Validation of the high-heparin confirmatory step for the diagnosis of heparin-induced thrombocytopenia

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Presented in abstract form at the 49th annual meeting of the American Society of Hematology, Atlanta, GA, December 10, 2007

Running title: HIGH HEPARIN CONFIRM OF HIT

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Abstract

The diagnosis of heparin-induced thrombocytopenia (HIT) requires detection of antibodies to the heparin/platelet factor 4 (PF4) complexes via enzyme-linked immunosorbent assay (ELISA). Adding excess heparin to the sample decreases the optical density (OD) by 50% or more and confirms the presence of these antibodies. One-hundred fifteen patients with anti-heparin/PF4 antibodies detected by ELISA were classified as clinically HIT-positive or HIT-negative, followed by confirmation with excess heparin. A multivariate logistic regression model was fitted to estimate relationships between patient characteristics, laboratory findings, and clinical HIT status. This model was validated on an independent sample of 97 patients with anti-heparin/PF4 antibodies. No relationship between age, race, or gender and clinical HIT status was found. Maximal OD and confirmatory positive status independently predicted HIT in multivariate analysis. Predictive accuracy on the training set (c-index 0.78, Brier score 0.17) was maintained when the algorithm was applied to the independent validation population (c-index 0.80, Brier score 0.20). This study quantifies the clinical utility of the confirmatory test to diagnose HIT. Based upon data from the heparin/PF4 ELISA and confirmatory assays, a predictive computer algorithm could distinguish patients likely to have HIT from those who did not. Prospective analyses are in progress to validate this strategy.
Introduction

Diagnosis of heparin-induced thrombocytopenia (HIT) requires that patients fulfill certain clinical criteria as well as demonstrate the presence of antibodies that bind to the complex of heparin and platelet factor 4 (PF4). Clinical criteria for HIT are generally well accepted, and include thrombocytopenia with or without thrombosis that develops in temporal association with heparin therapy and in the absence of other causes of platelet count decline. The diagnosis of HIT can be challenging, however, as critically ill patients can have multiple potential causes of thrombocytopenia. As many as half of the patients with HIT will have a thrombotic complication at presentation, and, from retrospective data, it has been demonstrated that an additional half of those without thrombosis at presentation will subsequently develop a thrombotic complication. Therefore, prompt recognition of this disorder is necessary so that appropriate treatment can be initiated to prevent the development of thrombotic sequelae.

Laboratory testing for HIT includes both antigen and functional (platelet activation) assays to detect heparin/PF4 antibodies. The $^{14}$C-Serotonin Release Assay (SRA), a functional assay which requires the use of radioactive material, is technically demanding, and is available at only a few reference laboratories. The most widely available test for HIT is the heparin/PF4 ELISA. This assay detects antibodies that bind to PF4 complexed to heparin (Diagnostica Stago, Parsippany, NJ, USA) or other negatively charged ligands (GTI Diagnostics, Waukesha, WI, USA) coated on microtiter plates. The test is very sensitive to the presence of anti-heparin/PF4 antibodies (> 97%),1 but it is less specific for the clinical syndrome of HIT (50-89% specificity), due to the detection of nonpathologic antibodies (antibodies present in the absence of clinical manifestations of HIT).
The manufacturer of one commercial immunoassay (GTI Diagnostics) recommends use of a high heparin confirmatory procedure to improve the specificity of the ELISA. In this particular assay, inhibition of a positive ELISA result by 50% or more in the presence of excess heparin (100 U/mL) is considered confirmatory of heparin-dependent antibodies. The significance of a negative confirmatory result is unknown, however, and there are data that suggest in the cardiac surgery patient population, the confirmatory result does not improve the diagnostic specificity of the heparin/PF4 ELISA. In a previous retrospective review of patients with a positive PF4 ELISA at our large university-based tertiary care center, we found that the majority of patients with antibodies and a positive confirmatory test met clinical criteria for HIT. This led to a hypothesis that the confirmatory assay provides additional useful information in the laboratory diagnosis of HIT.

To quantify the information contributed by the PF4 ELISA OD value and the confirmatory assay, we developed a predictive statistical model for HIT. The goal of this study was twofold: 1) to determine the diagnostic value of the heparin confirmatory test in the assessment of patients for HIT and, 2) to generate a clinically useful predictive tool to facilitate the diagnosis of HIT.
Patients, Materials, and Methods

Patients

This retrospective study was approved by the Institutional Review Board at Duke University Medical Center (DUMC). Using the data from the DUMC Coagulation Laboratory, all in-patients with a positive anti-heparin/PF4 antibody result determined by a commercial ELISA (GTI Diagnostics, Waukesha, WI, USA) during 2005 (training set) and the first 97 consecutive patients in 2006 (validation set) were included in this study. A threshold optical density (OD) measurement of 0.40 was defined as positive for the presence of anti-heparin/PF4 antibody.

Data Collection

All DUMC records were reviewed from the patient’s hospitalization and for up to 30 days when records were available, but patients were not contacted. Data collected for this study included: age, race, gender, medical service, dates of platelet count decline, platelet count nadir, platelet count increase and normalization, dates of anti-heparin/PF4 antibody testing, anti-heparin/PF4 antibody OD values, dates and types of heparin administration, dates and types of thromboembolic events, and patient death and cause of death.

All venous thrombotic events documented in patient records were confirmed by review of radiographic reports. Arterial thrombotic events, including stroke and myocardial infarction, were documented via radiographic studies (magnetic resonance imaging, computed tomography, or cardiac catheterization reports) but also included intra-operative assessment of bowel infarction, autopsy findings, and several cases of digital or limb ischemia based upon physical examination performed by vascular surgeons.
Chart reviews were systematically conducted using the diagnostic criteria summarized below by two of the investigators (N.L.W., T.L.O.). Since all of the patients included in the chart review had positive anti-heparin/PF4 ELISA results, the reviewers could not be blinded to assay results or that the diagnosis of HIT was clinically possible.

**PF4 Confirmation**

Patients with positive anti-heparin/PF4 antibodies were divided into Confirm+ and Confirm- groups for analysis. The confirmatory step with excess heparin was performed per manufacturer guidelines\(^9\). A positive confirmatory result was defined as >50% decrease in antibody binding in the presence of heparin (Confirm+). Likewise, a negative confirmatory test (Confirm-) was defined as a ≤50% decrease in antibody binding in the presence of heparin. For patients with more than one PF4 ELISA assay performed, the confirmatory test result of the initial positive PF4 ELISA (OD ≥ 0.4) designated the patient as Confirm+ or Confirm-. The maximal OD value of the positive test results for patients with multiple tests was utilized for data analysis.

**Definition of Clinical HIT Status**

Extensive chart review was undertaken to clinically diagnose all patients with anti-heparin/PF4 antibodies as having HIT or not, as previously described\(^8\). Criteria from the American College of Chest Physicians (ACCP) guidelines were used to make the clinical diagnosis of HIT: (1) thrombocytopenia defined as at least a 30% decline in the platelet count, with platelet count increase upon heparin cessation, (2) timing of platelet count fall between 4 and 14 days after heparin exposure or within 24 to 48 hours if recent (within last 100 days) heparin exposure, and
(3) lack of other, predominant causes of thrombocytopenia. For patients after cardiac bypass surgery, a platelet count drop of 50% or more from the highest post-operative value occurring between postoperative days 4-14 or persisting for six or more days after surgery was considered consistent with a diagnosis of HIT.

For this study, patients were classified as either having HIT (“HIT+”) or not (“HIT-”). Patients who met all three criteria for the clinical diagnosis of HIT were classified as HIT+. In addition, patients who met the first two criteria for HIT, but who also had potential alternative diagnoses to explain the thrombocytopenia were also categorized as HIT+. We used this approach for this study because many of these patients were in the ICU setting and had several potential causes for thrombocytopenia, but HIT could not be clearly eliminated from the differential diagnosis. Patients were also classified as HIT+ in the rare situation of an unexpected thromboembolic event after heparin exposure but prior to the development of thrombocytopenia. This was based on reports suggesting that anti-heparin/PF4 antibodies can result in a significantly increased risk for thrombosis and HIT in cardiac patients prior to the development of a platelet count decline.

Statistical Analysis

Statistical analysis and model fitting was performed using SAS 9.1 software (SAS Institute, Cary, NC, USA). Univariate analysis was performed on the 2005 patient population after the patients were clinically classified as HIT+ and HIT-. The initial goal was to determine if there was a relationship between clinical HIT status and age, race, gender, service of admission, PF4 OD value, or confirmatory assay result. Service categories were collapsed into Medical
(medicine, cardiology, neurology, bone marrow transplant/oncology) or Surgical (orthopedics, general surgery, cardiothoracic surgery). The *t*-test was utilized for continuous variables (age, PF4 OD value) and χ²-test for dichotomous variables (confirmatory positive, gender, race, medical service). A *P* value of <.05 was considered statistically significant.

Univariate logistic regression models were fitted to the 2005 data using clinical HIT status (+/-) as the dependent variable, and log anti-heparin/PF4 OD value, confirmatory positive status, and service category as the independent variables. Prior to constructing a multivariate model, collinearity diagnostics were performed. Collinearity exists when there is a pronounced association between two independent variables, making the effect of the independent variables upon the dependent variable in regression analysis difficult to quantify. In our study, collinearity diagnostics were necessary because a positive confirmatory test result might conceivably be associated with high anti-heparin/PF4 OD values. Collinearity is expressed as variable inflation factor (VIF), with large values (i.e. >10) implying that collinearity is present between two independent variables.

A multivariate logistic regression model was fitted to the 2005 data. The model was then validated on the independent sample of 97 patients with positive anti-heparin/PF4 antibodies in 2006. Predicted probabilities were generated for each patient using an inverse logit transformation. A nomogram summarizing the logistic regression model was developed using the Design library in R 2.9[15].
Assessment of Model Performance

Model performance was assessed using SAS 9.1 (SAS, Inc., Cary, NC) and R 2.9\(^15\). The most important property of a predictive model is discrimination, the ability of a model to distinguish between patients who are likely to have clinical HIT from those who are not\(^16\). Model discrimination was assessed using the concordance probability, or c-index\(^17\). The c-index is equivalent to the area under the receiver operating characteristic (ROC) curve\(^18\), and related to Somers’ \(D_{yx}\) rank correlation \([D_{yx} = (c-0.5)/0.5]\). In other words, the c-index is the probability that, of 2 patients drawn randomly from the population, one who had clinical HIT and the other who did not, the patient with HIT would have the higher prediction of HIT. A c-index of 0.5 would be equal to chance discrimination (e.g., a coin flip); a c-index of 1.0 would be a perfect predictor for HIT.

A second property of predictive models is reliability (precision). In a reliable model, if 100 patients were assigned an 80% probability of HIT, 80 of the patients should actually have clinical HIT. Generally, discrimination is valued over reliability. A poorly reliable model can be recalibrated, but there is no remedy for a model that discriminates poorly. The Brier score evaluates both discrimination and reliability on a scale from 0 to 1, with lower scores indicating better predictive performance\(^19\). Informative predictive models should not have Brier scores greater than 0.25. Harrell and Lee\(^20\) further developed methods for fitting a binary logistic model to a new sample to estimate the relationship between the predicted probability and the observed outcome in that sample. This fit provides a simple calibration equation that can be used to quantify unreliability (lack of calibration) and to calibrate the predictions for future use. This logistic calibration leads to indices of unreliability \((U)\), discrimination \((D)\), and overall quality \((Q)\)
= D – U) which are derived from likelihood ratio tests. Q is a logarithmic scoring rule which is comparable to the Brier score.
Results

Study Populations

Demographic characteristics of the two patient populations are summarized in Table 1. Patients from the cardiothoracic surgery and general medical services predominated. There were no patients identified from the obstetrics/gynecology or pediatric services. Slightly more than half the patients in each group met the clinical criteria for HIT (Table 1).

All patients underwent confirmation with excess heparin with the results for each patient group shown in Table 2, stratified by the presence of clinical HIT. In patients with a clinical diagnosis of HIT, 96% of patients in the training set and 88% in the validation set had positive confirmatory assays. After review of medical records, 36% (41/115) in the training set and 42% (41/97) in the validation set were felt to be HIT-.

Univariate Analyses

Univariate analysis of the training set demonstrated that the HIT- patients had significantly lower maximal anti-heparin/PF4 OD values than the HIT+ patients (1.36 vs. 0.75, \( P < .001 \); Table 3). The peak PF4 titer was distributed with considerable skewness to the right. Accordingly, it was log-transformed for the purposes of multivariate modeling. Analysis for collinearity resulted in a VIF of \(~1\), indicating an absence of correlation between anti-heparin/PF4 OD values and the high-heparin confirmatory test. In the training set, statistically significant univariate associations were noted with an increasing anti-heparin/PF4 OD, presence of a positive excess heparin confirmatory test, and being a surgical patient (Table 3). No significant relationship was detected between age, race, or gender and HIT status.
Logistic Regression Analyses

A series of univariate logistic regression models were fitted to the 2005 data using clinical HIT status as the dependent variable (Table 4). The log heparin/PF4 OD and the confirmation assay contributed similar quantities of information in univariate models, and more information than clinical service category (medical vs. surgical). In a stepwise multivariate logistic model, being a surgical patient did not meet the criterion ($P <.05$) for entry into the model. In contrast, both the log anti-heparin/PF4 titer and the confirmation assay independently contributed significant information to the multivariate model, with a c-index for the overall model of 0.783 ($P <.001$; Table 4). This prediction algorithm is graphically summarized as a nomogram in Figure 1.

Model Assessment and Validation

Predicted probabilities of HIT for the training and validation sets are shown in Figure 2. The ability of the model to discriminate between HIT+ and HIT- patients can be qualitatively appreciated by the degree of overlap in predictions for each group. Quantitatively, the discrimination of the algorithm fitted to the training set (c-index 0.783, Somer’s $D_{yx}$ 0.566, Brier 0.170) was maintained when the algorithm was applied to the independent validation set (c-index 0.799, Somer’s $D_{yx}$ 0.597, Brier 0.198). Receiver operator characteristic curves for both populations are shown in Figure 3.

Application of logistic calibration methods$^{17}$ yielded quality indices ($U=0.008$, $D=0.218$, $Q=0.210$) consistent with the c-index and Brier score measures (Figure 4). The calibrated risk distribution (histogram of logistic-calibrated probabilities) is shown on the x-axis (black vertical
bars). The ideal relationship (for a perfect predictor) between the predicted probabilities and the actual probabilities falls on the diagonal (blue). Using a smoothed nonparametric calibration curve, the reliability of the statistical model for predicting HIT is close to the ideal relationship (Figure 4, red line). This observation reflects that the prevalence of HIT in the validation set is close to the prevalence in the training set, and suggests that the overall need for recalibration is modest. However, the reliability of the model can be further improved using a logistic transformation, as shown by the fitted logistic calibration curve (Figure 4, green line). The fact that both curves are near the diagonal indicates that the model reliably predicts clinical HIT status based on the criteria defined in this study.
Discussion

HIT has been a recognized complication of heparin treatment for several decades, yet it remains difficult to diagnose because of other clinical contributors to thrombocytopenia. The diagnosis of HIT is a clinico-pathologic one\textsuperscript{1}, and several clinical findings essential to its diagnosis (such as the nadir platelet count) may take days to evolve. Patients with suspected HIT are often at increased risk for bleeding if treated with alternative anticoagulation, or at an increased risk for thrombosis if HIT is not recognized or misdiagnosed. Although specific, the SRA is time-consuming and often requires referral to a reference laboratory. The anti-heparin/PF4 and confirmatory assays are easier to perform and readily available at many hospitals.

The OD value of anti-heparin/PF4 antibodies detected by ELISA correlates with both a clinical diagnosis of HIT and a higher incidence of heparin induced thrombocytopenia and thrombosis (HITT)\textsuperscript{8,21,22}. A recent study examined whether the absolute OD value could predict the presence of HIT activating antibodies, defined by a strong positive SRA result. OD values of >2.00 resulted in a ~90\% probability of a strong SRA result whereas weaker OD values (0.4 to <1.00) had a < 5\% probability of being associated with a strong positive SRA result \textsuperscript{23}. Similarly, in univariate analysis, we found that higher absolute OD values correlated with a clinical diagnosis of HIT and in multivariate logistic regression analyses were predictive of clinical HIT. Nevertheless, not all patients who meet clinical criteria for HIT have markedly elevated anti-heparin/PF4 OD values\textsuperscript{8,22} and likewise, some high anti-heparin/PF4 OD values can be detected by ELISA in the absence of clinical manifestations of HIT. Consequently, identification of additional laboratory parameters would be potentially useful for the diagnosis of HIT.
Heparin-dependent binding is a defining characteristic of HIT antibodies in both serologic and functional assays. We first reported on the clinical utility of the heparin confirmation test in a retrospective study in which we demonstrated that patients with confirm+ test results were more likely to meet clinical criteria for HIT than patients who had confirm- results (72% versus 18% respectively; P<.001)\(^8\). In this study, we have extended this initial report by demonstrating that in patients who are clinically suspected of having HIT, a confirmatory step with excess heparin added significant diagnostic information to the maximal OD value of the anti-heparin/PF4 ELISA. Patients who were classified by clinical criteria as “HIT+” had a high likelihood of having a Confirm+ heparin/PF4 ELISA test result (96% and 88% in the two cohorts), whereas those who were classified as “HIT-” were much less likely to be Confirm+ (66%, 68%). Stated another way, 32 and 34% of patients who were clinically classified as “HIT-” demonstrated antibody binding in presence of excess heparin, and, therefore were considered as Confirm–.

Further, a predictive statistical model based upon both the maximal OD value and the confirmatory result discriminated well in our independent validation sample. Although neither test alone is a perfect predictor for HIT, these tests complement each other in the diagnosis of HIT as demonstrated by our model. For example, of the 41 patients in the training cohort who were clinically HIT negative, 27 of these patients were Confirm+. In univariate analysis, however, it was noted that patients who were HIT+ had a median anti-heparin/PF4 OD value of 1.36 vs. 0.75 for those patients who were HIT-. Although a correlation was seen with clinical diagnosis and OD, there was no correlation between OD and results of confirmatory testing. Therefore in these cases of clinically HIT negative patients with Confirm+ results, the absolute
The results of this study have the potential to aid in the diagnosis of HIT, yet there are limitations to the data presented. We did not use platelet activation assays to confirm or exclude the diagnosis of HIT in our study. A sensitive functional assay, such as the SRA would have buttressed the laboratory findings in this study. However, the SRA is not available at our institution and therefore, not routinely employed in the laboratory assessment of HIT. The diagnostic approach presented in this study is, nonetheless, particularly relevant for clinical practice, as most major medical centers do not offer the SRA or comparable sensitive functional assays. Second, the retrospective nature of the study allows only for the inclusion and study of patients who test positive for the presence of anti-heparin/PF4 antibodies. Knowing that a clinical suspicion exists for HIT also has the potential to bias the assessment. However, this is mitigated to a large extent in that only patients who are considered to be at risk for the development of HIT (exposure to heparin with thrombocytopenia) undergo anti-heparin/PF4 testing. Third, we did combine patients who met all criteria for HIT with patients who were thrombocytopenic in the correct timeframe in relation to heparin exposure, but who also had potential alternative explanations for their thrombocytopenia. Although a subset of these patients may have been subsequently assessed as not having HIT, the purpose of this study was to assess the value of the high-heparin confirmatory test in a “real-world” situation, and many patients with HIT may have more than one factor contributing to the overall thrombocytopenia. Lastly, we recognize that clinical requests for anti-heparin/PF4 ELISA assays may have introduced a selection bias on the prevalence of HIT, thus affecting our estimates of model
reliability. The maximum Brier score for a predictive model decreases as the prevalence of the predicted event decreases. Consequently, a disadvantage of the Brier score is that its interpretation depends on the frequency of the outcome (i.e., clinical HIT+ status). Application of the current predictive model to other patient populations (for example, obstetric patients, or pediatric patients) may require additional calibration and validation.

In summary, the confirmatory assay with excess heparin is a valuable adjunct in the laboratory diagnosis of HIT. Using a multivariate statistical model, the probability of being clinically HIT positive can be estimated for an individual patient using both the maximal anti-heparin/PF4 OD value and the confirmatory assay result. Accurate predictions of the probabilities of HIT will enable clinicians to initiate appropriate therapy rapidly thereby reducing complications of HIT.
Acknowledgements

This work was supported by grants from the Centers for Disease Control and Prevention (U01-DD000014) (T.L.O) and the following grants from the National Institutes of Health: T32-HL007057 (N.L.W.), 5K12-HL-087097-04 (A.D.M.), NIH RO1-HL081395 and Grant in Aid from the AHA (G.M.A.), and NIH UO1-HL072289 and U54-HL077878 (T.L.O).
Authorship

Contribution: N.L.W. and T.L.O designed and performed the research; D.F.K. analyzed the data and made the figures; N.L.W., T.L.O., G.M.A., and A.D.M reviewed the data and analyses, N.L.W., T.L.O., D.F.K., G.M.A, and A.D.M. wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

### Table 1. Demographic characteristics of the two patient populations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Training Set</th>
<th>Validation Set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=115</td>
<td>n=97</td>
</tr>
<tr>
<td>Median age, yr.</td>
<td>64</td>
<td>63</td>
</tr>
<tr>
<td>Male Gender (%)</td>
<td>52 (45%)</td>
<td>57 (59%)</td>
</tr>
<tr>
<td>White Race (%)</td>
<td>78 (68%)</td>
<td>66 (68%)</td>
</tr>
<tr>
<td>General Surgery Service (%)</td>
<td>17 (15%)</td>
<td>7 (7%)</td>
</tr>
<tr>
<td>Cardiothoracic Surgery Service (%)</td>
<td>39 (34%)</td>
<td>33 (34%)</td>
</tr>
<tr>
<td>Medicine Service (%)</td>
<td>35 (31%)</td>
<td>25 (26%)</td>
</tr>
<tr>
<td>Clinical HIT+ (%)*</td>
<td>74 (64%)</td>
<td>56 (58%)</td>
</tr>
</tbody>
</table>

* HIT indicates Heparin Induced Thrombocytopenia.
Table 2. Laboratory Evaluation of Anti-heparin/PF4 Antibody Positive Patients

<table>
<thead>
<tr>
<th>Laboratory Results</th>
<th>Training Set</th>
<th>Validation Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients, Confirm+ (%)</td>
<td>98/115 (85%)</td>
<td>77/97 (79%)</td>
</tr>
<tr>
<td>All patients, Confirm- (%)</td>
<td>17/115 (15%)</td>
<td>20/97 (21%)</td>
</tr>
<tr>
<td>HIT+ patients, Confirm+ (%)</td>
<td>71/74 (96%)</td>
<td>49/56 (88%)</td>
</tr>
<tr>
<td>HIT+ patients, Confirm- (%)</td>
<td>3/74 (4%)</td>
<td>7/56 (12%)</td>
</tr>
<tr>
<td>HIT- patients, Confirm+ (%)</td>
<td>27/41 (66%)</td>
<td>28/41 (68%)</td>
</tr>
<tr>
<td>HIT- patients, Confirm- (%)</td>
<td>14/41 (34%)</td>
<td>13/41 (32%)</td>
</tr>
</tbody>
</table>

PF4 indicates Platelet Factor 4; HIT, Heparin Induced Thrombocytopenia; Confirm+, High Heparin Confirmatory step positive; Confirm-, High Heparin Confirmatory step negative.

* Patients are classified as HIT+ and HIT- on the basis of clinical grounds, as described in the text.
**Table 3.** Training Population, Univariate Analysis*

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIT positive</th>
<th>HIT negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=74)</td>
<td>(n=41)</td>
<td></td>
</tr>
<tr>
<td>Mean age, yrs.</td>
<td>63</td>
<td>61</td>
<td>.430</td>
</tr>
<tr>
<td>White race</td>
<td>48 (64%)</td>
<td>26 (63%)</td>
<td>.400</td>
</tr>
<tr>
<td>Male gender</td>
<td>32 (43%)</td>
<td>20 (49%)</td>
<td>.570</td>
</tr>
<tr>
<td>Surgical service</td>
<td>42 (56%)</td>
<td>15 (37%)</td>
<td>.038</td>
</tr>
<tr>
<td>Maximal heparin/PF4 OD median, (25th, 75th percentiles)</td>
<td>1.36 (0.61, 1.99)</td>
<td>0.75 (0.47, 0.93)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Confirm+</td>
<td>71 (96%)</td>
<td>27 (66%)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* Confirm+ indicates High-Heparin Confirmatory positive; HIT, Heparin Induced Thrombocytopenia; PF4, Platelet Factor 4; OD, optical density.
Table 4. Univariate and Multivariate Logistic Regression Models*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Degrees of Freedom</th>
<th>Likelihood Ratio, $\chi^2$</th>
<th>c-index</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log anti-heparin/PF4</td>
<td>1</td>
<td>15.16</td>
<td>0.70</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Confirm+</td>
<td>1</td>
<td>18.60</td>
<td>0.65</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Surgical service</td>
<td>1</td>
<td>4.33</td>
<td>0.60</td>
<td>.037</td>
</tr>
<tr>
<td>Log anti-heparin/PF4+ &amp; Confirm+</td>
<td>2</td>
<td>34.42</td>
<td>0.78</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* Confirm+ indicates High-Heparin Confirmatory positive; HIT, Heparin Induced Thrombocytopenia; PF4, Platelet Factor 4
FIGURES

Figure 1. **Nomogram of Logistic Regression Model for Predicting HIT.** Anti-heparin/PF4 optical density (PF4 OD) and confirmatory assay (Neg = Negative; Pos = Positive) values are displayed on the outer scales. Connecting a pair of PF4 OD and confirmatory assay results using a straight edge allows one to estimate the probability of HIT from the center scale.

Figure 2. **Predicted Probabilities of HIT for the Training (n=115) and Validation (n=97) Data Sets.** Median, 25th, and 75th percentiles of the predicted probabilities for the HIT+ and HIT- patients using the log-anti-heparin/PF4 OD results and confirmatory status in the training (2005) and validation (2006) populations.

Figure 3. **Receiver Operating Characteristic (ROC) curves.** The discrimination of the algorithm fitted to the 2005 training set (n=115; blue) was maintained when the algorithm was applied to the independent test population (n=97; red).

Figure 4. **Reliability of logistic model in validation set.** The calibrated risk distribution (histogram of logistic-calibrated probabilities) is shown (black vertical bars.) The ideal relationship (for a perfect predictor) between the predicted probabilities and the actual probabilities falls on the diagonal (blue). The reliability of the statistical model for predicting HIT is illustrated by a smoothed nonparametric calibration curve (red) and a fitted logistic calibration curve (green).
Figure 2

Predicted Probability vs. Clinical HIT Status

- '05 HIT+: 0.915
- '05 HIT-: 0.745
- '06 HIT+: 0.907
- '06 HIT-: 0.755
- '05 HIT+: 0.703
- '05 HIT-: 0.529
- '06 HIT+: 0.540
- '06 HIT-: 0.535
- '05 HIT+: 0.164
- '05 HIT-: 0.141
Figure 3

- **Blue line**: 2005 data (n=115, c=0.780)
- **Red line**: 2006 data (n=97, c=0.772)

**Y-axis**: Sensitivity

**X-axis**: False Positive Rate (1 – Specificity)
Figure 4
Validation of the high-heparin confirmatory step for the diagnosis of heparin-induced thrombocytopenia

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