and the SRA results was 0.83 [95% CI, 0.80-0.87]. The prevalence of patients diagnosed with HIT was 14.7% and 14.5% in the derivation and the validation cohort respectively, while the diagnosis of likely or possible HIT according to the local investigators was 27.5% and 25.9%, respectively. Very few patients (n=22) were included while receiving fondaparinux, and none was diagnosed with HIT.

Development of the model - score construction and validation

Main characteristics of patients in the derivation cohort are shown in Table 2. Results of the univariate analysis are provided as supplementary information. In the multivariate analysis (Table

3), the following features were independently associated with the diagnosis of HIT: therapeutic dose of heparin, cardiac surgery with cardiopulmonary bypass (CPB), major trauma, time-interval from the initiation of anticoagulant treatment to clinical suspicion of HIT comprised between days 5 and 21, rapid decline in platelet count, and presence of a thrombotic event, either arterial or venous. More specifically the most suggestive temporal profile of the changes in platelet count was the following: a decrease of at least 40% over a maximum of six days; of note, it could be observed back to the 10 days preceding the day of the actual suspicion of HIT. There was a borderline statistical significance only for the association with HIT diagnosis of UFH as opposed to LMWH: OR 1.57 [95% CI, 0.99-2.48] (p-value 0.054). Bootstrapping analysis confirmed the features significantly associated with HIT diagnosis; specifically regarding to the type of anticoagulant, OR for UFH was 1.61 [95% CI, 1.01-2.58]. C-statistic of the model was 0.79 [95% CI, 0.76-0.82]; p-value: 0.71 (Hosmer-Lemeshow test).

A score was constructed, based on the regression coefficients (multivariate analysis); its calculation being performed by adding the points assigned to the selected features (Table 3). For a few patients (around 2% - 48/2280), the score could not be calculated since it was not possible to evaluate the item concerning the decrease in platelet count over time owing to missing data. The area under the ROC curve for the GFHT-HIT pre-test score was 0.79 [95% CI, 0.77-0.81] in the derivation cohort (Figure 2).

The score was then applied to the validation cohort (683 patients), of whom 14.5% were eventually diagnosed with HIT. The area under the ROC curve was 0.77 [95% CI, 0.74-0.80] (Figure 2).

Score groups

Grouping is often used to facilitate clinical use of a score. Patients were segregated to three pre-test probability groups: low, intermediate, and high when the GFHT-HIT pre-test score we have elaborated was ≤2, 3-8, >8, respectively. In total there were 137, 1,270 and 825 patients in the three groups respectively. The operating characteristics of the score at the selected cut-off points are shown for both cohorts in Table 4. In the validation cohort, the sensitivity and negative predictive value with the low (screening) cut-off were (with 95% CI) 99.0 [97.5; 100] and 97.4 [86.5; 99.9] respectively; the positive predictive value of a high score was 28.8 [23.4; 34.7]. The

observed prevalences of HIT in each group are shown for both cohorts in Figure 3: the prevalence of HIT increased with the diagnostic probability and reached almost 30% in the high-probability group, which comprises as much as one third of the patients with suspected HIT.

Post-hoc subgroups analyses

Scoring with the 4Ts score

The 4Ts score was routinely used in only seven participating centres, with a likelihood of HIT evaluated as low, intermediate and high in 34.5 %, 62.4 %, and 3.1 % of the 545 patients scored, respectively. Seventy of these patients were finally diagnosed as having HIT, including six with a low 4Ts score. Sensitivity and specificity of intermediate and high probability 4Ts scores (cut-off $\leq$ 3 for low HIT probability) were 91.4 [95% CI, 82.3-6.8] and 38.3 [95% CI, 33.9- 42.8] respectively.

Cardiac surgery with cardiopulmonary bypass

Five hundred and eighty-five patients (26.7%) had undergone cardiac surgery with cardiopulmonary bypass (CPB), and 143 (24.4%) were eventually diagnosed with HIT. For those patients, the Kappa coefficient of agreement between local investigators and the expert adjudicators was 0.77 [95% CI, 0.71-0.83], close to the one observed in the whole population; this indicates that the final adjudication was as good in this particular clinical setting characterized by platelet count changes due to CBP as well. Before surgery, 164 patients (28 %) had received heparin therapy, with either UFH (n = 142) or LMWH (n = 22), for a median duration of 2.5 days (Q1-Q3: 1.0-8.0) and 3.0 days (Q1-Q3: 1.0-6.0], respectively. After surgery, 312 (54%) patients received therapeutic doses of either UFH (n = 280) or LMWH (n = 32), and 273 (46%) patients received prophylactic doses of either UFH (n = 200) or LMWH (n = 70) or fondaparinux (n = 3). For those 585 patients, median time-interval between surgery and HIT suspicion was seven days (Q1-Q3: 4-10). A single patient received a low score (Table 5) and was not diagnosed with HIT; by contrast, 40% (55 out of the 136 patients with an available 4Ts score) presented a low probability 4Ts score, and three of those were diagnosed with HIT (Table 5). Moreover, with the GFHT-HIT score, 29.5% and 70 % of the CPB patients were classified as with intermediate and high probabilities respectively, while with the 4Ts score 56.6% and 3% were classified as with intermediate and high probabilities respectively. In addition, while almost all CPB patients (93%)

diagnosed with HIT were classified as with high HIT likelihood with the GFHT-HIT score, 17% only were classified as with high HIT probability using the 4Ts score (Table 5). Conversely, among the CPB patients classified as with high HIT likelihood using the GFHT-HIT pre-test score, HIT was diagnosed in a sizeable proportion of them: about one third.

Patients with sepsis and patients with shock without sepsis

For patients with sepsis (whether or not shock was present) and in patients with shock of other causes than sepsis, the prevalence of HIT was 6.7%, 8.1%, and 14.4 % respectively; the areas under the ROC curves for the GFHT-HIT pre-test score were in the derivation cohort, 0.73 [95% CI, 0.66-0.78], 0.74 [95% CI, 0.69-0.78], 0.73 [95% CI, 0.65-0.80], respectively; in the validation cohort, 0.84 [95% CI, 0.75-0.91], 0.86 [95% CI, 0.79- 0.92], 0.72 [95% CI, 0.60-0.83], respectively – thus not different from those of the full study set (supplementary material).

Discussion

This large prospective study, which included 2280 patients with suspected HIT, enabled us to construct a model based on eight clinical parameters found to be independently associated with HIT by multivariate analysis. A score, named GFHT-HIT pre-test score, was then derived using recommended statistical methods and was internally validated in an independent cohort of patients. Eventually we distinguished three groups with increasing pre-test diagnostic probability of HIT. The prevalence of HIT increased across the groups, reaching almost 30% in the high-probability group, which comprises as much as one third of the cases.

Independent adjudicators diagnosed HIT based on the prospectively collected data and SRA results. Up to 70 % of the patients with judged by local investigators as not having HIT were not submitted to the experts' adjudication. The way to rule out HIT diagnosis by local investigators relied on a structured approach, which was not uniform among centers however. The local diagnosis, recorded at the end of patients' hospital stay, was based on all clinical data, including heparin withdrawal or not, use of alternative non-heparin anticoagulant, and subsequent platelet count changes, and all laboratory data, including ELISA. In addition, the prevalence of HIT patients in a population with a low probability (according to 4Ts score) was reported to be near

0.7%.[14, 15, 46] Reviewing more than 1600 files to detect at most 10 HIT cases was considered very tough, time-consuming and poorly productive. Nevertheless, in order to check that the prevalence was that low indeed, 525 cases locally considered not being HIT were adjudicated, with no HIT.

The characteristics of included patients with HIT suspicion, with nearly 60% of patients receiving UFH, a quarter of patients having undergone cardiac surgery with CPB and a mean age above 65 years, are in agreement with previous reports, indicating that patients with those characteristics are at high risk of HIT.[31-33]

Some items identified in the present study, namely cardiac surgery with CPB, major trauma, and occurrence of arterial or venous thrombosis, have already been highlighted in previous reports,[32-35] supporting the validity of our results. The time to clinical suspicion of HIT, starting on day 5, was extended up to day 21. Such a time window was previously evoked, and recently confirmed when LMWH has been administered[36]. Of note, in everyday practice, HIT cannot always be suspected as soon as the platelet count starts to decline. Moreover, daily platelet counts are not always available, which makes it very difficult to capture the actual onset of thrombocytopenia. We have thus chosen to record the day of clinical suspicion of HIT by the clinicians in charge of the patients. Moreover, in previous studies on exposure duration to presumed onset of HIT, day 0 could have been set in such a way that duration of exposure was minimized: (i) in some surgical circumstances, such as cardiac surgery, day 0 was sometimes defined as the day of operation,[37,38] although the patient might have received heparin a few days before; (ii) day 0 is sometimes the one when platelet count was found the highest; (iii) since heparin treatment is sometimes interrupted for a couple of days (e.g. to perform a surgical procedure), day 0 can be placed either before or after such a discontinuation - we have considered that discontinuations of 48 hours or less did not require day 0 to be reset.

We found that therapeutic dose of heparin was associated with HIT diagnosis, while heparin dose regimen is not taken into account in the currently available scores. A higher incidence of HIT with therapeutic doses compared with prophylactic doses has been already contemplated in two studies.[39, 40] To our surprise, use of UFH as opposed to LMWH was only of borderline significance in our study. Since a greater risk of HIT with UFH compared to LMWH has been documented,[41] this criterion use was included in the GFHT score though.

There were a total of 70 patients in the derivation cohort (n= 1597) who presented with a platelet count lower than 20.10^9/L without a statistical significant difference whether or not HIT was ultimately diagnosed by the adjudicators or not. Of note, most of the HIT patients with such a severe thrombocytopenia had a high score, combining the items UFH, time to suspicion, and temporal profile of the decrease in platelet count. It is likely that many cases with an atypically very low platelet count were ascribed upstream to a very likely cause for thrombocytopenia, and HIT antibodies were not tested; those cases were therefore not included in the study.

Many experts agreed that speed at which platelet counts declined had to be taken into consideration and we found indeed that a platelet count decrease ≥40% over a maximum of six days was significantly associated with HIT diagnosis. Of note, since clinical suspicion could arise in a somewhat delayed manner as discussed above, such a decrease pace could be detected within the preceding ten days. Compared with a slow decline in platelet counts, a rapid fall is more suggestive, as illustrated by the typical pattern of platelet count changes associated with HIT after cardiac surgery[38], and as recently reported in a refinement of the 4Ts score as well.[44] In our study, a platelet count decline ≥ 40%, whether over three days or over six day, was associated with HIT, but the strongest association was found for a platelet count decline ≥ 40% over six days. To further refine this item about the most suggestive pace, daily platelet counting would have been required, which is not currently recommended.

Recent heparin exposure (within previous 100 days), noticed for 393 (24.6 %) patients of the derivation cohort, was not found to be associated with the diagnosis of HIT. Concerning rapid onset HIT, no patient with such a temporal profile was included. To the best of our knowledge its incidence is unknown since not reported in any prospective cohort studies; it is likely to be very uncommon. Concerning the use of the GFHT-HIT score in a patient with a rapid-onset profile, only the item concerning the pace of platelet count decline would be faulted.

The GFHT HIT pre-test score proved to be reasonably informative in both the derivation and validation cohorts, as assessed by the area under the ROC curves. The high negative predictive with the low cut-off would render resorting to HIT laboratory tests useless in most patients with a low score. However, due to the low number of patients with a low score, this remains to be studied in a prospective study. As a matter of fact, a substantial number of cases had been considered as so

unlikely that they were not included in the study.[28, 45] More specifically, most patients without a reasonably suggestive clinical picture and/or with clear evidence for another cause of thrombocytopenia (patients who would have been classified as low diagnosis likelihood according to the 4Ts score) were not investigated for HIT-antibodies. If the presence or absence of a possible alternative cause for thrombocytopenia (a frequent occurrence) were used for scoring, performance of the score would markedly depend on the experience of the physician making that judgement. On the other hand, the presence of such another cause has to be taken into account in addition to the score so that an experienced physician can decide either to switch to a non-heparin anticoagulant or continue heparin anticoagulation when facing with an intermediate pre-test probability combined with moderately increased OD values of an ELISA.

Considering the positive predictive value with the high cut-off, its appears sensible to immediately stop heparin therapy and initiate a non-heparin based anticoagulation without waiting HIT laboratory tests results for patients with a high score. As a matter of fact, thrombotic complications of HIT can be devastating and one third of patients with a high score will be eventually diagnosed with HIT.

Our study was not designed to compare the score we obtained with the well-known 4Ts score. Due to the sustained interest in and the critical appraisal of such a score designed for a sequential diagnostic approach (i.e. pre-test score), we nevertheless performed a post-hoc analysis in the subset of included patients for whom the 4Ts was available, as completed in real time by the local investigators. We observed a lower sensitivity of the 4Ts score (91.4%) than previously reported in a meta-analysis (99%)[46] but consistent with that previously reported (81.3%) in a similar population of 526 patients including 23 % with CPB surgery.[21]

Among the eight clinical parameters found to be independently associated with HIT, some of them (pace of platelet count decline, type and dose of heparin) are not taken into account in the 4Ts score. They had been already considered to discriminate HIT from thrombocytopenia due to other causes.[27, 44]. Whether the incorporation of those parameters into the 4Ts score or whether the score of the present study actually outperforms the 4Ts score can only be solved with a formal prospective comparison. The currently offered Bayesian analysis based on 4Ts and one based on the GFHT-HIT score, both combined with ELISA OD, deserve to be compared. Such an approach, while waiting for the result of a platelet activation test, could be of the greatest usefulness in specific populations such as patients with CPB. Indeed in such a setting, attending physicians

might be reluctant to switch to an alternative anticoagulation when there are both high haemorrhagic and thrombotic risks.

The highest prevalence of HIT is observed among patients having underwent CPB [28] and HIT was indeed diagnosed in one out of four such patients in the present study. Of note, low platelet counts within the first few days were not considered for the suspicion of HIT, and thus those patients were not included into the study. With the score offered by Lillo-Le Louët *et al*., failures to rule out HIT for CBP patients have been observed in a retrospective analysis of 84 patients - false negative rate: 3 % [12]. In the present study, two thirds of CPB patients were classified into the high pre-test diagnostic probability (as expected, cardiac surgery with CPB being in the GFHT score calculation) and HIT was diagnosed in one third of them. By contrast, because of the large number of other causes of thrombocytopenia post CPB, most (56.6%) such patients with suspected HIT and included in the study received an intermediate probability 4Ts score, when performed (n= 136 - see the corresponding results on table 5). In our opinion, the use of the GFHT HIT pre-test score for CPB patients with HIT suspicion could enable with a reasonable confidence to promptly switch to non-heparin anticoagulation in patients with a high score while waiting for the completion of the diagnostic work-up. For patients with an intermediate probability of HIT according to the score, great caution should be exercised about the decision to immediately stop heparin administration – again a decision that should involve an experienced physician. It is also well known that a positive immunoassay is observed in many patients after CPB even if they are not suspected of HIT.[47] Immunoassays are not very helpful in distinguishing HIT from non-HIT thrombocytopenia in that setting. We are currently analysing the results of a centrally performed immunoassay for HIT IgG antibodies and we hope that we will be able to find discriminant cut-off values (OD) to better estimate the post-test probability of HIT for each score group, as previously reported in non-CPB patients.[48]

To sum up, for the first time to our knowledge, an HIT pre-test score based on objective features identified by a multivariate analysis, named GFHT HIT pre-test score, has been constructed and validated in a large population of patients with a high prevalence of HIT. The diagnosis of HIT was based on independent adjudication by two experts per case, blinded with respect to each other's judgments: this is one of the strength of the study. There was a good agreement between the experts, with contradictory judgments in less than 10% of cases, which were all re-evaluated

by a third one. While it is likely that adjudicators used some of the variables that ended being selected by the model to make their judgment on HIT diagnosis, at the time of adjudication no statistical analysis had obviously been performed. Moreover, beyond the presence or absence of some risk factors or clinical features, the strength of the association (hence the weight carried by each item in the score), the independence from other predictors in the multivariate analysis, and the calibration of the model to identify groups of increasing prevalence of HIT are in our opinion important features from the scoring system. Although SRA is the gold standard platelet activation test to confirm the diagnosis of HIT, false positive or negative results are possible.[7, 49-50]. Thus we did not rely on SRA in isolation. Moreover the design of our study enabled us to diagnose HIT in case of 'indeterminate' SRA results as well. [51] Eight clinical parameters found to be independently associated with HIT by multivariate analysis. They are easy to identify and should not be vulnerable to inter-observer variability, facilitating reproducibility. However, the convenience and efficiency in clinical practice and thus the time required to score depend on many factors, including the full and accurate knowledge of the medical current and previous patient's histories. This knowledge itself depends on the ability to retrieve such data. The convenience of the GFHT-HIT score remains to be evaluated, as well as the inter-observer variability. We did not incorporate the item "other diagnosis of thrombocytopenia", which can be highly dependent on the physician's judgment and which may be inextricable despite careful scrutiny of complex cases. In addition to an external validation, the next step will be to elaborate a diagnostic algorithm for HIT that incorporates the GFHT-HIT pre-test score and a stratified interpretation of (semi-) quantitative immunoassays for HIT antibodies. As a follow-up of the GFHT-HIT study, we are analysing additional data concerning testing and titrating IgG HIT antibodies (centrally performed ELISA). We hope that we will be able to offer discriminant cut-off values (OD) for each score groups to better estimate the probability of HIT. After that, we intend to proceed to a Bayesian analysis.

In a few cases (<2%) only, it was not possible to assess the important criterion bearing on the temporal course of platelet count decline due to infrequent testing determination. Obviously, missing or unclear data concerning platelet counts and/or heparin administration remain a problem common to all manners of clinical appreciation of the probability of HIT. [52]

Our study has limitations. (1) As patients were considered for inclusion by experienced physicians of the local thrombosis unit, those with strong evidence for alternative diagnosis were not included. This explains the underrepresentation of low likelihood cases in the study.

(2) As a consequence of above, the study population does not fully align with the population to which the scoring system will eventually be applied. However, such a design enabled us to get enough clinically relevant events to be able to run the multivariate logistic regression analysis, and a not too low prevalence of HIT cases. It would not have been feasible to perform SRA for all cases referred to thrombosis units of tertiary care hospitals, and it was already well established that those patients are overwhelmingly tested negative for HIT antibodies and considered not suffering from HIT. Note that if anything, including a higher pre-test probability population likely made our task of identifying low risk patients harder and could actually be seen as a strength of our study.

(3) Relevant data pertaining to the follow-up after the inclusion day were available to the adjudicators and were of importance in their ultimate decision, but the interpretation of the criterion related to the changes in platelet counts after stopping heparin could have sometimes not been easy - it was left to adjudicator's' judgment.
Moreover, as for other HIT pre-test assessments, a pure Bayesian approach of pre-test likelihood assessment at the time of ordering laboratory tests and full results of the latter can be insufficient, and clinical features and changes in platelets counts having occurred meanwhile have to be taken into account in clinical practice. As previously envisioned for the 4Ts score, it could be wise to score again in order to take into account new data, and improved information on features of the pre-test phase.

(4) The expert's adjudication regarding HIT was based on patients' clinical and laboratory data. There was no formal way to incorporate laboratory results by the adjudicators, but the Kappa coefficients of agreement between experts and between experts and SRA results were very good.

(5) The absence of a standardized manner to consider alternative diagnoses represents an obvious challenge for any HIT scores. Recent efforts were made to standardize the 4th criterion of 4Ts score, which regards other potential causes

(6) The GFHT-HIT score cannot rule out the diagnosis of HIT in CBP (+2 points) and trauma (+3 points) patients does not seem possible. However, it is important to note that such patients were included only when they did not present strong evidence for an alternative cause of thrombocytopenia. For such patients with either the early expected fall in platelet count after cardiac surgery or in the setting of massive haemorrhage, HIT should not be among the main

differential diagnoses for thrombocytopenia.

(7) SRA was performed at four laboratories, and some interlaboratory variability is likely. Any effort was made to limit it though, and tests with 'indeterminate' results were referred to another laboratory in an attempt to get definite results.

(8) Similarly, the type of ELISA test was the one locally implemented without cross-validation between the participating centres. Without formal comparison between the three kits used in the study, some potential differences in their results may exist. Moreover, there was no uniform explicit manner on how the adjudicators should have taken into account the quantitative ELISA results (OD).

(9) The comparison with the 4Ts score was not in the design of the study. A head-to-head comparison is required to firmly establish the superiority of the score we propose over the previously developed ones. Whether our score would help rule out HIT by physicians in charge of the patient, thus preventing unnecessary laboratory testing with its attendant flow of false positive results, remains to be tested for all patients with declining platelet counts while receiving heparin. The study design as explained above, with the upstream involvement of experienced physicians of the thrombosis team, led to an underrepresentation of low likelihood cases in the GFHT study. Since further prospective investigations are required to externally validate, to assess the usefulness regarding clinical outcome, and to determine the best way to combine pre-test assessment with the help of the score with laboratory testing for HIT antibodies, there will be the opportunity for such a head-to-head comparison. In addition to that it could be a great opportunity to evaluate the interobserver variability with the GFHT-HIT score.

(10) Lastly, the study was carried out in 31 European tertiary centres (in three countries), and risk factors for HIT might somewhat vary between clinical settings, and depend on practices regarding the type of anticoagulant treatment and dosage.

In conclusion, we obtained a HIT diagnostic model with pre-test features identified with a multivariate analysis of prospectively collected data. We derived from the model a score based on eight easy-to-collect objective items and thus the score is less likely to depend on the physician's experience. The GFHT-HIT pre-test score deserves external validation and further investigation, in combination with laboratory testing, in management studies with clinical outcomes.

Authors Contributions: B.T-P., T.L. and B.T. designed the study. B.T-P., E.deM., M-A.G, D.L, C.M., A.S-S., A.B., F.M., J.P., P.M., L.G., I.E., and Y.G. enrolled patients. C.P., M.A-G., P.N. and I.E. performed the serotonin release assays. B.T-P., E.deM., D.L., M-H.H., G.LeG., A.L-LeL., A.G., and T.L. were the members of the adjudication committee. E.P. undertook statistical analysis. All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. B.T., T.L, A.G., P.M., G.LeG., F.M. and Y.G. drafted the manuscript. All authors reviewed the manuscript and approved the final version. B.T. is the guarantor.

Conflicts of interest

Dr. Mullier reports personal fees from Aspen, personal fees from Stago, grants from Werfen, outside the submitted work. Dr. Gruel reports personal fees from Sanofi, personal fees from LFB, other from Stago, personal fees from Aspen, outside the submitted work. Dr Greinacher reports grants and non-financial support from Aspen, Boehringer Ingelheim, MSD, Bristol Myers Squibb, Paringenix, Bayer Healthcare, Gore Inc., Rovi, Sagent, Biomarin/Provensa,, personal fees from Aspen, Boehringer Ingelheim, MSD, Macopharma, BMS, Chromatec, Instrumentation laboratory, non-financial support from Boehringer Ingelheim, Portola, Ergomed, GTh e.V. outside the submitted work.

None of the other authors report any conflicts of interest.

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Other contributors: Operation team (CIC1408): F Rancon (Project Manager); M Akrour (Clinical Research Assistant).

Investigators (by country and center; in order of decreasing numbers of enrolled patients): France: CHU Saint-Etienne, Saint-Etienne, (224): B Tardy-Poncet, E Chalayer, M Piot; CHU Besançon, Besançon, (196): E Racadot; Hôpital Bichat, AP-HP, Paris, (175): MG Huisse; CHU

Clermont-Ferrand, Clermont-Ferrand, (146): A Serre-Sapin, A Lebreton; CHU Nancy, Nancy, (145): M Toussaint-Hacquard, J Perrin; CHU Dijon, Dijon, (139): E de Maistre, A Grandvuillemin; CHU Bordeaux, Bordeaux, (137): C Mouton, A Bidet; CHU Lille, Lille, (118): A Bauters, N Trillot, A Vincentelli; AP-HM, Marseille, (111): W Cohen, P-E Morange, L Camoin; CHU Toulouse, Toulouse, (98): F Guerrero, S Voisin, P Sié; CHU Strasbourg, Strasbourg (86): L Grunebaum, D Desprez, L Sattler; Hôpital Henri Mondor, AP-HP, Créteil, (83): C Lavenu-Bombled; Hôpital Européen Georges Pompidou, AP-HP, Paris, (74): M Alhenc-Gelas; CHU Tours, Tours, (70): Y Gruel; CHU Reims, Reims (69): K Lafont; Hôpital Tenon, AP-HP, Paris (58): A Khaterchi; CHU Brest, Brest (53): MT Blouch; CHU Montpellier, Montpellier (40): E Boissier, C Biron; Hôpital Marie Lannelongue, Le Plessy Robinson (35): S Doubine; CHU Rouen, Rouen (27): MH Chretien; CHU Nantes, Nantes (24): C Ternisien; CH Colmar, Colmar (23): I Mazurier; Hôpital Antoine Béclère, AP-HP, Clamart (19): M Wolf ; HCL, Lyon (8): L Rugeri; CH Foch, Suresnes (5): D François; Hôpital Pitié Salpetrière (2): I Martin-Toutain. Belgium: CHU UCL Namur, Yvoir, Belgium (58): F Mullier, B Chatelain. Switzerland: Hôpitaux Universitaires de Genève, Geneva, Switzerland (18): F Boehlen; CHU Vaudois, Lausanne, Switzerland (12): A Angelillo-Scherrer.

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Figure legends

Figure 1. Flow chart of the study

The experts adjudicated all cases considered as likely or possible HIT by the local investigators (N=616), and 30% of cases (selected at random) considered as very unlikely HIT by the local investigators. Diagnosis of HIT was ruled in (+) or out (-) by the experts

Figure 2. Receiver operating characteristic (ROC) curves (with 95% CIs) for the HIT score in the derivation and validation cohorts

Figure 3. Prevalences of HIT according to score groups in the derivation and validation cohorts.

For a total of 2 % of patients (35 patients and 13 patients in the derivation and validation cohort respectively), the score was not established, as it was not possible to evaluate the item concerning the decrease in the platelet count over time owing to missing data.

		Derivation cohort	Validation cohort	P-value
		n= 1597	n = 683	
Age, years (SD)		66.8 (15.3)	67.6 (15.4)	0.31
Females (%)		670 (42)	277 (40.6)	0.54
Clinical context				
	Sepsis (%)	548 (34.3)	219 (32.1%)	0.30
	Active cancer (%)	288 (18)	134 (19.6)	0.37
	Major trauma* (%)	30 (1.9)	6 (0.9)	0.09
	Shock (%)	369 (23.1)	158 (23.1)	1.00
	Cardiac surgery with CPB (%)	399 (25)	186 (27.3)	0.27
	Orthopedic surgery (%)	58 (3.6)	23 (3.4)	0.80
	Multiple transfusion (%)	288 (18.1)	119 (17.4)	0.76
Intensive care unit (%)		918 (57.5)	377 (55.2)	0.33
Anticoagulant treatment				0.33
	UFH (%)	901 (56.4)	407 (59.6)	
	LMWH (%)	409 (25.6)	165 (24.2)	
	Fondaparinux (%)	16 (1)	6 (0.9)	
	Several * *(%)	271 (16.9)	105 (15.4)	
Anticoagulant regimen				0.81
	Therapeutic (%)	786 (49.2)	322 (48.6)	
	Prophylactic (%)	811 (50.8)	351 (51.4)	
Duration of exposure to anticoagulant$^{\alpha}$, days Median (Q1-Q3)		8 (5-14)	8 (4-14)	0.68
Platelet count nadir (G/L) Median, Q1-Q3)		76 (50-100)	75.5 (50-105)	0.51
Platelet count nadir		631 (39.7)	250 (36.9)	0.62

between 40 and 80 G/L				
Thrombotic event				
	Arterial (%)[ε]	73 (4.5)	45 (3.2)	0.05
	VTE (%)[ε]	136 (8.5)	60 (8.7)	0.75
	Dialysis filter (%)[ε]	20 (1.3)	6 (0.9)	0.94
	At least one event (%)[ε]	189 (11.8)	92 (13.5)	0.29
	Total (%)[β]	224 (15.4)	110 (16.2)	0.61
HIT eventually diagnosed		234 (14.7)	99 (14.5)	0.94
Death (%)		366 (22.9)	163 (23.9)	0.62

Table 1: Main characteristics of the patients in the derivation and validation cohorts

CPB: Cardiopulmonary bypass, UFH: unfractionated heparin LMWH: low-molecular weight heparin; *Major trauma is defined as the Injury Severity Score being greater than 15. **Some patients sequentially received two anticoagulants; α: the duration corresponds to the time-interval form the first day of heparin administration (including a possible interruption of heparin of no more than two days) until the day of suspicion of HIT. ε: observation extended beyond HIT suspicion up to the following 48 hours; β: thrombotic events that occurred during the entire period of follow-up. HIT eventually diagnosed: final diagnosis by the experts

		HIT (+)	HIT (-)
		n= 234	n = 1363
Age, years (SD)		67.1 (15.2)	66.7 (15.4)
Females (%)		111 (47.4)	529 (41)
Clinical context			
	Sepsis (%)	41 (17.5)	507 (37.2)
	Active Cancer (%)	23 (9.8)	265 (19.4)
	Major trauma* (%)	9 (3.8)	21 (1.5)
	Shock (%)	36 (15.4)	333 (24.4)
	Cardiac surgery with CPB (%)	96 (41)	303 (22.3)
	Orthopaedic surgery (%)	13 (5.6)	45 (3.3)
	Intensive care unit (%)	125 (53.4)	793 (58.2)
Anticoagulant treatment			
	UFH (%)	151 (64.5)	750 (55.0)
	LMWH (%)	30 (12.8)	379 (27.8)
	Fondaparinux (%)	0 (0.0)	16 (1.2)
	Several **(%)	53 (22.6)	218 (16)
Anticoagulant regimen			
	Therapeutic (%)	153 (65.4)	633 (46.4)
	Prophylactic (%)	81 (34.6)	730 (53.6)
Duration of exposure to the anticoagulant, days Median (Q1-Q3)		10 (8-13)	8 (4-14)
Platelet count nadir (G/L) (%)			
	Between 20 and 40	35 (15)	159 (11.7)
	Between 41 and 80	98 (41.9)	533 (39.3)

	Between 81 and 120	72 (30.8)	399 (29.4)
Timing of HIT suspicion			
	< 5 days and >21 days	17 (7.3%)	500 (36.7)
	Between 5 and 15 days	184 (78.6)	740 (54.3)
	Between 15 and 21 days	33 (14.1)	123 (9.0)
% of platelet count decrease			
	More 50% on day -5	101 (58.0)	385 (53.4)
	Between 40 and 50 % on day -5	22 (12.6)	92 (12.8)
	More 50 % on day-6	95 (56.2)	351 (55.5)
	Between 40 and 50 % on day -6	27 (16.0)	66 (10.4)
	≥50 % over 2 days during the last 6 days	99 (46.3)	482 (39.1)
	≥50 % at least over 3 days during the last 6 days	130 (59.6)	628 (49.6)
	≥40% over 3 days during the last 10 days	178 (77.1)	874 (65.6)
	≥40% over 6 days during the last 10 days	35 (15.2)	159 (11.9)
Thrombotic event			
	Arterial (%)[ε]	24 (10.3)	39 (2.9)
	VTE (%)[ε]	55 (23.5)	81 (5.9)
	Dialysis filter (%)[ε]	5 (2.1)	15 (1.1)
	At least one event (%)[ε]	189 (11.8)	92 (13.5)

Table 2: Main characteristics of patients of the derivation cohort with and without a diagnosis of HIT

CPB: Cardiopulmonary bypass, LMWH: low-molecular weight heparin; *Major trauma is defined as the Injury Severity Score being greater than 15. **some patients received sequentially two anticoagulants; ε: observation extended beyond HIT suspicion up to the following 48 hours; Day 0 in this table is the day of clinical suspicion of HIT

		Regression Coefficients (SD)	Odds Ratio (95% CI)	P Value	Scoring
Anticoagulant types	UFH	0.45 (0.23)	1.57 [0.99 ; 2.48]	P = 0.054	+ 1
Anticoagulant doses	Therapeutic	0.44 (0.17)	1.55 [1.12 ; 2.14]	P = 0.009	+ 1
Cardiac surgery with CPB		0.74 (0.17)	2.11 [1.51 ; 2.94]	P < 0.0001	+ 2
Major Trauma		1.38 (0.47)	3.98 [1.59 ; 9.96]	P = 0. 003	+ 3
Platelet count decrease	≥40% over ≤ 6 days during the last 10 days	1.14 (0.27)	3.12 [1.83 ; 5.30]	P < 0.0001	+ 3
Thrombotic event	Arterial	1.16 (0.30)	3.19 [1.76 ; 5.79]	P < 0.0001	+ 3
	Venous, or PE	1.49 (0.24)	4.45 [2.78 ; 7.12]	P < 0.0001	+ 3
Time to diagnosis suspicion	Between 5 and 21 days	1.94 (0.27)	6.95 [4.08 ; 11.8]	P < 0.0001	+ 4

Table 3: Results of the multivariate analysis

CPB: Cardiopulmonary Bypass; PE: Pulmonary embolism

C-statistic: 0.79 [0.76; 0.82]; p value with Hosmer-Lemeshow test = 0.71

	Cut-off	Sensitivity	Specificity	PPV	NPV
Derivation cohort, % [95% CI]	≤ 2	100.0 [98.4; 100]	7.4 [6.0; 8.9]	15.8 [13.9; 17.7]	100 [96.3; 100]
	> 8	72.3 [66.0; 78.0]	70.4 [67.9; 72.8]	29.8 [26.0; 33.5]	93.6 [91.9; 95.0]
Validation cohort, % [95% CI]	≤ 2	99.0 [97.5; 100]	6.65 [4.75; 9.00]	15.5 [12.8; 18.6]	97.4 [86.5; 99.9]
	> 8	76.8 [67.2; 84.7]	67.1 [63.1; 70.9]	28.8 [23.4; 34.7]	94.3 [91.6; 96.4]

Table 4: Operating characteristics of the score in the derivation and the validation cohorts

PPV: positive predictive value; NPV: negative predictive value

a	**HIT probability**	**Patients with CPB (%)**	**CPB Patients diagnosed with HIT (%)**	**Patients without CPB (%)**
Score of the present study	Low	1 (0.2)	0 (0)	136 (8.2)
	Intermediate	172 (29.5)	10 (7)	1098 (66.6)
	High	410 (70.3)	133 (93)	415 (25.2)
b				
4Ts score	Low	55 (40.4)	3 (13)	133 (32.5)
	Intermediate	77 (56.6)	16 (69.6)	263 (64.3)
	High	4 (3.0)	4 (17.4)	13 (3.2)

Table 5: Distributions of the 583 patients with CPB cardiac surgery into the three groups of HIT probability.

a) **Distribution of the 583 patients$^{\alpha}$ having undergone CPB cardiac surgery and of the 143 HIT patients among them into the three groups of HIT probability according to the score of the present study.**
b) **Distribution of the 136 patients having undergone CPB cardiac surgery and received a 4Ts score, and of the 23 HIT patients among them into the three groups of HIT probability according to the 4Ts score.**

With both scores three groups can be delineated with low, intermediate and high HIT probabilities; with the score of the present study: ≤ 2, 3-8, and >8 respectively; with the 4Ts score: ≤ 3, 4-6, and >6 respectively.

The distributions for the patients not having undergone CPB are shown in the far-right column, to enable comparison.

CPB: CardioPulmonary Bypass (cardiac surgery with); HIT: Heparin induced Thrombocytopenia

α: The score of the present study could not be calculated for two patients since the platelet count decrease criterion could not be assessed.

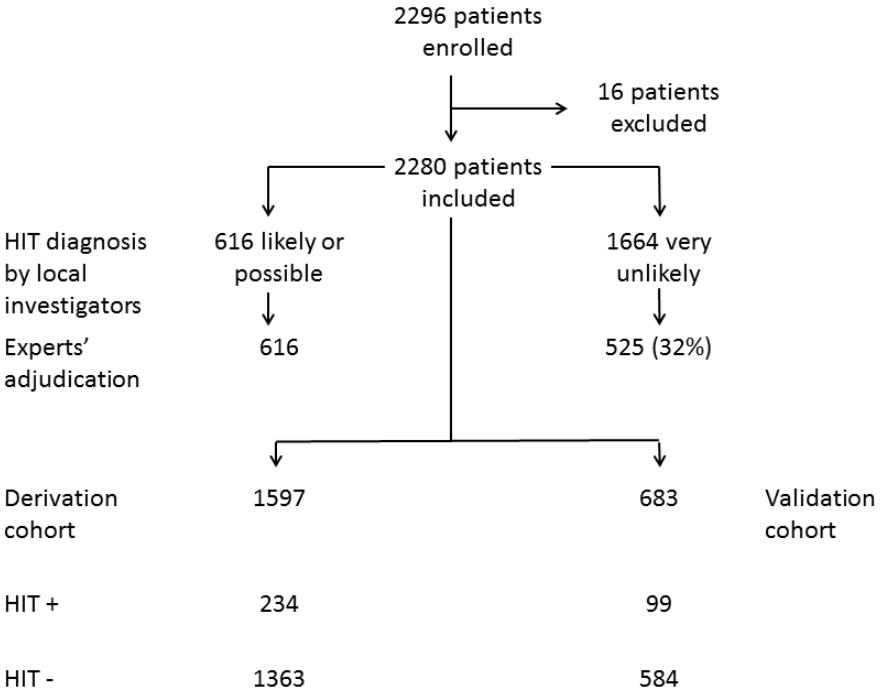

Figure 1

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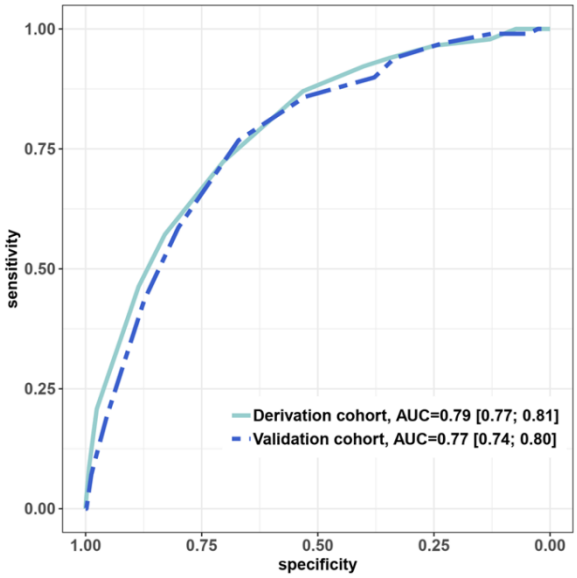

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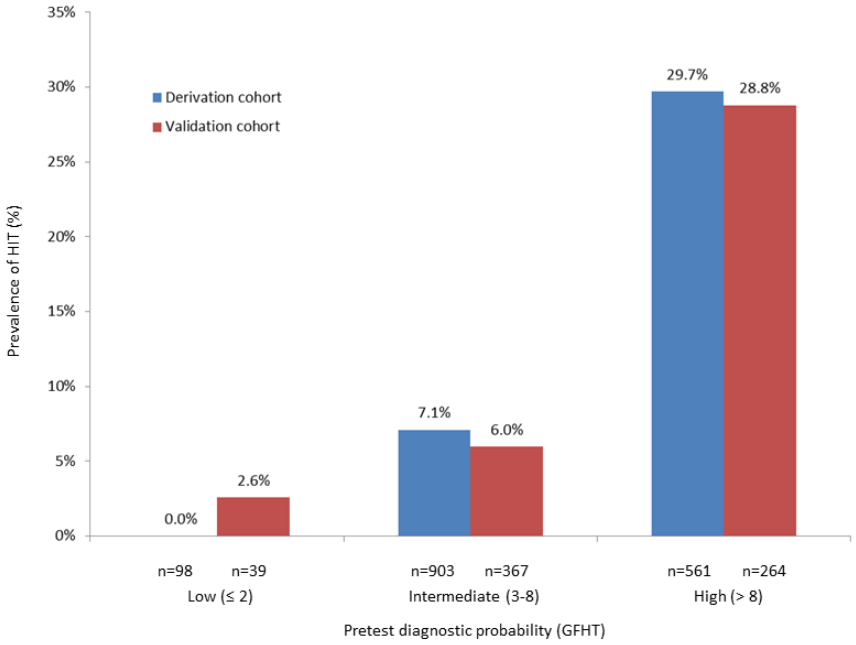

Figure 3

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