Heparin-induced thrombocytopenia

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Summary
Heparin-induced thrombocytopenia (HIT), typically occurring in the second week of heparin therapy, is an antibody-mediated adverse drug reaction associated with increased thrombotic risk. The most important antigens are located on platelet factor 4 (PF4)/heparin complexes. HIT is always caused by platelet-activating antibodies, but not all PF4/heparin-reactive antibodies cause HIT. Thus, tests have a high negative, but only a moderate, positive predictive value. Clinical suspicion of HIT requires cessation of heparin and substitution with an alternative anticoagulant. As these drugs have an increased bleeding risk, they should be used in therapeutic doses only if HIT is considered very likely. Avoiding/postponing coumarin is crucial in minimizing microthrombotic complications. Recent studies of HIT immunobiology suggest that HIT mimics immunology against repetitive antigens, as are relevant in microbial defense. Thus, understanding HIT may help unravel why host defenses can trigger autoimmunity.

Keywords
Heparin, heparin-induced thrombocytopenia, platelets, pathogenesis, immunology, anticoagulants

Schlüsselwörter
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Zusammenfassung

Heparin-induced thrombocytopenia (HIT) is an immunologic adverse effect of heparin therapy. Antibody-mediated platelet activation and consequent thrombin generation result in a fundamental paradox:

Despite thrombocytopenia induced by an anticoagulant, the major clinical effect in HIT is enhanced risk for venous and/or arterial thrombosis (22).

Although HIT is relatively rare, so many patients are exposed to heparin, that the absolute number of patients affected by HIT is likely one of the highest of all adverse drug effects (besides chemotherapy-induced thrombocytopenia) and certainly the highest of all immune mediated, drug-induced blood cell disorders.

Prompt diagnosis and introduction of alternative non-heparin anticoagulants are important to prevent further complications in HIT. However, diagnosis can be problematic, as the two leading symptoms of HIT are not specific for HIT:

- thrombocytopenia and
- thrombosis.

Particularly in patient populations with a high incidence of thrombocytopenia (e.g. critically ill patients) clinical diagnosis of HIT based on the platelet count is difficult. Up to 50% of intensive care patients develop thrombocytopenia, usually caused by many other reasons but not by HIT (15, 61). Also the second major symptom, new thrombosis, can cause a diagnostic dilemma. This is because the adverse drug effect HIT causes exactly the complications which should be prevented by heparin, i.e. new thrombosis and the challenge is to discriminate “heparin failure” (= underdosage) from HIT induced thrombosis (81). Testing for PF4/heparin antibodies can help to identify those patients in whom thrombocytopenia may be caused by HIT. However, PF4/heparin antibodies are much more frequent than clinical HIT.

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Thus, the negative predictive value of PF4/heparin antibody assays is high, while the clinical relevance of the detection of anti-PF4/heparin antibodies strongly depends on the clinical context.

As a general rule this applies to all laboratory tests for HIT. The positive predictive value of PF4/heparin antibody tests can be increased by several laboratory maneuvers and especially by interpretation of assay results in the context of the clinical presentation of the patient (= in the context of the pretest probability). This approach using the Bayes’ rule and the respective methodological background have been reviewed in detail elsewhere (71).

**Clinical presentation**

HIT usually features a platelet count fall >50% (from the highest value after day 4 of heparin treatment) and often new thrombosis, typically occurring 5–14 days after start of prophylactic or therapeutic dose heparin (5). Thromboembolic complications predominantly affect the venous system. Other rare complications include skin necrosis, adrenal haemorrhagic necrosis, or post-intravenous heparin bolus anaphylactoid reactions. The more unusual a new thrombosis during heparin treatment seems, the more HIT should be considered. HIT-associated thrombosis often occurs close to the 50% platelet count decrease and can even precede it by 1–2 days (~30% of thrombi) in a subset of patients (24). A platelet count fall within the first 4 days of heparin treatment is usually not HIT, unless the patient has been preimmunized and has circulating anti-PF4/heparin antibodies (46, 80). In these preimmunized patients the platelet count typically decreases within the first hours after start of heparin (= rapid onset HIT). Heparin-dependent antibodies tend to rapidly decrease in titers and become undetectable in >90% of patients within 100 days. In fact, in most patients, the heparin dependent, platelet activating antibodies disappear even much sooner, usually within one month.

**Rapid onset HIT** should be expected primarily in patients who did receive heparin within the preceding 30 days.

This time kinetic of the PF4/heparin immune response has implications for platelet count monitoring during heparin therapy. A platelet count should be obtained before start of heparin (and in patients with recent heparin exposure also 12–24 hours thereafter to recognize rapid onset HIT). Monitoring of platelet counts at days 5, 7 and 9 is then sufficient to recognize HIT in the vast majority of patients (33).

Especially in surgical patients it is not appropriate to compare the platelet count value at the time of suspicion of HIT with the baseline value, because surgical patients typically show a reactive increase in platelet counts with overshooting platelet counts in the second week after major surgery (Fig. 1). Comparison of the actual platelet count with the baseline value will underestimate the magnitude of the platelet count fall.

To make it even more complex, the term HIT by itself is somewhat misleading as severe thrombocytopenia is rare in HIT. Most patients have only moderately decreased platelet counts and they can present even with high normal counts, e.g. patients with myeloproliferative disorders (65).

The mean platelet count in patients with HIT is 60 × 10⁹/l and ranges from 15 to 150 × 10⁹/l in 90% of patients (72, 73).

Patients with platelet counts less than 15 × 10⁹/l rarely have HIT. In these patients other reasons for the platelet count decrease are much more likely, e.g. other
drug-induced immune thrombocytopenias, GPIIb/IIIa inhibitor induced thrombocytopenia, or post-transfusion purpura (8, 25, 44).

**Pathogenesis**

Most frequently, HIT antigens are found on PF4 bound to heparin or other sulfated polysaccharides (4), forming linear multimolecular clusters (57). Immunogenicity (UFH > LMWH > fondaparinux) is influenced by
- relative size,
- amount, and
- stability of the PF4/heparin complexes.

Anti-PF4/heparin antibodies bind to PF4 via their F(ab) domains. The pathogenic immunoglobulin (Ig) isotype in clinical HIT is IgG. As serial PF4 molecules become aligned, several IgG molecules bind, leading to formation of large immune complexes that cross-link the platelet FcγIIa receptors (37). This triggers
- generation of platelet microparticles,
- activation of monocytes and endothelial cells, and finally
- activation of the clotting system, which results in massive thrombin generation.

Thus, thrombocytopenia does not result from reticuloendothelial system phagocytosis, but rather from intravascular platelet activation (14), with release of procoagulant platelet-derived microparticles (catalytic surface for enhanced thrombin generation). Only PF4/heparin IgG antibodies can bind and activate the platelet FcγIIa receptor (†Fig. 2). This explains, why anti-PF4/heparin IgM and IgA antibodies have only a minor relevance in HIT as they have no Fc-part and cannot crosslink the FcγIIa receptor. The magnitude of thrombocytopenia correlates with thrombotic risk. Antibodies to other heparin-binding proteins (interleukin-8; neutrophil activating protein-2) have a minor role in HIT.

HIT antibodies also bind to endothelial cells, leading to procoagulant changes on microvascular endothelial cells (6). Further, HIT antibodies can activate monocytes (54) and neutrophils, forming platelet-monocyte-neutrophil aggregates. These pancellular activating effects could contribute to the prothrombotic nature of HIT.

PF4 complexes can be also be formed by other polyanions beside unfractionated heparin such as low molecular weight heparin (LMWH) or hypersulfated chondroitin-sulfate (32). In vitro also fondaparinux can induce complex formation of PF4 (26) and a few case reports have described HIT associated with fondaparinux treatment (58, 82). Furthermore, in some patients the B-cells producing the PF4/heparin specific antibodies start to produce antibodies with wider reactivity. The resulting antibodies then react also with PF4 bound to platelets in the absence of heparin. These antibodies can cause delayed onset HIT, which manifests several days after cessation of heparin (79). In rare cases, such antibodies can occur without exposure to heparin (70). These latter antibodies are true autoantibodies. They are usually present in very high titer and can persist for months.

**Diagnosis of HIT**

**The 4-T Score**

The frequency of HIT depends on the patient population(21,83). The typical features of HIT as described above have been summarized in a scoring system, which seems to be suitable for clinical application: see www.medizin.uni-greifswald.de/transfus/.

The 4-T score, validated by Lo et al. (43) (†Tab. 1, †Fig. 3), gives points according to four clinical criteria: thrombocytopenia, timing, thrombosis and other reasons for thrombocytopenia. According to the score the clinical probability of HIT is
- low (0–3 points),
- intermediate (4–6 points), or
- high (7–8 points).

For patients with a low score of less than 4 points, the probability of having immune HIT is less than 5% and testing for PF4/heparin antibodies is not recommended. A laboratory test for PF4/heparin antibodies
should be rather performed in patients with an intermediate score (4–6 points), while for patients with a high clinical probability (7–8 points) a direct switch of treatment to an alternative anticoagulant is recommended and laboratory testing should be performed in retrospect. However, the scoring system has to be applied carefully and sometimes requires “detective work” on the patient history.

### Laboratory testing

Beside application of the scoring system, testing for PF4/heparin antibodies is the second most important measurement to exclude or to confirm the diagnosis of HIT in patients with clinically suspected HIT. Two groups of serological assays are available. The first group are the antigen tests. They consist of ELISAs, particle gel immunoassays or particle immunofiltration assays, which recognize binding of antibodies to PF4/polyanion complexes. The second group are the washed platelet assays, which detect heparin-dependent activation of platelets by the patient serum. The big advantage of the functional HIT assays is that they reflect in vitro the pathogenic cascade of HIT one step further downstream than the antigen tests. Thus they have a higher positive predictive value for HIT (Figure 2). In fact only about 50% of patients testing positive in the antigen tests also test positive in the functional assays (27).

### PF4/heparin antigen assays

#### Solid phase ELISAs

Antigen assays detect the antibodies based upon binding to PF4/polyvinyl-sulfonate complexes or PF4/heparin complexes bound to a solid phase. Currently three commercial ELISAs are available: One from the Genetics testing institute (GTI, Waunakea, WIs), the Asserachrom PF4 (Diagnostica Stago, Parsippany, NJ) and the Zymunt-HIA (Hyphen BioMed, Neuville-sur-Oise, France). They detect anti-PF4/heparin antibodies of the IgG/IgA/IgM class, or IgG only, depending on the secondary antibodies used. These assays differ considerably in their structure. One ELISA uses PF4/polyvinyl-sulfonate complexes with PF4 purified from outdated platelets (GTI), the second uses recombinant PF4 complexed with heparin (Diagnostica Stago), and the third utilizes heparin-coated surfaces to which a platelet-leucocyte lysate is added allowing formation of PF4/heparin complexes but also of complexes of heparin with other chemokines such as IL-8 or NAP-2. These assays give slightly different results but show an overall acceptable concordance, especially with high titer PF4/heparin antibodies (17,59) (and unpublished observations).

#### Particle gel immunoassay

The Particle Gel Immunoassay (PaGIA, DiaMed, Biorad, Cressnien, Switzerland) uses PF4/heparin complexes bound to red, high-density polystyrene beads, which are agglutinated, if the patient’s serum contains anti-PF4/heparin antibodies. The particle gel immunoassay does not distinguish between IgG, IgA, and IgM antibodies (17) and is at best semiquantitative. However, serial dilution of the patient serum enables to some extent assessment of the antibody titer (1). Its biggest advantage is its fast turn-around time of less than 15 min. However, it does produce false negative results in a few patients with high probability of HIT (53). Therefore, in case of high clinical suspicion, a negative result in this assay should prompt testing of patient serum in another test system.

In contrast to the assays described above, the Particle Immuno Filtration assay produces considerable false negative and false positive results compared to the ELISA (86), and seem to have a poor correlation with the 4Ts score (19). The manufacturer claims to have improved test procedures after these publications. However, new data on the validation of this modified version are not available.

### Improving the predictivity of antigen tests for HIT

#### Detection of IgG-antibodies only

IgA and IgM anti-PF4/heparin antibodies are unlikely to cause clinical HIT as outlined above. Modifying the antigen test to detect IgG antibodies only is one strategy to...
increase specificity of the antigen tests for HIT (27, 41, 84).

**Increasing the PF4/heparin ELISA cut-off**

The probability of anti-PF4/heparin antibodies for being causative for the low platelet count or new thrombosis increases with the magnitude of the EIA OD result (35, 36, 59, 85, 88, 91). However, a subgroup of anti-PF4/heparin antibodies with platelet activating capacity show an OD < 1.0 in the PF4/heparin EIA, only (36). As higher the OD value is, as higher is the likelihood for anti-PF4/heparin IgG antibodies to be clinically relevant. However, there is no clear cut off to distinguish between clinically relevant and non-relevant antibodies.

Interpretation of ELISA test results according to a fixed OD cut off faces the problem that OD units are non-standardized arbitrary units. They might differ considerably between different photometers. This problem is similar to the issue of inter-laboratory comparison of the prothrombin time (or the Quick’s value). As long as there is no standard for anti-PF4/heparin antibodies, the OD of the ELISA could be used as an estimate for the likelihood of a clinically relevant antibody, only.

**Confirmatory step with high heparin**

Besides antibodies with specificity to PF4/heparin antibodies, some individuals have also antibodies against PF4 alone present in their serum (52). These antibodies seem to be especially prevalent in patients with anti-phospholipid syndrome (47, 52). However, their clinical relevance is unresolved. These antibodies bind to PF4 but also to PF4 within the PF4/heparin complexes. The PF4/heparin complexes to which the pathogenic complex-specific antibodies bind, form only at a certain stoichiometric ratio of PF4 and heparin (23). High dose heparin disrupts the multimolecular PF4/heparin complexes (30) by saturating the heparin binding sites of all PF4 molecules. This has the potential to identify antibodies which are not specific for PF4/heparin but recognize PF4 alone (89), as these antibodies will still bind to
non-complexed PF4. However, highly reactive sera may not be inhibited by high heparin, despite having platelet activating capacity and causing HIT in vivo (74). We assessed this systematically in 1000 consecutive samples of patients with suspected HIT and found that the high heparin inhibition step is helpful to differentiate between antibodies binding to PF4/heparin complexes (potentially pathogenic) and those binding to PF4 alone (clinical relevance unknown) as long as the reactivity of these antibodies is weak, as defined in our laboratory with an OD of < 1.0 (Fig. 3b). Sera of patients with higher reactivity in the ELISA seem to contain both, PF4/heparin complex antibodies and PF4-alone reactive antibodies. In about half of these sera a lack of inhibition by high heparin does not exclude relevant PF4/heparin antibodies (3).

**Platelet activation assays**

**PRP or whole blood aggregation assays**

Assays based on whole blood or platelet rich plasma of healthy donors are the least sensitive functional assays, which have a sensitivity of about 30% of the washed platelet assays discussed below. If a PRP or whole blood assay is positive, there is a high likelihood for the presence of clinically relevant antibodies. However, in critically ill patients, PRP based platelet activation tests sometimes give false positive results (76).

**Washed platelet assays**

Two assays are more widely used, the serotonin release test, which detects the release of radioactive serotonin from labelled donor platelets as a platelet activating endpoint (63) and the heparin-induced platelet activation assay (HIPA) (29), which detects platelet aggregation induced by patient serum. Both assays are performed on microtiter plates and allow for several quality controls. The most important is the two point approach, using low (0.1 to 0.3 IU/ml heparin) and high (100 IU/ml heparin) concentrations of heparin. Both tests are considered positive if a patient serum causes platelet activation at low but not at high heparin concentration. Other controls are the addition of Hirudin to quench all effects of residual thrombin in the patient sample and the use of a monoclonal antibody, which blocks the platelet Fc-receptor, to demonstrate that platelet activation is caused by a heparin and FcγⅠa receptor-dependent mechanism. Washed platelet assays have a higher predictive value for HIT compared to the antigen tests.

A diagnostic algorithm for HIT is given in Figure 3.

**Treatment**

If there is high clinical suspicion for HIT, stopping heparin alone is insufficient. To prevent new thrombosis, non-heparin anticoagulant therapy is required. Vitamin K antagonists (VKA) must not be given in acute HIT. They can induce venous limb gangrene because of the early effects on protein C, they precipitates venous limb gangrene and/or skin necrosis in the extreme hypercoaguable milieu of HIT. Therefore vitamin K should be given if HIT is recognized only after VKA treatment has been started (78).

Three drugs are approved for anticoagulation in HIT:
- the two direct thrombin inhibitors (DTIs), lepirudin and argatroban, and
- the heparinoid danaparoid.

Rational therapies for HIT (78) are also
- the DTI bivalirudin, and
- the anti-factor Xa inhibitor fondaparinux.

All alternative anticoagulants confer significant risk for major bleeding (0.8–1.25% per treatment day) and no antidote is available. In only 5% of patients clinically suspected to have HIT, the diagnosis is confirmed (even fewer in ICU patients). Therefore, in patients with low/moderate clinical probability for HIT, our practice to reduce the risk of bleeding is to use prophylactic dose alternative anticoagulation, pending results of laboratory testing.

**Direct thrombin inhibitors**

DTIs are usually monitored by aPTT. However, a major problem is inappropriate dose reduction, when low prothrombin levels (e.g., because of VKA therapy) result in falsely elevated aPTTs during DTI treatment (42). This often results in catastrophic outcomes. The carin chromogenic assay overcomes this issue by providing a linear dose-response curve for DTIs independent of prothrombin levels.

**Lepirudin** (recombinant, bivalent, irreversible DTI, t½ ~90 min) approved dosing is too high; recommended dosing must be adjusted downwards even further in patients with renal dysfunction (45, 78). Lepirudin induces antibodies in about 40–70% (re-exposure) of patients, which may reduce elimination, thereby prolonging lepirudin half-life. IgG-mediated anaphylactic reactions are rare and avoidable by omitting the bolus.

**Argatroban** (synthetic, monovalent, reversible DTI, t½ ~45 min) substantially increases the INR, complicating argatroban-VKA overlap. While cessation of DTIs before measuring INR and restart DTIs if the INR is not in the therapeutic range avoids major bleedings (9), the patient is at increased risk for thrombosis during this phase. Functional clotting assays cannot be interpreted readily during argatroban treatment (40). In patients with reduced liver perfusion, 75–90% dose reduction to 0.2–0.5 μg/kg/min is important (11).

**Bivalirudin** (synthetic, bivalent, reversible DTI, t½ ~25 min) (77) is cleaved by thrombin, which avoids major drug accumulation; the dose still needs ~50% reduction in patients with renal and/or hepatic dysfunction. Experience with bivalirudin in HIT is anecdotal(20, 38).

**Indirect FXa inhibitors**

**Danaparoid** is an AT-dependent heparinoid (13) with predominant anti-factor Xa activity (t½ ~24 hours). The dose-response relationship is predictable, thus monitoring of therapeutic-dose treatment (anti-FXa activity) is required in patients with severely impaired renal function. In vitro cross-reactivity with HIT antibodies is of minor clinical relevance. Therapeutic dosing is indicated when HIT is strongly suspected or confirmed, with prophylactic dosing appropriate when suspicion for HIT is moderate.

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Fondaparinux (AT-dependent FXa inhibitor; t 1/2 ~17 hours, considerably prolonged in patients with renal impairment) may be used in patients with low or moderate suspicion of HIT. Experience in treatment of acute HIT with thrombosis with therapeutic-dose fondaparinux is anecdotal (16).

Phenprocoumon

Most limb amputations in HIT result from microthrombosis due to severe DIC or (more often) VKA therapy. Thus, avoiding/postponing VKA use until platelet count recovery is a fundamental for HIT management. Inappropriate DTI dose reductions/cessation due to VKA-related aPTT prolongation is the rationale for giving vitamin K when HIT is recognized after VKA use (78).

New concepts of HIT

Special patient groups

The problem of HIT-antibody testing in cardiac surgery patients

Interpretation of a positive anti-PF4/heparin test is especially problematic in cardiac surgery patients. Up to 70% of these patients (depending on the test performed) test positive for anti-PF4/heparin antibodies during the first 10 days post-cardiac surgery, with only about 0.5–2% of patients presenting with clinically-evident HIT (55, 61, 68).

The problem of PF4/heparin antibody testing even starts before surgery. It has been a challenging observation that already before cardiac surgery anti-PF4/heparin antibodies are present in up to 20% of these patients (10, 12, 18, 50). However, the biological role of these antibodies is unclear. Particularly, it is controversial whether the presence of these antibodies predicts for adverse outcomes (18, 39, 50). We prospectively screened 591 patients before cardiac surgery for heparin-dependent antibodies by a PF4/heparin immunoassay (EIA) and platelet-activation test, and followed these patients for 30 days and found no evidence for an association of adverse outcomes post surgery and the presurgery anti-PF4/heparin IgG antibody status (60). Thus we strongly discourage testing for PF4/heparin antibodies in otherwise asymptomatic patients before cardiac surgery.

It is well accepted that the platelet count decrease occurring directly after cardiac surgery (due to hemodilution, platelet loss and consumption) (51) is not indicative for HIT, but rather if a patient develops a second episode of thrombocytopenia beginning within a narrow ‘window’ between postoperative days 5 and 10 (56). However, there is an additional group of patients, in whom it is very difficult to exclude HIT. These are those patients, in whom the early-onset thrombocytopenia after cardiac surgery persists beyond the first five days and thus extends into the period in which HIT typically occurs. When these patients test positive for anti-PF4/heparin antibodies, the crucial issue is whether the patient has developed ‘true’ HIT or whether a preceding – and persisting – surgery-related non-HIT thrombocytopenic disorder is simply associated with incidental and clinically-irrelevant seroconversion (75).

We systematically assessed this question in a prospective study and found early-onset and persisting thrombocytopenia in 4.3% of patients after cardiac surgery. 5 of these 25 patients tested positive for PF4/heparin antibodies and also positive in the HIPA test. But none of the 5 patients developed thrombosis and in none of the 4 patients who switched to a non-heparin anticoagulant did the platelet counts subsequently recover by day 10 (Fig. 4). Thus a causal role of platelet-activating antibodies for thrombocytopenia appeared very unlikely (61). This provides a rationale for considering continued heparin therapy in such patients even if a PF4/heparin antibody test is positive. Maintaining heparin should be particularly considered when there is a reasonable alternative explanation for the thrombocytopenia and there is no new thrombosis (which would increase the likelihood of ‘true’ HIT), and no additional...
platelet count decrease of at least 30% occurs during the characteristic HIT 'window' between days 5 and 10 (62).

**Chronic renal replacement therapy**

Patients under chronic renal replacement therapy test positive for anti-PF4/heparin antibodies in 0.26 to 10% (7, 34). However, in most studies these antibodies were not an independent risk factor for arterial cardiovascular events, venous thromboembolism, vascular access occlusion, or mortality (31). However, in a study of Matsuo T et al. (48) these antibodies predicted for adverse clinical outcomes. Based on our own experience three risk groups of patients have to be differentiated:

1. Patients in whom renal dialysis is started. These patients are at a considerable risk to develop HIT during the first 2–3 weeks of dialysis and new thrombotic complications in the context of a positive functional HIT assay should raise a high suspicion for HIT.

2. Patients under chronic hemodialysis. In those patients anti-PF4/heparin antibodies and heparin dependent platelet activating antibodies are usually an epiphenomenon with little, if any clinical relevance.

3. Patients under chronic renal replacement therapy, who undergo a surgical procedure. These patients are again at an increased risk to develop HIT within the next 2 weeks after surgery (67). It seems that the immune system of these patients is altered by the surgical intervention in a way that HIT becomes more likely.

A very interesting observation is the successful re-exposure of a dialysis patient with HIT to heparin. This patient had been treated with an alternative anticoagulant until the PF4/heparin antibodies disappeared and then received heparin again during dialysis without adverse effects (49). Preliminary data imply that this approach might be feasible in other patients, too (69).

**Anti-phospholipid syndrome**

The antibody-mediated disorders HIT and antiphospholipid antibody syndrome (APS) have remarkably similar clinical presentations. Both are associated with thrombocytopenia and a high risk of thrombosis. It is likely that HIT is overdiagnosed in APS (47) as about 70% of APS patients have anti-PF4-antibodies, which can cause false positive PF4/heparin antigen tests. Very recently, Sikara et al (64) reported that the main antigen in anti-phospholipid syndrome, β2-Glycoprotein 1, forms complexes with PF4. It might be that this protein-protein interaction requires new interpretation of test results in anti-phospholipid syndrome as well as in HIT, but this requires further studies.
Immunology of HIT

Currently, the most interesting aspect of HIT is its immunobiology. HIT neither exhibits features typical of a primary immune response (initial formation of IgM antibodies followed by a more delayed IgG response) nor the serological features of a secondary immune response (stronger and more persistent formation of IgG antibodies) (28). Most HIT patients form IgG antibodies between days 4–10, even with first heparin use. HIT antibodies do not persist, however (80). This profile seems more compatible with a non-T-cell-dependent B-cell response, as described for immune reactions against viral antigens with repetitive epitopes (90). Indeed, repetitive epitopes in HIT are expressed as structures with a distance of 4–6 nm contained within 100–150 nm size, linear, ridge-like clusters of PF4/heparin (26). This is within the range of repetitive viral epitopes found to cause T-cell-dependent B-cell activation. However, other arguments favor a T-cell-dependent immune reaction in HIT. T-cell-independent B-cell responses should be primarily IgM whereas in HIT IgG predominates, and in a mouse model the immune response against PF4/heparin was T-cell-dependent (66). This suggests there has been previous contact(s) between the immune system and the „HIT antigens“.

Our current working hypothesis is that early exposure to PF4 complexes, perhaps induced by endogenous non-heparin factors, leads to PF4 clustering and a T-cell-dependent antibody class switch of B-cells. Later in life, these B-cells (possibly, marginal zone B-cells (2)) become again transiently activated when PF4 clusters are induced by heparin treatment, together with a proinflammatory milieu. This would explain why major surgery is a risk factor for HIT (through PF4 release and inflammation). At the GTH 2010 meeting Krauel K et al. present data showing that gram positive as well as gram negative bacteria bind PF4 in a charge dependent manner. PF4 forms complexes on the bacterial surface, thereby exposing the same epitopes which are formed on PF4/heparin complexes. It was even possible to purify platelet activating, heparin dependent anti-bodies from sera of patients with clinical HIT by adsorption and elution using PF4-coated bacteria. This strongly indicates that PF4/polyanion antibodies have a role in anti-bacterial host defense. During heparin treatment, heparin binds to platelets, which allows rebinding of PF4 and formation of PF4/heparin complexes on the platelet surface. Thus platelets mimic PF4-coated bacteria. Potentially HIT is a misdirected host defense mechanism. Understanding the immunobiology of HIT may help explain why host defenses can result in autoimmunity.

Conflict of interest

A. Greinacher has given lectures sponsored by: Organon Int, Schering-Plough, Essex Pharma (drug: danaparoid), Mitsubishi Pharma (drug: argatroban), Glaxo-Smith-Kline (drug: argatroban, fondaparinux), Pharmion (drug: lepirudin). He has served as a consultant for: Organon Int, Schering-Plough, Essex Pharma, Mitsubishi Pharma, Pharmion, Bayer, Novartis. He has received funding for research projects by Organon Int, Schering-Plough, Mitsubishi Pharma, Pharmion.

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