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Review

Vaccine-Induced Immune Thrombotic Thrombocytopenia—Clinicopathologic Features and New Perspectives on Anti-PF4 Antibody-Mediated Disorders

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Abstract: Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare but severe adverse event that was first observed during the global vaccination campaign against SARS-CoV-2 infection, specifically in those receiving adenoviral vector-based vaccines for Coronavirus disease 2019 (COVID-19). VITT develops 4 to 42 days post-vaccination and is characterized by the development of platelet-activating anti-platelet factor 4 (PF4) antibodies, leading to thrombocytopenia and thrombosis at unusual sites. The rise in awareness and subsequent prompt recognition of VITT was paramount in reducing mortality. Moreover, as vaccination campaigns around the world continue, a better understanding of VITT not only has important clinical implications but is also crucial for future vaccine development. In this review, we summarize the clinical features, pathophysiology, and incidence rates of VITT as well as highlight other anti-PF4 antibody-mediated disorders of growing clinical significance.

Keywords: vaccine-induced immune thrombotic thrombocytopenia; adenoviral-vector based COVID-19 vaccines; platelet-factor 4; anti-PF4 antibody-mediated disorders; platelet; SARS-CoV-2

1. Introduction

Vaccine-induced immune thrombotic thrombocytopenia (VITT) has received significant attention from the hematology community. As global vaccination efforts persist, gaining an in-depth understanding of VITT is not only vital for clinical management but also holds crucial implications for future vaccine developments, and furthering our understanding about the pathophysiology of immune-mediated platelet disorders. The purpose of this literature review is to provide a comprehensive overview of the current research on VITT, including its epidemiology, clinical presentation, and management, as well as its pathophysiology and characteristics shared with other anti-PF4 antibody-mediated disorders. By providing an overview of VITT, we seek to identifying knowledge gaps in our current understanding and provide an outline for future investigations.

2. Clinical presentation and management of VITT

The earliest cases of thrombocytopenia and thrombosis in otherwise healthy individuals after receiving a dose of the ChAdOx1-S vaccine were documented in April 2021 by Greinacher *et al.*,[1] Scully *et al.*,[2] and Schultz *et al.*[3] The index patient reported by Greinacher *et al.*,[1] was hospitalized 10 days after vaccination and received various treatments, including anticoagulation (enoxaparin),

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transfusions of red blood cells and platelets, prothrombin complex concentrate and recombinant Factor VIIa.[1] Despite these interventions, this patient passed away 11 days post-vaccination.[1] The clinical resemblance of unexplainable thrombocytopenia and thrombosis to spontaneous heparin-induced thrombocytopenia (spHIT) prompted investigators to test patient serum for antibodies against platelet factor 4 (PF4/CXCL4),[1] a protein extensively studied for its role in the pathogenesis of HIT.[4] This unique clinical presentation[5] led to the first of many reported cases of thrombotic events caused by adenoviral vector-based vaccines, herein known as VITT.

VITT is a rare but life-threatening disorder characterized by the onset of arterial and venous thromboembolisms, often at unusual sites.[1,2] Current evidence suggests that VITT is induced by pathogenic antibodies that mediate platelet activation in the presence PF4, which is a chemokine released from activated platelets and is involved in normal coagulation and inflammatory processes.[6] VITT predominantly manifests following primary administration of certain replication-deficient adenoviral vector-based COVID-19 vaccines, namely the ChAdOx1-S (Oxford-AstraZeneca) and Ad26.CoV2.S vaccines (Johnson & Johnson/Janssen).[1,7] Despite its rarity, studies have also indicated the occurrence of VITT following subsequent booster doses of COVID-19 vaccines.[8] A recent case series in Brazil documented 11 VITT patients following either the third or fourth vaccine dose of ChAdOx1-S, with an incidence rate of 0.33 per million dose following these later doses, despite none of the patients having received an adenoviral vector-based vaccine but rather Sinovac or BNT162b2 as their primary dose.[8]

According to guidelines from the American Society of Hematology, a diagnosis of VITT should be made according to the following criteria: 1) COVID-19 vaccination 4 to 42 days prior to symptom onset; 2) thrombosis (evidence of venous or arterial thrombosis); 3) thrombocytopenia (platelet count below 150×10^9/L); 4) positive for anti-PF4 antibodies in immunoassays; and 5) significantly elevated D-dimer (exceeding 4 times the upper limit of normal).[9] When 1 or 2 of these criteria are unmet, “probable VITT” and “possible VITT” are ascribed, respectively.[9]

Advances in early diagnosis and enhanced treatment strategies have contributed to a notable reduction in its mortality rate of VITT, decreasing from 47% to 22% in cases with symptom onset after March 28, 2021, since the initial identification of VITT.[10] Moreover, due to its clinical similarities, insights into managing HIT have been instructive for the treatment of VITT.[5,11] Administration of high-dose intravenous immunoglobulin at 1g/kg daily for two days along with non-heparin-based anticoagulants is recommended for VITT management.[5,9] Although heparin is known to promote platelet activation in HIT, therapeutic doses of heparin have been found to inhibit VITT serum-induced platelet activation *in vitro*.[5,12–14] However, a clinical study involving 220 VITT patients indicated higher mortality rates among those treated with unfractionated heparin (20%) as compared to those administered non-heparin-based anticoagulants (16%).[15] For refractory cases of VITT, plasma exchange, corticosteroids, and monoclonal antibodies targeting CD20 on B-cells and complement protein C5 have been utilized, as reviewed in detail by Nadia *et al.*[12]

3. Incidence rate for VITT

Over 50 million doses of Ad26.COV2.S and 240.3 million doses of ChAdOx1-S have been administered globally as of April 2022 and August 2021, respectively.[16,17] The World Health Organization has documented a total of 109 cases of Thrombosis with Thrombocytopenia Syndrome, which includes VITT, following vaccination.[18] Of these, 70 were reported in the U.S. (3.7 per million doses), 35 were from Europe (1.7 per million doses), and 2 were from Brazil and South Africa (0.4 and 0.23 cases per million doses, respectively).[18] The incidence rate of VITT following ChAdOx1-S vary substantially by region: 17.6 cases per million doses in Nordic countries, and 10 cases per million doses in the UK, compared to 0.2 cases per million doses in Asian countries.[18] Despite the suspension of adenoviral vector-based vaccines by numerous countries, including Canada, United States, and multiple European nations,[19–21] as of September 2022, ChAdOx1-S and Ad26.COV2.S remain in active use as primary immunization options for adults in a considerable number of countries around the world.[22] This ongoing administration involves nine countries in Latin America, eight in Africa and the Middle East, and five in Asia.[22]

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Initially considered a risk factor for VITT, the higher incidence of VITT in females was later attributed to the demographic bias in early vaccine recipients, who were primarily healthcare professionals – a group that skews towards females in many countries and was given vaccination priority.[23] Subsequent analyses have identified age as a more relevant risk factor of VITT.[23] A meta-analysis of data from 10 countries showed VITT incidence was lowest at 1 in a million people over 65, increased to 1 in 300,000 among those aged 55 to 64, and peaked in the under-55 age group with a range from 1 in 20,000 to 1 in 60,000.[24] Geographic location may also represent another possible risk factors for developing VITT. Notably, countries in Asia, such as South Korea exhibit substantially lower incidence rates of VITT than Western countries; although, stronger investigations of this potential relationship are required.[25] The observed variation in VITT incidence rates across countries emphasizes the imperative for ongoing, vigilant monitoring and management of vaccine-associated complications, particularly as countries continue the administration of adenoviral-vector based vaccines.[22]

4. Mechanisms of VITT pathogenesis

VITT exhibits pathophysiological similarities to HIT, as both are pro-thrombotic disorders characterized by a pronounced decline in platelet count (relative to baseline platelet levels) and an elevated risk of thrombosis.[4,15] HIT is primarily attributed to the formation of antibodies directed against PF4 complexed with heparin (PF4/heparin), with symptom onset 5 to 10 days after initial heparin administration.[4] Conversely, VITT generally presents 4 to 42 days following the primary dose of adenoviral vector-based COVID-19 vaccine, with anti-PF4 specific antibodies directed against the heparin-binding site.[6,9,26] Given that symptoms in VITT can present as early as four days following vaccination, which is a timeframe insufficient for antibody class switching to IgG to take place, the rapid IgG-specific response seems to suggest a secondary immune reaction, a pattern also observed in HIT.[9,27] However, the onset time of VITT may be less precise as compared to HIT due to the reliance on self-reporting upon symptom development for VITT patients, as opposed to the routine monitoring conducted in hospital settings post-heparin administrations in HIT patients.

The prevailing theories behind VITT antibody development are based on immunogen formation caused by PF4 interaction with polyanionic vaccine constituents or induced by vaccination, drawing parallels with HIT pathogenesis. Several studies have demonstrated complex formation between PF4 and ChAdOx1-S, including free hexon proteins, and have been observed to associate with VITT anti-PF4 antibodies, supporting this proposed mechanism.[28–30] However, a study by Michalik *et al.*[30] indicates that while an interaction was observed with PF4 and the vaccine preparation of ChAdOx1-S, PF4 did not show binding to the purified virions of either ChAdOx1-S or Ad26.COV2.S.[30] Considering that ChAdOx1-S contains up to three-fold higher level of unassembled viral proteins than Ad26.COV2.S, the reactivity of PF4 may be associated with free hexon components rather than assembled virions.[30] It has been hypothesized that upon vaccine injection, the immunogenic PF4/polyanionic complexes may form and circulate systemically, engaging monocytes and neutrophils via FcγIIa receptor-dependent mechanisms.[31,32] Subsequently, these complexes could drive the differentiation of anti-PF4 B cells into plasma cells, a process proposed to be facilitated by a prior B-cell pre-priming event resulting from previous bacterial or viral exposure.[29,33] The formation of spHIT antibodies is believed to follow a similar process involving an incompletely understood seroconversion mechanism, which continues to be a subject of investigation.[34]

Complex formation between PF4 with vaccine excipients was also suggested to induce neoepitopes and anti-PF4 antibody generation, which include extracellular nucleic acids and proteins from human cell lines used for propagating the adenoviruses.[35] Proteomic analysis via liquid chromatography-mass spectrometry and subsequent bioinformatics assessments conducted by Krutzke *et al.* revealed substantial levels of proteins from human origin in ChAdOx1-S (44.3%-70.8%) as compared to Ad26.COV2.S (0.5%), perhaps explaining the different incident rates associated with the two vaccines.[36] Although aggregate formation was significant between PF4 and ChAdOx1-S,[30] Michalik *et al.* found no significant aggregation between PF4 and Ad26.COV2.S despite its implication in VITT.[30] Alternatively, Nicolai *et al.* suggested the potential occurrence of unintended

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intravenous vaccine injection, which resulted in direct adenovirus to platelet interaction and activation in mice treated with ChAdOx1-S, culminating in thrombocytopenia.[37] However, Azzarone *et al.* argues that viral load of COVID-19 adenoviral vector-based vaccines is not sufficient to cause coagulation in non-human primates.[38] Concerns have also been raised regarding the possibility of VITT antibody cross-reactivity due to sequence homologies between SARS-CoV-2 spike protein and two consecutive epitopes on PF4, intrinsic to the heparin-binding region.[39] Despite these homologies, Greinacher *et al.*[39] identified distinct immune pathways for the production of post-vaccination anti-spike antibodies and VITT-related anti-PF4 antibodies, as their investigation found no interaction between the spike protein and VITT antibodies.[39]

Beyond the initial role of neoepitope formation, studies also suggest the contribution of certain vaccine excipients in facilitating proinflammatory responses and promoting site-specific thrombosis. For instance, presence of ATPase activity in ChAdOx1-S, which could be due to the considerable amounts of heat shock proteins and chaperones, may also induce localized ADP production and subsequent platelet activation at the vaccine injection site.[36] EDTA found in ChAdOx1-S has also been shown to induce vascular hyperpermeability,[28,30] and the non-ionic surfactant polysorbate 80 found in both adenoviral vector-based vaccines has the capability to traverse the blood–brain barrier when complexed with nanoparticles.[40] Subsequent to gaining access to the brain, Kowarz *et al.* suggested that soluble spike protein variants produced by alternative splicing from DNA-based vaccines plus a lack of dural venous sinuses valves may increase spike protein variants' residence time at this site.[41] This extended presence might increase their likelihood of binding endothelial cells expressing angiotensin-converting enzyme 2, thereby elevating the risk of thrombi development in the cerebral sinuses[41] and potentially contributing to the overall immune responses observed in VITT. However, Eichinger *et al.*[42] noted that VITT onset occurs at a time when the adenovirus and vaccine excipients are likely absent from circulation, making these unlikely contributors of VITT.[42]

Alternatively, recombinant monoclonal VITT antibodies reverse-engineered using mass spectrometry have been helpful in expanding our mechanistic understanding of VITT.[43] Sequence analysis revealed a high concentration of acidic/negatively charged amino acids in the VITT paratope (antibody region involved in antigen binding), facilitating interactions with the heparin binding site on PF4 driven by electrostatic forces.[43] This electrostatic nature of epitope-paratope interactions plus the presence of an equatorial ring of cationic charges on PF4 allows one molecule of PF4 to link multiple VITT antibodies together, forming pathogenic complexes capable of inducing platelet activation without requiring a polyanionic scaffold.[43] While this behaviour deviates from the well-documented dependency of classical HIT antibodies on heparin, a similar model has been proposed for the atypical presentations of HIT (such as spHIT).[4,44] From these findings, Ivanov *et al.*[43] questioned the involvement of PF4 in initially triggering the formation of VITT antibodies, or whether it may merely contribute to the subsequent thrombi generation, potentially due to an induction of PF4 levels as a result of vaccination.[43] Despite being monoclonal, the charge-dependent binding behavior of these antibodies indicates a broad specificity, implying that they may associate with rather than specifically recognize PF4 as the antigen, whereas their initial target might still remain unidentified.[43]

5. Characterization of VITT antibodies

Central to gaining an understanding of the functional dynamics and pathophysiological roles in this rare condition is the characterization of these pathogenic anti-PF4 antibodies. Epitope specificity studies conducted by Huynh *et al.* have identified a restricted epitope of eight surface residues on PF4 critical for the binding of VITT antibodies, suggesting a limited B cell clonality in VITT.[6] Incorporating mass spectrometry analyses, Kanack *et al.* showed that anti-PF4 antibodies display either a monoclonal or oligoclonal profile,[45] standing in contrast to the polyclonal nature of antibodies associated with HIT, thus highlighting the distinct immunological profile of VITT.[46] Building upon these findings, Huynh *et al.*[6,26] have further revealed two distinct patterns of VITT antibody binding, potentially indicative of a two-site targeting on PF4.[6,26] The first involves antibodies binding to residues within the well-known heparin-binding site of PF4[5] and

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demonstrates PF4-dependent binding[26] while the second shows antibodies engaging additional residues recognized by HIT antibodies thus demonstrating PF4-indepentent binding.[26] This observation indicates immune responses heterogeneity in VITT patients, with variability in antibody binding to PF4 possibly influenced by genetic or physiological factors. However, the mechanisms underlying the development of one-site versus two-site binding is yet to be determined.[26]

To further investigate the molecular profile of VITT antibodies, two complementary studies employed mass spectrometry to sequence the variable region of VITT antibodies, with Wang *et al.*[52] analyzing the samples from five Australian VITT patients and Ivanov *et al.*[37] examining those from a Canadian VITT patient. Focusing on the third complementarity-determining regions (CDR3), known for embedding high level of diversity from somatic hypermutation and shaped by the unique immune histories of each individual, both groups identified a pattern of consensus sequence within the heavy chains of the CDR3 of these antibodies across six patients.[43,47] While the exact mutation may vary among individuals, the presence of this consensus sequence suggests the selection of specific clonotypes across various individuals in response to an immunogenic stimulus.[43,47] Furthermore, both studies consistently identified that the lambda light chain in VITT antibodies from all six patients was encoded by the same allele, pointing to a uniform antigen-binding specificity.[43,47] The sequence and structure uniformity likely contribute to the development of these pathogenic antibodies, implying a common immune response pathway consistently inducing a similar immune response amongst VITT patients, potentially indicative of a genetic predisposition.[43,47] Moreover, the epitope of reverse-engineered recombinant VITT antibodies from the five Australian patients[48] overlaps with the heparin-binding site, aligning with analyses conducted by Huynh *et al*. on antibodies isolated from VITT patient sera.[6] These studies highlight the importance of recombinant VITT antibodies with oligoclonal binding profiles as tools to investigate the underlying genetic makeup of clinical manifestations in these patients.[26]

6. Drivers of Thrombosis in VITT

Although the precise mechanism of thrombi formation in VITT remains unclear, recent evidence suggests that it may involve activated neutrophils via a process known as NETosis.[49] Upon activation, neutrophils release decondensed DNA coated with histones and various bactericidal proteins, forming structures known as neutrophil extracellular traps (NETs).[50] NETs serve an immunological role in capturing and destroying pathogens but have also been implicated in the development of thrombosis.[50] In fact, the role of neutrophils and thrombogenicity of NETs has been well characterized in HIT. Previous reports have shown that HIT antibody immune complexes can directly engage with FcγRIIa receptors on neutrophils, triggering their activation and the release of NETs.[32,51] Indirect pathways to NETosis in HIT can also be initiated through platelet-neutrophil interactions mediated by P-selectin and P-selectin glycoprotein ligand-1.[32,51,52] Consequently, NETs can significantly contribute to thrombi development by both trapping platelets within growing thrombi and by facilitating fibrin deposition.[32,51] Recent studies have demonstrated a consistent elevation in markers characteristic of NETosis in VITT patient sera, including levels of citrullinated histone H3, myeloperoxidase, and cell-free DNA, highlighting the critical role of NETs in thrombi formation, as evidenced in both *in vitro* assays and VITT mouse models.[28,49] As seen with HIT antibodies, VITT antibodies have also been shown to trigger neutrophil activation via FcγRIIa receptors, suggesting that the underlying process of thrombi development in VITT may be driven by similar mechanisms.[49,53]

Perhaps most importantly, recent evidence has implicated NETosis in the development of cerebral venous sinus thrombosis (CVST) in VITT patients. CVST is a rare cerebrovascular condition affecting ~3 per 100,000 in the general population, which can be accompanied by intracranial hemorrhaging, severe neurological dysfunction, and a high mortality.[54–56] Despite its rarity, CVST is found more frequently in VITT[57] and is associated with a 2.7-fold higher risk of mortality compared to patients presenting without CVST.[2] In a study by Jin *et al.*,[56] a high concentration of NETs were found both plasma and thrombi of CVST patients through examination of CVST tissue sections and quantifying plasma NETosis markers.[56] NETs not only contribute to thrombi

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formation, but were also shown to compromise the endothelial barrier and induce a procoagulant state in endothelial cells, possibly intensifying CVST pathogenesis.[56] Moreover, CVST patients were found to have enhanced platelet-neutrophil aggregation at thrombus sites, similarly to what is seen in HIT.[56] Released PF4 from activated platelets can subsequently induce NETosis through activating neutrophil autophagy pathways in a in a dose-depend manner,[56] which may hold further implications for anti-PF4 disorders such as VITT. Subsequent studies support these findings by demonstrating elevated NETosis markers in tissue sections obtained from VITT patients who developed CVST.[28,58]

Aside from the involvement of neutrophils and NETosis, there may be other factors contributing to CVST development in VITT. Expanding on the earlier mentioned binding patterns of VITT antibodies on PF4, Huynh *et al.*[26] found that PF4-independent antibodies, which exhibit a two-site binding profile, have a stronger association with the occurrence of CVST compared to PF4-dependent antibodies, which exhibit a single-site binding affinity.[26] This distinction is evident by a CVST incidence rate of 50.0% in VITT patients whose sera contained both PF4-dependent and -independent antibodies, compared to 5.9% in those whose sera contained PF4-dependent antibodies alone.[26] Based on both cellular and humoral findings, a more comprehensive understanding of CVST in VITT, one that encompasses pan-cellular activation, systemic inflammation, extensive neutrophil priming and activation, and various other novel mechanisms such as the potential role of antibody clonality and epitope specificity on PF4 in exacerbating NETosis may better explain the frequency of CVST development in VITT.

7. VITT-mimicking anti-PF4 antibodies in HIT and other disorders

As mentioned earlier in this review, evidence suggests a degree of resemblance between anti-PF4 antibodies in VITT and those identified in patients with atypical presentations of HIT, such as spHIT.[44] Despite the distinct antibody profiles and clinical presentations associated with HIT, it is a disorder involving anti-PF4 IgG antibodies that activate platelets by engaging FcγRIIa receptors and bind to residues in the heparin-binding region on PF4, a site known to be recognized by VITT antibodies.[6,59,60] The occurrence of spHIT is often observed post-infection or total knee arthroplasty without proximate exposure to heparin and associated with a high frequency of CVST.[61] Warkentin *et al.* have recently demonstrated that HIT antibodies exhibit an analogous reactivity profile to VITT antibodies in solid-phase laboratory diagnostic assays, while displaying a degree of variable reactivities in the recognitions of PF4 alone versus PF4/heparin complexes.[62] However, in fluid-phase EIAs where complexes of anti-PF4 IgG and PF4 with or without heparin are pre-formed before immobilization, spHIT antibodies exhibited a heparin-enhanced binding behavior while VITT antibodies display a significant heparin-inhibitory behavior, potentially elucidating the laboratory and clinical differences between these disorders.[62]

VITT-mimicking anti-PF4 antibodies were also found in two patients with monoclonal gammopathy of undetermined significance (MGUS), which is a neoplastic condition thought to be related to abnormal growth and proliferation of plasma cells in the bone marrow.[63] In both reported patients, the MGUS antibodies were produced from single clone of anti-PF4 plasma cells, and resulted in recurrent thrombosis and thrombocytopenia despite having no previous exposure to heparin.[64,65] However, the case reported by Kanack *et al.*[65] may represent an even more complex scenario: the patient has received an initial dose of Ad26.COV2.S followed by two doses of BNT162b2 mRNA vaccines.[65] Subsequent treatment via intravenous heparin administration led to a significant drop in platelet counts, though the timing of heparin administration relative to vaccinations was not indicated.[65] While no adverse effects were observed subsequent to these vaccinations, the precise impact of the adenoviral vector-based vaccine on the condition of the patient remains undetermined.[65] On the other hand, the patient documented by Greinacher *et al.*[64] had not received any COVID-19 vaccines at the time of reporting nor been given heparin in subsequent treatments, attributing their symptoms to pre-existing underlying conditions.[64]

Although no strong genomic link associated with the development of HIT, it may be possible that VITT is a result of genetic predisposition triggered by the administration of virus-based vaccines.

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For instance, three cases of VITT have been reported following vaccination with the mRNA-1273 (Moderna) vaccine, and another with the virus-like particle vaccine for nine-valent HPV (Gardasil-9).[66,67] The latter patient presented with platelet-activating anti-PF4 antibodies without history of thrombosis or exposure to heparin, nor had she received any COVID-19 vaccines.[67] A concurrent HPV infection, however, may have contributed to the formation of PF4/polyanionic complexes.[67] As such, anti-PF4 antibody-mediated hypercoagulability may also go beyond heparin and vaccine exposure. Warkentin *et al.*[60] recently reported two patients presenting with a VITT-like anti-PF4 disorder associated with adenovirus infection.[60] Both patients tested positive for adenovirus infection via nasopharyngeal swabs; one patient received two doses of Moderna COVID-19 vaccines 15 months earlier, while the other patient experienced a mild case of SARS-CoV-2 infection 16 months prior.[60] Serum samples from these two patients were positive for anti-PF4 antibodies in both solid and fluid phases assays, PF4-enhanced platelet-activation assays, and demonstrated binding to the heparin-binding region on PF4.[60] A similar finding was also reported by Schönborn *et al.*,[68] who identified nine patients with VITT-like clinical and humoral profiles from a retrospective analysis of repository serum samples dated before 2020.[68] These individuals experienced acute thrombocytopenia, significantly elevated D-dimer, and severe thrombotic events, including arterial strokes and CVST, without recent exposure to heparin or adenoviral vector-based vaccines.[68] Thus, the association of pro-thrombotic anti-PF4 antibodies with disorders showing clinical and/or pathological similarities to VITT may be indicative of an unknown trigger for pathogenic antibody production beyond our current scoop of understanding.

8. Longitudinal symptoms

While the acute clinical manifestations demand urgent attention, it is also critical to examine longitudinal characteristics of VITT, which could provide insights on long-term patient management strategies. Comparison to HIT often offers an invaluable perspective for the understanding of VITT due their clinical similarities. Unlike classical immunological memory, which leads to long-lasting heightened immunity upon re-exposure to an antigen, the development of anti-PF4/heparin antibodies in HIT is typically transient, only lasting a median of 50 – 80 days,[69,70] and lose their ability to activate platelets before becoming undetectable in serum.[71] HIT patients generally have no observed immune response following subsequent heparin treatments, suggesting the lack of immune memory from a clinical perspective.[70,72] The consensus recommendation suggests that for patients with a history of HIT who no longer have circulating platelet-activating anti-PF4/heparin antibodies, it is advisable to use heparin during surgical procedures and switch to an alternative anticoagulant post-operatively.[73] Interestingly, while HIT patients exhibit a higher propensity (8/17, 47%) for re-developing platelet-activating anti-PF4/heparin antibodies upon subsequent intra-operative heparin re-exposure, subsequent non-surgical heparin treatment does not appear to cause antibody redevelopment.[73] Warkentin *et al.*[73] report only 1 patient (1/17; 5%) who developed recurrent HIT (heparin-independent platelet activating HIT antibodies; thrombosis and thrombocytopenia) following intra-operative re-exposure to heparin.[73] Despite the higher-than-expected rate of seroconversion in patients with a history of HIT, the redevelopment of HIT is relatively rare.[73]

Like HIT antibodies, VITT antibodies lose their ability to activate platelets before becoming undetectable in serum.[74,75] However, preliminary evidence suggests that VITT may involve a more durable antibody response, potentially leading to prolonged clinical symptoms.[76–78] In a 6-month study conducted by Kanack *et al.*,[78] nine patients with VITT following Ad26.COV2.S vaccination showed no subsequent thrombosis and 78% did not experience thrombocytopenia after the acute phase.[78] Similarly, in a longitudinal study consisting of 71 VITT patients, Schönborn *et al.*[76] found that platelet-activating anti-PF4 antibodies became undetectable in 87% of patients over a mean of 79 weeks following initial symptom onset, with no further episodes of thrombosis or thrombocytopenia observed in 93% of patients, suggesting a generally low risk of VITT recurrence after the initial stage of symptom onset.[76]

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Antibody persistence was observed in a subset of patients. A case report by Roberge and Carrier describes a VITT patient who continues to exhibit persistent thrombocytopenia and tests positive for platelet-activating antibodies for more than 18 months following the initial VITT diagnosis.[77] Kanack *et al.*, also observed seven patients with antibodies persisting after 181 days; however, only one exhibited platelet-activating properties and two experienced mild thrombocytopenia recurrence related to VITT.[78] Schönborn *et al*.[76] found half (39/31; 55%) of all VITT patients exhibited EIA detectable anti-PF4 antibodies and 6/71 (8.5%) continued to produce platelet-activating anti-PF4 antibodies for greater than 18 months.[76] Moreover, the authors noted 2/71 patients (2.8%) with recurrent thrombocytopenia and 2/71 patients (2.8%) with recurrent thrombosis.[76] Patients with thrombocytopenia alone were receiving proper anticoagulation, while patients with thrombosis alone were not properly anticoagulated.[76] Only 1/71 (1.4%) patient in this study experienced recurrent thrombocytopenia and thrombosis despite anticoagulation, and they also tested positive for platelet-activating anti-PF4 antibodies, likely representing a case of persisting VITT.[76]

It remains unclear why some VITT patients exhibit persistent symptoms while others do not. One hypothesis is the involvement of long-lived plasma B cells and/or the development of immune memory, which could contribute to this persistence, contrast with the observations made in HIT,[72] although this remains speculative. As such, longitudinal follow-up and consistent anticoagulation for VITT patients remains of utmost importance. Along with the patient reported by Roberge and Carrier, 7/9 (77.8%) patients followed by Kanack *et al.* and 28/64 (43.8%) patients followed by Schönborn *et al*. have remained on anticoagulants during the study period to mitigate long-term symptoms.[76–78] While anticoagulation is effective in most cases, evidence suggests some VITT patients may be at risk of persistent and recurrent thrombocytopenia and/or thrombosis.[76–78] Based on these studies, there appear to be two antibody profiles of VITT patients: 1) those whose platelet-activating antibody diminishes over time and 2) those with persistent platelet-activating antibodies. Although the exact duration of this antibody persistence is still unknown, as there is drastic variation among individuals, both within and across various longitudinal studies, suggesting that there may be differences in immune responses between patients.[76–78] Nevertheless, these observations underscore the necessity to investigate the underlying causes of these persistence and emphasizes the importance of developing targeted long-term monitoring and management strategies for VITT patients.

9. Conclusions

The widespread use of adenoviral vector-based COVID-19 vaccines revealed VITT as a rare, yet significant adverse effect associated with this vaccine delivery platform. The occurrence of VITT underscores the challenges of monitoring and managing adverse events on a large scale, thus emphasizing the importance of continuous surveillance and improvement in vaccination strategies. Collaborative efforts within the scientific community are crucial to put forward guidelines to ensure safety during vaccine formulation, testing, and administration. Further research to provide evidence of adenoviral-vector or non-viral constituents involvement beyond preliminary studies to confirm these proposed mechanisms is therefore required. The sequence of events leading to VITT, reasons behind the differences in incidence rate of ChAdOx1-S and Ad26.CoV2.S, whether some individuals are predisposed to develop VITT IgG antibodies, persistence of VITT antibodies, as well as the prevalence of thrombi at specific sites, such as CVST, are questions that remain unanswered. Additionally, the identification of various VITT-like syndromes caused by antibodies in the absence of heparin or vaccine exposure represents a key area of future research that may provide us with novel insights into other anti-PF4 antibody-mediated disorders.

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Table 1. Proposed mechanism of VITT pathogenesis.

	Mechanism	Literature
VITT antibody formation	**The formation of neoantigens by adenovirus capsid hexon proteins or vaccine excipients (protein impurities or extracellular DNA) binding to PF4 triggers VITT antibodies production by anti-PF4 B cells.**	**Baker *et al.*[29]**
		Greinacher *et al.*[28]
	Although PF4 binding was not observed with purified vaccine virions.	**Michalik *et al.*[30]**
	Direct platelet activation and thrombocytopenia were observed in mice following intravenous injection of ChAdOx1-S.	**Nicolai *et al.*[37]**
	However, even if all the vaccine contents spill over into the bloodstream, viral load of COVID-19 adenoviral vector-based vaccines is unlikely to trigger such a response.	**Azzarone *et al.*[38]**
Proinflammatory and thrombotic events contributing to pathogenesis	ChAdOx1-S contains substantial amounts of human cell line impurities, including heat-shock proteins, that may mediate platelet activation at the injection site.	Krutzke *et al.*[36]
	DNA-based COVID-19 vaccines can lead to the production of soluble spike protein variants via splicing events. Due to the absence of dural venous sinus valves, prolonged exposure to spike protein variants may contribute to the development of thrombi in the cerebral sinuses.	Kowarz *et al.*[41]
	ChAdOx1-S stabilized with EDTA may increase vascular permeability and cause dermal vessel leakage, enhancing the spread of proinflammatory factors. Additionally, the surfactant polysorbate 80 in both ChAdOx1-S and Ad26.CoV2.S can cross the blood-brain barrier and enter brain endothelial cells when complexed with nanoparticles, possibly localizing thrombosis to the cerebral sinuses.	Greinacher *et al.*[28] Kowarz *et al.*[41]
	However, the replication-deficient adenovirus and other vaccine excipients are unlikely to be still circulating given the timing of VITT symptom onset.	Choi *et al.*[40] Eichinger *et al.*[42]

VITT antibodies were shown to activate neutrophils, leading to NETosis, which is the major driver of thrombosis in VITT, but does not significantly contribute to thrombocytopenia.	Leung *et al.*[49]
NETosis has also been implicated in CVST, potentially influencing its prevalence in VITT, although direct evidence of this connection is limited.	Jin *et al.*[56] Greinacher *et al.*[28]

References

1. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *N Engl J Med* 2021; **384**(22): 2092-101.
2. Scully M, Singh D, Lown R, et al. Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med* 2021; **384**(23): 2202-11.
3. Schultz NH, Sorvoll IH, Michelsen AE, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med* 2021; **384**(22): 2124-30.
4. Warkentin TE. Heparin-induced thrombocytopenia: pathogenesis and management. *Br J Haematol* 2003; **121**(4): 535-55.
5. Bourguignon A, Arnold DM, Warkentin TE, et al. Adjunct Immune Globulin for Vaccine-Induced Immune Thrombotic Thrombocytopenia. *N Engl J Med* 2021; **385**(8): 720-8.
6. Huynh A, Kelton JG, Arnold DM, Daka M, Nazy I. Antibody epitopes in vaccine-induced immune thrombotic thrombocytopaenia. *Nature* 2021; **596**(7873): 565-9.
7. See I, Lale A, Marquez P, et al. Case Series of Thrombosis With Thrombocytopenia Syndrome After COVID-19 Vaccination-United States, December 2020 to August 2021. *Ann Intern Med* 2022.
8. Mendes-de-Almeida DP, Mouta Nunes de Oliveira P, Bertollo Gomes Porto V, et al. Vaccine-induced immune thrombotic thrombocytopenia post COVID-19 booster vaccination in Brazil: a case series. *Research and Practice in Thrombosis and Haemostasis* 2023; **7**(8).
9. Bussel J, Cines D, Dunbar C, et al. Vaccine-induced Immune Thrombotic Thrombocytopenia. 2022. https://www.hematology.org/covid-19/vaccine-induced-immune-thrombotic-thrombocytopenia (accessed 7 December 2023).
10. van de Munckhof A, Krzywicka K, Aguiar de Sousa D, et al. Declining mortality of cerebral venous sinus thrombosis with thrombocytopenia after SARS-CoV-2 vaccination. *Eur J Neurol* 2022; **29**(1): 339-44.
11. Warkentin TE. High-dose intravenous immunoglobulin for the treatment and prevention of heparin-induced thrombocytopenia: a review. *Expert Rev Hematol* 2019; **12**(8): 685-98.
12. Gabarin N, Arnold DM, Nazy I, Warkentin TE. Treatment of vaccine-induced immune thrombotic thrombocytopenia (VITT). *Semin Hematol* 2022; **59**(2): 89-96.
13. Smith CW, Montague SJ, Kardeby C, et al. Antiplatelet drugs block platelet activation by VITT patient serum. *Blood* 2021; **138**(25): 2733-40.
14. Arnold DM. Heparin or nonheparin anticoagulants for VITT. *Blood* 2022; **139**(23): 3358-9.
15. Pavord S, Scully M, Hunt BJ, et al. Clinical Features of Vaccine-Induced Immune Thrombocytopenia and Thrombosis. *N Engl J Med* 2021; **385**(18): 1680-9.
16. The Janssen Ad26.COV2.S COVID-19 vaccine: What you need to know. 2022. https://www.who.int/news-room/feature-stories/detail/the-j-j-covid-19-vaccine-what-you-need-to-know. (accessed 23 October 2023).
17. Soboleva K, Shankar NK, Yadavalli M, et al. Geographical distribution of TTS cases following AZD1222 (ChAdOx1 nCoV-19) vaccination. *Lancet Glob Health* 2022; **10**(1): e33-e4.
18. Interim recommendations for use of the ChAdOx1-S [recombinant] vaccine against COVID-19 (AstraZeneca COVID-19 vaccine AZD1222 Vaxzevria™, SII COVISHIELD™). 15 March 2022. https://www.who.int/publications/i/item/WHO-2019-nCoV-vaccines-SAGE_recommendation-AZD1222-2021.1 (accessed 17 July 2023).
19. Wise J. Covid-19: European countries suspend use of Oxford-AstraZeneca vaccine after reports of blood clots. *BMJ* 2021; **372**: n699.

20. COVID-19 vaccines: Canadian Immunization Guide. 5 December 2023. https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-4-active-vaccines/page-26-covid-19-vaccine.html (accessed 8 December 2023).
21. Janssen COVID-19 Vaccine. 22 May 2023. https://www.fda.gov/vaccines-blood-biologics/coronavirus-covid-19-cber-regulated-biologics/janssen-covid-19-vaccine (accessed 8 December 2023).
22. Spinardi J, Dantas AC, Carballo C, et al. Narrative Review of the Evolution of COVID-19 Vaccination Recommendations in Countries in Latin America, Africa and the Middle East, and Asia. *Infect Dis Ther* 2023; **12**(5): 1237-64.
23. Pai M. Epidemiology of VITT. *Semin Hematol* 2022; **59**(2): 72-5.
24. Chan BTB, Bobos P, Odutayo A, Pai M. Meta-Analysis of Risk of Vaccine-Induced Immune Thrombotic Thrombocytopenia Following ChAdOx1-S Recombinant Vaccine. *medRxiv* 2021; doi: 10.1101/2021.05.04.21256613.
25. Boonyawat K, Angchaisuksiri P. Vaccine-induced immune thrombotic thrombocytopenia with ChAdOx1 nCoV-19 is rare in Asia. *Res Pract Thromb Haemost* 2022; **6**(1): e12644.
26. Huynh A, Arnold DM, Ivetic N, et al. Antibodies against platelet factor 4 and the risk of cerebral venous sinus thrombosis in patients with vaccine-induced immune thrombotic thrombocytopenia. *Journal of Thrombosis and Haemostasis* 2023.
27. Warkentin TE, Sheppard JA, Moore JC, Cook RJ, Kelton JG. Studies of the immune response in heparin-induced thrombocytopenia. *Blood* 2009; **113**(20): 4963-9.
28. Greinacher A, Selleng K, Palankar R, et al. Insights in ChAdOx1 nCoV-19 vaccine-induced immune thrombotic thrombocytopenia. *Blood* 2021; **138**(22): 2256-68.
29. Baker AT, Boyd RJ, Sarkar D, et al. ChAdOx1 interacts with CAR and PF4 with implications for thrombosis with thrombocytopenia syndrome. *Sci Adv* 2021; **7**(49): eabl8213.
30. Michalik S, Siegerist F, Palankar R, et al. Comparative analysis of ChAdOx1 nCoV-19 and Ad26.COV2.S SARS-CoV-2 vector vaccines. *Haematologica* 2022; **107**(4): 947-57.
31. McFadyen JD, Sharma P, Moon MJ, et al. Activation of circulating platelets in vaccine-induced thrombotic thrombocytopenia and its reversal by intravenous immunoglobulin. *Br J Haematol* 2022; **196**(1): 234-7.
32. Perdomo J, Leung HHL, Ahmadi Z, et al. Neutrophil activation and NETosis are the major drivers of thrombosis in heparin-induced thrombocytopenia. *Nature Communications* 2019; **10**(1322).
33. Greinacher A, Holtfreter B, Krauel K, et al. Association of natural anti-platelet factor 4/heparin antibodies with periodontal disease. *Blood* 2011; **118**(5): 1395-401.
34. Warkentin TE, Greinacher A. Spontaneous HIT syndrome: Knee replacement, infection, and parallels with vaccine-induced immune thrombotic thrombocytopenia. *Thromb Res* 2021; **204**: 40-51.
35. Greinacher A, Schonborn L, Siegerist F, et al. Pathogenesis of vaccine-induced immune thrombotic thrombocytopenia (VITT). *Semin Hematol* 2022; **59**(2): 97-107.
36. Krutzke L, Rosler R, Allmendinger E, Engler T, Wiese S, Kochanek S. Process- and product-related impurities in the ChAdOx1 nCov-19 vaccine. *Elife* 2022; **11**.
37. Nicolai L, Leunig A, Pekayvaz K, et al. Thrombocytopenia and splenic platelet-directed immune responses after IV ChAdOx1 nCov-19 administration. *Blood* 2022; **140**(5): 478-90.
38. Azzarone B, Veneziani I, Moretta L, Maggi E. Pathogenic Mechanisms of Vaccine-Induced Immune Thrombotic Thrombocytopenia in People Receiving Anti-COVID-19 Adenoviral-Based Vaccines: A Proposal. *Front Immunol* 2021; **12**: 728513.
39. Greinacher A, Selleng K, Mayerle J, et al. Anti-platelet factor 4 antibodies causing VITT do not cross-react with SARS-CoV-2 spike protein. *Blood* 2021; **138**(14): 1269-77.
40. Choi PY. Thrombotic Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med* 2021; **385**(3): e11.
41. Kowarz E, Krutzke L, Kulp M, et al. Vaccine-induced COVID-19 mimicry syndrome. *Elife* 2022; **11**.
42. Eichinger S, Warkentin TE, Greinacher A. Thrombotic Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. Reply. *N Engl J Med* 2021; **385**(3): e11.
43. Ivanov DG, Ivetic N, Du Y, et al. Reverse Engineering of a Pathogenic Antibody Reveals the Molecular Mechanism of Vaccine-Induced Immune Thrombotic Thrombocytopenia. *J Am Chem Soc* 2023; **145**(46): 25203-13.
44. Greinacher A, Selleng K, Warkentin TE. Autoimmune heparin-induced thrombocytopenia. *J Thromb Haemost* 2017; **15**(11): 2099-114.
45. Kanack AJ, Bayas A, George G, et al. Monoclonal and oligoclonal anti-platelet factor 4 antibodies mediate VITT. *Blood* 2022; **140**(1): 73-7.
46. Huynh A, Arnold DM, Kelton JG, et al. Characterization of platelet factor 4 amino acids that bind pathogenic antibodies in heparin-induced thrombocytopenia. *J Thromb Haemost* 2019; **17**(2): 389-99.
47. Wang JJ, Armour B, Chataway T, et al. Vaccine-induced immune thrombotic thrombocytopenia is mediated by a stereotyped clonotypic antibody. *Blood* 2022; **140**(15): 1738-42.

48. Wang JJD, van der Neut Kolfschoten MD, Rutten LD, et al. Characterization of reverse engineered anti-PF4 stereotypic antibodies derived from serum of VITT patients. *Blood* 2023.
49. Leung HHL, Perdomo J, Ahmadi Z, et al. NETosis and thrombosis in vaccine-induced immune thrombotic thrombocytopenia. *Nat Commun* 2022; **13**(1): 5206.
50. Mutua V, Gershwin LJ. A Review of Neutrophil Extracellular Traps (NETs) in Disease: Potential Anti-NETs Therapeutics. *Clinical Reviews in Allergy & Immunology* 2021; **61**(2): 194-211.
51. Gollomp K, Kim M, Johnston I, et al. Neutrophil accumulation and NET release contribute to thrombosis in HIT. *JCI Insight* 2018; **3**(18): e99445.
52. Leung HHL, Perdomo J, Ahmadi Z, Yan F, McKenzie SE, Chong BH. Inhibition of NADPH oxidase blocks NETosis and reduces thrombosis in heparin-induced thrombocytopenia. *Blood Advances* 2021; **5**(23): 5439-51.
53. Holm S, Kared H, Michelsen AE, et al. Immune complexes, innate immunity, and NETosis in ChAdOx1 vaccine-induced thrombocytopenia. *Eur Heart J* 2021; **42**(39): 4064-72.
54. Payne AB, Adamski A, Abe K, et al. Epidemiology of cerebral venous sinus thrombosis and cerebral venous sinus thrombosis with thrombocytopenia in the United States, 2018 and 2019. *Research and Practice in Thrombosis and Haemostasis* 2022; **6**(2): e12682.
55. Kehr S, Berg P, Müller S, et al. Long-term outcome of patients with vaccine-induced immune thrombotic thrombocytopenia and cerebral venous sinus thrombosis. *npj Vaccines* 2022; **7**(76).
56. Jin J, Qiao S, Liu J, et al. Neutrophil extracellular traps promote thrombogenicity in cerebral venous sinus thrombosis. *Cell & Bioscience* 2022; **12**(114).
57. Bissola AL, Daka M, Arnold DM, et al. The clinical and laboratory diagnosis of vaccine-induced immune thrombotic thrombocytopenia. *Blood Advances* 2022; **6**(14): 4228-35.
58. Carnevale R, Leopizzi M, Dominici M, et al. PAD4-Induced NETosis Via Cathepsin G-Mediated Platelet-Neutrophil Interaction in ChAdOx1 Vaccine-Induced Thrombosis-Brief Report. *Arterioscler Thromb Vasc Biol* 2023; **43**(10): e396-e403.
59. Warkentin TE. Heparin-Induced Thrombocytopenia and Vaccine-Induced Immune Thrombotic Thrombocytopenia Antibodies: Fraternal-Not Identical-Twins. *Thromb Haemost* 2021; **121**(12): 1558-61.
60. Warkentin TE, Baskin-Miller J, Raybould AL, et al. Adenovirus-Associated Thrombocytopenia, Thrombosis, and VITT-like Antibodies. *N Engl J Med* 2023; **389**(6): 574-7.
61. Warkentin TE. Platelet-activating anti-PF4 disorders: An overview. *Semin Hematol* 2022; **59**(2): 59-71.
62. Warkentin TE, Arnold DM, Sheppard JI, Moore JC, Kelton JG, Nazy I. Investigation of anti-PF4 versus anti-PF4/heparin reactivity using fluid-phase enzyme immunoassay for 4 anti-PF4 disorders: classic heparin-induced thrombocytopenia (HIT), autoimmune HIT, vaccine-induced immune thrombotic thrombocytopenia, and spontaneous HIT. *J Thromb Haemost* 2023.
63. Gkalea V, Fotiou D, Dimopoulos MA, Kastritis E. Monoclonal Gammopathy of Thrombotic Significance. *Cancers (Basel)* 2023; **15**(2).
64. Greinacher A, Langer F, Schonborn L, et al. Platelet-activating anti-PF4 antibodies mimic VITT antibodies in an unvaccinated patient with monoclonal gammopathy. *Haematologica* 2022; **107**(5): 1219-21.
65. Kanack AJ, Schaefer JK, Sridharan M, et al. Monoclonal gammopathy of thrombotic/thrombocytopenic significance. *Blood* 2023; **141**(14): 1772-6.
66. Padmanabhan A, Kanack AJ, Kaplan RB, Sangli S. COVID-19 mRNA-1273 vaccine induces production of vaccine-induced immune thrombotic thrombocytopenia antibodies. *Am J Hematol* 2022; **97**(6): E223-E5.
67. Johansen S, Laegreid IJ, Ernstsen SL, et al. Thrombosis and thrombocytopenia after HPV vaccination. *J Thromb Haemost* 2022; **20**(3): 700-4.
68. Schönborn L EO, Wesche J, Dobosz P, Broto M, Puig SR, Fuhrmann J, Torres R, Serra J, Llevadot R, Palicio M, Wang JJ, Gordon TP, Lindhoff-Last E, Hoffmann T, Alberio L, Langer F, Boehme C, Biguzzi E, Grosse L, Endres M, Liman TG, Thiele T, Warkentin TE, Greinacher A. Anti-PF4 immunothrombosis without proximate heparin or adenovirus vector vaccine exposure. *Blood* 2023.
69. Keeling D, Davidson S, Watson H, Haemostasis, Thrombosis Task Force of the British Committee for Standards in H. The management of heparin-induced thrombocytopenia. *Br J Haematol* 2006; **133**(3): 259-69.
70. Warkentin TE, Kelton JG. Temporal aspects of heparin-induced thrombocytopenia. *N Engl J Med* 2001; **344**(17): 1286-92.
71. Denomme GA. The platelet Fc receptor in heparin-induced thrombocytopenia. 6 ed: CRC Press; 2001.
72. Selleng K, Schutt A, Selleng S, Warkentin TE, Greinacher A. Studies of the anti-platelet factor 4/heparin immune response: adapting the enzyme-linked immunosorbent spot assay for detection of memory B cells against complex antigens. *Transfusion* 2010; **50**(1): 32-9.
73. Warkentin TE, Sheppard JA. Serological investigation of patients with a previous history of heparin-induced thrombocytopenia who are reexposed to heparin. *Blood* 2014; **123**(16): 2485-93.

 doi:10.20944/preprints202312.1578.v1

74. Lee CSM, Clarke LJ, Kershaw GW, et al. Platelet-activating functional assay resolution in vaccine-induced immune thrombotic thrombocytopenia: differential alignment to PF4 ELISA platforms. *Res Pract Thromb Haemost* 2023; **7**(3): 100128.
75. Schonborn L, Thiele T, Kaderali L, et al. Most anti-PF4 antibodies in vaccine-induced immune thrombotic thrombocytopenia are transient. *Blood* 2022; **139**(12): 1903-7.
76. Schonborn L, Seck SE, Thiele T, et al. Long-term outcome in Vaccine-induced Immune Thrombocytopenia and Thrombosis. *J Thromb Haemost* 2023.
77. Roberge G, Carrier M. Long VITT: A case report. *Thromb Res* 2023; **223**: 78-9.
78. Kanack AJ, Singh B, George G, et al. Persistence of Ad26.COV2.S-associated vaccine-induced immune thrombotic thrombocytopenia (VITT) and specific detection of VITT antibodies. *Am J Hematol* 2022; **97**(5): 519-26.