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The aetiopathogenesis of vaccine-induced immune thrombotic thrombocytopenia

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ABSTRACT

In the new science emanating from the COVID-19 pandemic, effective vaccine development has made a huge difference and saved countless lives. Vaccine roll-out led to the identification of rare cases of severe thrombotic and thrombocytopenic problems in some recipients. This apparent coupling of thrombosis with haemorrhagic potentiation might seem baffling but the ensuing clinical investigation rapidly shed important light on its molecular mechanism. This review outlines the current understanding on the role of adenovirus-based platforms, the immunogenic triggers and the immunothrombotic response underlying vaccine-induced immune thrombotic thrombocytopenia.

KEYWORDS: vaccine-induced immune thrombotic thrombocytopenia, COVID-19, adenovirus vaccines, platelet factor-4, Fc-gamma-RIIa

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Introduction

Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare syndrome identified after COVID-19 vaccination, particularly from vaccines that use adenovirus as a carrier, such as ChAdOx1 nCov-19 (AstraZeneca, University of Oxford and Serum Institute of India) and Ad26.COV2.S (Janssen; Johnson & Johnson).^{1–3} This syndrome is also called vaccine-induced prothrombotic immune thrombocytopenia and its clinical features are now well defined.^{4,5} Most cases of thrombotic thrombocytopenic syndrome (TTS) post-ChAdOx-1 nCov-19 were VITT.⁶ The incidence is estimated at about 1/100,000 in ChAdOx1 nCov-19 vaccination and 1/500,000 in Ad26.COV2.S. Since March 2021, VITT cases have been reported in different countries and its pathophysiological mechanisms explored in many laboratories with revelations propelled by similarities to heparin-induced thrombocytopenia (HIT).^{1,5,7,8} This review condenses available information to pose further questions.

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The adenovirus platform in VITT

Adenoviral (Ad)-based platforms in vaccine development offer many advantages, including ease of manipulation and manufacturing, coupled with their exceptional thermostability makes them invaluable in countries with limited ability to transport vaccines in a cold-chain appropriate manner.^{9,10} Furthermore, the ability of Ad-based platforms to induce potent antibody and T-cell responses against vector encoded antigens makes them durable and highly effective platforms for vaccine applications.^{11,12} Previous studies based on the use of Ad-based vaccines (in particular, the use of the common serotype, Ad5) identified high seroprevalence rates against the vector backbone resulting from natural infections as a major limitation to their efficacy.^{13,14} This was highlighted in the well-publicised Ad5-based STEP trial using an Ad5-based vector encoding the HIV-1 Gag, Pol and Nef to induce T-cell immunity against HIV-1. While the vaccine was effective in inducing CD8+ T-cell responses, the trial was unblinded and stopped in 2007 following an interim analysis that indicated that the vaccine failed to reduce HIV-1 acquisition or plasma HIV-1 RNA levels 3 months after diagnosis of HIV-1 infection.¹⁵

Concerns over the lack of efficacy of Ad5-based platforms against a backdrop of high seropositivity prompted development of novel serotypes with little or no pre-existing immunity in the population. Consequently, ChAdOx-1, a vector derived from a chimpanzee adenovirus was developed by the Jenner Institute and Ad26, a low seroprevalence species D adenovirus was developed by Crucell (now Janssen).^{16,17} Both platforms were rapidly modified in response to the COVID-19 pandemic, deployed worldwide and have proved to be highly effective in providing antibody and T-cell immunity against SARS-CoV-2. Evidence of VITT began to emerge only once the vaccines had been licensed and a sufficient number of individuals had been vaccinated to pick up these ultra-rare clotting events. While cases of VITT have been reported with low frequency with the ChAdOx-1 nCov-19 vaccine and Ad26.COV2.S vaccine, no cases have to date been reported with either the Russian Sputnik vaccine (which comprises a Ad26.S prime followed by an Ad5.S boost) or the Chinese Sinovac Ad5-based platform, which appears to offer weaker protection against SARS-CoV-2. It remains unclear as to whether these platforms do not induce VITT or whether cases of VITT have not been reported to date. It is also presently unclear why cases of VITT appear to be associated with a first priming dose of Ad-based vaccine, but rarely following the second boosting dose.

Immunogenicity in VITT

VITT appears to mimic autoimmune HIT whereby heparin sequesters platelet factor-4 (PF4) into an immunogenic complex, which can act as neoantigen to stimulate the immune system to produce anti-heparin and anti-PF4 immunoglobulin-Gs.^{18–22} These pathological antibodies cluster PF4 on platelet surfaces and activate platelets by binding Fc-gamma-RIIa receptors. Current reports on VITT have focused on PF4 and anti-PF4 antibodies. Recent studies have demonstrated the ability of both ChAdOx and Ad26, as well as Ad5, to bind PF4 on the viral capsid.²³ This could occur following microvascular damage during vaccine administration, with trace amounts of vaccine coming into contact with blood. It has been shown that the electrostatic nature of the adenovirus capsid coupled with shape complementarity provides an opportunity for the adenovirus to cluster PF4, potentially creating neoantigens, which trigger the production of anti-PF4 from memory B-cells.^{24,25} This 'clustering' of PF4 on the adenovirus capsid appears to occur primarily at the interface between the major capsid protein (hexon), and appears to be a relatively low affinity (~300 – 600 nM) and largely electrostatic interaction.

Previous studies have demonstrated that, following intramuscular administration, the hexon protein represents the immunodominant epitope recognised by the immune system, resulting in robust anti-hexon antibody responses.^{26,27} It is, therefore, tempting to speculate that this may provide an explanation for why VITT occurs only following the first dose. It is likely that, following immunisation with ChAdOx1 nCoV19 or Ad26.S, the vaccinated individual not only develops immunity to the encoded S protein but also to the adenovirus vaccine capsid components, thus generating an immune reaction towards the hexon protein. So, when a second dose is administered some 1–3 months later, PF4 binding to Ad in the blood will be out-competed by neutralising antibodies targeting hexon, thus opsonising any potential adenovirus exposed to the blood and preventing PF4 clustering to the capsid. Such a mechanism may also explain why VITT has not, to date, been reported with either the Sinovac vaccine (high rates of pre-existing natural immunity against Ad5 prevent PF4 binding) or with the Sputnik formulation (using two different Ad platforms for 'priming' and 'boosting').

Thrombogenicity in VITT

Complexes of PF4 and anti-PF4 activate platelets and also induce neutrophils to release neutrophil extracellular traps (NETs) to promote thrombosis along with thrombocytopenia in a manner similar to HIT (Fig 1).²¹ Adenovirus vaccines contain the vector and other constituents, such as proteins from packaging cells. The latter may cause systemic inflammation to increase PF4 expression. PF4 binds to hexon, a packaging protein, and clusters on the surface of the adenovirus to stimulate the immune system to produce anti-PF4 antibodies. PF4 and anti-PF4 form large immune complexes that activate platelets and induce NETosis via Fc-gamma-RIIa receptors. Extensive activation of platelets will lead to thrombocytopenia and promote thrombosis. NETs act as scaffolds for promoting and propagating thrombosis. PF4 is part of the CXC chemokine family and also known as chemokine (C-X-C motif) ligand 4 (CXCL4).²⁸ It is released from alpha-granules of activated platelets to promote coagulation and inhibit local

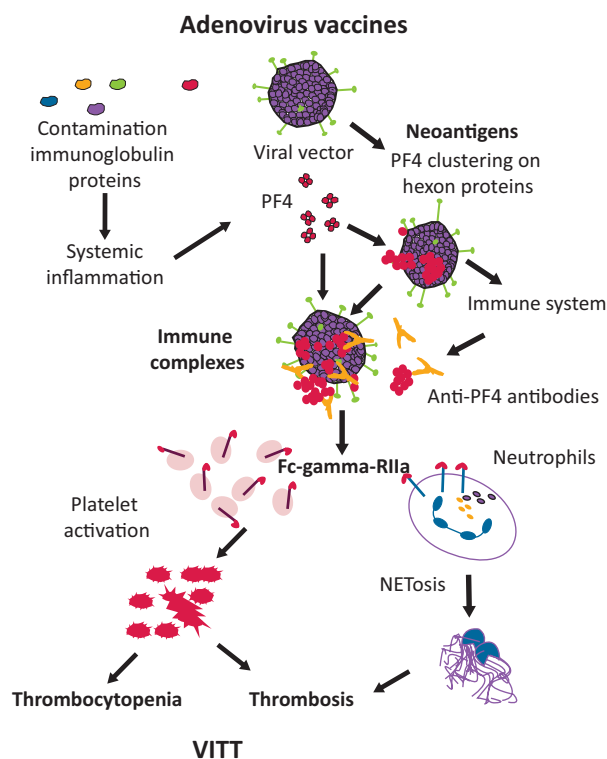


Fig 1. Possible aetiopathogenesis of vaccine-induced immune thrombotic thrombocytopenia. PF4 = platelet factor-4; NETosis = neutrophil extracellular traposis; VITT = vaccine-induced immune thrombotic thrombocytopenia.

antithrombin activity.^{29–31} PF4-containing immune complexes can further induce platelet activation and consequent calpain-dependent platelet death.³² Autoantibodies that arise can also induce platelet destruction via raft-associated glycoprotein Ib-alpha and Fc-gamma-RIIa.³³ In VITT, platelet activation by patients' sera containing anti-PF4 autoantibodies is mediated via Fc-gamma-RIIa, which can be blocked by inhibitors to Fc-gamma-RIIa or downstream signalling pathways.^{1,2,34,35} Anti-PF4 levels decrease over time after diagnosis, but do not become negative for up to 200 days post-treatment.^{36,37} These sera continue to activate platelets, thereby suggesting that anti-PF4 production continues for a long period after initiation by vaccination.³⁷ Notably, previous studies have also demonstrated that intravenously administered Ad bind directly to platelets, resulting in thrombocytopenia.^{38,39} However, direct interaction between Ads and platelets is unlikely to underpin VITT as the timing of onset of VITT (being typically >10 days post-vaccination) would not be compatible with such a direct mechanism. Similarly, the secretion of splice protein variants lacking the membrane tethering domain into blood and direct activation of endothelial cells has also been proposed as a potential mechanism underpinning VITT.⁴⁰ Again, timing of VITT does not appear compatible with such a model, since maximal adenovirus-induced spike protein expression would be achieved typically 2–3 days post-intramuscular vaccination, yet VITT is typically not seen until later time points, minimally 5 days but normally >10 days post-vaccination.

The immunoglobulin receptor is mainly expressed on platelets and neutrophils, and activation of Fc-gamma-RIIa is also able to induce NETosis in HIT.²¹ NETs have been demonstrated to play important roles in thrombosis by extruding DNA fibres that are laced with procoagulant enzymes and histones.^{41–43} Excessive NETs can potentiate microvascular occlusion, development of venous thrombosis and trigger disseminated intravascular coagulation (DIC).^{44–46} Incubation of plasma from patients with VITT induces NETs in *ex vivo* assays.^{24,47} In addition, in specimens of thrombi from patients, NETs were more prominent in VITT than in non-VITT patients.²⁴ These data strongly suggest that PF4-anti-PF4-induced NETs play important roles in the development of VITT.³⁴

In addition, ethylenediaminetetraacetic acid (EDTA) present in the ChAdOx1 nCov-19 vaccines (about 0.1 mmol) has been described in one report to potentially enhance microvascular leakage, induce systemic inflammation and enhance production of PF4 and anti-PF4.²⁴ In addition, the presence of proteins from packaging cells may also contribute to systemic inflammation.²⁴ Further research is required on both these possible factors in the pathogenesis of VITT.

Current insight on VITT pathogenesis

- > VITT occurs 5–20 days after vaccination.⁵ Neoantigens stimulate the host immune system to produce antibodies, ie anti-PF4 antibodies.
- > Complexes of PF4-hexon/viral particles have been demonstrated in *in vitro* experiments.²⁴ This raises the possibility that viral particle-PF4 may form neoantigens to stimulate the immune system to produce anti-PF4 antibodies and the potential to modify the adenovirus particle to prevent such interactions.
- > In blood samples from most VITT patients, PF4 and high titers of anti-PF4 have been detected.^{48–50} PF4 circulates at higher concentrations in younger than in older people with enhancement during inflammatory situations.⁵¹ Anti-PF4 antibodies are not normally detectable but high titers are present in VITT.
- > Plasma from patients with VITT can induce platelet activation.^{24,34,52} The results could partly explain the development of thrombocytopenia. Components in plasma that activate platelets are mainly believed to be the PF4-anti-PF4 complexes. However, it does not rule out other factors.
- > Co-incubation of plasma with platelets from VITT patients induces NETs via the Fc-gamma-RIIa.^{24,47,53} Isolated anti-PF4 can also induce NET formation in *ex vivo* assays to suggest that anti-PF4 is the most likely ligand for Fc-gamma-RIIa-mediated NETosis.
- > Within thrombi, NETs can be detected by immunofluorescent staining.²⁴ NETs breakdown products (such as cell free DNA (cfDNA), citrullinated histones and myeloperoxidase (MPO)) are elevated in the circulation of VITT patients.²⁴ These data indicate that NETs, which act as scaffolds to potentiate thrombosis, form in VITT patients.

Current gaps in understanding VITT

- > Why is the incidence as low as 1/100,000? Are there intrinsic factors that predispose individuals to develop VITT? Might genome sequencing provide further information?

- > Is there an accessory role for the SARS-CoV-2 spike protein in VITT? Could spike protein-induced T-cell immunity be involved?
- > Is there overlap with the immunothrombosis of acute COVID-19 and/or autoantibodies in critically ill patients? Could there be co-factors or vascular site-specific properties that amplify or propagate the process, leading to catastrophic events?
- > What is the role of other constituents that make up the vaccines implicated in VITT, which contribute to its immunogenicity via PF4?
- > Can we establish an *in vivo* (or even *in vitro*) model to robustly test for VITT and interrogate new/modified agents for their ability, or lack of, to induce VITT?
- > Why does VITT only occur after the first dose? Does induced anti-vector immunity prevent PF4 binding and clustering?

The importance of further understanding VITT

Rolling out vaccines to the entire world population will be critical in ending the COVID-19 pandemic; indeed, the current wave of infections sweeping the world embodies the fact that ‘nobody is safe until everybody is safe’. To achieve effective vaccination, it will be critical to vaccinate populations in countries where cold chain delivery is not practical and reliance on Ad-based COVID-19 vaccines will therefore be essential. Although VITT is now better recognised and treated, at least in high-resource settings, there remains a moral responsibility to define the mechanistic basis of VITT in order to build vaccine confidence and develop safe, VITT-resistant Ad-based vaccines for rollout in such populations. Importantly, useful information will be developed from further research to minimise the risk in future development of vaccines and gene therapies that use an adenovirus as vectors. ■

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