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Evaluation of a diagnostic algorithm for Heparin-Induced Thrombocytopenia

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ABSTRACT

Introduction: Heparin-Induced Thrombocytopenia (HIT) is a rare but serious immune-mediated complication of heparin treatment. HIT is characterized by an acute, transient prothrombotic state combined with thrombocytopenia and is caused by platelet-activating IgG antibodies that bind to complexes of heparin and platelet factor 4. The diagnosis of HIT relies on clinical presentation and the demonstration of HIT antibodies. One approach to improve the efficacy of laboratory analysis is to use a diagnostic algorithm.
Aim: To evaluate one diagnostic algorithm for HIT where the 4 T's clinical risk score is combined with immunochemical and/or functional assays.
Materials and methods: The quality of the diagnostic algorithm was retrospectively evaluated in 101 patients with suspected HIT. Laboratory results obtained from the diagnostic algorithm were compared to Heparin-Induced Platelet Aggregation (HIPA) and clinico-pathological evaluation of patients' medical records.
Results: We found that the algorithm had a diagnostic efficacy of 94%, with specificity of 94% and sensitivity 94%. Positive likelihood ratio (LR+) was 16.0, and the negative likelihood ratio (LR−) 15.5. The efficacy of PaGIA ($n = 95$) was 0.46, and IgG-specific HPF4-abELISA ($n = 54$) was 0.87.
Conclusions: The diagnostic algorithm for HIT is sufficiently accurate and leads to in overall faster results and decreased cost of analysis. The weakest link of the algorithm is the risk of miscalculated 4 T's scores, which is inevitably exacerbated by the insufficient experience most clinicians have with HIT. This highlights the importance of clear instructions from the laboratory and coagulation clinic.

1. Introduction

Heparin-Induced Thrombocytopenia (HIT) is a serious immune-mediated reaction to heparin and low-molecular-weight heparin, affecting 0.25–3% [1] of treated patients. HIT is initiated by the formation of complexes between heparin and platelet factor 4, causing susceptible individuals to produce antibodies against the heparin-platelet factor 4 complex (HPF4). HIT occurs if these transient antibodies activate platelets. Platelet activation leads to aggregation, thrombocytopenia and a prothrombotic state that results in potentially limb- and/or life-threatening thrombosis in 20–30% of cases [2]. In suspect HIT, heparin treatment needs to be replaced by an alternative anticoagulant, after which an urgent verification of the diagnosis is needed. There is a considerable risk of doctors' delay, because patients affected by HIT commonly have a number of other possible causes of thrombocytopenia and thrombosis, since the incidence of HIT is highest in patients with activated platelets, such as in trauma or surgical patients.

Diagnosis of HIT is based on the detection of HPF4 antibodies by functional or immunochemical assays. However, although all instances of HIT are caused by platelet-activating antibodies, not all HPF4 antibodies cause HIT [3]. HPF4 antibodies of non-IgG class are often not clinically relevant [4] and anti-protamine antibodies can

Abbreviations: 4 T's, The 4 T's score for the estimation of clinical pretest probability; LR+, Positive Likelihood Ratio; LR−, Negative Likelihood Ratio; HIT, Heparin-Induced Thrombocytopenia; HIPA, Heparin-Induced Platelet Activation assay; HPF4, Heparin-Platelet Factor 4 Complex; HPF4-abELISA, Enzyme-linked immunosorbent assay against HPF4 antibodies; PaGIA, Rapid particle-based immunoassay against HPF4 antibodies; PF4, Platelet Factor 4; PLT, Platelet; SEM, Standard error of the mean; SRA, 14C-Serotonin Release Assay; OD, Spectrophotometric Optical Density.

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be falsely detected as HIT antibodies [5]. This occurrence of clinically irrelevant HPF4 antibodies in patients without HIT is referred to as the iceberg effect [3]. Depending on the choice of cohort, clinically irrelevant HPF4 antibodies can be found in 3–50% of heparin-treated patients, and in ~0.5–18% of asymptomatic patients even HPF4 antibodies with the potency to activate platelets are found by functional assays [1]. The fact that detection of HPF4 antibodies alone is not sufficient for diagnosis has led to the development of analytical algorithms comprised of multiple assays.

Assays for HIT are either immunological or functional. Immunological assays detect HPF4 antibodies and are divided into enzyme-linked immunosorbent assays (HPF4-abELISAs) and particle-based rapid immunoassays. The most sensitive assay currently is HPF4-abELISA, with a sensitivity >97% and a negative predictive value (NPV) of >95% [6–8]. Diagnostic specificity is increased if the immunological assay is IgG specific [2,9], but the functional assays naturally excel in specificity because they exclusively detect HPF4 antibodies with platelet-activating capacity. The gold standard for detecting clinically relevant HIT antibodies are the functional assays 14C-Serotonin Release Assay (SRA-method) [7,10] and heparin-induced platelet aggregation (HIPA), both with a high sensitivity (>90%) as well as specificity (77–97%) [7,11]. HIPA is preferred because it measures platelet aggregation, whereas SRA has the release of radioactive serotonin as an endpoint.

Since no single assay is optimized for both sensitivity and specificity, a combination of functional and immunological assays seems to be the optimal laboratory diagnostic approach. Diagnostic algorithms with functional and immunological assays in combination with the clinical 4 T's score (Table 1) have previously been proposed by Pouplard [12], Greinacher [13], Lassila [14] and Cuker for the American Society of Hematology [15,16], but the optimal diagnostic pathway is yet to be established.

Karolinska University Hospital has used one such algorithm for more than five years (see Material and methods). As part of internal quality control, we previously examined all cases investigated in the first five years after implementation of the algorithm, and could verify that the implementation of the diagnostic algorithm had resulted in a faster obtainment of HIT results (see Results). The aim of this study was to evaluate and improve the diagnostic algorithm for HIT used at Karolinska University Hospital in a Swedish population based on prospectively collected and analyzed samples.

2. Material and methods

2.1. Patients and samples

Samples from 101 patients with clinically suspected HIT were collected between 2010 and 2014 (66 consecutive negative patients and 35 consecutive positive patients). The inclusion period for positive samples was longer than for negative samples; this was to attain a high rate of positive samples in order to adequately examine the risk of over diagnosis. The citrated plasma samples were stored at −70 °C.

Patient consent was not required according to Swedish law, since the project was an internal quality assessment using coded samples from routine testing.

2.2. Diagnostic algorithm for HIT

The diagnostic algorithm adapted at Karolinska University Hospital is based on the 4 T's score combined with immunochemical and/or functional assays with a Bayesian approach (Fig. 1). The 4 T's score for the estimation of clinical pretest probability of HIT [6,12,17,18] is a useful tool in combination with laboratory assays in order to establish a HIT diagnosis (Table 1).

The 4 T's score determines the pathway of each sample through the algorithm. Samples with low risk according to the 4 T's score are not analyzed, since their low probability of HIT (NPV for HIT 98.9% [17]) would be low even if HPF4 antibodies would be demonstrated [12]. The laboratory will in these instances inform the clinicians forthwith that the samples will not be analyzed, and the algorithm states that the samples should not be submitted to the laboratory. Samples with intermediary or high 4 T's are primarily analyzed by a rapid particle-based immunoassay (ID-PaGIA heparin/PF4). Intermediary-scored samples are then considered negative if PaGIA is negative, and high-scored samples are considered positive if PaGIA is positive. Intermediary-scored samples with positive PaGIA as well as high-scored samples with negative PaGIA are further analyzed by IgG-specific HPF4-abELISA. Reports of the HPF4-abELISA are released in all cases except for intermediary-scored samples that are positive for HPF4 antibodies, which are finally analyzed by HIPA. Preliminary results are released after each assay in the diagnostic pathway to give clinicians a continuous indication of the risk for HIT.

2.3. Study design

The validity of the diagnostic algorithm was assessed retrospectively against analysis of HIPA, and any non-concurring results were further investigated. PaGIA (HPF4-ab) and HPF4-abELISA were analyzed at the Karolinska University Laboratory, and HIPA was analyzed in the Institute for Immunology and Transfusion Medicine in Ernst-Moritz-Arndt University, Greifswald. No additional HIPA was performed on samples where HIPA had previously been performed as part of the algorithm ($n = 9$).

In the 83 samples where the functional reference method confirmed the algorithm results, the diagnosis was considered correct. In the 18 samples where results were not confirmed by HIPA, the presence of HIT was determined after the assessment of the patients' medical records in combination with lab results by two independent medical doctors (one coagulation specialist (TF) and one laboratory physician (MF)). Special emphasis was put on timing of heparin treatment in relation to the onset of platelet decrease, on whether platelet counts were normalized after the cessation of treatment, and on other causes of thrombocytopenia and thrombotic conditions. If a false diagnosis from the algorithm could not be ruled out with high certainty, the results of

Table 1
The 4 T's score for the estimation of clinical pretest probability of HIT. 0–3 points equals low probability of HIT; if patient gets a low pretest probability of HIT, no laboratory analysis is needed. 4–5 points equals intermediary, and 6–8 points equals high pretest probability of HIT. In both cases, laboratory investigation is needed.
Plt: Platelet count ($\times 10^9$/L); Hep: first heparin administration.

	2 points	1 point	0 points
Thrombocytopenia	Plt decreased > 50% nadir > 20 × 10^9/L	Plt decreased 30–50% nadir 10–19 × 10^9/L	Plt decreased < 30% nadir < 10 × 10^9/L
Timing	Debut 5–10 days after initiation of Hep (if no recent heparin)	Debut > 10 days after initiation of Hep	Debut ≤ 4 days after initiation of Hep (if no recent heparin)
Thrombosis	New thrombosis	Suspected thrombosis or progression	None
Other Causes of Thrombocytopenia	None apparent	Possible	Definite