New Developments in Diagnosis and Treatment of Heparin-induced Thrombocytopenia

Norbert Lubenow

Institut für Immunologie und Transfusionsmedizin, Ernst-Moritz-Arndt-Universität Greifswald, Germany

Key Words

Thrombocytopenia · Heparin · Thrombosis

Abstract

Heparin-induced thrombocytopenia (HIT) is a drug induced immune mediated thrombocytopenia that affects up to 3% of patients treated with unfractionated heparin (UFH). It is less frequent when low molecular weight heparins (LMWH) are used. Fondaparinux does not seem to induce HIT. A functional and an antigen assay should be performed to confirm the clinical diagnosis of HIT. Immediate cessation of heparin and start of compatible anticoagulant is mandatory when HIT is suspected clinically. Danaparoid (a heparinoid) and the direct thrombin inhibitors lepirudin and argatroban are available for this purpose. Short-term reexposure with heparin, for example during cardiopulmonary bypass, is possible in patients with history of HIT, provided HIT antibodies are no longer detectable. In children systematic data on treatment of HIT are lacking.

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Introduction

Heparin-induced thrombocytopenia (HIT) is the most important and most frequent drug-induced, immune-mediated thrombocytopenia.

In HIT platelet counts usually decrease by >50% of the highest preceding value [1] or below 100,000 Gpt/L after day 5 of heparin treatment. In case of reexposure within 100 days this drop can occur earlier [2]. Thrombocytopenia of <20,000 μ l occurs in less than 10% of HIT-patients [3]. Typical thromboembolic complications (TECs) associated with HIT are deep venous thrombosis, pulmonary embolism [4], venous limb gangrene [5], cerebral vein thrombosis [6], arterial thrombosis [3]. Heparin-induced skin lesions [7] and acute systemic reactions can also occur. Bleeding complications may occur in patients with concomitant uremic thrombocytopenia but are not typical for HIT.

Studies screening symptomatic as well as asymptomatic patients [8-13] showed that most patients who form HIT antibodies do not develop clinical HIT. Clinically significant HIT, however, occurs in up to 3% of patients treated with unfractionated heparin [8,14]. Different patient populations can have greatly differing incidences of clinically inapparent HIT antibodies as well as clinically manifest HIT.

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1424-8832/04/0336-0407\$21.00/0

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Norbert Lubenow
Ernst-Moritz-Arndt Universität
Institut für Immunologie und Transfusionsmedizin
Klinikum/Sauerbruchstrasse
17489 Greifswald, Germany

Tel: +49 38 34 865458; Fax +49 3834 865489; E-mail: lubenow@uni-greifswald.de

Pathogenesis

Platelet factor 4 (PF4) is the most important protein involved in the immune response of HIT [15]. After binding of heparin to PF4 a cryptantigen or autoantigen is exposed [16,17]. Larger and higher sulfated heparins are more able to expose this cryptantigen than low molecular weight heparin [18].

In symptomatic HIT patients, the antibodies involved are of the IgG class in > 80% of patients. Antibodies of the IgG1 class were found to be predominant among HIT-IgG [19]. But patients with HIT antibodies of the IgM or IgA class only have also been described [20].

Fig. 1. Scales-model of hemostatic balance: The steady state of the healthy individual (1.) is tipped towards anticoagulation in the patient receiving heparin (2.). HIT-related clotting activation is increasing the risk of thrombosis (3.), especially if heparin is stopped (4.) without switching to an alternative anticoagulant (5.). Importantly, as HIT related clotting activation decreases, anticoagulation has to be adjusted accordingly (6.).

The most important glycoprotein of the platelet membrane involved in HIT is the platelet FcγIIa receptor [21]. Binding of HIT antibodies to the PF4-heparin complexes results in large multimolecular immune complexes [15]. These complexes crosslink the FcγRIIa and cause platelet activation [22].

Thrombin plays a central role in HIT - thrombin generation is enhanced in HIT [5,23] by concomitant activation of platelets [24], generation of platelet microparticles [25], and alteration of endothelial cells [26]. Activated endothelial cells upregulate tissue factor expression. Platelet microparticles and anti- PF4/heparin antibodies also cause expression of tissue factor on monocytes [27].

Laboratory Testing

In vitro demonstration of HIT-antibodies by functional (i.e. activation) and immunological assays [28] can help to confirm the clinical diagnosis of HIT.

The serotonin release assay (SRA), regarded as the gold standard, is, however, technically cumbersome and requires platelet labeling with radioactive compounds. The heparin-induced platelet activation (HIPA) assay - while still technically demanding - is better suited for routine testing [28]. Results of other platelet activation dependent assays measuring e.g. luminescence, platelet derived microparticle generation, or annexin V expression do not differ largely as long as washed platelets are used. However, sensitivity is reduced, if performed with platelet-rich plasma.

Antigen assays use surface-bound target antigen (PF4/heparin- or PF4/polyvinylsulphate-complexes respectively) to detect the antibodies. A new gel-column system utilising this test principle has recently been introduced and evaluated, showing its suitability for HIT-antibody testing [29].

Regardless which assay is used it is important to incorporate clinical information in the interpretation of test results and to combine a sensitive functional assay with an antigen assay. Antigen assays may have greater sensitivity for HIT antibody seroconversion. Thus the functional assays' positive predictive value for clinical HIT might be greater. False negative results are estimated to be less than 5% if both test systems are applied.

Therapy

When HIT develops, immediate cessation of heparin is mandatory, but this will not stop continuing thrombin generation, nor avoid subsequent thrombotic events, which occur in as many as 40-50% of patients over the next several days or weeks [4,30]. Wallis et al. [30] showed that heparin cessation alone is not an appropriate treatment for

HIT, and that even in HIT, heparin's anti-IIa-activity may have a beneficial impact (figure 1).

Non-heparin anticoagulants that do not cross-react with HIT antibodies, such as hirudin, argatroban or danaparoid are approved for alternative anticoagulation in HIT. Notably, hirudin and argatroban, but not danaparoid, have antithrombin activity even inside clots.

There is emerging evidence that LMWH cause HIT less often when compared to UFH [8,31], they must not however be used in patients with acute HIT.

Treatment of HIT

With respect to treatment it is important to differentiate patients groups depending on the clinical presentation [32]:

- **Isolated seroconversion:** presence of HIT-antibodies without clinical signs such as thrombocytopenia or thrombosis, does not mandate special treatment, nor discontinuation of heparin.
- **HIT without thrombosis** ("isolated thrombocytopenia"): about 50% of patients with HIT do not have a new HIT-associated thrombosis at the time HIT is first clinically suspected on the basis of thrombocytopenia alone. In a retrospective cohort study of 62 such patients with "isolated thrombocytopenia" the subsequent cumulative thrombotic event rate over 30 days was high (52.8%) [4]. These patients should therefore be anticoagulated with an alternative drug. Furthermore, patients should be carefully assessed for lower limb DVT [30]. Anticoagulation should at least be continued until recovery of platelet counts to a stable plateau [32]. Farner et al presented data suggesting that HIT patients without thrombosis still require therapeutic anticoagulation [33].
- **HIT with thrombosis:** HIT patients with acute thrombosis require systemic anticoagulation. Whereas anticoagulation can probably be continued just until resolution of thrombocytopenia in a patient with isolated HIT, HIT-associated thrombosis requires longer-term anticoagulation (several months) in most patients. It is important to start coumarins only after therapeutic anticoagulation has been achieved with a compatible anticoagulant and a substantial platelet count recovery to a stable plateau has occurred to avoid coumarin induced venous limb gangrene [5]. This is an iatrogenic complication in which warfarin therapy leads to microvascular thrombosis, and limb loss, by causing acute depletion of a Vitamin K-dependent natural anticoagulant (protein C), while at the same time failing to down-regulate the increased thrombin generation caused by acute HIT.

In case of strong clinical suspicion of HIT, patients should immediately be treated with an alternative anticoagulant. Awaiting laboratory confirmation would cause a significant delay in most cases, enhancing the risk for the patient substantially.

Alternative Anticoagulants in HIT

Danaparoid

The heparinoid danaparoid-sodium [34] mainly exhibits anti-Xa activity, with a half life of approx 24 hours. Its antithrombin activity has a half-life of about 2-4 hours. As danaparoid is mainly eliminated renally, dose reduction of about 30% is required to maintain aFXa levels within the therapeutic range in patients with renal failure [34].

Bioavailability is nearly 100% after s.c. injection and plasma levels are usually predictable. Monitoring is recommended in substantial renal impairment, unusually high or low body weight, life- or limb threatening thrombosis, unexpected bleeding and critically ill or unstable patients [34]. Danaparoid does not significantly prolong aPTT, activated clotting time or INR, thus measurement of anti-FXa-activity for monitoring using a calibration curve with danaparoid is required.

Danaparoid has been assessed in one compassionate use program and in one small prospective, randomized clinical trial [34]. In the named patient program, 667 HIT patients with 708 treatment episodes were included. The treating physicians judged treatment to be successful in 93% because of platelet count recovery or clinical improvement of patients. In 1.7% of patients new thromboembolic complication occurred. Of 114 (17.1%) deaths, 14 (12.2%) were thought to be related to the danaparoid treatment.

Danaparoid cross-reacts with HIT-antibodies in-vitro at a rate of 7-50% depending on the sensitivity of the assay used [34]. Despite anecdotal reports of unfavorable outcomes, two studies found no difference in clinical outcomes comparing HIT patients treated with danaparoid with or without in-vitro cross-reactivity [35]. It therefore seems reasonable to limit testing for in-vitro cross-reactions to patients who develop new, progressive or recurrent thrombocytopenia or thrombosis during treatment with danaparoid [34]. In case of a positive result in such a patient, anticoagulation should be switched to a different drug.

Hirudin

Hirudin is a recombinant direct thrombin inhibitor. Lepirudin is the most extensively investigated drug in HIT [36]. Terminal plasma elimination half-lives ($t_{1/2\beta}$) are 0.8 - 1.7 hours after intravenous (i.v.) injection of bolus lepirudin doses of 0.01 - 0.5 mg/kg, and 1.1 - 2.0 hours after continuous i.v. infusions over 6 hours.

Renal clearance accounts for approximately 90% of the systemic clearance. The $t_{1/2\beta}$ of lepirudin is lengthened with deterioration of renal function [37]; in nephrectomized humans it is prolonged to up to 150 hours.

For monitoring, the aPTT shows considerable variability between patients. At higher hirudin plasma levels (aPTT values >70 seconds), the concentration-aPTT curve flattens and the correlation becomes poor. The ecarin clotting time (ECT) should be used in patients requiring high hirudin doses, for example during cardio-pulmonary bypass [38]. There is no antidote available for hirudin.

Two prospective, multicenter, historically-controlled trials, HAT-1 (n=82) and HAT-2 (n=112) were conducted in patients with the clinical diagnosis of HIT confirmed by laboratory tests [36].

Both studies had similar mortality rates (7.3% and 9.8%); however, limb amputations (3.7% and 8.9%) and new thromboembolic complications (9.8% and 17.9%) differed between the studies.

The combined endpoint (new TECs, limb amputation, death) at day 35 was 52.1% in a historical control (n=120) and 25.4% in the HAT-1- (P=0.014; adjusted risk ratio 0.508; 95% CI, 0.290-0.892) and 30.9% in the HAT-2 study (P=0.15; adjusted risk ratio 0.709; 95% CI, 0.44-1.14).

In both studies more bleeding events occurred in the lepirudin-treated group as compared to controls (39.1% [HAT-1], 44.6% [HAT-2] vs. 27.2%), but not more bleeding events requiring transfusion (9.9% [HAT-1], 12.9% [HAT-2] vs 9.1%).

In a meta-analysis of both studies, patients with HIT and ongoing thrombosis treated with lepirudin showed a clear reduction of the combined endpoint compared to the historical control group (P=0.004) [23]. An aPTT ratio of 1.5 to 2.5 is the recommended target range [23].

Patients with acute HIT (N=91) were also shown to benefit from lepirudin treatment in a study using a contemporaneous control group (N=47): new TECS 4.4% vs. 14.0%, P=0.02; combined end-point 19.8% vs. 29.8%, P=0.0281 [55].

Whereas a change in the prothrombin time observed at the start of treatment with lepirudin is of minor clinical relevance, no evidence was found indicating that cessation of lepirudin causes a change of the INRs [23].

Hirudin can induce anti-hirudin antibodies [39,40]. Of 198 HIT patients treated for 5 days with lepirudin, 45% developed anti-hirudin antibodies of the immunoglobulin G (IgG) class [39].

In 2-3% of patients with anti-hirudin antibodies, the hirudin dose had to be decreased by more than 60% to maintain the aPTT within the target range. [39] This enhanced anticoagulant effect may be caused by decreased renal elimination of the hirudin-anti-hirudin complexes. In a few

patients, development of anti-hirudin antibodies was paralleled by a decrease of the aPTT.

Nine anaphylactic reactions to lepirudin have been reported in ~ 35,000 treatments, 4 of them fatal. Five of these reactions occurred during reexposure (at a reexposure rate of ~7.5%) [41]. Consequently, lepirudin should be started in a setting with access to treatment of anaphylaxis, and the bolus should be omitted unless there is life- or limb-threatening thrombosis [41].

Argatroban

Argatroban (MW 526.66 Da) is a synthetic direct thrombin inhibitor. It distributes mainly in the extracellular fluid (distribution volume 174mL/kg), and is 54% serum protein-bound. Its elimination half-life is 39-51 minutes [42]. The main route of metabolism is the liver. Steady state levels are reached within 1-3 hours.

Argatroban is routinely monitored by the aPTT. It also increases the ACT, INR, thrombin time, and ecarin clotting time. As argatroban prolongs the INR, during change from argatroban to oral anticoagulation special precautions have to be followed [43].

There is no specific antidote available for argatroban. Across 3 studies [43-45], 754 patients, with the clinical diagnosis of HIT, received argatroban therapy [2µg/kg/min i.v. (up to 10 g/kg/min, max) aPTT adjusted].

In the first study ARG-911, 304 patients having HIT (n=160) or HIT with thrombosis (n=144) received argatroban. Compared to 193 historical controls (HIT, n=147; HIT with thrombosis, n=46) the composite endpoint was reduced in argatroban-treated patients by time-to-event analysis in HIT (hazard ratio=0.60; 95% CI, 0.40-0.89, P=0.010) and HIT with thrombosis (hazard ratio=0.57; 95% CI, 0.36-0.90, P=0.014).

In the other 2 studies, argatroban treatment was also favored, compared with controls, by time-to-event analysis [43]. The overall major bleeding rate was 6.6% for argatroban-treated patients and 6.7% for the historical control.

Fondaparinux and HIT

HIT-antibody seroconversion occurred in patients treated with fondaparinux following knee- and hip-replacement (n=364 and 989 respectively), at a rate similar to patients treated with LMWH. However, no thrombocytopenia indicating clinical HIT was observed in this study in fondaparinux treated patients [46]. Furthermore, HIT-sera of 39 patients did not cause platelet activation with fondaparinux in various functional assays, indicating absence of cross-reactivity [47]. Successful treatment of 5 HIT-patients with fondaparinux was reported recently [48], but systematic data on treatment of HIT-patients with fondaparinux are lacking.

Reexposure of Patients with a History of HIT to Heparin

Short term reexposure to heparin in patients with a history of HIT may be considered in special clinical settings as only patients who had received heparin within the past 100 days seem to develop early onset of HIT (< 5 days) [2]. This temporal pattern offers treatment options in patients with a history of HIT who are in need of surgery necessitating cardiopulmonary bypass. Provided no circulating HIT-antibodies are detectable, these patients can receive heparin during CPB if heparin exposure is strictly avoided pre- and postoperatively. With this strategy, complications during CPB with anticoagulants for which no antidote is available, i.e. hirudin, argatroban or danaparoid, can be avoided [38,49,50].

Children

Schiffmann et al. [51] described a 12-year-old patient with HIT and multiple TECs. Using a bolus of 0.2 mg/kg lepirudin followed by a continuous infusion of 0.1-0.7

mg/kg/h, the aPTT was prolonged to 45-85 sec. Planned surgical interventions were possible after discontinuing lepirudin for 4 hours.

Danaparoid has been used in children successfully [52]. For thrombosis prophylaxis in children below 55 kg, the manufacturer [53] recommends subcutaneous injections of 10 aFXaU/kg bid, in acute thrombosis a bolus of 30 aFXaU/kg followed by 1.2-2.0 aFXaU/kg/h with a target range of 0.4-0.6 aFXaU/mL or 0.5-0.8 aFXaU/mL respectively.

Klenner et al (2004) recently showed that newborns and infants <4 years undergoing cardiac surgery (incidence ~1-2%), and adolescents receiving heparin for treatment of thrombosis are the two main risk groups for HIT in children and that the 'adult cut-off' in antigen assays is probably appropriate for children [54].

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