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REVIEW

Clinical and laboratory diagnosis of heparin induced thrombocytopenia: an update

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Summary

Heparin remains a commonly used anticoagulant in prophylaxis and treatment of venous and arterial thrombosis, in addition to ensuring patency of artificial blood circuits such as cardiopulmonary bypass (CPB). Heparin induced thrombocytopenia (HIT) is a rare but potentially fatal complication of heparin therapy that results from production of polyclonal antibodies to heparin in complex, usually with platelet factor 4 (PF4). In a proportion of patients, this causes platelet activation and thrombin generation, which may result in thrombosis. However, identification of patients with HIT can be complicated as thrombocytopenia is common in hospitalised patients receiving heparin, and is usually due to other causes. Clinical assessment of the likelihood of HIT is paramount in order to make appropriate decisions regarding laboratory investigations and ongoing anticoagulation, especially given clinically expressed pro-thrombotic states. However, clinical assessment, on its own, cannot guarantee diagnosis or exclusion of HIT, and therefore is facilitated by laboratory testing, although unfortunately, this is frequently limited by local availability of assays and delay in availability of results. Nevertheless, there are an increasing number of available laboratory tests that can be used to identify antibodies causing HIT, including both immunological and functional assays. This narrative review will discuss the existing tools for clinical assessment in addition to evaluating the advantages and disadvantages of the available laboratory assays for HIT.

Key words: Heparin induced thrombocytopenia; HIT; diagnosis; laboratory testing; clinical identification.

Abbreviations: 4Ts, 4 T score; APTT, activated partial thromboplastin time; AUC, area under the curve; CPB, cardiopulmonary bypass; ELISA, enzyme linked immunosorbent assay; GAG, glycosaminoglycan; HEP, HIT expert probability; HIPA, heparin induced platelet activation; HIT, heparin induced thrombocytopenia; HPLC, high pressure liquid chromatography; IL-8, interleukin 8; LMWH, low molecular weight heparin; LTA, light transmission aggregometry; NAP-2, neutrophil-activating peptide 2; NPV, negative predictive value; OD, optical density; PaGIA, particle gel immunoassay; PF4, platelet factor 4; PMP, platelet derived microparticles; PPV, positive predictive value; PRP, platelet rich plasma; ROC, receiver operating characteristic; SRA, serotonin release assay; UFH, unfractionated heparin; WBA, whole blood aggregometry.

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INTRODUCTION

Heparin induced thrombocytopenia (HIT) represents a significant complication of heparin therapy, with potential for high morbidity and mortality, due to platelet activation, thrombin generation, and resulting thrombosis. Despite the recent introduction of novel oral anticoagulants,[1] medical use of heparin remains extensive, especially in a hospital setting, including its use as prophylaxis and initial treatment of venous thrombosis,[2] and to ensure patency of artificial blood circuits used for cardiac surgery and haemodialysis. It is counter-intuitive but nonetheless pertinent that a drug used to prevent and treat thrombosis is itself a potential cause of thrombosis in a subset of treated patients. Heparin comprises either unfractionated heparin (UFH), a heterogeneous mixture of sulfated polysaccharides with a molecular weight range of 8,000 to 24,000 Da, or low molecular weight heparin (LMWH) (2,000–8,000 Da). HIT can arise from use of either UFH or LMWH, but is more common when using UFH. For example, a large meta-analysis determined the incidence of HIT in patients treated with UFH was 2.6%, compared with 0.2% for patients treated with LMWH.[3] The diagnosis of HIT, and indeed the identification of the subset of patients that will more likely develop serious consequences related to platelet activation and thrombosis is challenging for many reasons, and potentially leads to its over-diagnosis, under-diagnosis, and misdiagnosis (summarised in Table 1). Primarily, both thrombocytopenia and heparin exposure are common in hospitalised patients, and therefore may often co-exist and represent unrelated variables. Thus, the frequency of HIT in heparin exposed critically ill patients has been estimated to be close to 1%, perhaps 0.3–0.5%, compared to a much higher background frequency of 30–50% thrombocytopenia in such patients.[4,5]

Even if related, and antibodies to heparin are produced, the pathophysiology of HIT is complex, and only a proportion of patients who develop antibodies will develop a serious complication, as related to the ability of the generated antibodies to activate platelets. Accordingly, the initial clinical identification of candidate HIT patients is difficult, and then the difficulty is further compounded when trying to identify the subset of patients likely to have an adverse event such as thrombosis. This represents a very difficult scenario for clinicians, having to decide if it is safe for patients to continue critical anticoagulant therapy using heparin, or else to cease heparin therapy and use an alternative non-heparin

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Table 1 HIT: a summary of possible reasons for over-diagnosis, under-diagnosis and misdiagnosis

Over-diagnosis of HIT?	Under-diagnosis of HIT?	Misdiagnosis of HIT?
Thrombocytopenia is common in hospitalised ill patients. Heparin is used extensively in such patients. Thus, thrombocytopenia and heparin may be coincidental findings. Most patients with thrombocytopenia, even whilst on heparin therapy, will not have HIT	Thrombocytopenia is common in hospitalised ill patients. Heparin is used extensively in such patients. Due to the common use of heparin and the relative rarity of HIT as the aetiology of thrombocytopenia, HIT may not be considered by the inexperienced clinician	Differentiating HIT from other pathologies is not always easy. A number of frequent clinical problems such as sepsis and treatments such as antibiotics and transfusion of blood products can be associated with thrombocytopenia
Clinicians may not be aware of, or be able to effectively utilise, HIT scoring systems such as 4Ts	Exposure to heparin may not be always obvious (e.g., heparin locks/flushes)	Other diagnoses to be considered include drug-induced thrombocytopenia, post-transfusion purpura, and DIC
Immunological assays detect both pathogenic and non-pathogenic HIT antibodies. Most positive test results using immunological assays will not be pathogenic in nature	Most laboratory assays will occasionally give a negative result in HIT, either because of lack of sensitivity, or selection bias (e.g., HIT occasionally caused by non PF4-heparin antibodies)	Clinical pretest scores provide a useful framework to direct testing and differentiate HIT from other causes. However, clinical judgment is often required to take into account the complexities of today's hospital medicine

DIC, disseminated intravascular coagulation; HIT, heparin induced thrombocytopenia; PF4, platelet factor 4.

anticoagulant. This latter course of action is itself problematic, since the choices of safe anticoagulants other than heparin may be limited depending on the particular clinical situation and/or jurisdiction, and the use of alternate anticoagulants can itself lead to further complications, including increased bleeding risk and greater difficulty in monitoring therapy, and thus managing patients. Clinicians therefore often rely on laboratory testing for guidance, to provide evidence, or lack of evidence, of 'heparin related antibodies'. However, as for clinical assessment, laboratory testing for HIT is also imperfect. Indeed, although a plethora of test methodologies are now available for laboratories to assess HIT, all tests suffer several limitations, related to limited sensitivity and specificity, or to complexity and/or temporal unavailability.

The purpose of this narrative review is to provide an update of the clinical and laboratory process for identifying or excluding HIT. This review also briefly covers the pathophysiology of HIT, as this has clear relevance to its diagnosis. Management of HIT *per se* is not covered in this review, being considered outside the scope, and otherwise deserving of a separate publication.

PATHOPHYSIOLOGY OF HIT

The pathophysiology of HIT has been recently extensively reviewed by McKenzie and Sachais.[6] In brief, the pathophysiological process is initiated when polyclonal antibodies are developed to heparin in complex, usually with platelet factor 4 (PF4). Typically, IgG antibodies against the heparin-PF4 complex bind to the platelet FcγRIIa receptor, and this subsequently activates platelets. PF4 is a protein normally released from the alpha-granules of activated platelets and binds with high affinity to heparin. A strong chemoattractant for neutrophils and fibroblasts, PF4 probably has a role in inflammation and wound repair, but otherwise its major physiological role appears to be neutralisation of heparin-like molecules on the endothelial surface of blood vessels, thereby inhibiting default antithrombin activity. However, in a pathophysiological process, PF4-heparin binding may initiate HIT, which is then further propagated by activated platelets, monocytes, endothelial cells and the coagulation system. Such IgG antibodies develop in only a proportion of patients exposed to heparin, some 4–14 days after exposure (a temporal association utilised in HIT scoring systems; see below), but only a subset of these antibodies are capable of activating platelets. In susceptible patients, the process generates thrombin, which further activates platelets, and platelet-fibrin thrombi are formed. Although IgA and IgM antibodies to heparin/PF4 have also been described, IgG antibodies are believed to be most relevant to the pathogenesis of HIT.[6]

IgG antibodies to heparin and PF4 are detected in many more patients than will develop clinical manifestations of HIT.[3–7] Antibody titres disappear after 90 or so days in most patients. The clinical manifestations of HIT are believed to be caused by antibodies that recognise an ultra-large complex of heparin and PF4 tetramers. HIT antibodies preferentially bind to PF4 when heparin is present in a narrow molar ratio of 'reactants' (meaning antibodies, PF4 and heparin). Activation of platelets causes a cascade of events: platelets secrete more PF4, which in turn creates more antigen for the antibodies to bind; platelets aggregate via activated platelet integrin $\alpha_{IIb}\beta_3$; a proportion of activated platelets become procoagulant with an exposed phophatidylserine surface that facilities coagulation and shedding of procoagulant microparticles. The end result is thrombin generation, consumption of platelets (exacerbating the apparent 'thrombocytopenia'), and most importantly platelet-fibrin thrombi, which may occlude blood vessels and/or induce limb or organ damage.

CLINICAL IDENTIFICATION OF HIT

As noted above, thrombocytopenia and heparin are coincidently present in many hospitalised patients, and this co-occurrence is not always causally related. Indeed, most cases of thrombocytopenia, even in patients on heparin therapy, are not related to heparin, and will not cause clinical manifestations of HIT. Making a correct clinical diagnosis of HIT is crucial as decisions regarding management may be required prior to results of laboratory investigations being available and anticoagulant therapy cannot simply be withdrawn, due to high risk of thrombosis. Furthermore, the options for replacing heparin are currently limited, are more expensive, often require more frequent or complex laboratory monitoring and may be associated with an excess bleeding risk.[8–10] In addition there is often a lack of clinician familiarity with these agents.

In order to improve the diagnosis of HIT, clinicians should undertake a formal assessment of the probability of HIT, as well as potentially ordering laboratory investigations for heparin-PF4 or heparin-platelet antibodies. In some cases, the