

SEED Haemostasis



Heparin-induced thrombocytopenia (HIT)

Introduction

Heparin is a naturally occurring glycosaminoglycan isolated from animal tissue (cattle), which serves as an effective and economic anticoagulant in clinical practice.

The large heterogenic polymer is negatively charged (unfractionated heparin (UFH)) and can be depolymerised to smaller polymers known as low-molecular-weight heparins (LMWH). Both heparins and the even smaller heparinoids primarily act as indirect anticoagulants as they amplify the efficacy of antithrombin a 1000-fold by binding to it. The different sizes and structures of heparins and heparinoids result in distinct activity patterns, for instance, UFH inhibits thrombin and coagulation factor Xa, while LMWH primarily affects factor Xa.

Administered intravenously or subcutaneously, heparins act fast and reliable in a wide range of clinical indications such as venous thromboembolism, acute coronary syndrome, atrial fibrillation, cardiac surgery and other states and conditions with increased

thrombotic risk. Heparins are also used to prevent clotting in external devices exposed to blood, such as the extracorporeal membrane oxygenation (ECMO) circuit for extracorporeal life support or haemodialysis and filtration.

Heparin-induced thrombocytopenia (HIT) is a severe complication that can occur in patients exposed to heparins, UFH and LMWH. Patients experiencing HIT may develop thromboembolic complications that are associated with significant morbidity and mortality.

HIT can be classified into two types: Type I, in which thrombocytopenia is caused by the mild platelet aggregation effect of heparin. This is a mild, non-immune mediated reaction. It is not associated with any complications and the platelet count will normalise even if heparin is continued. Type II, is an immune, antibody-mediated reaction in which transient heparin-dependent autoantibodies activate platelets and cause thrombocytopenia. (Table 1)

It is Type II that causes serious complications as a side effect of heparin administration. In Type II HIT, the production of antibodies against the complex of platelet factor 4 (PF4) and heparin (anti-PF4–heparin complex) plays a central role in the pathogenesis of the disease.

Some anti-PF4/heparin complexes exhibit strong platelet activating capacity. This immune complex leads to activation of platelets, mononuclear cells and vascular endothelium, ultimately resulting in overproduction of thrombin, which induces thrombocytopenia and eventually, thromboembolism. (1-4)

Table 1: HIT classification

| | HIT type I | HIT type II |
|--|------------------------------|---|
| Incidence | 30 – 40 % | 0.2 – 5 % |
| Time to onset after first heparin exposure | 1 – 4 days | 5 – 10 days |
| Immune-mediated? | No. Just platelet clumping | Yes. Antibodies against heparin–PF4 complex |
| Platelet count | ca. 100 x 10 ⁹ /L | 30 – 60 x 10 ⁹ /L |
| Thrombosis? | No associated risk | In 30 – 60 % of cases |
| Mortality | Benign | In 20 – 30 % of cases |

The mechanism of HIT type II

The cause of HIT type II is a misinterpretation of the adaptive immune system, which falsely identifies heparin–PF4 complexes as pathogenic. This misguided recognition leads to the formation of antibodies against heparin–PF4 complexes.

When these antibodies bind to their targets, the resulting complexes can activate platelets via their FcγRIIIa-receptor, which has severe consequences,

such as thrombin formation, platelet aggregation and thrombus formation.

The systemic, non-localised platelet activation causes arterial and venous thrombotic events, as well as a sharp drop in platelet counts due to consumption. (1-5) The mechanism of HIT type II is depicted and explained in Figure 1.

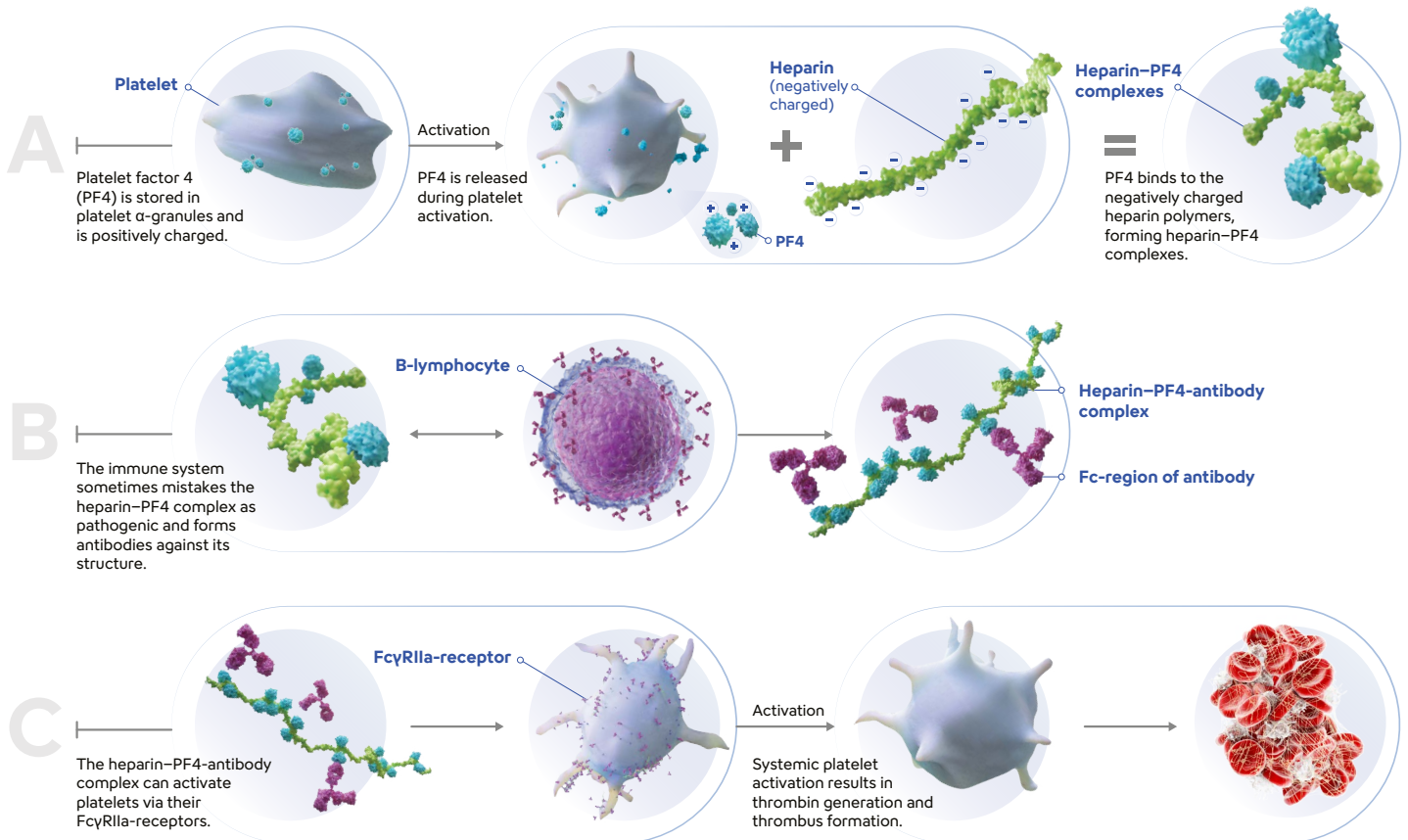


Figure 1: Mechanism of HIT type II. A: Formation of heparin–PF4 complexes. B: Production of heparin–PF4 antibodies by the misinterpreting immune system resulting in heparin–PF4–antibody complexes. C: Activation of platelets by heparin–PF4–antibody complexes via their FcγRIIIa-receptor, causing systemic thrombin and thrombus formation as well as thrombocytopenia.

Not all antibodies against heparin–PF4 complexes cause HIT type II. Only a subset of anti-heparin–PF4 complex antibodies (HIT IgG) can activate platelets. While the absence of HIT IgG antibodies can securely rule out HIT type II, their detection does not necessarily indicate the presence of HIT type II.

Platelet factor 4-related disorders

HIT type II is not the only thrombotic disorder involving platelet factor 4 and antibodies. (5–7) The major characteristics of the different PF4-related disorders are summarised in Table 2.

Essentially, anti-PF4 antibodies can be classified in three types:

- Type 1: non-pathogenic, non-platelet activating
- Type 2: heparin dependent, platelet activating
- Type 3: heparin independent, platelet activating

Type 3 anti-PF4 antibodies, which were originally described in VITT, are now increasingly found to be relevant in cases previously assigned as autoimmune HIT or previously diagnosed as thrombosis of unknown cause.

Like in HIT type II, those cases require anticoagulation and measures to reduce the impact of platelet activation. (3,5,6)

Incidence of HIT type II

Most importantly, the incidence of heparin–PF4 antibodies is much higher than the incidence of clinical HIT. Furthermore, clinical HIT is more prevalent in patients treated with UFH as compared to LMWH. (4) In addition, drug dosing and the type of intervention, as well as injury, impact the frequency of clinical HIT.

Therefore, the incidence of clinical varies ranging from 0.2% with LMWH in predominantly orthopaedic patients to up to 6.6% in patients supported with ECMO. (3) A Cochrane review on patients following surgery concluded HIT was present in 2.2% of patients treated with UFH, and 0.5% in patients treated with LMWH. (8) Independent of patients, therapies and dosing, HIT is a common complication of therapy and the use of heparins, which need to be considered and addressed.

Table 2: PF4-related thrombotic disorders

| Disorder | Principle | Trigger | Treatment |
|--|--|---|---|
| HIT type II | Onset of 5–10 days after heparin exposure due to antibodies against heparin–PF4 complexes. FcγRIIIa-receptor mediated activation of platelets and the coagulation system by heparin–PF4-antibody complexes | Heparin-dependent antibodies against PF4-containing complexes | <ul style="list-style-type: none"> ■ Stop heparin (removes the antigen) ■ Therapeutic dose non-heparin anticoagulation |
| Autoimmune HIT | Onset more than 10 days (up to 6 months) after heparin exposure or after cessation of heparin exposure due to antibodies against heparin–PF4 complexes, which can activate platelets via their Fcγ-receptor independent of the presence of heparin. | Heparin-dependent and heparin-independent antibodies against PF4-containing complexes | <ul style="list-style-type: none"> ■ Stop heparin ■ Therapeutic dose non-heparin anticoagulation ■ High dose Intravenous Immunoglobulin (IVIG) |
| Vaccine-induced thrombocytopenic thrombosis (VITT) | Onset 5 – 10 days after exposure to a vaccine with an adenovirus vector due to antibodies against vaccine component–PF4 complexes. Heparin-independent Fcγ-receptor mediated activation of platelets and the coagulation system by vaccine component–PF4-antibody complexes resulting in sinus or splanchnic vein thrombosis | Heparin-independent antibodies against vaccine component–PF4 complexes | <ul style="list-style-type: none"> ■ Therapeutic dose anticoagulation ■ High dose Intravenous Immunoglobulin (IVIG) ■ Plasma exchange/immune suppression |
| VITT-like disorders | Onset 5–10 days after exposure to a compound forming a complex with PF4 (adenovirus infection, paraprotein), which can initiate antibody production. Heparin-independent Fcγ-receptor mediated activation of platelets and the coagulation system by trigger component–PF4-antibody complexes. | Heparin-independent antibodies against trigger component–PF4 complexes | <ul style="list-style-type: none"> ■ Therapeutic dose anticoagulation ■ High-dose Intravenous Immunoglobulin (IVIG) ■ Plasma exchange/immune suppression |

Diagnosis of HIT type II

The American Society of Haematology Guideline (4) as well as the British Society of Haematology Guideline (3) clearly recommend, as a first step in a patient suspected of HIT type II, to perform a pretest probability assessment utilising the 4Ts score (Table 3), which is available online*.

Based on guideline recommendations and expert opinions, no further testing is indicated if a low probability has been assessed (score ≤ 3). (1,3,4,7) A 4Ts score of 4 -8 not only triggers the determination of the presence of HIT IgG antibodies but also the immediate stop of heparin therapy and a switch to an alternative anticoagulant suitable for the patient’s original indication for anticoagulation therapy.

For the assessment of thrombocytopenia, it is recommended, prior to the initiation of heparin therapy, to obtain a baseline platelet count. (3,4) A drop in platelet count by more than 50 % is a better indicator of HIT than evaluating the absolute platelet count.

Up to 50 % of HIT patients present with associated thrombosis, and most commonly with VTE, but arterial or atypical thrombosis can also occur. (3,4)

For laboratory diagnosis, the detection of heparin–PF4-specific antibodies is required through either using classic ELISA assays or modern, faster chemiluminescence immunoassays, such as the new, automated, state-of-the-art Sysmex HISCL HIT IgG Assay Kit on Sysmex CN-3500/6500 analyser. The assays use a heparin–PF4 complex to capture the HIT IgG antibodies and then antibodies connected to an enzyme to detect the captured antibodies. The linked

enzyme is finally used to create a signal by releasing a chromophore (ELISA) or a fluorophore (CLIA), which is proportional to the amount of captured HIT IgG.

If the HIT IgG testing cannot detect HIT antibodies, the presence of HIT type II can be ruled out with a high probability. (1-4) HIT IgG assays detect those antibodies binding to PF4 when it is bound to heparin. Thus, other antibodies against PF4 may not be detectable by these specific assays due to the steric hindrance of the heparin. For instance, VITT or VITT-like PF4-antibodies are often not detected by specific HIT IgG assays. (10)

The detection of HIT IgG antibodies does not prove the presence of clinical HIT type II, which led to the suggestion of the so-called “iceberg” model. (9) The iceberg model views HIT type II as being caused only by a subset of anti-PF4–heparin antibodies, which can activate platelets via their Fc γ R11a-receptors. This model illustrates why functional assays detecting antibodies capable of binding and cross-linking platelet Fc gamma R11A and triggering platelet activation, such as the heparin-induced platelet activation (HIPA) assay or the serotonin-release assay (SRA), are crucial for the diagnosis of HIT.

Both assays use washed platelets from healthy donors, serum from a patient suspected of HIT and heparin to trigger platelet activation, which is then measured either by turbidimetric detection of platelet aggregation (HIPA) or the release of radioactive labelled serotonin with which the platelets were loaded before washing. Only those functional assays are able to distinguish non-pathogenic, non-activating antibodies from those actually causing HIT type II.

Table 3: The 4Ts score pretest probability assessment

| Category | 2 points | 1 point | 0 points |
|-----------------------------------|--|--|--|
| Thrombocytopenia | > 50% reduction AND platelet nadir ≥ 20 | 30–50% reduction OR platelet nadir 10–19 | < 30% reduction OR platelet nadir < 10 |
| Timing of platelet count fall | 5–10 days OR platelet fall ≤ 1 day | > 10 days OR platelet fall ≤ 1 day | Prior to day 4 without recent heparin exposure |
| Thrombosis or other sequelae | New | Progressive | None |
| Other causes for Thrombocytopenia | No other cause | Other possible cause | Confirmed other cause |



*Scan to learn more about the 4Ts score.

Unlike the automated enzyme immunoassays (EIA), the functional assays are laborious and complex (e.g. washing platelets, loading with radioactive serotonin), and involve manual methods requiring skilled personnel and special equipment. However, while HIT IgG assays and functional assays share a similar high sensitivity to HIT antibodies, the functional assays are far more specific for HIT. (3,4)

Thus, fast and automated HIT IgG assays, such as Sysmex HISCL HIT IgG Assay Kit on CN-3500/6500 analyser, can sensitively exclude HIT but if antibodies are detected, require a functional assay to confirm clinical HIT. Figure 2 summarises the diagnostic workflow leading to the diagnosis of HIT type II. (3,4,9)

Treatment of HIT

If the probability of HIT type II is high (4Ts score >3) and/or a strong signal in an HIT IgG assay is recorded, heparin therapy or exposure should be immediately stopped and depending on the patient's initial indication for heparin treatment, switching to prophylactic or therapeutic anticoagulation therapy with a non-heparin anticoagulant is strongly recommended. (3,4)

As HIT is a pro-thrombotic disorder, therapeutic anticoagulation with an alternative non-heparin anticoagulant is warranted if the diagnosis is highly likely or confirmed. Currently, the British Society of Haematology recommends therapeutic anti-coagulation for 3 months in patients following clinical HIT with a thrombotic complication and for 4 weeks without thrombotic complications. (3)

In patients with a low probability of HIT, it is not only recommended to refrain from further laboratory testing, but also to continue with heparin therapy as indicated. (3,4) It is only if the low probability scoring for HIT is uncertain that laboratory testing for HIT (e.g. HIT IgG assay) and discontinuation of heparin therapy, followed by an appropriate non-heparin anticoagulant therapy, are suggested.

Severe cases of HIT as well as other PF4-related immune-mediated disorders may require more drastic therapeutic approaches such as intravenous immunoglobulins (IVIG), which likely act by competitively blocking FcγRIIa-receptors or plasma exchange and thus, removes the antibodies against heparin–PF4 complexes. (3)

The existing guidelines provide detailed recommendations and suggestions how to efficiently manage HIT as well as VITT patients. (3,4)

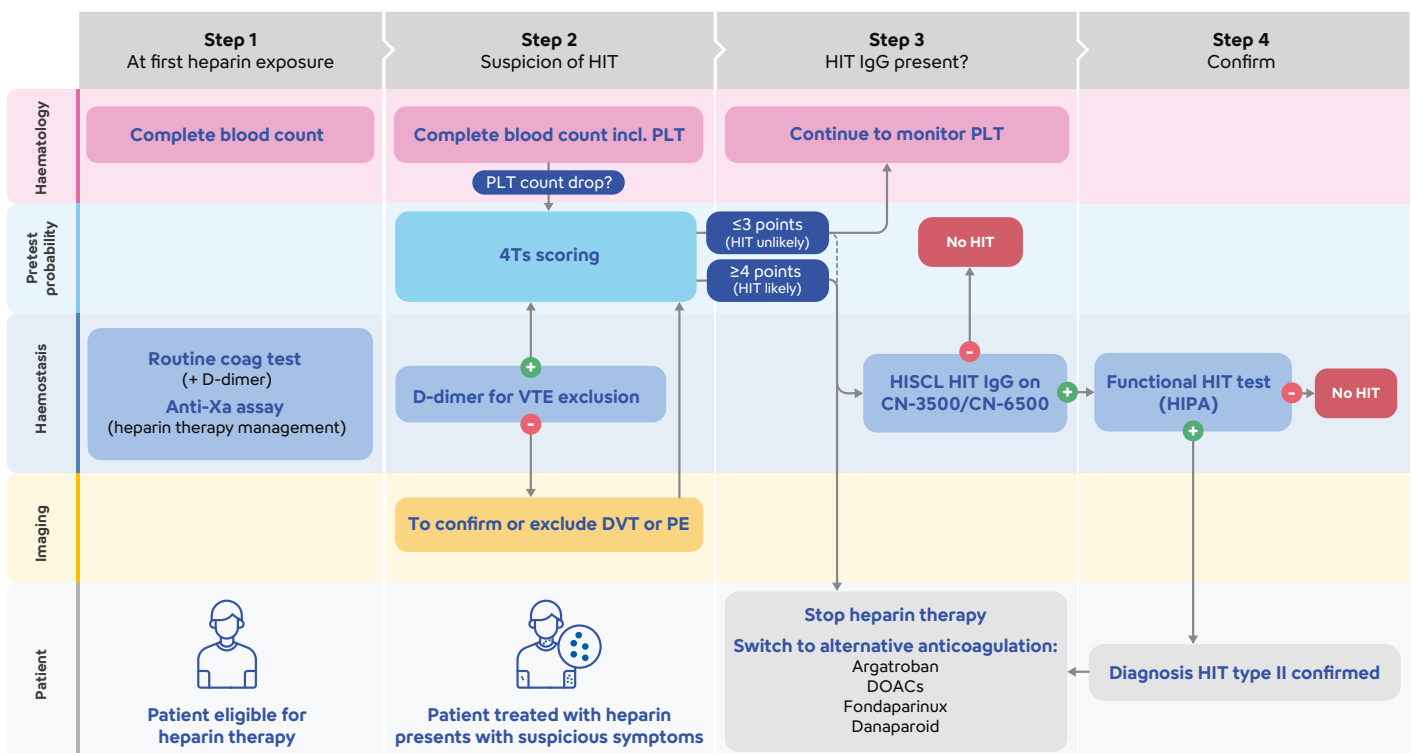


Figure 2: A guideline-based Sysmex variant of the workflow for diagnosing HIT type II.

Conclusion

Heparin-induced thrombocytopenia (HIT) is a common complication of heparin therapy. While HIT type I is harmless, immune-mediated HIT type II can be fatal if not diagnosed and managed. Other PF4-related and immune-mediated diseases, such as autoimmune HIT, vaccine-induced thrombotic thrombocytopenia (VITT) and disorders similar to VITT, present newer mechanistically related disorders.

If a heparin-treated patient presents with suspicious symptoms, an evaluation of pre-test probability (the 4Ts score) should be performed. Measuring HIT IgG levels in plasma or serum can exclude HIT type II or if positive, trigger functional HIT testing to confirm the presence of HIT type II.

The new and easily accessible Sysmex HISCL HIT IgG Assay Kit* provides fast and accurate results with excellent sensitivity and specificity.

Take-home message

- HIT type II is a severe, potentially fatal, but manageable complication of heparin therapy
- Pretest probability tools such as the 4Ts score and sensitive, fast, and reliable assays such as the new Sysmex HISCL HIT IgG Assay Kit help to diagnose, exclude and manage HIT.

*Product availability depends on the country. Please contact your local Sysmex representative.

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