

Mechanism and Clinical Presentation of Heparin-Induced Thrombocytopenia

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Heparin-induced thrombocytopenia (HIT) is a potentially life-threatening complication of heparin anticoagulation, occurring in approximately 3% of patients treated with unfractionated heparin.

Heparin and platelet factor 4 (PF4) are capable of forming multimolecular complexes. Given stoichiometric concentrations of heparin and platelet factor 4 (PF4), heparin may induce conformational changes in the PF4 molecule, rendering it antigenic. The subsequent immune response generates antibodies against heparin–PF4 complexes (HIT antibodies). Binding of these antibodies to FcγIIA receptors on the surface of platelets results in potent platelet activation. Binding of HIT antibodies to heparan sulfate–PF4 complexes on the surface of endothelial cells (ECs) causes EC activation with subsequent expression of tissue factor. Activation of platelets and of ECs together leads to marked thrombin generation, resulting in the hypercoagulable state in HIT.

Clinically, HIT presents with two major sequelae: thrombocytopenia and thrombosis. Thrombocytopenia—that is, a platelet count below $150 \times 10^9/L$ —is present in 85%–90% of HIT patients and typically occurs between day 5 and day 10 of heparin treatment. The mean platelet count nadir is approximately $60 \times 10^9/L$. Alternatively, HIT may be associated with a marked fall in platelet count ($\geq 50\%$ of the initial value) whose nadir is not below $150 \times 10^9/L$. Despite the low platelet count, thrombosis rather than bleeding predominates. In HIT, the risk for thrombosis is 5%–10% in the first 2 days; the 30-day cumulative risk is approximately 50%. Thromboses most often occur in deep veins of the lower limbs, frequently leading to pulmonary embolism. If thrombosis is severe or if it is detected in an unusual location in heparin-treated patients, HIT should be suspected.

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Key words

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Introduction

Heparin-induced thrombocytopenia (HIT) is a paradoxical clinicopathologic syndrome encountered in patients treated with heparin. In HIT, an anticoagulant turns procoagulant.

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Although small reductions in platelet count upon treatment with heparin were described shortly after the drug had been introduced into clinical medicine [1], the pathogenesis of this intriguing disease has been elucidated only recently. It is now clear that HIT is the most common drug-induced thrombocytopenia, occurring in approximately 3% of patients treated with heparin [2]. This review summarizes the current knowledge of the pathogenesis of HIT, subsequently focusing on the main clinical sequelae of this puzzling disease.

Mechanism of HIT

Structural components in the pathogenesis of HIT

To understand the mechanism of HIT, knowledge of the structural components critically involved is needed. Apparently heparin is one key factor in the pathogenesis of the disease. Heparin is not a single pharmacologic agent; instead, it comprises a polydisperse mixture of negatively charged glycosaminoglycans (GAGs) with molecular weights ranging from 5 kDa to 40 kDa [3]. Heparin is composed of D-glucosamine residues linked either to L-iduronic acid or D-glucuronic acid. Owing to sulfation of many of these residues, heparin is the GAG with the highest charge density, resulting in a degree of sulfation (number of sulfate groups per disaccharide unit) of 2.0–2.5 [3].

Within the circulation, heparin binds to platelets, which are likewise critically involved in the pathogenesis of HIT. Binding of heparin to these cells is specific and saturable, yet does not depend on unique heparin or platelet structures [4]. Instead, negative charge density, molecular size, and binding affinity of the ligand are key determinants [4]. Heparin-binding sites on platelets provide mainly a complementary positive charge.

Upon binding of heparin to platelets, subtle cell activation may occur. This subtle activation is the case in nonimmune heparin-associated thrombocytopenia (nonimmune HAT; formerly known as heparin-induced thrombocytopenia type I), which is attributed to a direct platelet-activating effect of heparin. Nonimmune HAT is frequently seen in up to 30% of patients receiving heparin [5]. It is characterized by a fall—typically mild and transient—in platelet count within the first few days of receiving heparin. Nonimmune HAT does not require that heparin anticoagulation be stopped.

Heparin and platelet factor 4 are capable of building multimolecular complexes

Heparin also binds to proteins that are secreted from activated platelets. One of these proteins is platelet factor 4

(PF4), which is released from platelet α -granules and which binds heparin with high affinity. Platelet factor 4 is a homotetrameric globular protein (70 amino acid residues per subunit) containing a number of positively charged amino acids. Its tetrameric structure is stabilized by non covalent associations—electrostatic and hydrogen-bonding interactions.

Within the COOH-terminal α -helices, lysine residues are predominantly located on one side and form a ring of strong, positive charge. These residues are thought to be critically involved in heparin binding [3]. The PF4 is able to neutralize heparin action, but it does not inhibit heparin binding to platelets. In fact, heparin can bind to platelets in complexes with PF4 [6]. The PF4 may bind not only to heparin, but also to GAG expressed on the surface of endothelial cells (ECs) [7]. Here, the GAG most often involved is heparan sulfate. Owing to the relatively weak affinity between PF4 and heparan sulfate, heparin releases PF4 into the fluid phase, allowing their further interaction.

Multimolecular complexes of heparin with PF4 are the major target antigen in HIT

When PF4 and heparin are present in specific stoichiometric concentrations (that is, 27 IU of heparin per milligram of PF4), multimolecular heparin–PF4 complexes (H–PF4) are built [7]. These multimolecular complexes play a crucial role in the pathogenesis of HIT; they have recently been identified as the major target antigen for heparin-dependent antibodies in HIT [8–10]. With these specific stoichiometric concentrations of PF4 and heparin, heparin induces conformational changes in the PF4 molecule, resulting in the formation of so-called cryptic autoantigens or neoantigens. The autoantigens then stimulate an immune reaction that ultimately leads to generation of anti–H–PF4 antibodies (HIT antibodies).

Most HIT antibodies recognize a noncontiguous epitope on the PF4 molecule, which is produced when 4–8 PF4 molecules are bound to heparin [11]. In some cases, other PF4-related CXC chemokines, such as interleukin-8 (IL-8) or neutrophil-activating peptide-2 (NAP-2), are involved in antibody formation [12]. In patients with clinical HIT, HIT antibodies are most often of the IgG isotype (80%); in the remaining cases, only antibodies of the IgA or IgM isotype (or both) are observed [7,13].

Further insights into the immunopathogenesis of HIT

Studies addressing the role of GAG in the pathogenesis of HIT were able to define specific structural characteristics required to induce HIT antibody formation: (a) The saccharide chain must be in a flexible, relatively unconstrained state [14]. (b) The heparin molecule must contain more than 10 saccharide residues. Optimal antibody recognition has been reported to require fragments containing at least 12 saccharide residues [15]. (c) The heparin molecule must be highly sulfated, containing more than 1.0 sulfate group per disaccharide unit [3].

These observations help to explain why certain GAG preparations show a reduced capacity to induce HIT antibody formation. Their chain length being shorter than that of unfractionated heparins (UFHs), and their affinity for PF4 being reduced, the low molecular weight heparins (LMWHs) are less frequently likely to induce an immune response than are the UFHs [3]. This behavior has been convincingly demonstrated in a recent study prospectively evaluating HIT antibody formation and clinical HIT in patients requiring hip surgery [5]. Once HIT antibodies are generated, however, the cross-reactivity between UFHs and LMWHs is close to 100%. For this reason, LMWHs must not be given to patients suffering from acute HIT [2].

Danaparoid is a heparinoid consisting of a depolymerized mixture of GAG predominantly comprising low molecular weight heparan sulfate (80%). It shows only low cross-reactivity to HIT antibodies, which is assumed to be clinically relevant in about 5% of patients [2]. The low cross-reactivity of danaparoid is due both to its low molecular weight and its low degree of sulfation (between 0.5–0.7) [3]. In this regard, characteristics have been defined for a carbohydrate-based anticoagulant that should have a negligible risk for inducing HIT antibodies [3].

Further dissection of the immunopathogenesis of HIT has revealed that HIT antibodies are not specific for “compound epitopes” consisting partly of GAG and partly of peptide sequence [15]. Instead, they are reactive with neo-epitopes within the PF4 tetramer, which are exposed owing to configuration changes resulting from the binding of linear polyanions. Molecular analysis of the PF4 tetramer hints that the region between the third and fourth cysteine residue may constitute a major antigenic determinant for HIT antibody binding. In particular, amino acid P37 was addressed [14]. However, studies with PF4 mutants suggest the existence of multiple (neo)antigenic PF4 epitopes; at least three dominant HIT antibody recognition sites can be distinguished [14].

Apart from inducing conformational changes in the PF4 molecule, heparin also seems to be critically involved in the uptake or antigen processing, or both, of PF4 by antigen presenting cells, the exact mechanism of which awaits further clarification. The detection of immunoglobulin isotypes other than IgG indicates antibody class switching, which is likely to require T-helper cells. With regard to T-cell activation, a common V β T cell receptor family in HIT was not demonstrated. In addition, the current data do not support HLA restriction on HIT antibody formation [15].

Binding of H–PF4–IgG complexes to platelets leads to their activation

Once immune complexes (ICs) consisting of H–PF4 and immunoglobulins have been formed, a further step in the pathogenesis of HIT is IC binding to the surface of platelets. Because PF4 receptors are known to be present on the platelet surface, they are, at first sight, favored to be involved in IC binding to the platelet surface. However, attachment of ICs to platelets

mainly occurs by binding of heparin to its binding sites [7]. Mere IC attachment to platelets does not constitute a sufficient trigger for platelet activation. Instead, platelet activation results from the binding of the Fc portion of the immunoglobulins involved to Fc γ RIIA receptors (Fc γ RIIA) on the platelet surface [7,16]. The subsequent cross-linking of Fc γ RIIA activates the platelets. Here, IgG Fc moieties can interact with Fc γ RIIA on the same platelet (intraplatelet activation) or on other closely located platelets (interplatelet activation) [16].

In this regard, Fc γ RIIA have low affinity for IgG and predominantly interact with multivalent antigen-antibody complexes [17]. The HIT antibodies were found to potently induce the production of procoagulant platelet-derived microparticles, the capacity of which was exceeded only by calcium ionophore [16]. The HIT-IgG complex also caused the generation of thromboxane A₂ associated with platelet granule release. In addition, upon platelet activation by HIT-IgG, adenosine diphosphate (ADP) seems to constitute an important autocrine stimulator. Pretreatment of platelets with a potent ADP receptor antagonist completely blocked the activity of HIT sera [18].

Recently, Fc γ RIIA Arg/His131 polymorphism has been addressed with respect to an increased susceptibility for clinical HIT. However, because studies published on this topic have so far not yielded uniform results, the role of this polymorphism in contributing to the pathogenesis of HIT remains controversial [16].

Binding of H-PF4-IgG complexes to endothelial cells leads to expression of tissue factor

The HIT antibodies do not bind only to H-PF4 complexes in the fluid phase or on platelets; HIT antibodies also recognize PF4 complexed with heparan sulfate molecules on the EC surface [10]. This HIT antibody binding leads to EC activation, which in turn results in the expression of tissue factor. Tissue factor increases the production of thrombin. Moreover, vascular injury mediated by ICs is likely to contribute to thrombotic complications in HIT [19].

Activation of platelets and endothelial cells: the central role of thrombin generation

The marked platelet activation induced by HIT antibodies involves several distinct but interrelated mechanisms, of which cross-linking of Fc γ RIIA appears to be the most prominent. Platelet-derived microparticles markedly increase the phospholipid surface, which catalyzes formation of the prothrombinase complex. Thus, platelet activation by HIT antibodies ultimately leads to profound thrombin generation. Tissue factor expression on ECs enhances thrombin production. As platelet activation leads to further PF4 release, new H-PF4 complexes can form, thus increasing the quantity of pathogenic antigen in HIT, with platelet consumption thereafter. This process may lead to a sort of vicious cycle within the development of HIT.

The central role of thrombin in the pathogenesis of HIT provides the rationale to use potent thrombin inhibitors for alternative anticoagulation in this intriguing disease. Fig. 1 summarizes the main pathogenetic characteristics of HIT.

Clinical presentation of HIT

Knowing the pathogenesis of HIT provides a basis for understanding its main clinical sequelae. Emphasis has been placed on the fact that the definition for HIT should include both clinical and laboratory criteria, for which reason the disease has been termed a clinicopathologic syndrome [20].

Apart from the specific pathogenetic conditions favoring HIT antibody formation, the risk for developing clinical HIT depends on the clinical state of the patient, which determines the extent of platelet and endothelial cell activation [7]. For example, after cardiac surgery, patients often are positive for HIT antibodies; however, in comparison with patients after orthopedic surgery, they are less likely to develop clinical HIT [21]. Because determination and interpretation of data focusing on the frequency and incidence of HIT in various patient populations is a complicated matter, the reader is referred to the literature that discusses this topic in detail [21].

Here, the general clinical features with which HIT can present are primarily addressed, with a subsequent focus on

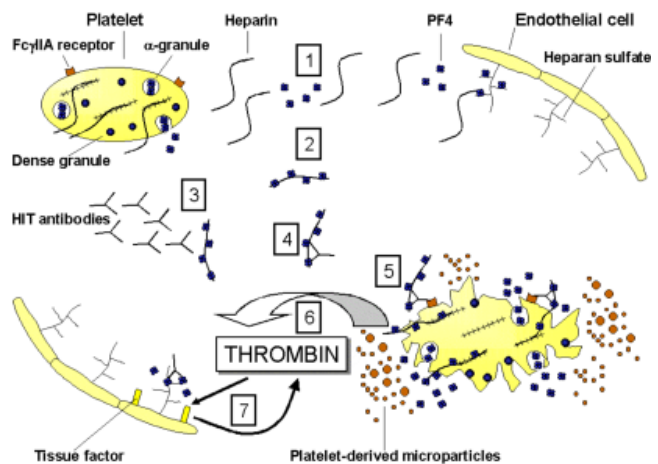


FIGURE 1 Mechanism of heparin-induced thrombocytopenia. (1) Heparin releases platelet factor 4 (PF4) from platelet α -granules and heparan sulfate glycosaminoglycans on the surface of endothelial cells (ECs). (2) Heparin and PF4 form multimolecular complexes (H-PF4 complexes). Upon specific stoichiometric concentrations, heparin induces conformational changes in the PF4 molecule. (3) PF4 neoantigens induce an immune response with subsequent generation of antibodies against H-PF4 complexes (HIT antibodies). (4) HIT antibodies bind to H-PF4 complexes. (5) Binding of HIT antibodies to Fc γ RIIA receptors on the platelet surface leads to their cross-linking (not shown), resulting in potent platelet activation with marked granule release and generation of procoagulant platelet-derived microparticles. (6) Marked thrombin generation occurs. (7) Binding of HIT antibodies to heparan sulfate-PF4 complexes on the surface of ECs causes their activation with subsequent expression of tissue factor. Further thrombin generation results.

TABLE I Main clinical characteristics associated with heparin-induced thrombocytopenia (in part modified from [2]).

Thrombocytopenia
Platelet count falls below $150 \times 10^9/L$ starting between day 5 and day 10 of heparin treatment
Platelet count falls $\geq 50\%$ from initial value starting between day 5 and day 10 of heparin treatment
More rapid fall in platelet count if patient was exposed to heparin within 3 months before actual heparin treatment
Main thrombotic events
Venous
Deep vein thrombosis
Pulmonary embolism
Adrenal hemorrhagic infarction secondary to adrenal vein thrombosis
Cerebral vein or cerebral dural sinus thrombosis
Arterial
Lower limb arterial thrombosis
Thrombotic stroke
Myocardial infarction
Vascular graft occlusion
Skin lesions occurring predominantly at subcutaneous heparin injection sites
Potential characteristics in hemodialysis patients
Occlusion of the vascular access (native fistula or prosthetic graft)
Premature clotting of the hemodialyzer or extracorporeal circuit
Thrombosis at insertion site of central venous catheter

HIT presentation in hemodialysis patients. Table I lists the main clinical characteristics of HIT.

General clinical sequelae

THROMBOCYTOPENIA

Defining thrombocytopenia as a platelet count below $150 \times 10^9/L$, thrombocytopenia is the most common clinical effect of HIT, being present in 85% – 90% of patients [22]. It must be emphasized, however, that suspicion of HIT must not only rest upon the fall in platelet count below this threshold in patients treated with heparin. Independent of absolute value, a fall in platelet count by $\geq 50\%$ of the value measured at the start of heparin treatment may likewise indicate HIT. In this regard, it should be emphasized that a marked drop in platelet count may especially be missed if the patient initially presents with platelet counts in the upper normal range or even with thrombocytosis. Assessment of platelet count must be performed sufficiently frequently to detect early the drop upon heparin treatment.

In HIT, platelet counts typically start to fall 5 – 10 days after initiation of heparin therapy [5]. An earlier fall in platelet count upon heparin treatment may be due to nonimmune HAT (discussed earlier), and a decrease in platelet count after 10 days of continued heparin treatment most often results from other pathologic conditions [22]. Yet, following recent catheter or surgical interventions, HIT may develop independent of the typical time frame [23]; this condition has been termed “resetting the clock” [22].

Because HIT antibodies often cannot be detected 100 days after a first positive test [24], HIT antibody formation appears to be transient. If the platelet count drops shortly after initiation of heparin therapy and is due to HIT rather than to nonimmune HAT or some other reason, the drop is most often the result of residual circulating HIT antibodies and is less likely due to a secondary immune response [24]. In this regard, occult heparin exposure (for example, heparin flushes) may change the typical time frame. Also, heparin flushes are capable of inducing HIT [25].

Often, the platelet count nadir in HIT is not as low as in other forms of drug-induced immune thrombocytopenia. A recent study evaluating platelet counts in 142 patients with laboratory-proven HIT showed a median platelet count nadir of approximately $60 \times 10^9/L$ [26]. Similar data have been obtained in a meta-analysis of 113 HIT patients suffering from thromboembolic complications [27]. In HIT, the platelet count nadir ranges from $20 \times 10^9/L$ to $150 \times 10^9/L$, with a median nadir of approximately $50 \times 10^9/L$ [2].

In HIT, the platelet count will not recover unless heparin is discontinued. The median duration until the platelet count increases above the threshold of $150 \times 10^9/L$ is about 4 days [22,27]. But platelet count recovery in HIT additionally depends on the severity of the disease; recovery may therefore take weeks in a given patient [22].

THROMBOSIS

Severe thrombocytopenia is often associated with bleeding, which may present as petechiae or spontaneous hemorrhage. In HIT, however, this rarely is the case. A prospective study demonstrated no increase of bleeding complications in HIT patients as compared with controls [5]. Instead, HIT is a prothrombotic state for which an odds ratio (OR) of 36.9 for any thrombosis (arterial and venous) has been prospectively determined [5]. This odds ratio for thrombosis far exceeds those reported for the most prominent hypercoagulable states, such as congenital antithrombin deficiency (OR: 24.1) or protein C deficiency (OR: 14.4) [22]. The unique pathogenesis in HIT with marked release of procoagulant platelet-derived microparticles and profound thrombin generation may sufficiently explain this paradoxical finding.

The first reports of thrombotic events during heparin treatment focused on arterial emboli requiring embolectomy. Thromboemboli were found to be rich in fibrin and platelets, which gives them a pale, salmon-colored macroscopic appearance, for which reason HIT was also termed “white clot syndrome” [28]. For a long time following these reports, HIT was regarded to predispose to arterial thromboembolism [28]. Indeed, current data show an odds ratio for arterial thromboembolism of 40.9 [95% confidence interval (CI): 0.6 – 831] in HIT patients as compared with control patients [22].

Arterial thrombosis in HIT most often occurs as lower limb arterial occlusion, followed by stroke and myocardial infarction, a pattern different from that observed in the gen-

eral (non HIT) population [20]. In contrast to the initial assumption that HIT-associated thrombosis occurs preferentially in arterial vessels, large case series now suggest that venous thrombotic complications predominate in HIT [22]. The likelihood of HIT to constitute the underlying disease seems to be associated with the severity of thrombosis, which is reflected in the respective odds ratios recently reported: distal deep vein thrombosis (DVT) (OR: 1.9; 95% CI: 0.3 – 10.4) < proximal DVT (OR: 27.0; 95% CI: 5.4 – 141) < bilateral proximal DVT (OR: 46.6; 95% CI: 3.5 – 380) < pulmonary embolism (OR: 93.4; 95% CI: 5.7 – 1374) [22].

Despite thrombotic events in HIT predominantly occurring in the deep veins of the lower limb, HIT may also present with thromboses in unusual locations: for example, cerebral dural sinus thrombosis, or adrenal hemorrhagic infarction due to thrombosis of adrenal veins [22]. Heparin-induced skin lesions—including skin necrosis, especially at the site of subcutaneous heparin injection—can be clinical manifestations of HIT. Recent intra-arterial punctures and central venous catheter insertions may also predispose for local thrombotic events [20].

Thus, because HIT may present with unusual manifestations, close monitoring not only of platelet count, but also of the clinical condition of patients treated with heparin is necessary. Lastly, a need to increase the heparin dose required to reach an intended target range for anticoagulation (“heparin resistance”) may indicate HIT in individual patients.

Given the specific role of heparin in initiating and maintaining HIT, mere heparin discontinuation could theoretically be assumed sufficient to avoid HIT-associated thromboses. A retrospective cohort study analyzing HIT patients presenting with isolated thrombocytopenia determined a cumulative risk for thrombosis of approximately 50% within the following month, regardless of heparin anticoagulation being simply stopped or substituted by warfarin [29]. A prospective trial likewise found a high initial thrombotic event rate of about 5% – 10% per day [30]. Therefore, it is now generally agreed that alternative anticoagulation should be considered also in those patients who present with HIT asymptomatic for thrombosis [2].

Clinical sequelae in the hemodialysis population

Anticoagulation with unfractionated heparin is the standard method for preventing clotting of the extracorporeal circuit in blood purification procedures such as hemodialysis (HD). Defining the potential role of HIT in contributing to morbidity and mortality in patients with chronic renal failure is therefore important. In a recent study investigating 154 consecutive patients newly treated with HD, 6 patients (3.9%) were clinically suspected of having developed HIT owing to a fall in platelet count accompanied by clotting of the dialyzer and the extracorporeal circuit [31]. The clinical diagnosis was confirmed by the detection of HIT antibodies in all but one patient. In a cross-sectional study of 165 patients undergoing HD with unfractionated heparin (UFH), 7 patients (4.2%) were

identified as having HIT antibodies [32]. However, in that study, no difference was seen in the incidence of thromboembolic events in HIT antibody-positive patients as compared with HIT antibody-negative patients [32]. Similar findings have also been reported by others (summarized in [33]).

One may conclude from these studies that only a few HD patients who form HIT antibodies develop clinical events. Moreover, it appears as if HIT-related events are more likely to be clotting of the dialyzer and extracorporeal circuit than symptomatic thrombosis directly affecting the patient [33]. However, patients can develop HIT even after years of chronic HD treatment: one patient developed life-threatening HIT following parathyroidectomy after 9 years of uneventful HD treatment with UFH anticoagulation [23]. Here, surgery may have contributed to HIT antibody formation, given that the highest reported rates of HIT are in postoperative patients receiving UFH (compare [21]).

With regard to thrombocytopenia being the clinical sign most often found in HIT, it is worth remembering that the HD procedure itself is associated with a relative decrease in platelet count [33]. Furthermore, the fall in platelet count in HD patients developing HIT may be only moderate [34].

One of the most serious complications in HD patients, occlusion of the vascular access (the “Achilles heel” of HD), may indicate HIT. This complication has been described for both native fistulae and prosthetic grafts [35,36]. Fibrin formation or clotting of the extracorporeal circuit despite sufficient anticoagulation should strongly suggest the possibility of HIT [37]. However, these clues are not specific. Thus, the clinician must consider other factors that could compromise patency of the extracorporeal circuit (for example, low blood flow owing to access or catheter malfunction, high ultrafiltration rate, excess turbulence, and foam formation in the drip chambers [33]). In addition, patient-related factors such as low arterial blood pressure or high hematocrit should be considered. Apart from insufficient anticoagulation, these factors should first be ruled out as potential underlying causes of clotting within the extracorporeal circuit before HIT is considered in the differential diagnosis.

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