Anti-platelet factor 4 immunothrombotic syndromes

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Anti-platelet factor 4 immunothrombotic syndromes comprise a group of disorders that include heparin-induced thrombocytopenia and vaccine-induced immune thrombocytopenia and thrombosis. These are highly prothrombotic, immunological disorders characterised by specific clinical and pathological criteria which include thrombocytopenia and thrombosis. While they are predominantly triggered by heparin and the adenoviral vector vaccines, respectively, other provoking factors have been described, as well as spontaneous forms. The unexplained co-occurrence of thrombocytopenia with thrombosis should raise suspicion and prompt testing. This nutshell review discusses the pathophysiology, presenting features and diagnostic criteria for these conditions.

KEYWORDS
heparin-induced thrombocytopenia, immunothrombosis, PF4 antibodies, platelet factor 4, vaccine-induced immune thrombocytopenia and thrombosis

PF4-MEDIATED IMMUNOTHROMBOTIC SYNDROMES (PFITS)

Vaccine-induced immune thrombocytopenia and thrombosis secondary to ChAdOx1 nCoV-19 and Ad26.COV2.S appeared as a new condition in early 2021 during the national vaccination programmes in the fight against the COVID-19 pandemic. The similarities to HIT were promptly identified.

Both are characterised by anti-PF4 antibody-mediated platelet activation, giving rise to microvascular or large-vessel thrombosis, and thrombocytopenia due to platelet consumption. Presentation is five or more days after exposure to heparin and adenoviral vector vaccines, respectively, but other provoking factors and spontaneous forms have also been described (Table 1).1,2 The presentation, diagnostic criteria and confirmatory testing of these disorders are summarised in Table S1.

<table>
<thead>
<tr>
<th>HIT and HIT-like syndromes</th>
<th>VITT and VITT-like syndromes</th>
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<tr>
<td>HIT</td>
<td>VITT</td>
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<tr>
<td>Rapid onset HIT</td>
<td>Occurs 5–30th days after adenoviral vaccination</td>
</tr>
<tr>
<td>Delayed onset HIT&lt;sup&gt;ª&lt;/sup&gt;</td>
<td>Occurs without vaccine exposure. Can be provoked by adenovirus or other infection, and can be chronic.</td>
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<tr>
<td>Persisting (refractory) HIT&lt;sup&gt;ª&lt;/sup&gt;</td>
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<tr>
<td>Spontaneous HIT</td>
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<td>Fondaparinux induced HIT&lt;sup&gt;ª&lt;/sup&gt;</td>
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<tr>
<td>Abbreviations: DVT, deep vein thrombosis; HIT, heparin-induced thrombocytopenia; PE, pulmonary embolism; PF4, platelet factor 4; VITT, vaccine-induced immune thrombocytopenia and thrombosis.</td>
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<tr>
<td>&lt;sup&gt;ª&lt;/sup&gt;Considered autoimmune (aHIT) as both heparin-dependent and heparin-independent PF4 antibodies are present. Clinical outcomes of aHIT are often more severe.</td>
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<tr>
<td>&lt;sup&gt;ª&lt;/sup&gt;Up to 42 days if isolated DVT/PE.</td>
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TABLE 1 PF4-mediated immunothrombotic syndromes (PFITS).
PATHOPHYSIOLOGY

Platelet factor 4 is a chemokine that forms a globular, tetrameric molecule characterised by an equatorial band of strong positive charge (Figure 1A).

HIT antibodies

Heparin, a long chain sugar molecule known as a glycosaminoglycan, carries a very high negative charge. In HIT, heparin binds to positively charged amino acids on PF4 (Figure 1B) neutralising PF4’s positive charge. This allows: (1) close approximation of PF4 molecules and (2) exposure of otherwise hidden sites at the north and south poles of PF4 to which anti-PF4/heparin antibodies can bind5 (Figure 1C). This leads to the formation of ultra-large immune complexes which activate platelets and leukocytes through interaction of antibody Fc regions with cellular Fc receptors.6,7 Platelet and leukocyte activation leads to thrombosis, inflammation, platelet consumption and thrombocytopenia.

Innate immunity and prevalence of anti PF4-heparin antibodies

The interaction with sugars on bacterial surfaces may be the evolutionary reason for the production of anti PF4-heparin antibodies.

Abbreviations: DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assays; HIT, heparin-induced thrombocytopenia; LMWH, low molecular weight heparin; PE, pulmonary embolism; PF4, platelet factor 4; UFH, Unfractionated heparin; VITT, vaccine-induced thrombotic thrombocytopenia.

Rapid VITT test in development.

QUICK HITS

<table>
<thead>
<tr>
<th>HIT</th>
<th>VITT</th>
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<tr>
<td><strong>Incidence</strong></td>
<td>~1% of patients receiving UFH. Incidence increases with dose and duration. Less common with LMWH</td>
</tr>
<tr>
<td><strong>Presentation</strong></td>
<td>Drop in platelet count ≥30%, 5–10 days from start of heparin exposure (can be sooner if recent heparin exposure) Thrombosis in ~50% at diagnosis</td>
</tr>
<tr>
<td><strong>Sites of thrombosis</strong></td>
<td>Venous or arterial thrombosis depending on the clinical setting of the patient. Predominantly DVT/PE but ~30% risk of arterial thrombosis Bleeding is uncommon</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td>4Ts score to guide antibody testing: Timing after exposure to heparin, thrombocytopenia—severity of thrombosis, and other causes of thrombocytopenia unlikely</td>
</tr>
<tr>
<td><strong>Diagnostic assays</strong></td>
<td>Positive rapid HIT tests and ELISA. Gold standard: demonstration of platelet activating antibodies in functional assays</td>
</tr>
<tr>
<td><strong>Antibody specificity</strong></td>
<td>Anti PF4-heparin antibodies Polyclonal</td>
</tr>
<tr>
<td><strong>Atypical presentations</strong></td>
<td>Can occur spontaneously. Rare but under-recognised. If new thrombosis with thrombocytopenia and no other explanation for thrombocytopenia test using HIT and VITT diagnostic assays</td>
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</table>

[Figure 1] Crystal structure of the tetrameric PF4 molecule.3 (A) positively charged amino acid residues (blue), (B) heparin-binding sites (orange), (C) binding sites of HIT antibodies (yellow) and (D) binding sites of VITT antibodies (green and red). In VITT, antibodies are usually detected against the equatorial, heparin binding region (green) with some patients also having antibodies against other regions (red).4 HIT, heparin-induced thrombocytopenia; VITT, vaccine-induced thrombotic thrombocytopenia.
antibodies, enabling the adaptive immune system to recognise negative charge associated with gram-negative bacteria in particular. This is exaggerated in HIT where heparin therapy floods the system with more negative charge than would ever be seen in nature. Anti PF4-heparin antibodies are frequently seen in patients undergoing cardio-thoracic surgery and extracorporeal life support (~50%) but platelet activating antibodies and clinical HIT are uncommon (~5%). Low-level anti PF4-heparin antibodies are detectable in ~5% of healthy individuals but these antibodies do not activate platelets in vitro.

Ongoing thrombotic risk

Even with the withdrawal of heparin, PF4 continues to bind glycosaminoglycans similar to heparin, which coat cellular surfaces. This PF4 can also be recognised by anti PF4-heparin antibodies, leading to thrombotic risk persisting for days to weeks. Delayed or persistent HIT may also occur due to development of PF4 antibodies capable of activating platelets in the absence of heparin (autoimmune HIT).

VITT antibodies

In VITT, anti-PF4 antibodies bind at the heparin-binding sites, clustering PF4 molecules without the need for heparin. In some patients, antibodies to regions away from these sites can also be found. Intriguingly, the occurrence of CVT is associated with the presence of these antibodies that bind to regions outside the equatorial region (Figure 1D). The clustering of PF4 molecules leads to the formation of immune complexes which activate leukocytes and platelets (Figure 2). While HIT is a consequence of perturbing a physiological immune response caused by a short-lived polyclonal antibody response, VITT can be considered as a more typical autoimmune disease with a mono- or oligoclonal antibody response that persists for months to years.

CLINICAL PRESENTATION

Both HIT and VITT are intensely prothrombotic conditions with high mortality if not promptly treated. Clinical manifestations begin after 5 days from the initial stimulus; thrombosis becomes rapidly widespread and can affect any vascular bed, affecting arterial, venous, microvascular circulations and frequently a combination of these.

Timing and severity of clinical presentation

While HIT usually presents within 5–10 days of initial heparin exposure, VITT can present much later—median 14 days...
post-vaccine. Although those affected by HIT are already undergoing medical care and monitoring, 50% still present with thrombosis. Thus, HIT should be suspected, considered, investigated and treated at the earliest opportunity to prevent thrombosis.

Conversely, VITT involves healthy vaccine recipients, and thus presentation is not until symptoms of thrombosis are manifest. For both, the magnitude of decline in platelet count is key, with ≥30% and ≥50% fall typical of HIT and VITT, respectively; the platelet count may be normal at presentation.

### Sites of thrombosis

Thrombosis in HIT depends on the patient’s clinical profile. DVT and PE occur more frequently in postoperative patients, while arterial thrombosis occurs in ~30% and is the predominant site in patients undergoing cardiovascular surgery.

In contrast, VITT is striking due to the preponderance for CVT, an otherwise rare site of thrombosis. CVT has also been noted in HIT but in less than 2% of patients. The reason for this difference is unknown. Spontaneous HIT-like syndromes manifest with a wide range of sites of thrombosis. While there are only a few reports, CVT seems to also be common in spontaneous VITT-like syndrome.

### Diagnosis

Diagnosis of HIT and VITT is based on clinical suspicion and the demonstration of anti PF4 antibodies. The gold standard is to demonstrate platelet activation by patient serum in functional testing as these assays reveal the pathological platelet activating potential of anti PF4-heparin antibodies. Examples include the heparin-induced platelet activation assay and the serotonin release assay. However, the assays require modification to diagnose VITT as the addition of heparin reduces platelet activation to VITT antibodies. In atypical cases such as spontaneous presentations, careful communication with the laboratory is required. These assays are also time consuming, require specialist equipment and expertise, and are thus only available in a few specialist centres. ELISA is widely used for both HIT and VITT, where optical densities (OD) are typically ≥1.0. Positive OD of >0.4 but <1.0 is less specific and may represent background non-platelet activating antibodies. Rapid tests such as standard chemiluminescent assays (CLA) using PF4-heparin-coated particles are sensitive for HIT but poorly specific and typically negative in VITT. A modified CLA using PF4-only particles to identify VITT antibodies is in development and requires further validation.

### Management

Upon suspicion, while awaiting confirmatory tests, patients should be immediately commenced on full-dose non-heparin anticoagulation, although in VITT, heparin can be used if alternative anticoagulants are not available. Intravenous immunoglobulin should be given without delay for VITT and severe cases of HIT to inhibit antibody-induced platelet activation. Plasma exchange should be considered for severe HIT and can be lifesaving in patients with VITT and poor prognostic features.

### Conclusion

Understanding of PFITS has increased over recent years and research is ongoing into mechanisms and treatments. In the meantime, clinical awareness and early diagnosis is crucial given the high mortality if treatment is delayed.

### Author Contributions

Richard J. Buka and Sue Pavord contributed equally to writing the manuscript.

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### Conflict of Interest Statement

The authors declare no conflicts of interest.

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### References


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