

## Theme Issue Article

# Heparin-induced thrombocytopenia (HIT II) – A drug-associated autoimmune disease

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### Summary

Autoimmune thrombocytopenia (ITP) is an acquired autoimmune disease characterised by isolated persistent thrombocytopenia and normal megakaryopoiesis. This definition also applies to heparin-induced thrombocytopenia (HIT II), a frequent side effect of heparin treatment. In HIT II, the immunogen is a coagulation active complex of heparin and platelet factor 4 (PF4). By now, diagnostics of HIT II is often material and time consuming. Three groups of patients were investigated for HIT II antibodies (HIT II-AB): 54 hospitalised stroke patients, 87 hospitalised cardiac patients, and 71 patients on chronic haemodialysis, all treated with heparin. Furthermore, 100 healthy volunteers were investigated. For detection of HIT II-AB the innovative whole blood test PADA-HIT (PADA: platelet adhesion assay) was used. PADA-HIT quantifies the interaction of IgG

antibodies with Fc $\gamma$ IIA receptors by comparing the activation state of platelets in citrated and heparinised whole blood. The occurrence of HIT II-AB in blood was very high with 44 % of stroke patients, 69% of cardiac patients and 38% of haemodialysis patients compared to only 15% of healthy volunteers. This demonstrates a high incidence and a rapid onset of HIT II-AB in patients being acutely treated with heparin. HIT II is one of the most frequent and severe autoimmune diseases bearing a great thrombosis risk. PADA-HIT represents an innovative diagnostic method for detection of autoimmune antibodies of IgG type that are directed against platelet factor 4 (PF4)-heparin-complex. By early and fast diagnostics and appropriate treatment severe complications of HIT II can be prevented.

### Keywords

HIT II, IgG antibody, PADA-HIT, autoimmune disease

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## Definition and pathophysiology of HIT II

Heparin induced thrombocytopenia type II (HIT II) is a severe and common side effect of acute, subchronic or chronic use of heparins (1–4). HIT II is antibody-mediated (5, 6). The indirect anticoagulant heparin causes a paradoxical thrombophilia that presents in severe venous and arterial thrombotic states (2, 7). All patients who develop HIT II antibodies (IgG) during heparin treatment possess a considerably increased thrombosis risk, most of all when they suffer from severe or life-threatening primary disease (stroke, cardiac arrest, big surgical interventions, tumors) (1, 3, 8). HIT II is a drug-induced thrombocytopenia meeting the criteria of an autoimmune disease. The classical autoimmune disease with a strongly decreased platelet number, the ITP, is defined as follows: Immune thrombocytopenic purpura (ITP) is an acquired autoimmune disease characterised by isolated persistent thrombocytopenia (<150 k/ $\mu$ l platelets) with normal megakaryopoiesis (9). This definition applies to HIT II as well. ITP and HIT II differ only by the immunogens causing thrombocytopenia. In ITP this are endogenous proteins that are

modified due to inflammatory reactions or other causes resulting in an immunogenic response. In HIT II, the complex of heparin and platelet factor 4 (PF4) is the immunogen that causes release of immunoglobulins, predominantly IgG (6). In Table 1 direct heparin-platelet-interactions are listed. An important fact is that platelets can be activated directly by heparin. Heparin interacts with platelet integrins (platelet "heparin receptor"), especially integrin  $\beta$ III that is part of the heterodimeric platelet fibrinogen receptor GPIIb/IIIa (integrin  $\alpha$ IIb $\beta$ III). This interaction of heparin with integrin  $\beta$ III of platelets causes a stimulatory effect and increased adhesiveness of platelets. In the following the contents of  $\alpha$  granula, also the heparin-neutralising factor PF4, a basic protein, are released. This basic protein can interact not only with circulating heparin but also with heparan sulphates of glycoalyx of endothelial cells (3, 10). Immunocompetent cells in the circulation phagocytise the PF4-heparin complexes and they can produce antibodies against heparin-PF4 complex (11, 12). Most of all, these are antibodies of IgG type as immediate immunologic reaction. IgG antibodies, also those against heparin-PF4 complex, occupy the specific IgG receptors on platelets (Fc $\gamma$ IIA).

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**Table 1: Platelet-heparin-interactions.**

- direct heparin binding site on the platelet membrane
- Heparin binds to platelet  $\beta$ III integrins  $\rightarrow$  direct stimulation of the platelets + enhancement of fibrinogen binding
- after activation and release reaction, also of PF4, heparin-PF4-complexes occur in blood = strong immunogenic structures inducing specific immunoglobulin synthesis in the immunocompetent cells
- first-line answer: release of IgG's
- IgG's join the immunogenic heparin-PF4-complex and are bound by the IgG receptor Fc $\gamma$ IIA to the platelet surface
- the thereby initiated *in vivo* platelet aggregation results in micro white clots and the immune thrombocytopenia
- due to the dual thrombogenic platelet activation and aggregation the complete clinical picture of HIT II with arterial and venous thrombosis and thromboembolism often ends lethally
- even after discontinuation of heparin administration heparin molecules can be detected in the patient's blood for up to four weeks

Occupancy of platelet membrane by IgG antibodies induces platelet activation. The greater the occupancy of Fc $\gamma$ IIA receptors with IgG antibodies the more intensive is the platelet activating and thrombogenic effect. When heparan sulphates of endothelial cells interact with free PF4, the resulting complexes can be neutralised by IgG as well (13). These endothelium-bound IgG antibodies also interact with Fc $\gamma$ IIA receptors of platelets and bind platelet aggregates to the endothelium (10). It is of importance that the HIT II-IgG complex differs from other immunologic IgGs. IgG antibodies of other genesis do not have endothelial affinity and do not cause a direct thrombogenic endothelial surface. In Figure 1 graphic presentation of pathophysiology of HIT II is given.

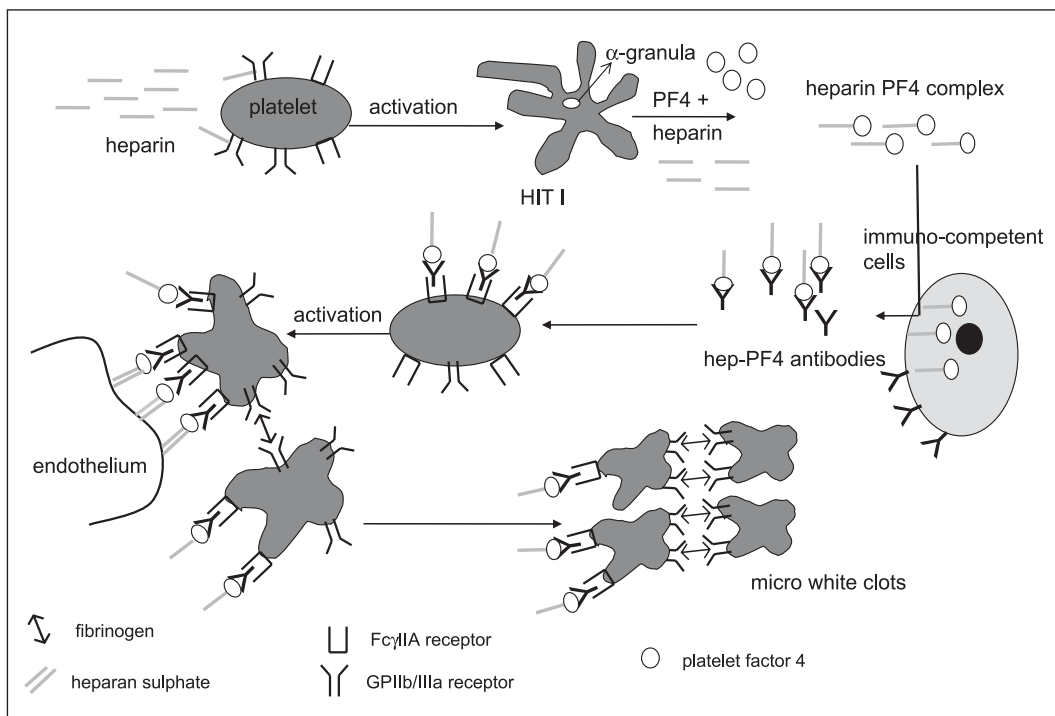
ITP differs from HIT II. ITP causes micro bleedings (cutaneous purpura) which are induced by micro white clots of acti-

vated IgG loaded platelets in microcirculation (9). In HIT II, thrombocytopenia is the indication of direct micro- or macro-thrombotic reaction in peripheral vascular regions and organs (2, 14).

## Diagnostics of HIT II

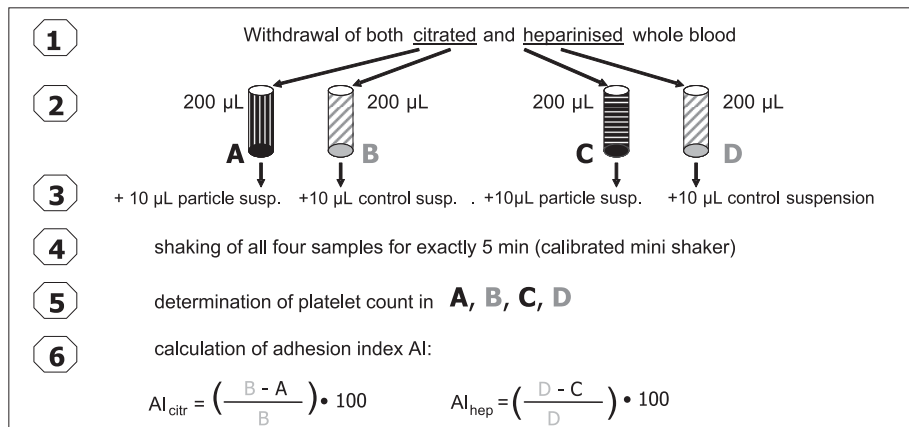
Diagnostics of HIT II antibodies, especially of IgG antibodies, is performed similar to diagnostics of autoimmune diseases. Nearly all methods use patient's serum.

- Most frequently ELISA is used for detection of HIT II antibodies in serum of patients. Results can be obtained within 2–3 hours (h). Sensitivity is low, specificity and the costs are moderate.
- For HIPA (heparin-induced platelet activation) platelet-rich plasma (PRP) of three healthy volunteers is needed. Agglutination of these platelets in presence of patient's serum and heparin is optically evaluated. Results are obtained within 4–8 h. Specificity and sensitivity of HIPA are acceptable. The assay is both time- and material-consuming with high costs.
- For  $^{14}\text{C}$  serotonin release assay ( $^{14}\text{C}$  SRA) PRP of a healthy volunteer is needed besides serum of the patient. Tests must be done in a special laboratory by specialised staff. Costs are very high. Results are available after 12 h.  $^{14}\text{C}$  SRA is extremely sensitive and has a high specificity. By now it has been considered as gold standard of HIT II antibody detection.
- A gel permeation test can be performed very fast; results are available within 1 h. The required material is plasma or serum of the patient. The test has a low specificity and sensitivity. Special equipment is needed, costs are moderate.
- Furthermore, flow cytometric methods can detect HIT II antibodies in plasma; however, they are not suited for routine

**Figure 1: Pathophysiology of HIT-II.**

Principle of PADA-HIT:

- comparative determination of platelet adhesiveness in citrated and heparinised whole blood
- blood owned fibrinogen attaches to the surface of special polymer particles that are added to the anticoagulated blood samples
- following a defined shear stress (= shaking period), activated platelets adhere to protein coated particle surface
- unattached platelets are counted and are compared to platelet count in control sample (= without particles)
- adhesion index AI is calculated from both citrated and heparinised blood
- diagnostics of HIT II:  $AI_{hep} \leq AI_{citr} \Rightarrow$  HIT II - antibody positive  
 $AI_{hep} > AI_{citr} \Rightarrow$  HIT II - antibody negative



**Figure 2: Principle of PADA-HIT.**

diagnostics as they are very time and material-consuming and need a specialised staff.

The fundamental drawback of all these methods is that antibodies must be present in free form in the patient's serum or plasma. The first line fraction of platelet-bound IgG antibodies cannot be detected by these methods. It is assumed that only above a threshold concentration IgG antibodies are also present in serum or plasma. Whether free IgG antibodies and platelet-bound IgG antibodies are in distribution equilibrium is not known.

The only commercial test that detects HIT II antibodies bound on platelet receptors in whole blood is PADA-HIT (PADA: platelet adhesion assay). PADA-HIT (JenAffin GmbH, Jena, Germany) is a functional platelet-specific test that quantifies the interaction of IgG antibodies with FcγIIA receptors in fresh whole blood. The characteristics and the flow chart of PADA-HIT are presented in Figure 2. This test has bedside quality. Besides a calibrated mini-shaker only a blood cell counter is needed. PADA-HIT makes use of the surprising finding that after occupation of FcγIIA receptors immune antibodies of IgG type induce a lower activation of platelets in heparinised blood due to shear stress. For PADA-HIT both citrated and heparinised whole blood of the patient is needed. To both samples special porous polymer particles (Ø 6.5 µm) are added which then coat spontaneously with fibrinogen of the blood sample. Following a defined shear force by shaking the samples, activated platelets are bound to the fibrinogen-coated particle surface. After counting free platelets in both blood samples adhesion indices are calculated and compared. The lower the ad-

hesion index in heparinised blood, the more the platelets are loaded with IgG antibodies.

In case of acute or chronic heparin treatment, it is assumed that the IgG antibodies have been induced predominantly by the anticoagulant ("suspicion of HIT II"). The finding of a HIT II antibody reaction is definite when the adhesion index in heparinised blood is as high as or even lower than the adhesion index in citrated blood.

PADA-HIT has a very low cut-off. Already a partial occupancy of FcγIIA receptors on platelet surface indicates the presence of IgG antibodies in PADA-HIT. The missing specificity for heparin-induced antibodies may be disadvantageous for the test. On the other hand, the detection of autoimmune antibodies of IgG type on platelet surface is an important warning for patients. Already by a low immunoreaction to PF4-heparin-induced antibodies these patients would be stressed with the HIT II typical pathophysiological reactions. Whereas IgG antibodies of other genesis only induce platelet activation and removal of these platelets mainly in the spleen, the HIT II-induced IgG antibodies cause a severe and often life-threatening activation of coagulation. As shown in Figure 1, antibodies against PF4-heparin-complex are also bound to heparan sulphate chains of glycocalyx of endothelial cells, where also PF4 is bound, by their IgG immunoreactive site. The activated platelets are coupled by FcγIIA receptors and thereby platelet aggregates are attached to the endothelial surface. This results in a severe clot formation, mostly in microcirculation of organs with intense blood supply but also in vascular periphery in extremities.

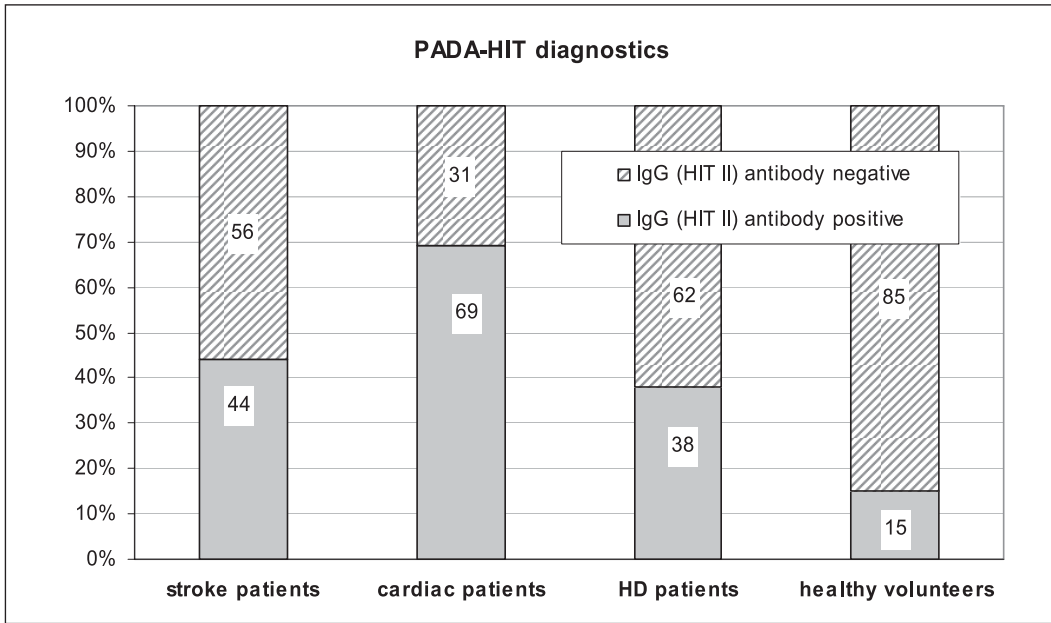


Figure 3: PADA-HIT results in patients and healthy volunteers.

### Clinical investigations of incidence of HIT II antibodies

In clinical studies using PADA-HIT we investigated the occurrence of IgG antibodies in stroke patients, cardiac patients, patients on chronic intermittent haemodialysis and 100 healthy volunteers. The patient's characteristics are as follows:

- **54 stroke patients** (28 female, 26 male; Sophien- und Hufelandklinikum, Weimar, Germany) were investigated on day 3 after heparinisation.
- **87 cardiac patients** (61 male, 26 female, mean age 69 years, University Hospital Jena, Germany, Clinic of cardiology)

were investigated after hospitalisation due to several acute cardiac disorders; acute heparin administration.

- **71 patients on chronic intermittent haemodialysis (HD)** (43 male, 28 female, mean age 62, KfH Nierenzentrum, Jena, Germany) were investigated after at least half a year of chronic HD with heparin anticoagulation; performance of PADA-HIT both before and after a HD session.
- **100 healthy volunteers** (45 male, 55 female, mean age 33 years); no current heparin administration.

All investigated patients received heparin, in case of HD patients even over a long period. In Figure 3 the PADA-HIT results of the

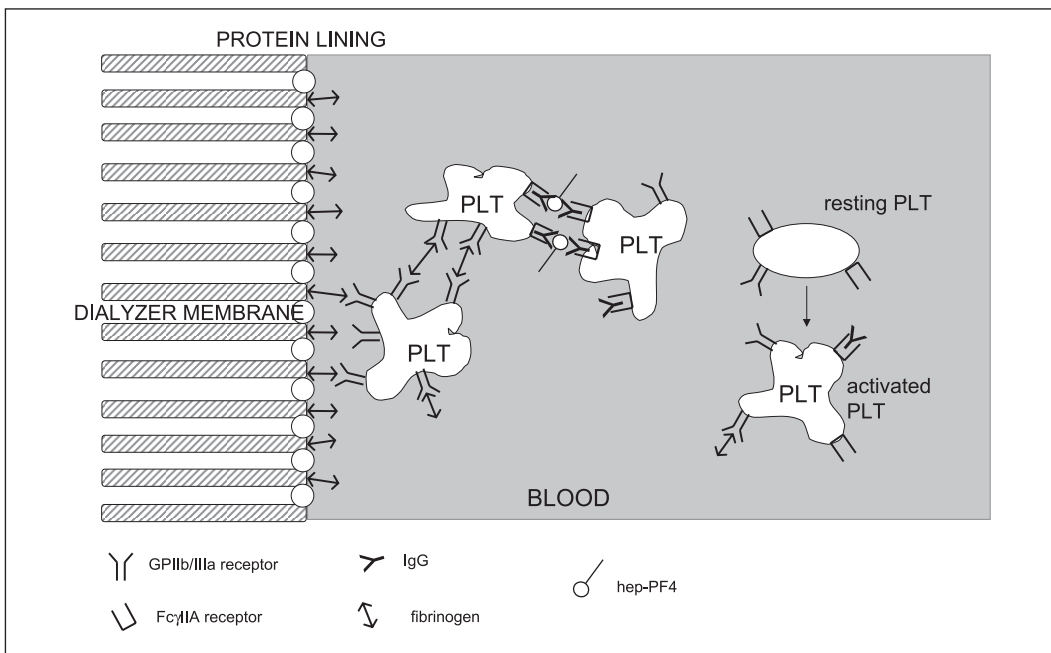


Figure 4: HIT-II antibodies in HD patients.

three patient groups are presented in comparison to healthy volunteers.

The occurrence of HIT-II antibodies in blood was very high with 43.6% of stroke patients, 69% of cardiac patients and 38% of HD patients compared to only 15% of healthy volunteers. These results clearly demonstrate that a great number of patients acutely treated with heparin (stroke and cardiac patients) have developed HIT-II antibodies within few days. In HD patients the occurrence of HIT II antibodies is lower. Although in cases of chronic HD high heparin doses are given for a long period, the picture of classical HIT II is rare (16). An explanation is depicted in Figure 4. The dialyser membranes are quickly coated with plasma proteins, e.g. fibrinogen. This fixed fibrinogen can "fish" for activated platelets or platelet-antigen-antibody-complexes. This way the HIT II IgG antibodies that are bound on the platelets are removed at the end of HD session by discarding the dialyser.

How is the finding in healthy volunteers to be rated? Even in the group of apparently healthy volunteers IgG antibodies were detected using the very sensitive PADA-HIT. These antibodies are not associated with heparin treatment but possibly indicate other immune dysfunctions. Further detailed diagnostics was not possible within this study.

In the scope of the study presented here it could not be clarified whether the high number of patients with HIT II antibodies was affected by other autoimmune reactions. It is to be investigated whether autoimmune diseases or post-infection hyperimmunaemia of IgG type represent comorbidities for platelet-derived coagulation disease (15).

The PADA-HIT presented here has a high sensitivity for IgG antibodies but alone it does not provide the assured diagnosis of

the disease HIT II. Only in association with clinical signs of HIT II this diagnosis can be confirmed. Besides antibody detection further investigations are necessary to measure an increased clotting activity or thrombosis risk in order to diagnose this life-threatening side effect of heparin treatment.

In first longitudinal investigations of concerned patients it was demonstrated that PADA-HIT is also suited for quantitative follow-up of antibody titer. By repeated measurements the gradual decrease of the antibody titer can be detected when the patient is no longer treated with heparin.

## Conclusion

In conclusion, it is stated that HIT II is a frequent and severe autoimmune disease. In comparison to other platelet-associated autoimmune diseases HIT II is dangerous due to the fast incidence of a thrombosis risk. Due to the potentiating effect of platelet adhesion and aggregation HIT-II is often lethal. For this reason a reliable diagnosis of HIT II is necessary, preferably using a test with bedside quality and high sensitivity. PADA-HIT represents the test of choice for fast HIT II diagnostics. This assay can diagnose the autoimmune disease also before a thrombocytopenia is fully developed. This way an immediate discontinuation of heparin administration and onset of the imperative HIT II therapy using alternative anticoagulants (e.g. r-hirudin, argatroban) becomes possible. In spite of all diagnostic and therapeutic feasibility – the most effective prophylaxis of HIT II consists in a life-long avoidance of heparin and heparin derivatives.

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