Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Advances in the treatment of heparin-induced thrombocytopenia: latest clinical data

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Heparin-induced thrombocytopenia (HIT) is an immune-mediated adverse drug effect caused by platelet-activating antibodies to a complex of platelet factor 4 and heparin. Increasing evidence from clinical and laboratory studies suggests that HIT is overdiagnosed due to a high prevalence of nonactivating platelet factor 4/heparin antibodies. This is especially true in patient populations where thrombocytopenia is frequent due to other reasons, such as intensive care patients and patients after cardiovascular surgery. Several scoring systems have been proposed that, in combination with laboratory assays, facilitate the diagnosis of HIT. Beside danaparoid and the direct thrombin inhibitors argatroban, lepirudin and bivalirudin, several case series suggest that fondaparinux is also a relatively safe option for anticoagulation in acute HIT.

Keywords: alternative anticoagulant • clinical risk score • heparin-induced thrombocytopenia • laboratory assays • platelet factor 4

Antibody-mediated heparin-induced thrombocytopenia (HIT) is an adverse effect of heparin treatment. HIT usually occurs if platelet-activating antibodies targeting an antigen composed of polyanions, such as unfractionated or low molecular weight heparin, and platelet factor 4 (PF4) are formed [1–3]. Platelet activation results from crosslinking of the platelet Fcγ receptor IIa by the polyanion–antibody immune-complexes [4]. Intravascular platelet activation causes platelet consumption, as indicated by a fall in the platelet count and/or thrombocytopenia, and leads to a paradox prothrombotic state. *In vitro* data suggest that the endothelium and monocytes might contribute to the prothrombotic state [5]. The mechanism leading to thrombocytopenia in HIT by intravascular platelet activation is fundamentally different from the mechanisms leading to thrombocytopenia by autoantibodies or other drug-dependent antibodies. In the latter, antibodies bind via their F(ab)-domain to the platelet, while the Fc-domain of the antibodies binds to Fc-receptors on macrophages, causing enhanced clearance of platelets [6].

Only IgG antibodies can crosslink platelet Fcγ receptor IIa. Thus, IgG antibodies to PF4/heparin are primarily responsible for HIT, while PF4/heparin IgA and IgM antibodies are not platelet activating and are probably of little clinical relevance. In addition, antibodies to other heparin-binding proteins (e.g., neutrophil-activating protein-2 and IL-8) play only a minor role in the development of HIT (<2%).

Although the incidence of HIT decreased during the last years and may decrease further as heparin and low molecular weight heparin are replaced by novel anticoagulants, heparin is still required in a variety of indications such as cardiac surgery, hemodialysis and especially for therapeutic dose anticoagulation in critically ill patients.

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When HIT develops, the risk for the development of new thrombosis is very high with an absolute risk of 30–75% and an odds ratio of a 20–40-fold increased risk, respectively [7]. Therefore, if HIT is suspected, heparin should immediately be replaced by an alternative anticoagulant. Alternative anticoagulants approved for the treatment of HIT include danaparoid, lepirudin, argatroban and bivalirudin. Other alternative anticoagulants not yet approved but often considered in the treatment of HIT include various factor Xa and thrombin inhibitors. This review provides an overview on HIT with a focus on recent clinical data, which impact diagnosis and treatment of HIT.

Diagnosis of HIT

Heparin-induced thrombocytopenia should be suspected if the platelet count decreases by 50% (≤30%) during or shortly after administration of unfractionated heparin low molecular weight heparin, or fondaparinux. The platelet count typically starts falling 5–14 days after the start of heparin. However, patients who have been recently pre-exposed to heparin may have circulating PF4/heparin antibodies. In these patients the platelet count falls within hours after re-exposure. In a subset of these patients who receive a heparin bolus, generalized reactions that are mediated by immune complex formation may occur. PF4/heparin antibodies are transient and usually decline within a few weeks, therefore, such rapid-onset HIT needs to be considered only in patients with recent heparin exposure, typically within the previous 2–4 weeks. Because HIT is a prothrombotic condition, bleeding complications are rare. In addition, the platelet count in HIT usually does not decline below 20 G/l. In patients with very low platelet counts and especially in bleeding patients, other causes of thrombocytopenia are much more likely than HIT.

A clinical determination of the probability that HIT is present should always precede laboratory assays for the disorder. Clinical scoring systems are often helpful in assessing the pretest probability if HIT may be the cause for the low platelet count. In general, laboratory assays may not be required in patients with a low pretest probability in a clinical scoring system. If the clinical scoring system indicates an intermediate or high risk for HIT, laboratory assays for the presence of antibodies directed against PF4/heparin can help to determine whether HIT is likely present or not.

Clinical scoring systems

The 4 T-score (Table 1) is a risk score taking clinical and laboratory information into account. The score was first validated in two populations of mixed patients with suspected HIT in Canada and Germany in 2006 [8], and subsequently validated in ICU patients [9]. The 4 T-score results in a score of 0–8 points if applied to a given patient with thrombocytopenia or a falling platelet count. HIT should be considered unlikely if the resulting score is equal to or lower than 3 points. If the 4 T-score is greater than 3 points, HIT is moderate (4–6 points) or very likely (7–8 points) and laboratory work-up should be initiated to eventually rule out HIT.

Recently, a novel risk score termed the HIT expert probability (HEP) score was introduced by Cuker *et al.* [10]. For the development of this score, eight clinical and laboratory features with potential importance for the diagnosis of HIT were identified (Table 2). These features were then weighted by 26 experts in the field who assigned each feature a score ranging from -3 (arguing strongly against HIT) to +3 (arguing strongly for HIT). The median weights determined by the experts were then incorporated into a clinical pretest score that was

Table 1. 4 T-score.

Category	2 points	1 point	0 point
Thrombocytopenia	Platelet count fall >50% and platelet nadir ≥20 × G/l	Platelet count fall 30–50% or platelet nadir 10–19 × G/l	Platelet count fall <30% or platelet nadir <10 × G/l
Timing of platelet	Clear onset between days 5 and 10 or platelet count fall ≤1 day (prior heparin exposure within 30 days)	Consistent with days 5–10 fall, but not clear (e.g., missing platelet counts) or onset after day 10, or fall ≤1 day (prior heparin exposure 30–100 days ago)	Platelet count fall <4 days without recent heparin exposure
Thrombosis or other sequelae	New thrombosis (confirmed) or skin necrosis at heparin injection sites or acute systemic reaction after intravenous heparin bolus	Progressive or recurrent thrombosis or non-necrotizing (erythematous) skin lesions or suspected thrombosis (not proven)	None
Other causes for thrombocytopenia	None apparent	Possible	Definite

A total score of >6 points indicates a high probability of heparin-induced thrombocytopenia (HIT), 4 to 6 points indicates an intermediate probability of HIT and ≤ 3 points a low probability of HIT.

validated in 50 consecutive patients. The HEP score was compared with the 4 T-score and showed greater inter observer agreement (0.88 vs 0.71) and a greater area under the receiver operator curve (0.91 vs 0.74; p = 0.017); thus, it showed greater sensitivity and specificity. The authors proposed a cut-off of 2 points for 100% sensitivity (i.e., identification of all patients where HIT was present), which was comparable to 4 points for a 100% sensitivity in the 4 T-score. The authors were able to rule out HIT in 26 patients with the HEP score and in 19 patients with the 4 T-score. Conversely, HIT was suspected in 24 and 31 patients, respectively, in the two scores, eight of which truly had HIT demonstrated by a functional assay.

In contrast to the 4 T-score and the HEP-score, which aim to select patients in whom HIT antibodies are likely present, Messmore *et al.* proposed a very simple score to exclude HIT [11]. They evaluated their score (Table 3) retrospectively by chart review of 100 patients, most of whom (99) were male. Their score is dichotomous, resulting in scores of 0 (HIT unlikely) or 1 (HIT likely), and achieved a specificity of 89 and 98% for two functional assays (platelet aggregation and serotonin release, respectively), that is, in other words, capable of correctly identifying most patients who did not have HIT. The authors even propose a management strategy, although they do not report outcomes such as new thrombosis or bleeding in their patient cohort.

Both of these novel scores need to be validated in larger prospective studies before firm conclusions can be drawn.

Laboratory assays

■ Screening assays

The screening tests for PF4/heparin antibodies are based on measuring antibody binding to immobilized

Table 2. Heparin-induced thrombocytopenia expert probability score.

Clinical feature	Score
% magnitude of fall in platelet count (measured from peak platelet count to nadir platelet count since heparin exposure)	
<30	-1
30–50	1
>50	3
Timing of fall in platelet count	
For patients in whom typical-onset HIT is suspected	
- Fall begins <4 days after heparin exposure	-2
- Fall begins 4 days after heparin exposure	2
- Fall begins 5–10 days after heparin exposure	3
- Fall begins 11–14 days after heparin exposure	2
- Fall begins >14 days after heparin exposure	1
For patients with previous heparin exposure in last 100 days in whom rapid-onset HIT is suspected	
- Fall begins <48 h after heparin re-exposure	2
- Fall begins >48 h after heparin re-exposure	-1
Nadir platelet count (x G/l)	
≤20	-2
>20	2
Thrombosis (select no more than one)	
For patients in whom typical-onset HIT is suspected	
- New VTE or ATE ≥4 days after heparin exposure	3
- Progression of pre-existing VTE or ATE while receiving heparin	2
For patients in whom rapid-onset HIT is suspected	
- New VTE or ATE after heparin exposure	3
- Progression of pre-existing VTE or ATE while receiving heparin	2
Skin necrosis	
Skin necrosis at subcutaneous heparin-injection sites	3
Acute systemic reaction	
Acute systemic reaction after intravenous heparin bolus	2
Bleeding	
Presence of bleeding, petechiae or extensive bruising	-1
Other causes of thrombocytopenia (select all that apply)	
Presence of a chronic thrombocytopenic disorder	-1
Newly initiated non-heparin medication known to cause thrombocytopenia	-2
Severe infection	-2
Severe DIC (defined as fibrinogen <100 mg/dl and D-dimer >5.0 µg/ml	-2
Indwelling intra-arterial device (e.g., IABP, VAD and ECMO)	-2
Cardiopulmonary bypass within previous 96 h	-1
No other apparent cause	3

A total score of ≥2 points might be 100% sensitive for HIT, that is, identify all patients where HIT is present.
ATE: Arterial thromboembolism; DIC: Disseminated intravascular coagulation; ECMO: Extracorporeal membrane oxygenation; HIT: Heparin-induced thrombocytopenia; IABP: Intra-aortic balloon pump; VAD: Ventricular assist device; VTE: Venous thromboembolism.

Table 3. A simple clinical scoring system for the diagnosis of suspected heparin-induced thrombocytopenia.

Score	Clinical criteria	Clinical management
0	Heparin therapy not present for 5 days preceding platelet count drop, or platelet count did not fall by 30%, or significant competing cause for thrombocytopenia (e.g., recent coronary artery bypass [2–3 days], sepsis, shock, balloon pump or drugs other than heparin)	Continue heparin therapy if clinically indicated, while waiting for heparin-induced thrombocytopenia laboratory test results
1	On heparin therapy No significant competing cause for thrombocytopenia Platelet count fall by >30% New thrombosis	Discontinue heparin therapy, while waiting for heparin-induced thrombocytopenia laboratory test results Administer alternative anticoagulation if clinically indicated

PF4–polyanion complexes. There are two types of screening assays, enzyme immunoassays and agglutination assays. These screening assays are available at most larger hospitals.

All enzyme immunoassays are based on the recognition of a PF4–heparin or a PF4–polyanion complex by antibodies. The antibodies are then detected by anti-human-immunoglobulin enzyme-linked antibodies, which only bind to IgG, or recognize IgG, IgA, and IgM combined. IgG-specific assays have a greater specificity for clinically relevant, that is platelet-activating, antibodies.

The commercially available antigen assays can be divided into solid-phase enzyme-linked immunoassays, particle-based assays and automated assays based on latex particles agglutination or chemiluminescence.

Several enzyme-linked immunoassays are commercially available, and vary in their design. They recognize PF4/heparin IgG alone or detect IgG, IgA and IgM antibodies. They also differ in the type of antigen, which could be recombinant, purified from platelets or containing platelet/leukocyte lysates. These proteins are complexed to heparin or polyvinyl-sulfonate. Despite these differences, the overall comparability of these assays is reasonable [12,13], although none of the assays recognize all PF4/heparin antibodies. It seems, however, that most of the clinically relevant antibodies are reactive in all the different assays.

The results of the enzyme immunoassays are usually reported in optical densities. As a general rule, greater optical density levels indicate a greater likelihood that the antibodies are platelet activating and clinically relevant. However, optical density values between different laboratories can vary considerably due to different reagents and photometers [14]. Therefore the range (0–maximum value) of the optical density has to be considered if cut-off levels from different laboratories are compared. In particular if the reactivity strength is used for clinical decisions on whether alternative treatment is required or not, the reactivity profile of the assay of the respective laboratory needs to be determined. This, however, is difficult as no standard for PF4/heparin antibodies exists. Therefore we recently proposed to standardize report test reactivity by dividing the absolute optical density into ten equal steps and reporting in which decile a specific measurement falls.

Zwicker *et al.* found in a retrospective study that a higher optical density of the enzyme immunoassay correlated with the development of thrombosis in patients with suspected HIT [15]. In 2008 and 2009, Warkentin *et al.* and Bakchoul *et al.*, respectively, found that a very high optical density predicted HIT in approximately 90% of patients [16,17]. However, Althaus *et al.* recently showed that considering only high optical density (OD) of the enzyme-linked immunoassays (defined by an OD >1.0) as positive, will cause an unacceptable loss of sensitivity for functional active antibodies by approximately 15% [18]. While HIT becomes much more likely in patients with very high optical density in the EIA, the test results must always be interpreted in the context of the clinical presentation. The strong correlation of high ODs with the presence of platelet-activating antibodies indicates that the OD need to be reported to the treating physician.

The particle-based immunoassay uses gel centrifugation technology, which is widely applied for detection of red cell antibodies in immunohematology. The sensitivity and specificity of this assay seems to be intermediate between the enzyme immunoassays and the washed platelet-activation assays [19]. Specificity can be increased by titrating the patient serum, with sera being positive at a 1:8 dilution having a very high likelihood for indicating HIT [20]. However, the sensitivity of the particle gel immunoassay reaches only approximately 95%; thus, up to 5% of HIT patients might be missed. Therefore, patients with a high clinical probability and a negative particle-based immunoassay should be evaluated in another test system.

In contrast to the above described assays, the particle immunofiltration assay showed poor sensitivity and specificity and should not be used in the diagnosis of HIT [21].

Two automated assays, one based on the agglutination of latex particles and the other on chemiluminescence, have recently been introduced. In the latex agglutination assay, agglutination of PF4/heparin coated beads by a monoclonal anti-PF4/heparin-antibody is inhibited in the presence of human PF4/heparin antibodies [22]. The chemiluminescence assay shows a wide range of reactivity and might, thus, provide additional information compared with the enzyme-linked immunoassays [23]. These automated assays will allow greater standardization and better comparability of results obtained in different laboratories. However, they are just being introduced and experience under daily practice conditions is still limited.

In summary, with the one exception of the particle immunofiltration assay, the antigen-based assays are good in ruling out HIT. However, they have a very limited specificity for functionally active (platelet-activating) antibodies. If the diagnosis of HIT is based solely on these antigen assays, HIT will be overdiagnosed by at least 50% because less than half of the antibodies recognized by the enzyme immunoassay have platelet-activating properties that cause HIT [24].

■ Functional assays

Positive results in the antigen-based screening assays should be followed up with a functional assay with greater specificity for clinically relevant antibodies, such as the platelet serotonin-release assay or the heparin-induced platelet-activation assay. These functional assays are not commercially available. They are technically demanding and require access to freshly obtained and washed platelets from healthy donors. Sensitivity of these assays depends strongly on the preparation of platelets and on donor characteristics. Functional assays are usually limited to specialized laboratories and obtaining the final results – including shipping of the sample – can take several days. Therefore, the treating physicians are often faced with a positive screening assay, but without a definite diagnosis of HIT. Since the development of thrombosis due to platelet-activating PF4/heparin antibodies is potentially limb or life threatening, patients with a positive screening assay are often switched to an alternative anticoagulant until the results of the functional assays are obtained and the diagnosis of HIT is established or ruled out. Very recently it was proposed that impedance aggregometry using whole blood (Multiplate™) also reaches high sensitivity for platelet-activating antibodies [25,26]. This interesting observation requires further validation. If confirmed, it might be a practicable approach for many laboratories because freshly obtained washed platelets would no longer be required.

Combination of clinical scores & laboratory assays

To facilitate the management of patients in whom HIT is suspected, but not yet proven by a functional assay, Ruf *et al.* proposed to incorporate the optical density from an enzyme immunoassay together with the 4 T-score in the decision of whether alternative anticoagulants are required or not [27]. They retrospectively evaluated their algorithm in 83 patients in whom HIT was suspected and achieved a sensitivity of 90% and a specificity of 82% compared with the functional serotonin-release assay.

Another approach using the 4 T-score and the particle immunoassay was proposed by Pouplard *et al.* in 2007 [28]. By applying this approach in 213 consecutive patients with suspected HIT, the authors were able to exclude HIT in patients with moderate risk in the 4 T-score and a negative particle gel immunoassay. However, a positive result in the particle gel immunoassay did not improve the diagnostic accuracy for platelet-activating antibodies.

Table 4 indicates an overview of a diagnostic strategy and the probability that HIT is present. We suggest a determination of the clinical probability by a clinical risk score followed by a screening and a functional assay. To determine whether this approach combining the clinical risk score with laboratory assays is feasible and safe requires a prospective management study evaluating the outcomes of patients with suspected HIT.

Treatment of HIT

■ Rationale of anticoagulant treatment despite low platelet counts

In HIT, PF4/heparin antibodies cause intravascular platelet activation. This results in a prothrombotic state, characterized by increased thrombin generation, which is indicated *in vivo* by elevated levels of thrombin–antithrombin complexes [29]. Tardy-Poncet *et al.* recently confirmed the concept of increased thrombin generation in HIT indirectly, by demonstrating *in vitro* increased thrombin generation in platelet-rich plasma if PF4/heparin antibodies and low doses of heparin were present [30]. Increased thrombin generation in HIT provides the rationale for the current therapeutic concepts, which all aim to decrease thrombin generation by anticoagulation with a compatible anticoagulant. Thrombin generation is decreased either by direct thrombin inhibitors (argatroban, lepirudin and bivalirudin) or indirectly by factor Xa inhibitors

Table 4. Proposed scheme for interpretation of heparin-induced thrombocytopenia test results in the context of the clinical presentation

Clinical risk score	Screening assay† (OD)	Functional assay	Likelihood of HIT
High	High	Positive	Very likely
	High	Negative	Unlikely, but repeat functional assay
	Low	Positive	Likely
	Low	Negative	Unlikely
Intermediate	High	Positive	Very likely
	High	Negative	Unlikely
	Low	Positive	Likely
	Low	Negative	Very unlikely
Low	High	Positive	Revaluate clinical score. If still low, this is unlikely to be HIT
	High	Negative	Very unlikely
	Low	Negative	HIT excluded

The clinical risk score should be categorized into high, intermediate and low probability of HIT where appropriate, while the functional assay should be categorized into positive (platelet-activating with low but not with high heparin) and negative (non-activating) antibodies.
†As maximal ODs differ depending on photometer and test design, no precise cutoff can be given. High ODs are usually ODs >1.0–1.5.
HIT: Heparin-induced thrombocytopenia; OD: Optical density.

(danaparoid and fondaparinux). Of these drugs, all but fondaparinux are currently approved for the treatment of HIT (approval status may differ between medical jurisdictions). None can be antagonized and most share a relatively high risk of bleeding. For lepirudin and argatroban the risk of major bleeding was approximately 1% per day of treatment in the trials, leading to approval of the respective drugs [7].

■ Factor Xa inhibitors

Danaparoid

Danaparoid is a mixture of anticoagulant glycosaminoglycans (heparansulfate ~80%, chondroitinsulfate ~15% and dermatansulfate ~5%). Its main mode of action is antithrombin-mediated inhibition of factor Xa and to a far lesser extent of factor IIa via heparin cofactor II. Danaparoid also seems to support fibrinolysis by rendering the fibrin network more permeable and consequently less resistant to fibrinolysis, an effect that is shared with the direct thrombin inhibitors argatroban and bivalirudin [31].

In contrast to all other alternative anticoagulants, danaparoid has a property that makes it unique in the treatment of HIT. Danaparoid displaces PF4 from the platelet surface, disrupts PF4/heparin complexes, and is thereby shutting off the immune-mediated intravascular platelet activation, which is the primary reason for increased thrombin generation [32]. In rare cases (2–5%) antibodies to PF4/heparin can crossreact with danaparoid, which may result in treatment failure. Thus, if the platelet count does not increase after 3–5 days of treatment with danaparoid, cross-reactivity should be considered and an alternative anticoagulant should be given.

The dose of danaparoid is adjusted by anti-FXa levels with a target range of 0.5 to 0.8 anti-FXa U/ml in a danaparoid calibrated anti-Xa assay. Monitoring of danaparoid is independent of prothrombin levels, which allows correct dose estimation in patients with low prothrombin levels, for example, in disseminated intravascular coagulation. Danaparoid has a long half-life and its pharmacokinetic elimination depends (by ~30%) on renal function. Although it is the only drug which has been evaluated in a prospective randomized trial in HIT, only a few patients were included in the trial and danaparoid was compared with dextrane only [33].

Fondaparinux

Fondaparinux is a synthetic pentasaccaride that specifically inhibits factor Xa in an antithrombin-dependent manner. Although fondaparinux is negatively charged and can bind to PF4, HIT antibodies usually do not bind to this complex. There are several small observational case series successfully using fondaparinux in the treatment of HIT, all of which were recently summarized [34]. None of these studies had been conducted in a well controlled setting. Fondaparinux can be used to treat venous thromboembolism at weight-adjusted once-daily doses without monitoring, which is a considerable advantage. However, in patients with impaired renal function, fondaparinux accumulates

and its long half-life may increase the risk for bleeding problems in patients requiring invasive diagnostic or therapeutic procedures.

There is some uncertainty regarding potential clinically relevant crossreactivity of PF4/heparin antibodies with fondaparinux. It has been shown in a prospective trial that anti-PF4/heparin antibodies occur and develop at similar rates in orthopedic patients treated with fondaparinux or enoxaparin [35]. However, *in vitro* crossreactivity of PF4/heparin antibodies with fondaparinux is weak and can be demonstrated only in a small subset of HIT sera [36], while *in vivo* crossreactivity of fondaparinux has not been shown convincingly. Salem *et al.* published one case and summarized two others where HIT occurred in fondaparinux-treated patients [37]. HIT occurred after orthopedic surgery in all three patients. It remains unclear whether fondaparinux was truly the cause for the thrombotic complications, or whether fondaparinux triggered an immune response in which the PF4/heparin antibodies became autoantibodies recognizing PF4 bound to chondroitinsulfate at the platelet surface and, thereby, caused clinical sequelae independently of fondaparinux .

Direct FXa inhibitors

Rivaroxaban is an oral direct factor Xa inhibitor, which is independent of antithrombin and has been shown to be noninferior to low molecular weight heparin followed by vitamin K antagonists in the treatment of venous thromboembolism [38]. *In vitro* data demonstrated that rivaroxaban did not crossreact with PF4/heparin and enoxaparin antibodies [39].

Otamixaban is an intravenous direct factor Xa inhibitor. Preliminary data indicate no crossreactivity of PF4/heparin and PF4/enoxaparin antibodies with otamixaban [40].

Rivaroxaban, and potentially also other direct factor Xa inhibitors, might prove useful in the treatment of HIT, but clinical data are yet lacking.

■ Direct thrombin inhibitors

Four direct thrombin inhibitors have been used for the treatment of HIT. All are usually monitored by a prolongation of the activated partial thromboplastin time (aPTT) with a target range of a 1.5–3-fold prolongation. The aPTT is strongly dependent on the prothrombin concentration. This dependency can cause severe dosing issues of the direct thrombin inhibitors in patients with low levels of prothrombin (e.g., caused by: liver impairment, disseminated intravascular coagulation or recent treatment with vitamin K antagonists), which can cause a false prolongation of the aPTT. This can result in under-dosing and development of thromboembolism. The direct thrombin inhibitors also cause a prolongation of the prothrombin time and the international normalized ratio (INR). As a general rule, interpretation of clotting parameters is difficult in patients treated with these drugs. Hereby, the effects are greatest in argatroban, intermediate in bivalirudin, and smallest in lepirudin- or desirudin-treated patients.

Dosing of all direct thrombin inhibitors is sometimes challenging even for experienced physicians. Kiser *et al.* reported that the implementation of a nomogram compared with the physician's discretion for the dosage of direct thrombin inhibitors can significantly decrease the time to therapeutic range, but also found that the rates of major bleeding and new thrombosis were similar in both groups [41]. However, the cohorts were too small to draw firm conclusions for clinical outcomes. As the nomogram improved the time in therapeutic range, implementation of comparable nomograms should be considered.

Lepirudin

Lepirudin is a recombinant hirudin that directly inhibits thrombin by forming irreversible complexes. The recommended dose of lepirudin is a continuous infusion of 0.1 mg/kg in patients with normal renal function [7]. Lepirudin is primarily excreted by renal filtration. Therefore, extreme caution needs to be applied if the drug is used in patients with renal insufficiency and substantial dose reduction in case of renal impairment is necessary. A recent case report describes the use of lepirudin in a patient with HIT-associated thrombosis and acute renal failure. Lepirudin was used in very low doses and was titrated using the aPTT and an anti-factor IIa assay [42]. While this approach is feasible it requires experience and close monitoring.

As lepirudin is a xenogenous protein, antibody formation can be induced. These antibodies can cause anaphylactic reactions if the immune system has been pre-exposed to the drug. Contrary to IgE-mediated anaphylactic reactions, the anaphylactic reactions to lepirudin are caused by IgG antibodies. IgG mediated anaphylactic reactions require a high antigen load, which is usually only achieved if a large starting dose is given. More frequently, antibodies to lepirudin are formed *de novo* during treatment and can cause reduced clearance of the drug with the risk of accumulation, but without affecting lepirudin's anticoagulant properties. Therefore, the aPTT needs to be monitored daily while the drug is administered.

Bivalirudin

Bivalirudin is a synthetic congener of hirudin consisting of only 20 amino acids. It is a direct thrombin inhibitor, but contrary to lepirudin it can be cleaved by thrombin. This causes reversible binding, although the intact

drug binds very strongly to thrombin. The half-life of the drug is short (20 min) and it is eliminated largely enzymatically (80%). Bivalirudin was introduced in the treatment of HIT after one case series of 54 patients [43] undergoing coronary intervention. Two subsequent trials showed in 49 [44] and 51 [45] patients, respectively, that bivalirudin can be used in patients with coronary artery bypass surgery. If the drug is used in cardiac surgery, it is important to avoid any stasis of blood in the extracorporeal circuit, since in nonflowing blood, such as in reservoirs, thrombin rapidly cleaves bivalirudin resulting in clotting [46].

Skrupky *et al.* compared bivalirudin to argatroban in a retrospective analysis of 138 patients in whom HIT was suspected. No differences were found between bivalirudin and argatroban in the frequency of thromboembolic events or bleeding. Furthermore, both drugs were comparable for the time in therapeutic range defined as a 1.5–3-fold increase of the aPTT. Perhaps the most important finding was that patients were more frequently supratherapeutic in the argatroban group than in the bivalirudin group, suggesting that the current dose recommendations for argatroban are too high [47].

Desirudin

Desirudin is also a recombinant hirudin differing from lepirudin in two functionally nonrelevant amino acids, with very similar characteristics as lepirudin. It has originally been studied in patients undergoing orthopedic hip-replacement surgery [48,49]. In an open label trial of 16 patients with suspected HIT, patients were randomized to receive either argatroban at therapeutic dose or desirudin at prophylactic dose (15 mg subcutaneously every 12 h) in patients without thrombosis, and desirudin at greater doses (30 mg subcutaneously every 12 h) in patients with thrombosis [50]. The authors found that the efficacy of the two drugs was comparable. Interestingly, desirudin was applied without dose adjustment although the aPTT was used to avoid overdosing of the drug. However, as desirudin is also renally excreted, there is still a risk of accumulation if the renal function is impaired or anti-desirudin antibodies are formed. Further evaluation in a larger cohort is needed to determine if a fixed subcutaneous dose of desirudin with minimal monitoring is safe.

Argatroban

Argatroban is a small molecule with specific binding to the catalytic site of thrombin, which is dosed at 2 or 0.5 µg/kg/min (the latter dose in patients with hepatic insufficiency) [51]. The main advantages of argatroban are its relatively short half-life, hepatic elimination and its independence of renal function. Observational studies assessing argatroban in intensive care patients strongly suggested to decrease the dose from 2 to 0.2–0.5 µg/kg/min [52,53]. These data were confirmed in a recent retrospective study, which showed that some patients with hepatic impairment require argatroban doses of less than 0.25 µg/kg/min [47].

Beside the aPTT, no other test for monitoring argatroban has been systematically evaluated. This makes dosing difficult in patients with spontaneous a PTT prolongation, for example, caused by disseminated intravascular coagulation, and especially during transition to vitamin K antagonists (see below).

Dabigatran-etixilate

No *in vitro* or clinical studies have been carried out for the orally administered direct thrombin inhibitors dabigatran-etixilate in HIT, but since the chemical structure of dabigatran-etixilate differs greatly from the negatively charged polysaccharides of heparin, crossreactivity seems unlikely. Similar to argatroban, dabigatran blocks thrombin *in vivo,* making it a drug that might be useful in the treatment of HIT.

Platelet concentrates in HIT

Platelet transfusions should be avoided in acute HIT patients without major bleeding because the transfused platelets might become activated by still circulating HIT-antibodies. Prophylactic platelet transfusions are not indicated before embolectomy of an arterial thrombus in acute HIT unless there is increased peri-operative bleeding.

However, very low platelet counts or bleeding (e.g., for surgical reasons) occasionally require the transfusion of platelet concentrates. Two retrospective chart reviews identified four patients in whom HIT was proven by functional assay [54] and 37 patients in whom HIT was suspected due to a positive screening assay [55]. Platelet transfusions were given for prophylactic reasons (n = 25) and for bleeding (n = 16). None of these patients developed new thromboembolism and bleeding was controlled in ten of 16 patients. This indicates that platelet transfusions can be considered in HIT patients if the platelet count is very low (<10 G/l) or if bleeding occurs.

Outlook: tyrosine kinase inhbition

The platelet Fcγ receptor IIa mediates platelet activation by phosphorylation of its cytoplasmatic domain by tyrosine kinases. Reilly *et al.* demonstrated in a mouse model that inhibition of the spleen tyrosine kinase (syk) by a selective inhibitor (PRT-318) prevented platelet activation *in vitro* as well as thrombocytopenia and thrombosis *in vivo* without causing a bleeding diathesis. These results indicate a novel approach in the treatment of HIT where platelets are directly inhibited and which

might not require therapeutic antithrombotic drugs. However, the Fcγ IIa receptor is widely expressed in various tissues and although mice did not show major side effects during treatment, it is unclear whether this is also true in humans [56].

Long-term treatment of HIT associated thrombosis

Venous and arterial thromboembolism are typical complications of HIT. Vitamin K antagonists are still the mainstay of long-term secondary prophylaxis in HIT patients with venous thrombosis. However, vitamin K antagonists must be avoided in the acute phase of HIT as they induce transient protein C deficiency due to a shorter half-life of protein C than the vitamin K dependent procoagulant factors II, IX and X. This intensifies the prothrombotic state in HIT thereby enhancing the risk for microvascular thrombosis and limb loss. Therefore, vitamin K antagonists should only be initiated at a maintenance dose in HIT and after the platelet counts have been normalized to a steady plateau under bridging with a compatible anticoagulant.

The initiation phase of vitamin K antagonists can be challenging if a direct thrombin inhibitor is given. The INR is dependent on the activity of thrombin, which is decreased if direct thrombin inhibitors are used. This effect is greatest for argatroban, less for bivalirudin and lowest for lepirudin [57]. Therefore, a falsely elevated INR is often measured if direct thrombin inhibitors are used and the INR does not reflect the true anticoagulant activity of the vitamin K antagonist *in vivo*. If argatroban is used in combination with vitamin K antagonists, INR values of greater than 5 occur without an increased risk of bleeding complications [58]. Alternatively, assays that are not clot-based and therefore not influenced by the direct thrombin inhibitor argatroban can be applied to monitor the vitamin K antagonist effect [59]. Most practical is the chromogenic determination of factor X activity [60], where a factor X activity of approximately 30% usually indicates an INR in the therapeutic range (2–3) [61]. Currently, no data on the new anticoagulants such as dabigatran-etixilate or rivaroxaban are available for the long-term treatment of HIT associated thrombosis.

Pitfalls in the diagnosis of HIT

■ Atypical onset of HIT

As discussed above, the platelet count typically starts falling 5–10 days after the start of heparin in HIT patients and venous or arterial vascular occlusion may occur during this time period. Although the term HIT implies thrombocytopenia, the platelet count might still be in the normal range, even if platelet-activating PF4/heparin antibodies are present. For example, a fall in the platelet count from 400 to 200 G/l would result in a platelet count within the normal range, however, a relative fall in platelet counts of 50% has occurred and HIT might be considered. In some patients, the platelet count decrease can occur within the first day of heparin treatment, so called 'rapid-onset HIT'. These patients have usually been exposed to heparin within the last 4 weeks (rarely <100 days) and PF4/heparin antibodies are still circulating in their plasma. Very rarely, naturally occurring functional antibodies may be present even if the patient has not been pre-exposed to heparin. Furthermore, PF4/heparin antibodies can develop in fondaparinux-treated patients, and if these patients are then switched to heparin, for example when renal-replacement therapy becomes necessary, the platelet count can also drop very fast.

Heparin-induced thrombocytopenia can also manifest several days after cessation of heparin, so called delayed-onset HIT. In these patients, the antibodies recognize platelet bound-PF4 even if heparin has been withdrawn for several days that is in the absence of heparin. This autoimmune disease probably develops by epitope spreading of B cells, leading to antibodies with a wider reactivity profile. Affected patients require prolonged therapeutic dose alternative anticoagulation until the platelets count normalize and the autoreactive antibodies disappear, which can last several weeks and sometimes months [62,63].

When to expect HIT & PF4/heparin antibodies

■ General hospital patients

Data on the frequency of HIT are usually retrospective and depend on the composition of the cohort, which is either surgical or medical patients. In a retrospective analysis of all patients admitted to a tertiary care hospital during 1.5 years, Ban-Hoefen *et al.* found that HIT was likely in 0.48% of patients treated with unfractionated heparin, 0.08% in patients treated with low molecular weight heparin and 0.33% in patients treated with both drugs [64]. Due to the local policy of the institution, the diagnosis of HIT was established not by a functional assay but by PF4/heparin–antibody assays. Therefore, the real prevalence of HIT is probably lower than in this cohort of medical and surgical patients. Unfortunately, the authors did not analyze surgical and medical patients separately, although the prevalence of HIT differs between these two groups.

■ HIT in patients with acute venous thromboembolism

In a *post hoc* analysis of a study enrolling 3994 patients with acute venous thromboembolism, who were randomized to fondaparinux treatment compared with either unfractionated heparin [65] or low molecular

weight heparin [66], Warkentin *et al.* found that 3% of patients had PF4/heparin antibodies even before enrollment [67]. In both studies, almost 40% of patients had a history of surgery/trauma within the previous 90 days or active/a history of cancer. Thus, some of these patients were almost certainly exposed to heparin or low molecular heparin within 100 days before the thrombosis did develop.

In total, 90% of the PF4/heparin antibodies present in these patients at study entry were only reactive in a PF4/heparin antigen assay, but were not platelet activating. One of the most important findings of the study is that in the 14 patients with platelet-activating PF4/heparin antibodies, the outcomes differed depending on the anticoagulant used for treatment of the acute thrombosis. All four patients who were treated with unfractionated or low molecular weight heparin developed HIT, while none of the ten patients treated with fondaparinux did [67]. This provides further evidence that fondaparinux might be safe if platelet-activating antibodies are present. This study also strongly corroborated the concept that only platelet-activating PF4/heparin antibodies are of clinical relevance. Furthermore, the study shows that patients with recent heparin exposure are at an increased risk for HIT if treated with heparin or low molecular weight heparin.

■ Intensive care unit (excluding cardiovascular surgery) patients

In an observational study, antibodies to PF4/heparin were present in approximately 10% of patients at admission to the intensive care unit, regardless of the underlying disease (medical, neurotrauma or shock trauma patients). The frequency of PF4/heparin antibodies differed between the three groups 7 days after admission. Antibodies were more prevalent in shock trauma patients (almost 40%) than in neurotrauma (25%) or in medical (22%) intensive care unit patients [68]. In addition, the authors found that only approximately 20% of the PF4/heparin antibodies were platelet activating. Thus, HIT is relatively rare in intensive care unit patients, although it is frequently considered in the differential diagnosis of thrombocytopenia in these patients [69]. The overall prevalence of HIT caused by platelet-activating antibodies in mixed surgical/medical ICU populations seems to be approximately 0.5% [9,70,71].

■ Cardiovascular surgery

The prevalence of antibodies to PF4/heparin is particularly high in patients who undergo cardiovascular surgery, but the biological role of these antibodies is not well understood. PF4/heparin antibodies of the IgG, IgM and IgA class can be found in 30–70% of patients after cardiac surgery; while PF4/heparin IgG antibodies are found in up to 50% of patients [72,73]. Approximately 10–20% of the detectable antibodies are platelet activating [74], but only 1–2% of patients develop HIT.

The high prevalence of PF4/heparin antibodies in patients after cardiac surgery is a particular diagnostic problem, because the platelet count usually drops within the first 3 days after cardiac surgery. This drop in the platelet count after surgery is nearly exclusively caused by platelet consumption. Nevertheless, HIT is often wrongly suspected in these patients, if only the drop in the platelet count without the timing is considered. HIT is then diagnosed if PF4/heparin antibodies are found in a screening assay, although these antibodies are usually not platelet activating and patients are then switched to an alternative anticoagulant. Therefore, the use of a clinical score and the use of functional washed platelet assays are important to avoid substantial overdiagnosis of HIT in cardiac surgery patients.

Several authors investigated if the platelet count time course over several days after cardiac surgery can be used to identify patients in whom HIT is likely. They identified two characteristic patterns of the platelet count over several days after cardiac surgery that are highly suspicious of HIT. The first pattern consists of a transient fall of the platelet count 2–3 days after surgery followed by a rise and a second fall 5–10 days after surgery. The second pattern is persisting thrombocytopenia beyond day 5 after surgery without a transient rise of the platelet count [75,76]. However, the second pattern is only predictive for HIT, if there is a second superimposed decrease in platelet counts by at least 30% from on day 5 [77]. Based on these findings, the proposed management scheme for patients after cardiac surgery [78] should be slightly modified, taking into account the second, superimposed platelet count decrease in patients with early and persistent thrombocytopenia after cardiac surgery. During recent years, several studies evaluating the presence of PF4/heparin antibodies before surgery as a risk factor for adverse outcomes were published. While some studies found that the presence of antibodies to PF4/heparin is predictive of adverse outcomes [79–82] other studies did not find this association [83–85]. However, only two studies differentiated the PF4/heparin antibodies according to immunoglobulin class [82,85]. Both studies found that PF4/heparin IgG antibodies present before surgery are not a risk factor for any adverse outcome. Interestingly, one study showed that PF4/heparin IgM antibodies were associated with an increased risk for nonthrombotic adverse events. The authors speculate that the IgM antibodies are a marker for additional comorbidities or bacterial infection [82]. In this regard it is noteworthy that periodontits, which is caused by chronic bacterial infection, is associated with a higher prevalence of PF4/heparin IgM antibodies [86].

Hemodialysis

Patients under chronic renal replacement therapy usually receive heparin over a long period of time. Cross sectional studies in hemodialysis patients indicate that 6 months after initiation of hemodialysis PF4/heparin antibodies are found in approximately 10% of patients [87]. Still, the presence of PF4/heparin was not a risk factor for adverse cardiovascular events or bleeding in hemodialysis patients [88].

These cross-sectional analyses are misleading because HIT typically manifests within the first 2 weeks after start of heparin treatment. Especially during the first 2–3 weeks of chronic hemodialysis, the development of PF4/heparin antibodies can cause adverse events such as thrombocytopenia and/or clot formation [89], and HIT should be ruled out if these symptoms occur. PF4/heparin antibodies occurring more than 3 weeks after onset of chronic hemodialysis are usually an epiphenomenon without clinical relevance. It is important to keep in mind that an acute inflammatory event, such as surgery, can retrigger the immune reaction to PF4/heparin [90] and patients should be monitored for a new decrease in platelet count or newly occurring clots in the extracorporeal circuit during the first 2 weeks after surgery, even if they had been enrolled in a dialysis program for years.

The unusual immunobiology of HIT has important implications for patients who depend on chronic renal replacement therapy and who developed HIT. As almost all patients will test negative for PF4/heparin antibodies 100 days after the withdrawal of heparin, heparin re-exposure can be attempted when the PF4/heparin antibodies are no longer detectable. A Japanese group showed first in a case report [91] and then in 2010 in a case series [92], that re-exposure to heparin is feasible and does not re-induce PF4/heparin antibodies.

Future perspective

The introduction of nonheparin anticoagulants will further reduce the incidence of HIT. Nevertheless, unfractionated heparin will not be replaced in some patient populations due to the drug's short half-life, reversibility and easy monitoring. These patients groups are typically severely sick patients with a high prevalence of thrombocytopenia [79]. Especially in these patients, HIT is currently overdiagnosed due to two main reasons: a relatively high prevalence of clinically irrelevant PF4/heparin antibodies; and, the use of screening laboratory assays for PF4/heparin antibodies that cannot distinguish between platelet activating and nonactivating antibodies. Currently, patients in whom HIT is (often wrongly) diagnosed, are then immediately switched to alternative anticoagulants, which are associated with a high bleeding risk.

Therefore, the main goal of clinical research in HIT should focus on making assays available for clinically relevant PF4/heparin antibodies, for example functional assays, with a rapid turn-around time in order to avoid the dilemma of the presence of PF4/heparin antibodies without knowledge of whether these antibodies are platelet activating. This has been achieved, for example, in Germany where results of a functional HIT assay are usually available within 24 h (at least Monday–Friday) for all hospitals due to a network of laboratories offering washed platelet functional assays. Such an approach will lead to a substantial reduction in the use of alternative anticoagulants for the 'niche-indication' HIT.

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Executive summary

- Heparin-induced thrombocytopenia (HIT) is a rare but potentially severe adverse effect of heparin treatment.
- A combination of a clinical risk score and laboratory testing improves diagnosis of HIT.
- Antigen tests for platelet factor 4/heparin antibodies have a high negative predictive value, they can exclude HIT.
- Functional assay should be used for confirming HIT.
- Danaparoid, lepirudin, argatroban and bivalirudin are approved for alternative anticoagulation in acute HIT in several jurisdictions.
- Some of these alternative anticoagulants have a high risk of bleeding.
- There is increasing evidence that fondaparinux is a treatment option in HIT.
- The new anticoagulants might also emerge as new treatment options.

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