



Heparin-induced thrombocytopenia: a clinical paradox

Heparin has many applications in managing thrombotic diseases and their risks. However, under certain circumstances, heparin anticoagulant therapy can induce life-threatening thrombosis, and herein lies a paradox, as Jean Amiral, Graham Jones and Anne Marie Vissac explain.

Ever since the use of heparin was introduced to treat thrombotic complications more than half a century ago, countless patients have benefited from this anticoagulant therapy, which has dramatically improved the outcome of thrombotic diseases. Anticoagulant properties of unfractionated heparin (UFH), a heterogeneous sulphated polysaccharide with a molecular weight ranging from less than 10 kDa to more than 30 kDa, were demonstrated at the beginning of the 20th century. This led to the introduction of this compound as an anticoagulant drug in the early 1950s. Since then there has been a continuous evolution of heparin-like substances, with the introduction of low molecular weight heparin (LMWH) and, recently, pentasaccharide (the shortest polysaccharide sequence of heparin showing anticoagulant activity).

The anticoagulant activity of heparin is expressed through its binding to the coagulation inhibitor protein antithrombin III (AT III), which has a molecular weight of 58 kDa and is present at a concentration of approximately 150 µg/mL. When bound to heparin, AT III becomes a fast-acting inhibitor of coagulant serine esterases generated when blood coagulation occurs via factor IXa, factor Xa and finally thrombin. This final-stage blood clotting enzyme converts fibrinogen into a fibrin clot. The action of AT III in the presence of heparin prevents the propagation of blood clotting

and inhibits blood procoagulant pathways.

Therefore, heparin has many invaluable applications in managing thrombotic diseases and their risks. Its introduction has revolutionised the prognosis in these disorders. Unfractionated heparin inhibits the various serine esterases, LMWH preferentially inhibits factor Xa, while pentasaccharide inhibits factor Xa alone.

Heparins now have many curative and preventive applications and are the most extensively used therapeutic agents in the world. The basic material (UFH) is extracted from bovine lung or pig intestine, is cheap and is produced in large amounts. Chemical or enzymatic cleavage of UFH permits the preparation of LMWH (MW <3 to >10 kDa, average 3.5 to 6.5 kDa). These preparations are better defined pharmacologically, but they are significantly more expensive.

In addition to treating cardiovascular diseases and venous thrombosis (eg deep-vein thrombosis or pulmonary embolism),

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heparins (mainly LMWH) are used preventively in surgical procedures that have an associated increased risk of post-surgical thrombosis (eg orthopaedic and gastric surgery) or in some cases of bone fracture or in malignancy.

However, despite its invaluable benefits, heparin may have severe adverse effects in some cases. The paradox of this anticoagulant drug is that it can induce thrombocytopenia and sometimes thrombosis as a sudden and acute life-threatening complication, which may result in limb amputation or death.^{1,2} It then becomes absolutely necessary to withdraw heparin and replace it with another anticoagulant drug, selected according to the patient's clinical situation and immediate clinical risk.³

HISTORICAL PERSPECTIVE

This complication of heparin therapy was suspected at the end of the 1950s and confirmed subsequently by many studies. It differs from the mild thrombocytopenia frequently associated with heparin therapy and which reverses spontaneously. It was named heparin-induced thrombocytopenia (HIT) type II in order to distinguish it from the reversible form (type I). A drop in platelet count is the first indication of the presence of the disease,⁴ and it can be associated with the presence of an arterial white clot, which contains many platelets.

More recent studies, however, have shown that thrombosis occurs in the veins in about 70% of cases.¹ Normalisation of platelet count within a few days of cessation of heparin therapy confirms the heparin dependence of the HIT complication.

Figure 1 shows an example of the course of the platelet count in a patient who developed HIT, and typical normalisation after therapy is stopped.

Hundreds of scientific articles are

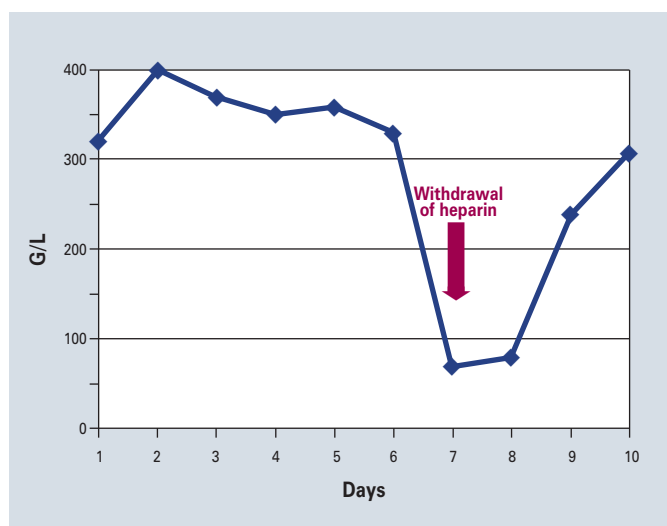


Fig 1. Kinetics of the platelet count in a patient with HIT, and normalisation at the cessation of heparin therapy.

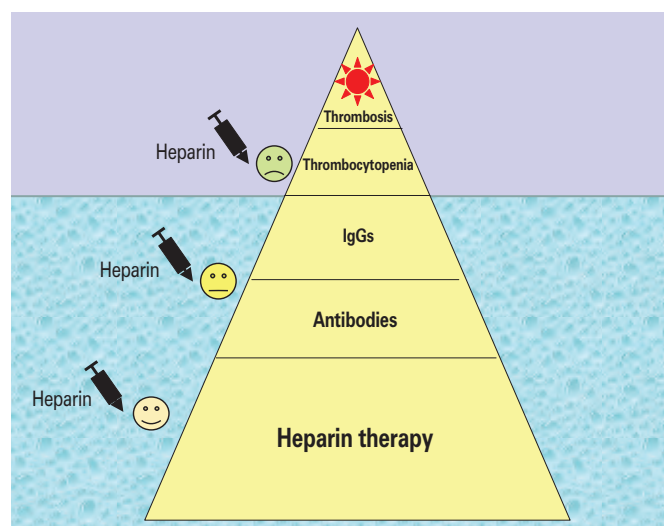


Fig 2. The 'iceberg model' for heparin-induced thrombocytopenia.

available on this topic. An immunological origin was suggested in the 1970s, when it was proposed that patients develop a heparin-dependent antibody, which causes platelet activation and thrombocytopenia, probably through the interaction of heparin-antibody complexes with the platelet IgG receptor Fcγ-RIIIa.⁵

Various functional assays, based on platelet activation, were then proposed.⁶ The first is the platelet aggregation test (PAT) in which assayed patient plasma is incubated with normal platelets at a low (0.1–1 iu/mL) or high (10–100 iu/mL) heparin concentration. The assay diagnoses HIT only if aggregation occurs at low heparin concentration. Although useful, this test lacks sensitivity and is highly dependent on the donor who supplied the normal platelet preparation.

In 1986, Sheridan and Kelton introduced a more sensitive assay, the C¹⁴-serotonin release assay (SRA),⁷ which is frequently considered to be the gold standard for diagnosing HIT. This very sensitive assay works with washed platelets, but can be used only in specialised laboratories that use radiochemicals. Among the functional assays is the heparin-induced platelet aggregation (HIPA) test, proposed by Greinacher, which is based on the use of washed platelets, which improves sensitivity and specificity.⁶

In the early 1990s the target antigen of heparin-dependent antibodies was identified in the vast majority of HIT cases as platelet factor 4 (PF4) complexed with a stoichiometric concentration of heparin (about 27 iu heparin, or approximately 150 µg per mg of PF4 tetramer).⁸ This allowed the design of specific diagnostic tests for measuring the heparin-dependent antibodies, and allowed elucidation of the mechanisms involved.⁹

Today, heparins (mainly LMWH) have expanding applications as they remain the drug of choice for the acute-phase treatment of thrombosis and for its prevention in high-risk surgical or clinical contexts, even with the risk

of HIT.¹⁰ Heparin-induced thrombocytopenia occurs in about 0.5–2% of patients treated with UFH and in less than 0.2% of those treated with LMWH, although seroconversion (generation of heparin-dependent antibodies) is far more frequent.^{11–14}

Heparin-induced thrombocytopenia is now better recognised and managed, as clinicians are more aware. However, this disease remains difficult to diagnose in many clinical contexts, as its onset may be delayed^{15,16} and other causes of thrombocytopenia may be present.¹⁷ In addition, thrombosis can occur in some patients in the absence of thrombocytopenia, which can be masked by the presence of an elevated platelet count (thrombocytopenia) or may coincide with thrombosis.

CLINICAL CONTEXT

Typically, HIT develops in some patients between five and 15 days following the onset of heparin therapy, although it can develop earlier (rapid onset) within hours or days when there has been previous exposure to the drug.^{1–3} This confirms the immune origin of this clinical complication. In a few cases delayed-onset thrombocytopenia has been reported,^{15,16} possibly associated with thrombosis, which can develop days after cessation of heparin therapy, or can persist despite withdrawal.

Heparin-induced thrombocytopenia usually occurs due to an abrupt but moderate drop in platelet count, falling below 150 G/L or decreasing by more than 30% between two

successive counts. Other causes of thrombocytopenia (infections, antibiotics etc) must be excluded for accurate diagnosis; however, occurrence of thrombosis, triggered by heparin-dependent antibodies, without significant thrombocytopenia (platelet count >150 G/L) has been reported in some cases. In these circumstances, the blood procoagulant effect induced by platelet activation is immediate, and thrombosis develops, unless thrombocytopenia is present.

Clinical context can be an additional risk factor for development of HIT.^{17–19} Occurrence is more frequent following cardiovascular or orthopaedic surgery,^{17,18} or when there is infection, inflammation or associated malignancy. These conditions are known to induce a high level of blood activation, which favours development of heparin-dependent antibodies and subsequent HIT. Usually, thrombocytopenia precedes thrombosis; however, not all patients with thrombocytopenia develop thrombosis if heparin treatment is replaced rapidly by a substitute anticoagulant therapy.

Seroconversion occurs more frequently. Many patients on heparin therapy develop heparin-dependent antibodies but are asymptomatic.¹¹ Most are IgM isotypes but some IgA and/or IgG isotypes develop, and this becomes a complementary risk factor for developing the disease.²⁰ Warkentin proposed the 'iceberg model' because 20–50% (depending on the clinical context) of patients receiving heparin develop asymptomatic antibodies, and only a subgroup of them develop IgG isotypes (isolated or associated with other isotypes) and thrombocytopenia,²¹ and only about half of these develop thrombosis (Fig 2). However, there is some overlapping between groups, as thrombocytopenia can be seen in the presence of only IgM and/or IgA,²⁰ and very occasionally thrombosis can occur without apparent thrombocytopenia.

Diagnosis of HIT is of prime importance and special care must be taken when treating

'The anticoagulant activity of heparin is expressed through its binding to the coagulation inhibitor protein antithrombin III'

those patients in high-risk clinical situations. Thrombotic events associated with HIT are variable. Arterial thrombi can form (identified as white clots), but venous thrombosis predominates. Pulmonary embolism is frequent. Venous limb gangrene can develop. Classic central skin necrosis can be present (especially at subcutaneous injection sites), with a paradoxical pathogenic role for coumarin anticoagulation, which induces necrosis at limb extremities.²²

PATHOLOGICAL MECHANISMS

Heparin-induced thrombocytopenia is usually generated following immunisation of heparin-treated patients with complexes of heparin and PF4.⁹ Origin of the heparin appears to have little bearing, although bovine material is reported to be more immunogenic.²³ The route of heparin administration also appears to have no effect on the prevalence of HIT. The sulphation grade of heparin, and the length of oligosaccharide chains, should correlate with its ability to generate antibodies and subsequently produce HIT (UFH is more immunogenic than LMWH).

Present knowledge allows us to speculate about how heparin-dependent immunisation can occur. At the beginning of heparin therapy, large amounts of PF4 are released from the endothelial storage pool. This PF4 binds to endothelial cell glycosaminoglycans, from which it is displaced by heparin, which exhibits a higher affinity for PF4. PF4 concentration in blood is then high (>200 ng/mL, compared to an average 5 ng/mL in normal individuals). At pathological sites, where inflammation or blood activation develops, much higher amounts of PF4 can be present, and can form complexes with heparin. When stoichiometric conditions are met (about 0.15 µg or 0.027 iu heparin per µg PF4), PF4 forms a tight complex with heparin, which induces the modification of PF4 structure through intramolecular constraints, and cryptic epitopes or neo-epitopes are then exposed on PF4 (Fig 3).⁹ PF4 concentrations in the µg range can be present at pathological sites where platelet activation occurs.

As heparin concentration in blood varies between injections, many possibilities exist as to why stoichiometric PF4 and heparin complexes develop and why antibodies subsequently are formed. Heparin requires at least 12–14 sulphated oligosaccharide chain lengths to wrap around the PF4 tetramer and render it antigenic; however, the pentasaccharide is not expected to form immunoreactive complexes with PF4.²⁴

Negatively charged sulphated groups of heparin interact with the positively charged lysine and arginine groups of PF4. If heparin therapy is continued, heparin can be present on platelets and other blood or endothelial cells, onto which it binds. PF4 can then form complexes with heparin on these cell surfaces, and can bind heparin-dependent antibodies targeted to complexes of PF4 and

'Heparin-induced thrombocytopenia occurs frequently in complex clinical situations in which other causes of thrombocytopenia and thrombosis can be present'

heparin. This can induce platelet activation,²⁵ especially through the binding of the Fc fragment of IgG to the platelet Fcγ-RIIa receptors,⁵ but also through platelet–leucocyte interactions,^{26–29} activation of endothelial cells,^{30,31} release of tissue factor and of procoagulant microparticles, all converging to induce a potent procoagulant state that can result in abrupt thrombosis.

Interestingly, procoagulant and platelet-derived microparticles, as well as platelet–leucocytes aggregates can be seen in the blood of patients with HIT (Fig 4). Antibodies exhibiting the highest affinity are the more pathogenic.³³ The presence of heparin-dependent antibodies targeted to heparin and PF4 complexes must be considered as a short-term, high-risk factor for HIT, especially if they are of the IgG isotype and present at high concentration.

However, IgM and/or IgA isotypes should not be disregarded as they can predict evolution to HIT, and can precede IgG antibodies. When present at high concentration, IgM and/or IgA isotypes might be harmful themselves through their targeted binding of platelets and blood cells,³⁴ although this possibility is still under debate.

Interestingly, heparin-dependent antibodies are observed frequently in patients on extra-corporeal circulation (ECC),^{12–14} but less so with HIT, although IgG isotypes are often observed. In such cases, high heparin concentrations (>2 iu/mL) are perfused and these concentrations could be high enough to prevent the formation of PF4-heparin complexes on the surface of blood cells in some patients. Furthermore, at the end of the ECC process, heparin is neutralised rapidly with protamine sulphate.

In a few cases of clinically apparent HIT, associated with a positive platelet functional assay (PAT, SRA or HIPA), no antibody to PF4–heparin complexes is present.³⁵ Further studies have demonstrated the presence of antibodies to non-PF4 antigens (eg interleukin-8 [IL-8]),^{35–37} although these could have been present prior to the heparin therapy. This anticoagulant treatment could render the antibodies pathogenic by targeting them to IL-8–heparin complexes present on blood cell surfaces, thereby exposing heparin or IL-8 receptors.

DIAGNOSIS

The risk of developing HIT must always be considered when heparin therapy is used, especially in surgical or medical situations where this risk is enhanced (bone fracture, multiple trauma, sepsis, malignancy), or in patients who are exposed repeatedly to heparin. The first indication of HIT is a drop in platelet count (<150 G/L or a decrease >30%) on two successive counts. In heparin-treated patients at risk of HIT, platelet count every two days is recommended, and every day in clinical situations where this risk is increased. When thrombocytopenia occurs, heparin therapy must be stopped immediately and replaced with another anti-thrombotic treatment.

When HIT is suspected, the first clinical decision is to stop the patient's contact with heparin, which will suppress the formation of heparin-dependent target antigens, and therefore limit the production of antibodies. The occurrence of heparin-dependent antibodies can be investigated with functional assays (PAT, SRA or HIPA performed at low and high heparin concentration) or by testing antibodies to PF4–heparin complexes or to heparin–protein complexes.^{6,8,9,18}

Antibodies to heparin–PF4, also reactive with PF4 complexed with polysulphated polystyrene, are measured by enzyme-linked immunosorbent assay (ELISA) or using coloured (red) latex particle (covered with PF4 complexed to heparin) agglutination, which are separated from non-agglutinated particles by gel migration using centrifugation. If antibodies are present, the latex particles agglutinate and are unable to enter the gel (they form a red layer on the top) during centrifugation, while free latex particles form a sediment at the bottom.

Recently, a new assay (Zymutest HIA) has been introduced which mimics the binding of antibody to blood cell surfaces exposed to heparin. This assay measures all heparin- and PF4-dependent antibodies, their circulating complexes and also heparin-dependent antibodies targeted to non-PF4 antigens. Briefly, an ELISA plate is coated with a complex of protamine sulphate and heparin (in excess), then stabilised and blocked with an animal serum. Patient's plasma (or serum) is then introduced. When antibodies are present, an immune complex is formed with heparin, PF4 or another chemokine (eg IL-8), and the heparin-dependent antibody is

'A sudden drop in platelet count, or any sign of skin necrosis or of thrombosis during heparin therapy, requires immediate intervention'

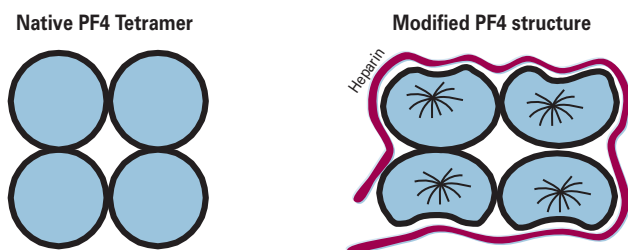


Fig 3. PF4 tetramer and modification by its interaction with heparin.

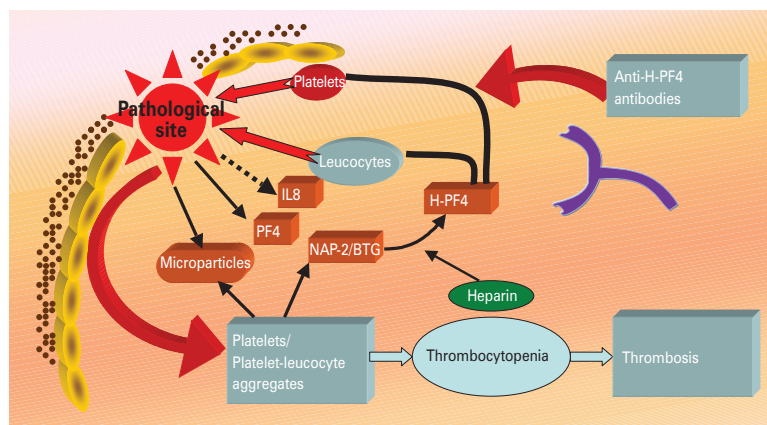


Fig 4. The hypercoagulable state in heparin-induced thrombocytopenia.



Fig 5. Principle of the new assay for heparin-dependent antibodies.

immobilised by the heparin, which is biologically available. The principle of the assay is depicted in Figure 5.

Immobilised antibodies are measured in a second step using an anti-human immunoglobulin labelled with horseradish peroxidase (HRP) and tetra-methyl-benzidine (TMB) with hydrogen peroxide (H_2O_2) as substrate. Depending on the HRP-labelled antibody used, all immunoglobulin subclasses (IgG, IgA and IgM) can be measured, or specifically only individual isotypes (IgG or IgM or IgA). Usually, measuring all immunoglobulins (IgG, IgA and IgM) provides the best indicator for assessing the risk of developing HIT in heparin-treated patients, as the presence of any antibody can precede development of the disease.

Measuring only IgG isotypes when platelet count is decreased offers the highest specificity for HIT. However, the presence of IgM and/or IgA isotypes should not be disregarded, as they can undergo seroconversion to IgG²¹ and result in the rapid development of HIT.

THERAPEUTIC OPTIONS

Cessation of heparin therapy is mandatory when HIT is suspected, although subsequent therapeutic choices are limited. Alternative anticoagulants included sodium danaparoid (Orgaran), which does not cross-react with heparin-dependent antibodies in the majority of patients with HIT, although it is not available in all countries for treating HIT. Another anticoagulant, hirudin (Lepirudin, Bivalirudin),³⁸ has been proposed but it must be handled with great care as it has no antidote and some anaphylactic reactions

'Not all patients with thrombocytopenia develop thrombosis if heparin treatment is replaced rapidly by a substitute anticoagulant therapy'

(which can be fatal) have been reported. Recently, in some countries, Argatroban,³⁹ a synthetic specific thrombin inhibitor, has been introduced for treating HIT. Short heparin drugs such as pentasaccharide (Fondaparinux or Arixtra) offer some promise as they contain only five oligosaccharides, which is the minimum sequence required for inhibition of factor Xa in the presence of AT III.

CONCLUSIONS

The importance of HIT, the life-threatening complication of heparin therapy, must not be underestimated. Heparin is used widely in medicine and the clinical incidence of HIT is not negligible. A sudden drop in platelet count, or any sign of skin necrosis or of thrombosis during heparin therapy, must be regarded as a sign that requires immediate intervention. Heparin must be stopped and replaced by another antithrombotic agent, and the diagnosis of HIT must be confirmed by platelet functional tests or the measurement of heparin-dependent antibodies.

Heparin-induced thrombocytopenia occurs frequently in complex clinical situations in which other causes of thrombocytopenia and thrombosis can be present. Combining laboratory methods to confirm a diagnosis is especially helpful, but results sometimes can remain inconclusive. However, the urgent clinical decision is always to remove the cause of HIT by stopping heparin, while protecting the patient with another anticoagulant drug. ■

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