Thrombotic anti-PF4 immune disorders: HIT, VITT, and beyond

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Antibodies against the chemokine platelet factor 4 (PF4) occur often, but only those that activate platelets induce severe prothrombotic disorders with associated thrombocytopenia. Heparin-induced thrombocytopenia (HIT) is the prototypic anti-PF4 disorder, mediated by strong activation of platelets through their FcγIIa (immunoglobulin G [IgG]) receptors (FcγRIIa). Concomitant pancellular activation (monocytes, neutrophils, endothelium) triggers thromboinflammation with a high risk for venous and arterial thrombosis. The classic concept of HIT is that anti-PF4/heparin IgG, recognizing antigen sites on (cationic) PF4 that form in the presence of (anionic) heparin, constitute the heparin-dependent antibodies that cause HIT. Accordingly, HIT is managed by anticoagulation with a nonheparin anticoagulant. In 2021, adenovirus vector COVID-19 vaccines triggered the rare adverse effect "vaccine-induced immune thrombotic thrombocytopenia" (VITT), also caused by anti-PF4 IgG. VITT is a predominantly heparin-*independent* platelet-activating disorder that requires both therapeutic-dose anticoagulation and inhibition of FcγRIIa-mediated platelet activation by high-dose intravenous immunoglobulin (IVIG). HIT and VITT antibodies bind to different epitopes on PF4; new immunoassays can differentiate between these distinct HIT-like and VITT-like antibodies. These studies indicate that (1) severe, atypical presentations of HIT ("autoimmune HIT") are associated with both HIT-like (heparin-dependent) and VITT-like (heparin-independent) anti-PF4 antibodies; (2) in some patients with severe acute (and sometimes chronic, recurrent) thrombosis, VITT-like antibodies can be identified independent of proximate heparin exposure or vaccination. We propose to classify anti-PF4 antibodies as type 1 (nonpathogenic, non–platelet activating), type 2 (heparin dependent, platelet activating), and type 3 (heparin independent, platelet activating). A key concept is that type 3 antibodies (autoimmune HIT, VITT) require anticoagulation plus an adjunct treatment, namely high-dose IVIG, to deescalate the severe anti-PF4 IgG-mediated hypercoagulability state.

LEARNING OBJECTIVES

- Classify the different prothrombotic disorders induced by platelet-activating anti–platelet factor 4 antibodies
- Define 3 types of anti-PF4 antibodies
- Review treatment for patients with anti-PF4 disorders and the requirement for therapeutic-dose anticoagulation and high-dose intravenous immunoglobulin (IVIG)

Introduction

Anti–platelet factor 4 (PF4) antibodies are the underlying cause of the prothrombotic disorders heparin-induced thrombocytopenia (HIT) and vaccine-induced immune thrombotic thrombocytopenia (VITT). It is increasingly recognized that anti-PF4 antibodies can also cause severe prothrombotic disease independent of ongoing heparin treatment or adenovirus vector vaccination.[1] However, only a subset of anti-PF4 antibodies are pathogenic.

Within the emerging concepts of thromboinflammation and immunothrombosis,[2] we describe the clinical presentations, serological characteristics, and pathogenesis of HIT and VITT and address the emerging evidence of prothrombotic anti-PF4 antibody disorders beyond HIT and VITT. We also propose a new nomenclature for anti-PF4 antibodies. Type 1 antibodies are non–platelet activating and usually of no pathological relevance. In contrast, type 2 and type 3 antibodies are pathogenic and activate platelets via FcγIIa receptors (FcγRIIa). Type 2 antibodies cause classic HIT and require the concomitant presence of PF4 and pharmacological concentrations of heparin (or another polyanion) to effect pathogenicity. Type 3 antibodies cause thromboinflammation by binding to PF4 alone and have been identified to underlie VITT. An

important new development is that anti-PF4 type 2 and type 3 antibodies are increasingly identified as causes of severe, atypical HIT ("autoimmune HIT") as well as thrombotic disorders beyond HIT and VITT.

Favorable outcomes in thrombotic anti-PF4 antibody disorders require early recognition and aggressive treatment. It is increasingly being recognized that the often extreme hypercoagulability state of patients with predominant anti-PF4 type 3 antibodies will not abate with therapeutic-dose nonheparin anticoagulation. An adjunctive strategy to deescalate the prothrombotic state is paramount. This is currently achieved by high-dose intravenous immunoglobulin (IVIG).

CLINICAL CASE

In 2015 a 35-year-old woman presented with severe headache and thrombocytopenia (49×10^9/L); D-dimer levels were greatly elevated (>35 000 µg/L fibrinogen equivalent units [FEU]). Cerebral vein sinus thrombosis (CVST) was confirmed by nuclear magnetic resonance. Her past medical history included a recent upper respiratory tract infection about 2 weeks prior; otherwise, she was healthy and did not take any medications other than hormonal contraception. Heparin was started, but the CVST progressed. The combination of unexplained thrombosis and thrombocytopenia prompted testing for anti-PF4 antibodies; a PF4/heparin immunoglobulin G (IgG) microtiter plate assay was strongly positive, but the confirmatory heparin-dependent platelet activation test was negative. Although platelet counts increased, the patient developed fatal secondary intracerebral bleeding.

HIT and VITT

HIT

Fifty years ago (1973), HIT was recognized as a prothrombotic disorder associated with heparin-dependent, platelet-activating antibodies. Later, the antigen target was identified as a multimolecular complex of the cationic chemokine PF4 (also named CXCL4) and negatively charged heparin. Until the early 1990s, the predominant clinical presentation of HIT was believed to be arterial thrombosis.[3] But during the era of widespread heparin thromboprophylaxis, it became evident that HIT was more often associated with venous thrombosis and pulmonary embolism, particularly after major surgery. HIT antibodies activate platelets and leukocytes via FcγRIIa and trigger massive thrombin generation. The classic view of HIT is that of a predominantly heparin-dependent, platelet-activating disorder, managed by heparin cessation and substitution with a nonheparin anticoagulant. This includes direct thrombin inhibitors (argatroban, bivalirudin) and agents with exclusive (fondaparinux, rixaroxaban, apixaban) or predominant (danaparoid) anti–factor Xa activity.[4] In addition, IVIG is emerging as an adjunct treatment to inhibit FcγRIIa-mediated platelet activation.[5] In contrast, warfarin treatment during acute HIT increases the risk for limb ischemic necrosis and amputation due to the progressive microvascular thrombosis associated with severe protein C depletion.[6] A clinical suspicion of HIT is based on clinical features, as reflected by the 4Ts scoring system (Table 1).

VITT

In 2021, recognition of a rare (1-2/100 000 vaccinations) adverse effect of adenovirus vector–based COVID-19 vaccines, VITT, greatly increased interest in anti-PF4 IgG-mediated disorders.[7-9] The characteristics of VITT are summarized in Table 2. In comparison to HIT, the risk for CVST or splanchnic vein thrombosis seems to be especially increased in VITT. Anti-PF4 antibodies in VITT differ substantially from HIT antibodies: they bind to an epitope on PF4 distinct from the HIT heparin-dependent antigen sites.[10] Indeed, heparin usually *inhibits* VITT antibody-mediated platelet activation. The current view of VITT is that of a predominantly heparin-*independent* platelet-activating disorder that requires high-dose IVIG to decrease FcγRIIa-mediated platelet activation and associated hypercoagulability together with therapeutic-dose anticoagulation.[11]

The iceberg model

A striking feature of the anti-PF4/heparin immune response is that a high proportion of heparin-exposed patients form nonpathogenic (anti-PF4 type 1) antibodies. Also, in 5% to 8% of the normal population, nonpathogenic anti-PF4 type 1 antibodies are found after COVID-19 vaccination.[12] This has enormous diagnostic relevance, given that anti-PF4 antibodies detectable by immunoassays do not necessarily indicate clinical disease. The "iceberg" model (graphical abstract) represents this concept[13]: the "tip" of the iceberg represents the clinically evident manifestations (thrombocytopenia, thrombosis) of the anti-PF4 response, in association with anti-PF4 type 2 and/or type 3 antibodies.

"Functional" (platelet activation) tests detect anti-PF4 platelet-activating antibodies (Table 3).[14] They differentiate between anti-PF4 type 1, type 2, and type 3 antibodies. For detection of anti-PF4 type 2 antibodies in HIT, heparin is added. First-generation platelet-rich plasma assays were later supplanted by washed platelet assays, with various readout modifications, mainly: the serotonin-release assay (SRA), the heparin-induced platelet activation assay (HIPA), and flow cytometry-based assays. For the detection of anti-PF4 type 3 antibodies in VITT, PF4 (instead of heparin) is added. All functional assays are restricted to reference laboratories.

The seminal discovery by Jean Amiral that HIT antibodies target PF4 paved the way for developing widely applicable enzyme immunoassays (EIAs) for detecting IgG that recognize PF4/heparin (polyanion) complexes. With widespread EIA availability, HIT entered the diagnostic mainstream. Microtiter plate-based HIT assays are also sensitive for anti-PF4 type 3 (VITT) antibodies, while commercially available rapid immunoassays generally only recognize anti-PF4 type 2 HIT antibodies (Table 3).[15]

Pathogenesis of HIT and VITT

The clinical characteristics of HIT have long puzzled clinicians and scientists. The notion that the 2 dominant anticoagulants of the 1970s and 1980s—heparin and vitamin K antagonists—induce or aggravate thrombotic complications, in the setting of thrombocytopenia, was highly counterintuitive. Today, HIT and VITT can be seen as prototypic examples of immunothrombosis and thromboinflammation.[2] These 2 evolving concepts combine immunity and hemostasis, especially the network between coagulation and the innate immune system, involving platelets,

Table 1. The 4Ts score for heparin-induced thrombocytopenia

	Score=2	Score=1	Score=0
Thrombocytopenia Compare the highest platelet count within the sequence of declining platelet counts with the lowest count to determine the % of platelet fall	• >50% platelet fall AND a nadir of ≥20×10⁹/L AND no surgery within preceding 3 days	• >50% platelet fall but surgery within preceding 3 days OR • Any combination of platelet fall and nadir that does not fit criteria for score 2 or score 0 (eg, 30% to 50% platelet fall or nadir • 10 to 19×10⁹/L	• <30% platelet fall • Any platelet fall with nadir <10×10⁹/L
Timing (of platelet count fall or thrombosis[a]) Day 0=first day of most recent heparin exposure	• Platelet fall days 5 to 10 after start of heparin • Platelet fall within 1 day of start of heparin AND exposure to heparin within past 5 to 30 days	• Consistent with platelet fall days 5 to 10 but not clear (eg, missing counts) • Platelet fall within 1 day of start of heparin AND exposure to heparin in past 31 to 100 days • Platelet fall after day 10	• Platelet fall ≤4 days without exposure to heparin past 100 days
Thrombosis (or other clinical sequelae)	• Confirmed new thrombosis (venous or arterial) • Skin necrosis at injection site • Anaphylactoid reaction to IV heparin bolus • Adrenal hemorrhage	• Recurrent venous thrombosis in a patient receiving therapeutic anticoagulants • Suspected thrombosis (awaiting confirmation with imaging) • Erythematous skin lesions at heparin injection sites	• Thrombosis not suspected
oTher cause for thrombocytopenia	• No alternative explanation for platelet fall is evident	• Possible other cause is evident: • Sepsis without proven microbial source • Thrombocytopenia associated with initiation of ventilator • Other:	• Probable other cause is present: • Within 72h of surgery • Confirmed bacteremia/fungemia • Chemotherapy or radiation within past 20 days • DIC due to non-HIT cause • Posttransfusion purpura • Thrombotic thrombocytopenic purpura • Platelet count <20×10⁹/L and given a drug implicated in causing drug-induced immune thrombocytopenia • Nonnecrotizing skin lesions at LMWH injection sites

[a]Key features of HIT are a platelet count decrease of more than 50% but, uncommonly, less than 20×10⁹/L; a typical onset in the second week of heparin treatment (between days 5 and 10; first day of immunizing heparin exposure=day 0); the occurrence of new thrombotic complications (arterial and/or venous); and the absence of another compelling explanation for the clinical features observed. Risk for HIT: score less than or equal to 3=low; 4–5=intermediate; 6–8=high.

LMWH, low-molecular-weight heparin.

Data modified from Warkentin and Cuker.[37]

monocytes, neutrophils, and—in the case of anti-PF4 antibody disorders—anti-PF4 IgG antibodies as key players. Immunothrombosis was originally designed through evolution to defend against microbial infection. It locally confines an infection by facilitating the recognition, containment, and destruction of pathogens. When these defense mechanisms get out of control, thromboinflammation develops. If misdirected, for example, by anti-PF4 type 2 and especially type 3 antibodies, thromboinflammation results in activation of the endothelium, complement, and innate immune cells, particularly granulocytes and monocytes, causing immunothrombosis.[16]

Figure 1 summarizes the self-enhancing activation cascade involving platelets, the coagulation cascade, and the innate immune system by platelet-activating anti-PF4 antibody types 2 and 3.

While the downstream effects of thromboinflammation and immunothrombosis are rather similar in HIT and VITT, the mechanisms triggering initial immunization differ. In HIT, PF4 binds to the polyanion, heparin; PF4 thereby undergoes conformational changes, expressing neoepitopes, which trigger the activation of B cells and the production of anti-PF4/polyanion antibodies. For VITT, the region on PF4 to which anti-PF4 type 3 antibodies bind is well characterized.[10] It overlaps with the binding site of heparin to PF4 and is distinct from the binding site of HIT antibodies. Which vaccine constituent(s) trigger(s) the anti-PF4 immune response and why tolerance is broken after vaccination, resulting in anti-PF4 type 3 antibodies, remain(s) unresolved. Patches of negative charge on the adenovirus vector have been suggested as PF4 binding sites,[17] but it remains unclear whether these or other vaccine constituents interact with PF4 to trigger the aberrant immune response. The ChAdOx1-nCoV-19 vaccine contains more than 2000 different proteins, many derived from the cell line in which the vaccine vector is propagated.[18] The usual straightforward approach to identify the binding partner of PF4 that causes the conformational changes inducing the immune reaction would be to incubate PF4 in the presence and absence of different potential binding partners and measure the binding of anti-PF4 antibodies to putative complexes. This

Table 2. Characteristics of VITT

Thrombocytopenia (<150×10⁹/L) or documented platelet count decrease by more than 50%
AND
D-dimer >8-fold the upper normal limit, in most assays corresponding to >4000 µg/mL (FEU)
AND
Presence of thrombosis OR typical headache that may precede CVST as described below: • severe persistent headache starting 4 or more days after vaccination (in about 10% of VITT patients, severe headache precedes CVST). Of note, headache during the first 2 days after vaccination is a common, harmless adverse event.
AND
Positive anti-PF4 antibody EIA assay and positive functional assay for PF4 dependent antibodies: • if no functional assay is available, a strong OD in the PF4 ELISA of >2.0 can be used as a surrogate marker, as this is associated with a >95% probability for the presence of platelet-activating PF4-dependent antibodies.[39] • Of note rapid assays for HIT are insensitive for VITT antibodies and a negative rapid test does not exclude VITT
AND
Onset of symptoms 4 to 30 days after vaccination (day of vaccination=day 0) with the exception of isolated DVT/PE, which may occur up to 42 days after vaccination
VITT should not be diagnosed
If an alternative diagnosis is more likely (eg, tumor, sepsis)

DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay; OD, optical density; PE, pulmonary embolism.

Data modified from Pavord et al.[38]

approach, however, cannot be used in VITT because the antibodies became autoantibodies that bind to and cluster PF4 by themselves with very high affinity.[19] This phenomenon is also seen in patients with atypical clinical presentations of HIT, such as when thrombocytopenia begins, worsens, or persists in the absence of heparin.

The anti-PF4 type 2 antibody immune response in HIT (and most likely also the anti-PF4 type 3 antibody response in VITT) is a *secondary* immune response. The onset of thrombocytopenia in HIT occurs typically between days 5 and 10 (median, day 7), even in patients who have never been treated with heparin and without anti-PF4 IgM precedence. A primary immune response would require considerably more time for B-cell activation, and an immunoglobulin class switch from IgM to IgG, before the production of high-titer antibodies. One explanation for primary immunization against PF4/polyanion complexes is PF4 binding to bacteria. Gram-positive and gram-negative bacterial surfaces are strongly negatively charged, at least twice as much as eukaryotic cells. Indeed, this charge difference represents a fundamental difference between prokaryotic and eukaryotic cells. Strong negative charges are highly efficient activators of innate immunity, including the complement system, the contact phase of coagulation, and the granulocytes. However, the adaptive immune system has no receptors for negative charges. We have proposed that one of the biological roles of PF4 is to "translate" negative charge into structure.[20] After binding to strong negatively charged molecules on bacterial surfaces, PF4 undergoes conformational changes that induce anti-PF4 antibodies. The interesting evolutionary concept is that these anti-PF4/polyanion IgG antibodies can opsonize any bacterium that binds PF4, even if the immune system has never encountered that particular organism before. During treatment with heparin, this strongly charged polyanion binds to cell surfaces, and subsequently, PF4 binds and undergoes its conformational changes, exposing the "danger" epitopes to which pathogenic anti-PF4 type 2 antibodies bind.

Another unusual feature of the anti-PF4 immune response is antibody transience (rapid seroreversion). In both HIT and VITT, platelet-activating antibodies disappear in the majority of patients within 3 to 6 months,[21,22] and in some patients even faster. This feature, however, does not fit a typical secondary immune response. In addition, even PF4 knockout mice can produce anti-PF4 antibodies upon microbial challenge despite their immune system never having encountered PF4 before. Furthermore, B cells that produce anti-PF4 antibodies after nonspecific in vitro stimulation are found in the blood of nearly all humans (including newborn cord blood). Thus, at least the IgM immune response against PF4 is part of the innate immunoglobulin repertoire. Both the transience of the IgG response and the antigen-independent production of antibodies indicate that the involved B cells are most likely either B1 or marginal zone B cells,[23] as they typically produce natural antibodies that react with endogenous proteins. Many healthy individuals have natural, polyreactive IgM antibodies, which bind to PF4/heparin complexes.[24] This allows complement factor C3 binding to the natural IgM bound to PF4 complexes. B cells express the receptor for C3, allowing binding of PF4/natural IgM-C3 complexes to nearly all B cells. This also brings PF4 into close proximity to the cognate receptor on anti-PF4 antibody-producing B cells.[25]

Despite the many similarities, there is a striking difference between HIT and VITT anti-PF4 antibodies. Anti-PF4 IgG antibodies in HIT are polyclonal[26]; in VITT, the resulting antibodies appear to be mono- or oligoclonal, with a unique restriction to one haplotype of the hypervariable IgG light chain region.[27] This may hint toward a genetic predisposition for VITT, while in HIT no genetic predisposition has been identified.

Beyond HIT and VITT

The classic picture of HIT as a primarily heparin-dependent disorder was challenged over 20 years ago when patients were

Table 3. Platelet antigen and activation assays for detecting HIT and VITT antibodies

Assay	Comment
Enzyme-immunoassays (EIAs) *(None of the EIAs detect all HIT and/or all VITT antibodies)*	
PF4/heparin (in-house and commercial)	Sensitive (≥99%) for HIT and VITT
PF4/polyvinylsulfonate	Sensitive (≥99%) for HIT and VITT
Platelet lysate/heparin	Sensitive for HIT (>99%), slightly reduced sensitivity for VITT in comparison to other EIAs
Aeskulisa HIT II	Sensitive for HIT; slightly reduced sensitivity for VITT in comparison to other EIAs
Rapid immunoassays	
Particle gel immunoassay	Sensitivity for HIT >95%, but ~45% sensitivity for VITT
Lateral flow assay	Sensitivity for HIT ~90%, but ~10% sensitivity for VITT
Latex enhanced immunoturbidimetric assay	Sensitivity for HIT ~95%, but <5% sensitivity for VITT
Chemiluminescence immunoassay for anti-PF4/heparin antibodies	Sensitivity for HIT ~95%, but <5% sensitivity for VITT
Chemiluminescence immunoassay for anti-PF4 antibodies[15] (only available as research assay; status October 2023)	Sensitivity for VITT ~95%, but ~30% sensitivity for HIT (possible marker for autoimmune HIT [aHIT])
Washed platelet activation assays	
SRA	Sensitivity for HIT antibodies ~95%; ~50% sensitivity for VITT antibodies Read out: measurement of ^{14}C-radiolabeled serotonin (or other methods of serotonin measurement) released from platelets
PF4-SRA	PF4-SRA more sensitive than SRA for detecting HIT and VITT antibodies Read out: measurement of ^{14}C-radiolabeled serotonin (or other methods of serotonin measurement) released from platelets[14]
PF4/H-SRA	PF4/H-SRA is more sensitive than SRA for detecting HIT antibodies and less sensitive for VITT antibodies than PF4-SRA Read out: measurement of ^{14}C-radiolabeled serotonin (or other methods of serotonin measurement) released from platelets
HIPA	Sensitive for HIT antibodies (>95%); ~50% sensitivity for VITT antibodies Read out: platelet aggregation, assessed visually on microtiter plates
PIPA	PIPA more sensitive than HIPA for detecting VITT antibodies; sensitivity of PIPA increases when sera are tested undiluted and 1:4 diluted Read out: platelet aggregation, assessed visually on microtiter plates
PEA	PEA is more sensitive than SRA for detecting VITT antibodies Read out: flow cytometry (detection of P-selectin as platelet activation marker)
Whole-blood platelet activation assays	
PIFPA	PIFPA has high sensitivity and specificity for VITT Read out: flow cytometry (detection of P-selectin as platelet activation marker)
Multiplate	Minimal experience reported to date for diagnosis of VITT Read out: impedance aggregometry performing using multiplate instrument
Platelet procoagulant assay	Exploits synergistic platelet activation by PAR-1 agonist and HIT/VITT antibodies Read out: flow cytometry (detection of P-selectin as platelet activation marker and annexin V binding)
Platelet-rich plasma (citrated) platelet activation assay	
HitAlert	Minimal experience reported to date for diagnosis of VITT Read out: flow cytometry (detection of P-selectin as platelet activation marker)

PAR-1, protease activated receptor 1; PEA, PF4-enhanced P-selectin expression assay; PF4/H-SRA, PF4/heparin-SRA; PF4-SRA, PF4-enhanced SRA; PIFPA, PF4-induced flow cytometry–based platelet activation assay.

Data modified from Warkentin and Greinacher.[14]

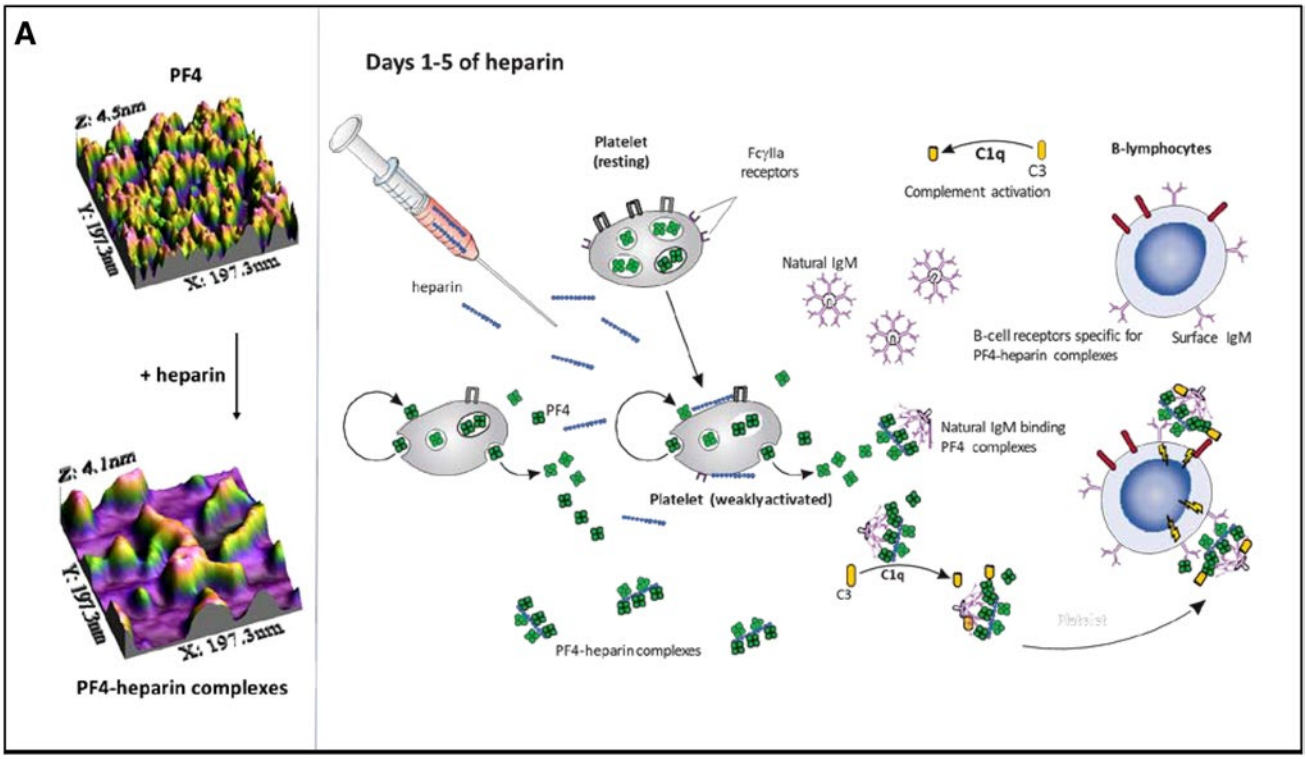

Figure 1. Current concepts of the pathogenesis of HIT and VITT. The schematic presentation shown in panel B is speculative and in large part inferred from experiments in HIT. The schematic presentation of the downstream prothrombotic process shown in panel C is largely substantiated by experimental data, some performed with VITT antibodies, others with HIT antibodies. (A) After heparin exposure, positively charged PF4 binds to negatively charged heparin; PF4/heparin complexes are formed. Natural IgM binds to the complexes, and complement factor C3 binds to the natural IgM. Complexes of PF4/heparin, natural IgM, and C3 bind to B cells via the complement receptor CR2 (CD21). B cells expressing the receptor for the HIT antigen on PF4 also bind to the PF4/heparin complexes, thus activating B cells to produce anti-PF4/heparin antibodies. The far left of the panel shows atomic force microscopy images of PF4 alone (*upper*) and formation of PF4/heparin clusters when PF4 is incubated with heparin (*lower*). (B) After vaccination, PF4 comes in contact with vaccine constituents and activates B cells. *Left:* It has been proposed that a direct inadvertent breach in the microvasculature at the vaccination site by IV injection or by disruption of VE-cadherin tight junctions by EDTA in ChAdOx1 nCoV allows vaccine constituents to enter the circulation.[19] Within the circulation, adenovirus particles can bind to platelets and can also bind PF4 released by activated platelets or from the microvascular endothelium.[17] Platelets may become activated (i) by vessel injury caused by injection of vaccine, (ii) after binding of the virions to the cell surface, or (iii) by immune complexes formed between contaminating host cell line proteins in the vaccine and natural IgG antibodies against these proteins. Whether the virions themselves or another yet unknown constituent in the vaccine causes the conformational change in PF4 is unknown. *Middle:* Once complexes with PF4 have formed, natural IgM antibodies activate complement (as it has been shown for PF4/heparin complexes[25]), which enhances their proximity to B-cell receptors. In a mouse model, upon IV injection of ChAdOx1, platelet-bound adenoviral particles are transported to the marginal zone of the spleen, where B cells are activated upon direct contact.[33] However, electron microscopy and superresolution microscopy revealed complexes between PF4 and anti-PF4 VITT antibodies with amorphous constituents of the vaccine rather than virus particles.[19] Beside the virions, other potential partners for PF4 include unassembled hexons and host cell-line proteins. However, there is little overlap in the proteins contaminating ChAdOx1 and Ad26.COV2 vaccines,[18] which both induce anti-PF4 VITT antibodies. *Right:* Eventually, complexes of PF4 and vaccine come in contact with B cells, expressing a cognate Ig receptor for PF4, either as fluid-phase complexes, as virion-PF4 complexes, or as complexes presented by platelets. (C) *From right to left:* After clonal expansion and isotype switching of one or a few B-cell clones in VITT, or polyclonal B cells in HIT, high-titer IgG anti-PF4 antibodies are released into the circulation. Immune complexes containing PF4 (or PF4/heparin complexes) and anti-PF4 IgG cluster and signal through FcRγIIA, which generates procoagulant platelets, induces platelet/neutrophil aggregates,[34] and stimulates NETosis by neutrophils.[35] DNA released by NETosis amplifies immune injury and activates complement, which deposits on the endothelium. Endothelial cells become activated, expressing tissue factor and releasing von Willebrand factor (VWF). VWF binds PF4 and subsequently anti-PF4 antibodies,[36] which in turn further activates neutrophils and further propagates thrombin generation. To reduce complexity, multimolecular PF4 complexes in VITT are shown and not the multimolecular PF4/heparin complexes in HIT as shown in panel A. HS, heparan sulfate; MPO, myeloperoxidase. Data modified from Cines and Greinacher.[32]

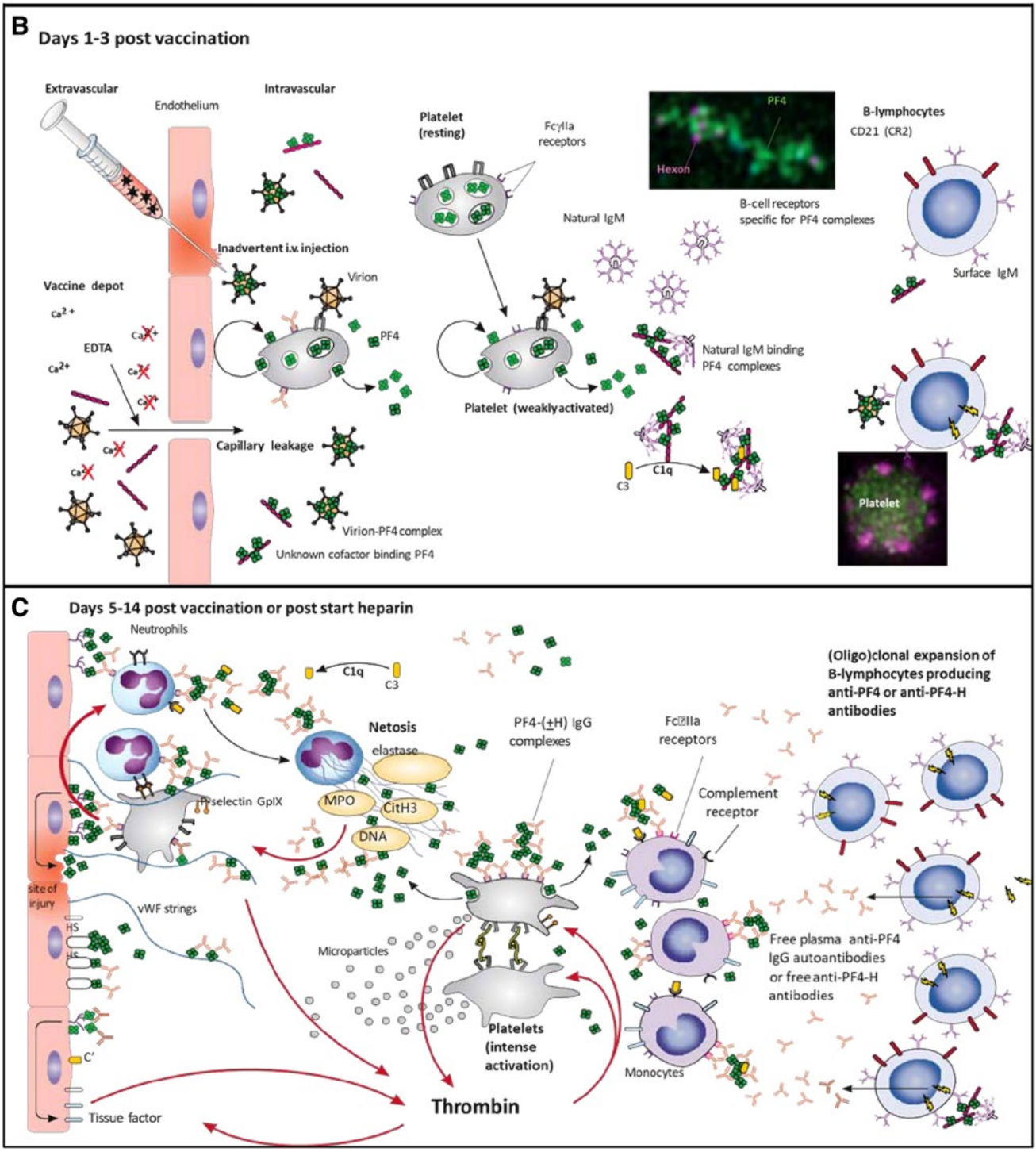

Figure 1. Continued

recognized with atypical clinical presentations. These included patients in whom the platelet count fall began or worsened after stopping heparin (delayed-onset HIT) or persisted for more than a week after stopping heparin (persisting, refractory HIT) and HIT associated with trivial exposures to heparin (heparin "flush" HIT).[28,29] We introduced the term "autoimmune HIT" (aHIT) in 2017 to indicate this group of patients, emphasizing their more severe clinical course (including overt disseminated intravascular coagulation [DIC], a higher risk of thrombosis including microthrombosis) and need for adjunct high-dose IVIG treatment.[29]

A unifying feature is that aHIT sera cause strong platelet activation in functional assays in the absence of heparin. Recent studies show that in these patients anti-PF4 type 3

antibodies are present in addition to anti-PF4 type 2 antibodies.[30] This might also explain how the additional application of PF4 enhances functional assays for HIT.[14] Sporadic reports since 2008 indicate that some patients develop thrombocytopenia and thrombosis in association with platelet-activating anti-PF4 antibodies even in the absence of proximate heparin exposure (spontaneous HIT; for review[1]). Applying assays developed for VITT, preliminary evidence indicates that in most of these patients both anti-PF4 type 2 and type 3 antibodies are found,[15,30] while in other patients, only anti-PF4 type 3 antibodies are present. The majority of spontaneous HIT patients are recognized after knee (not hip) replacement surgery, suggesting that polyanions released during this procedure may initiate the anti-PF4 response (for example, DNA and RNA released from degraded cells during application of the tourniquet). Most of the remaining patients appear to have developed this anti-PF4 antibody disorder following apparent viral infection. In other patients with monoclonal gammopathy and associated thrombocytopenia/recurrent thromboses, the paraprotein has features of anti-PF4 type 3 antibodies.[31] However, in other patients with anti-PF4 antibody-induced thrombosis, no clear proximate precipitating event is apparent. It is likely that these patients are underrecognized given that testing for anti-PF4 antibodies is usually not initiated in the absence of proximate heparin exposure or COVID-19 vaccination.

CLINICAL CASE (continued)

In 2023 we reevaluated the patient's case using repository samples: the positive anti-PF4/heparin IgG EIA and negative heparin-dependent functional assay for HIT were reconfirmed. However, the PF4-dependent functional assay (PIPA) was strongly positive. In hindsight, this patient had anti-PF4 type 3 antibodies and a VITT-mimicking severe prothrombotic disorder. The trigger was most likely the preceding upper respiratory tract infection (this case preceded the COVID-19 pandemic/vaccination campaign by several years). Today, a similar case would be managed by adjunctive high-dose IVIG in addition to anticoagulation.

Summary and future considerations

The role of pathogenic anti-PF4 antibodies is well established for HIT and VITT, among the most prothrombotic acquired disorders in medicine. Pathogenic anti-PF4 antibodies have in common the property of activating platelets strongly via the low-affinity FcγRIIa. This is fundamentally different from the nonpathogenic, non–platelet-activating antibodies found in a considerable percentage of heparin-exposed patients as well as in the normal population. We propose to name these nonpathogenic, non–platelet-activating antibodies "anti-PF4 type 1 antibodies." Pathogenic anti-PF4 antibodies most often recognize PF4/polyanion complexes and induce classic HIT (anti-PF4 type 2 antibodies). In contrast, antibodies causing VITT recognize the same site on PF4 to which polyanions (eg, heparin) bind and can cluster PF4 in the absence of polyanions (anti-PF4 type 3 antibodies). Increasingly, prothrombotic disorders caused by anti-PF4 antibodies independent of the presence of heparin are being recognized. Here, anti-PF4 type 2 and type 3 antibodies, in variable proportions, are usually identified (Table 4). Currently, these antibodies can be differentiated by functional assays.

Fifty years of HIT research facilitated the rapid identification of anti-PF4 antibodies as the underlying cause of VITT and provided guidance for diagnosis and effective treatment. The lessons learned from VITT indicate that in patients who present with thrombocytopenia and severe venous and/or arterial thrombosis, the presence of anti-PF4 type 2 and especially type 3 antibodies should be considered as indicating a

Table 4. Anti-PF4 antibodies and associated disorders

	Antibody target (PF4/heparin and PF4) and type of antibodies		
Nonpathogenic antibodies	unknown sites on PF4		**Type 1**
Platelet-activating anti-PF4 disorders	PF4/H	PF4	
HEPARIN TRIGGER			
Classic HIT (induced by heparin, low-molecular-weight heparin, and other polyanions)	√		**Type 2**
aHIT Delayed-onset HIT (onset or worsening of thrombocytopenia after stopping heparin) Persisting (refractory) HIT (delay in platelet count recovery after stopping heparin, eg, 7 or more days) Heparin "flush" HIT (HIT triggered by exposure to small concentrations of heparin) Unusually severe HIT (severe thrombocytopenia with overt DIC)	√	√	**Type 2+3**
NONHEPARIN (OR UNKNOWN) TRIGGER			
VITT		√	**Type 3**
Spontaneous thrombosis and thrombocytopenia	√	√	**Type 2+3**
Paraprotein-associated chronic thrombocytopenia/thrombosis		√	**Type 3**
Adenovirus-associated thrombosis and thrombocytopenia		√	**Type 3**

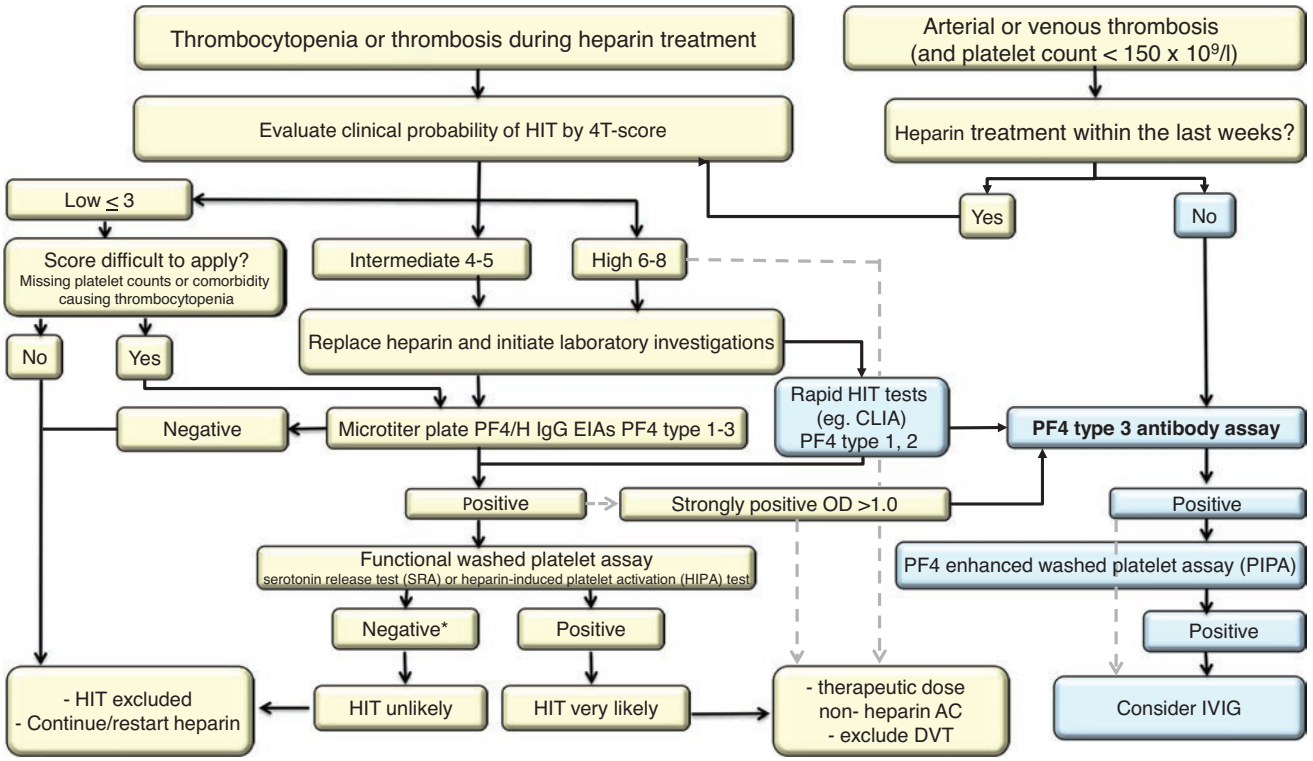

Figure 2. Flow chart for the diagnosis of anti–PF4-antibody-induced prothrombotic disorders. Anti–PF4 antibody-mediated disorders should be clinically diagnosed and laboratory testing only performed upon reasonable clinically suspicion. The yellow part of the figure presents the current diagnostic flow for heparin-induced thrombocytopenia. Anti-PF4/heparin antibody antigen tests are differentiated between microtiter plate-based EIAs and rapid HIT tests (Table 2). The light-blue part of the figure shows the workflow when clinically applicable immunoassays for anti-PF4 type 3 antibodies are available (currently under development). As the blue section of the workflow is untested, the decision to test for anti-PF4 antibodies should include consideration of the pretest probability: if there are other obvious reasons to explain thrombosis and thrombocytopenia, such as cancer or intensive care unit treatment, testing is probably not indicated. The dotted lines show the workflow based on clinical considerations, bypassing some diagnostics steps; for example, a typical presentation of HIT with a high 4Ts score of 6, 7, or 8 points should immediately prompt therapeutic-dose alternative anticoagulation, and laboratory test results are used to confirm the diagnosis. *Functional assays can be more sensitive if PF4 is added. Clinically nonrelevant anti-PF4 type 1 antibodies (detected in up to 20% of heparin-treated patients and up to 8% of COVID-19 vaccinated individuals) do not require treatment change. AC, anticoagulation; CLIA, chemiluminescence-based immunoassay; DVT, deep vein thrombosis; OD, optical density.

potential IgG-mediated prothrombotic disorder even in the absence of proximate heparin exposure or vaccination (Figure 2).

The challenge is now to identify how frequently anti-PF4 type 3 antibodies contribute to severe complications in HIT, including atypical forms of HIT, and in patients who present with thrombosis and/or thrombocytopenia independent of heparin treatment or vaccination. New immunoassays can differentiate between HIT-like and VITT-like antibodies in the research laboratory. Widely applicable assays that can differentiate among these various anti-PF4 disorders are needed to evaluate systematically the prevalence of these antibodies in different patient cohorts. From VITT we have learned that the outcome of patients with anti-PF4 type 3 antibodies can be improved (in addition to anticoagulation) by adjunctive treatment with IVIG to deescalate the FcγRIIa-dependent cell activation that triggers massive hypercoagulability. Potentially, the inhibition of FcγRIIa signal transduction might be an attractive new therapeutic approach in patients with thrombotic anti-PF4 type 3 antibody immune disorders beyond HIT and VITT.[40]

Note added in proof

Recent studies[40–42] have further implicated VITT-like anti-PF4 antibodies in chronic autoimmune anti-PF4 disorder and acute post-adenovirus infection anti-PF4 disorder.

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Conflict-of-interest disclosure

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Off-label drug use

Apixaban, bivalirudin, danaparoid, fondaparinux, rivaroxaban, and high-dose IVIG are "off-label" for treatment of HIT.

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