The clinical correlates of vaccine-induced immune thrombotic thrombocytopenia after immunization with adenovirus vector-based SARS-CoV-2 vaccines

Eleanor R. Gaunt* & Neil A. Mabbott*

*Correspondence: The Roslin Institute & Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, United Kingdom
Email: Elly.Gaunt@ed.ac.uk & neil.mabbott@roslin.ed.ac.uk

Abbreviations used

Ab, antibody
Ad26.COV2.S, Johnson & Johnson/Janssen adenovirus-based COVID-19 vaccine
BNT162b2, Pfizer–BioNTech mRNA-based COVID-19 vaccine
ChAdOx1 nCov-19, Oxford-AstraZeneca adenovirus-based COVID-19 vaccine
C3, complement component 3
C4, complement component 4
CR2, complement receptor 2
COVID-19, disease caused by infection with Severe Acute Respiratory Syndrome CoronaVirus 2

© The Author(s) 2021. Published by Oxford University Press on behalf of the British Society for Immunology. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
CXADR, coxsackie-adenovirus receptor
CXCL4, chemokine (C-X-C motif) ligand 4
CVST, cerebral venous sinus thrombosis
FcγRIIa, Fcγ receptor IIa
GAG, glycosaminoglycan
Gam-COVID-Vac, Gamaleya adenovirus-based COVID-19 vaccine
HIT, heparin-induced thrombocytopenia
IL-8, interleukin-8
MERS, Middle Eastern Respiratory Syndrome
MHRA, Medicines and Healthcare products Regulatory Agency
mRNA-1273, Moderna mRNA-based COVID-19 vaccine
NETs, neutrophil extracellular traps
PF4, platelet factor 4
SARS-CoV-2, Severe Acute Respiratory Syndrome CoronaVirus 2
VITT, vaccine-induced immune thrombotic thrombocytopenia
vWF, von Willebrand factor
Abstract

We are at a critical stage in the COVID-19 pandemic where vaccinations are being rolled out globally, in a race against time to get ahead of the SARS-CoV-2 coronavirus and the emergence of more highly transmissible variants. A range of vaccines have been created and received either emergency approval or full licensure. To attain the upper hand, maximum vaccine synthesis, deployment and uptake as rapidly as possible is essential. However, vaccine uptake, particularly in younger adults is dropping, at least in part fuelled by reports of rare complications associated with specific vaccines. This review considers how vaccination with adenovirus vector-based vaccines against the SARS-CoV-2 coronavirus might cause rare cases of thrombosis and thrombocytopenia in some recipients. A thorough understanding of the underlying cellular and molecular mechanisms that mediate this syndrome may help to identify methods to prevent these very rare, but serious side-effects. This will also help facilitate the identification of those at highest risk from these outcomes, so that we can work towards a stratified approach to vaccine deployment to mitigate these risks.

Keywords: SARS-CoV-2; COVID-19; Coronavirus; vaccination; heparin-induced thrombocytopenia (HIT); vaccine-induced immune thrombotic thrombocytopenia (VITT)
Introduction

Currently in the UK (August, 2021), relatively low numbers of patients with severe disease, hospitalisations and deaths after infection with Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) have been attained in recent weeks, largely attributable to a phenomenal vaccine deployment effort. However, an emerging problem is vaccine hesitancy (1). A contributing factor towards this is the association of vaccinations, specifically those using an adenovirus based delivery system, with rare instances of severe outcomes including clotting syndromes that have been collectively referred to as vaccine induced immune thrombotic thrombocytopenia (VITT). In this review we consider how adenovirus vector-based vaccines, including those against SARS-CoV-2, might cause rare cases of thrombosis and thrombocytopenia in some recipients. We introduce the relevant biology underpinning development of these vaccines, describe analogous clotting syndromes including heparin induced thrombocytopenia (HIT) and compare VITT with HIT. Finally, we emphasise the importance of the continued use of adenovirus-based vaccines. Detailed analysis of the underlying cellular and molecular mechanisms that mediate this syndrome may help to identify methods to identify those at higher risk of these outcomes and ultimately prevent these very rare, but serious side-effects.

Several different vaccines have been produced in response to the SARS-CoV-2 pandemic. The most common immunogenic approach used in the vaccines developed so far has been based on the induction of specific immunity to the SARS-CoV-2 spike glycoprotein antigen, using a range of platforms for its delivery. Several spike-targeting vaccines have successfully passed safety and efficacy trials, and these have received emergency approval and licensing for use in many countries (Table 1). The development, approval and implementation of these vaccines has been undertaken at an astonishing pace. Indeed, by April 27, 2021, just 16 months since the first infections with the SARS-CoV-2 coronavirus were identified (2), over a billion vaccine doses had been administered to 570 million individuals around the world (3).

At the time of writing (August 2021) the most frequently used SARS-CoV-2 vaccines are based on mRNA or adenovirus vector systems that deliver genetic material encoding the coronavirus spike protein into cells. These include the BNT162b2 (Pfizer–BioNTech) (4) and mRNA-1273 (Moderna) (5) vaccines that contain mRNA encoding the coronavirus spike glycoprotein encased in lipid nanoparticles. These mRNA-based vaccines represent the first licensed vaccines using this type of platform.

The ChAdOx1 nCov-19 (Oxford–AstraZeneca) vaccine, in contrast, comprises a non-pathogenic recombinant, replication-deficient chimpanzee Ad5 adenovirus vector (originally called ChAdY25 and later renamed ChAdOx1; (6)) encoding the spike glycoprotein (7). The Ad26.COV2.S (Johnson & Johnson/Janssen) vaccine similarly utilises a human replication-deficient Ad26 adenovirus-based vector for spike protein delivery (8). Adenovirus vectors are also used in the Gam-COVID-Vac (Sputnik V, Gamaleya) vaccine, but in this instance they are applied in a heterologous prime/boost approach. An Ad26 vector encoding the spike glycoprotein is used for the first (prime)
injection, and this is followed by a booster injection comprising a spike glycoprotein-encoding human-derived Ad5 vector (9).

It was possible to conceptualise and produce the ChAdOx1 nCoV-19 vaccine so quickly because the same platform had previously been used for the development of a vaccine targeting Middle Eastern Respiratory Syndrome (MERS)–CoV. The ChAdOx1 MERS vaccine provided protective immunity to MERS-CoV infection in rhesus macaques (10), and was deemed safe in Phase I clinical trials in humans (11). The rapid application of this platform to target SARS-CoV-2 instead was therefore straightforward; this vaccine was designed over a weekend (12). In the ChAdOx1 nCoV-19 vaccine, the Ad5 vector has been engineered to also encode the full-length SARS-CoV-2 spike glycoprotein downstream of a tissue plasminogen activator leader sequence that directs the target protein into the secretory pathway, under a CMV promoter. The spike sequence was also codon optimised for more efficient translation (7, 13). The CHAdOx1 vector has been rendered replication incompetent through deletion of the E1 and E3 genes (14).

For the ChAdOx1-nCoV vaccine, a dose of 5 x 10¹⁰ viral particles (corresponding to the highest dose of ChAdOx1 MERS tested in humans) is administered by intramuscular injection during both the first and second vaccinations. An error in virus quantification during clinical trials resulted in second doses being tested at both full and half dose regimen (15). Lower antibody titres were seen in individuals boosted with the half-dose compared with full dose, and no differences in side effects were detected between individuals given a first or second injection at half dose, compared with those given standard doses at both times (15, 16).

The rapid deployment and widespread uptake of the described vaccines has had a dramatic impact in reducing the incidence of SARS-CoV-2 infections, transmissions and associated serious illnesses, especially in countries such as Israel and the UK where large proportions of adults have been vaccinated (17-19). A study of more than 365,000 UK households released in April 2021 reported that the ChAdOx1 nCov-19 and BNT162b2 vaccines were effective in reducing SARS-CoV-2 transmissions to others in the same household by 40-50% even after a single dose (17), although vaccine efficacy is lower against the rapidly emerging delta SARS-CoV-2 variant (20, 21). Analysis by Public Health England published in July 2021 has also estimated that the UK COVID-19 vaccination programme had prevented between 21.3 - 22.9 million infections and 57,500 – 62,700 deaths (22).

A large UK community cohort study revealed that side effects after vaccination with either a single dose of the BNT162b2 or ChAdOx1 nCoV-19 vaccines were experienced in <30% of recipients, and these were typically mild and short-lived (23). In these instances the most-commonly reported side effects included a sore arm at the injection site, headache and systemic flu-like symptoms lasting approximately 1-2 days. Similar findings were reported in clinical trials using the Gam-COVID-Vac vaccine (9). This was consistent with the side effects observed during clinical trials. However, following the administration of these vaccines to millions of recipients around the world some extremely rare but serious instances of potentially vaccine-induced side effects have been reported. These include reports of thrombosis (blood clots) accompanied by depletion of platelets (thrombocytopenia) within 5-24 days after injection with either the ChAdOx1 nCov-19 or the Ad26.COV2.S adenovirus-vector based vaccines (24-27). For the ChAdOx1 nCoV-19 vaccine, the
Medicines and Healthcare products Regulatory Agency (MHRA) in the UK estimated the incidence of thromboembolic events accompanied by thrombocytopenia to be 14.9 per million first (or unknown) doses (28, 29). For Ad26.COV2.S, six cases of cerebral venous sinus thrombosis (CVST) with thrombocytopenia, one of which was fatal, were reported in 7.2 million vaccinees (30). This novel syndrome of combined thrombosis and thrombocytopenia observed in these vaccine recipients has been termed vaccine-induced immune thrombotic thrombocytopenia (VITT) (31). Very rare instances of thrombocytopenia and bleeding but without thrombosis were also reported after injection with the BNT162b2 and mRNA-1273 mRNA-based vaccines, with 17 cases reported in over 20 million vaccinees (32). The BNT162b2 and mRNA-1273 vaccines have also been associated with other very rare side effects including allergic reactions and anaphylaxis (33, 34), and myocarditis or pericarditis (35) (Table 1).

Amongst the patients that had received adenovirus-based SARS-CoV-2 vaccines and experienced clotting shortly thereafter, the majority had thromembolic events in unusual locations in the body. These events included cerebral venous sinus thrombosis (CVST in the brain), splanchnic vein thrombosis (abdomen), and hepatic vein thrombosis (liver). These patients also presented with moderate to severe thrombocytopenia, and some unfortunately died. The incidence of CVST in adults in the general population is extremely rare (approximately 1% of all stroke forms), with estimates suggesting there are between 2 to 15.7 cases annually per million individuals (36-39). The similarly rare incidence of these cases of thrombosis in combination with thrombocytopenia in UK recipients of the ChAdOx1 nCov-19 vaccine (28) makes identifying a definitive link with vaccination difficult. However, the available evidence suggests this should be considered a possible, but very rare side effect of these vaccines. This assessment has prompted some medical health agencies around the world to take a cautious and approach and update their safety guidance, and in some instances, to halt the use of adenovirus vector-based SARS-CoV-2 vaccines, or restrict their use to certain age groups (28).

Vaccines and viruses associated with clotting

Many viral infections including SARS-CoV-2 can themselves induce thrombocytopenia (40, 41). Additionally, potential links between the use of vaccines (other than those targeting SARS-CoV-2) and rare instances of thrombocytopenia have been described. The best-evidenced association is that of the live measles, mumps and rubella vaccines with thrombocytopenic outcome reported to occur in 1 in 21,000 to 1 in 40,000 vaccine recipients (42, 43). However, it is important to stress that this rate is considerably lower than the occurrence of thrombocytopenia following natural infection with measles or rubella (44). Thrombocytopenia has also long been recognised as a potentially serious adverse reaction following use of adenovirus-based DNA vectors (45). Nevertheless, for the vast majority of individuals the benefits of using adenovirus vector-based vaccines to provide protection against the hospitalisation and death that COVID-19 can cause, far out-weigh the risks associated with these very rare side effects. Clotting events are also far more likely in COVID-19 patients than as a result of
inoculation with the vaccines developed against this disease. For example, clotting outcomes in COVID-19 patients admitted to intensive care units in the Netherlands occurred in approximately 49% of patients (46, 47).

These rare cases of vaccine-associated thrombosis accompanied with thrombocytopenia have (so far) only been reported in recipients within 5 to 24 days of injection with the ChAdOx1 nCov-19 or Ad26.COV2.S adenovirus vector-based vaccines, and not after injection with mRNA-based vaccines (24, 48). This implies that there may be features of these vaccines, independent of the coronavirus spike glycoprotein antigen that can trigger these serious side effects in extremely rare circumstances.

Mechanistic insights into adenovirus-vector associated clotting events

In order to understand the possible mechanisms underpinning VITT, let us first briefly consider the sequence of events following vascular injury that lead to the formation of a clot (thrombosis). Clotting in response to vascular injury and bleeding is an essential process of tissue repair known as haemostasis. Vascular injury causes vasoconstriction (to reduce blood flow) and exposes collagen at the lesion site. This induces the adherence, activation and aggregation of platelets that form a platelet plug in order to seal off the lesion in the injured vessel (49). von Willebrand factor (vWF) constitutively secreted by platelets and endothelial cells adheres to the exposed collagen and mediates the recruitment and adhesion of platelets to the lesion site. Multiple signalling cascades then converge to result in cross-linking of vWF and/or fibrinogen to receptors on platelets, resulting in their activation. Platelet activation leads to changes in their shape, which stimulates the release of their secretory granules. This triggers the production of further coagulation factors and the activation of multiple signalling cascades that converge on the formation of thrombin. The generated thrombin then mediates the conversion of circulating fibrinogen into an insoluble fibrin network that helps to strengthen the platelet plug. Although an essential process to help repair injured blood vessels, clots can cause pathology by restricting blood flow through the affected tissue.

Platelets are highly granular, and upon activation (e.g. during a clotting response) release the contents of a specific subset of granules termed ‘α-granules’ into the circulatory system. Of the hundreds of different α-granule constituents, one of the most abundant factors released during platelet activation is chemokine (C-X-C motif) ligand 4 (CXCL4), also known as platelet factor 4 (PF4). Aside from its roles in clotting pathways, PF4 is involved in inter-cellular innate immune signalling, including various activities such as activating neutrophil degranulation, monocyte recruitment and facilitating monocyte differentiation into macrophages. PF4 has been suggested as a pivotal factor that mediates the aberrant clotting events that have occurred after immunization with adenovirus-vector-based vaccinations (25, 50).

Platelet factor 4 is positively charged (cationic) and can complex with negatively charged (polyanionic) molecules such as heparin and glycosaminoglycans (GAGs) present in blood, forming complexes on the surfaces of platelets (51). The first hints at a possible mechanistic link between
adenovirus-based vaccination and clotting outcomes came from the analysis of sera from a cohort of German and Austrian patients that developed moderate-to-severe thrombocytopenia with unusual thromboses 5-16 days after immunization with the ChAdOx1 nCov-19 vaccine. Each candidate in the study had sera with high-titres of antibodies (Abs) that could bind to cationic PF4 in complex with other polyanionic molecules (25, 31). Subsequently, a similar association was also reported in a small number of recipients of the Ad26.COV2.S vaccine (27, 30, 52).

Parallels with heparin-induced thrombocytopenia (HIT)

As in VITT, PF4 has been implicated in aberrant clotting morbidities occurring during heparin-induced thrombocytopenia (HIT). HIT is a pathological syndrome that occurs when Abs are generated that bind heparin in complex with PF4, resulting in the activation of platelets and leading to a hypercoagulative state (53). The clinical events that occur during HIT bear similar hallmarks to those observed during adenovirus-associated thromboses. During HIT, several interlinked mechanisms may contribute to the pathology, with PF4 central to all of them. We describe four positive feedback loops that may be important in triggering HIT-associated pathology.

The central role of PF4 in platelet activation and clearance. In its regular morphology PF4 is not immunogenic. However, when PF4 is released from platelets (Fig. 1.1) it can bind to heparin or other polyanionic molecules and form ‘ultra-large’ complexes (Fig. 1.2). This aggregation causes conformational changes to PF4 which exposes epitopes that can induce the formation of PF4-heparin/polyanion-specific Abs (Fig. 1.3), and these can activate platelets via the binding of Abs to γ (FcγRIIa) expressed on their surfaces (Fig. 1.4). The PF4-heparin ultra-large complexes also activate the complement system leading to their opsonisation by complement components C3 and C4 (Fig. 1.3). This mediates the uptake and retention of these complement-opsonised complexes by complement receptor 2 (CR2/CD21)-expressing B cells (54). The PF4-heparin-containing complexes are then delivered to B cell follicles within secondary lymphoid organs where anti-PF4-heparin-specific Abs are produced.

Platelet activation triggers the release of more PF4, further enhancing the formation of PF4-heparin/polyanion-containing complexes (Fig. 1.4 → 1.2) (55). This creates a positive feedback-loop whereby PF4 released from activated platelets amplifies the production of anti-PF4-heparin-specific Abs. During this process, Abs with particularly strong binding may be generated that can bind PF4 in the absence of polyanions – i.e. PF4-specific autoantibodies (56).

The combined actions of platelets and mononuclear phagocytes leads to the development of a severe prothrombotic state (Fig. 1.5) that is accompanied by increased removal of platelets (thrombocytopenia) (Fig. 1.6); this can be lethal (57).

The central role of PF4 in endothelial cell damage. During HIT, the increased availability of PF4 can also activate endothelial cells (Fig. 2.1), and this can stimulate the recruitment and activation of monocytes and neutrophils. For example, anti-PF4 Abs from HIT patients can bind to PF4-complexed with GAGs on the surfaces of activated endothelial cells (Fig. 2.2), especially microvascular endothelial cells (58, 59). This can cause further activation and damage to endothelial cells accompanied by the release of tissue factor, thrombodulin, and vWF – the latter of which can
also bind PF4 (Fig. 2.3) (58-60). A positive feedback loop is similarly created whereby the enhanced formation of Ab-PF4-GAG-containing immune complexes cause further activation and injury to local endothelial cells (Fig. 2.4), indicating that microvasculature damage may contribute to the development of HIT.

The central role of PF4 in monocyte activation and thrombin-mediated platelet aggregation. The release of PF4 from activated platelets during HIT can also stimulate the recruitment of monocytes (Fig. 3.1). Here, PF4 also complexes with the GAG side-chains on the surfaces of monocytes (Fig. 3.2). The binding of anti-PF4 Abs to these PF4-GAG-containing complexes can then activate the monocytes via FcγRIIa stimulating the production of pro-coagulant factors, tissue factor and interleukin-8 (IL-8) (Fig. 3.3) (61-63). Activation-induced changes to the GAGs on the monocyte surface further enhance this response (62). The pro-coagulant activity of the monocytes generates thrombin (Fig. 3.4), which can contribute to HIT through the trans-activation and consequent aggregation of platelets (63).

The central role of PF4 in NETosis. Neutrophils provide an important first line of defence in the clearance of invading pathogens. Neutrophil activation is typically induced via the binding of Abs to Fcγ receptors on their cell surfaces, but they can also be activated via stimulation from platelet-derived P-selectin. Physical interactions between neutrophils with platelets (Fig. 4.1), or Ab-mediated stimulation via Fcγ receptors (including PF4-heparin-Ab complexes; Fig. 4.2) trigger the release of fibrous complexes from neutrophils called neutrophil extracellular traps (NETs) through a process known as NETosis (Fig. 4.3). These complexes comprise DNA and associated proteins that ‘trap’ extracellular pathogens and destine them for phagocytosis (64). While the release of NETs from neutrophils may provide protection against infection with certain pathogenic microorganisms, their excessive formation can contribute to the pathology of some inflammatory and autoimmune diseases (65). NETs could also play an important role in the development of the thrombosis in HIT (66), since neutrophils can be activated after in vitro treatment with Abs specific for PF4 (64). Immune complexes containing heparin, PF4 and Abs can activate NETosis in neutrophils either directly via stimulation of their FcγRIIa by anti-PF4 Ab, or indirectly via the FcγRIIa-mediated activation of platelets (66). The large amounts of negatively charged DNA within these NETs may then complex with PF4 (Fig. 4.4), enhancing platelet activation and coagulation and the production of anti-PF4-heparin Abs (67) (Fig. 4.4 → Fig. 4.1).

The central role of PF4 in pathogen clearance. Many individuals, including children <6 months old (68), have PF4-heparin-specific B cells, and so it is reasonable to expect that they have a beneficial role that outweighs their involvement in the development of HIT. For example, the binding of PF4 to the polyanionic surfaces of the Gram positive bacteria *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Listeria monocytogenes* or the Gram-negative bacteria *Escherichia coli* and *Neisseria meningitidis* may provide innate immune protection by mediating their removal from the circulation by natural PF4-heparin-specific Abs (68, 69) (Fig. 1.7). Nevertheless, although the presence of PF4-heparin-specific B cells may be relatively common, the prevalence of HIT is not,
indicating that in rare cases the dysregulation of this activity may lead to HIT. It is unclear what events lead to this dysregulation, but the availability of PF4 antigen is central to the process (70).

**How might vaccination cause VITT?**

HIT and VITT share several common pathological features. The sera from individuals diagnosed as potential VITT patients that had received the ChAdOx1 nCov-19 vaccine could activate platelets in the presence of PF4 (as in Fig. 1.4), and this was inhibited by the addition of a blocking FcγRIIa-specific Ab (31). These characteristics share notable similarities with HIT, whereby the binding of anti-PF4 Abs to complexes of PF4 and polyanionic heparin, activates platelets via their FcγRIIa, ultimately leading to thrombosis and thrombocytopenia (Fig. 1) (57). However, in contrast to patients with HIT, none of the patients that developed thrombosis and thrombocytopenia post-vaccination were known to have been treated with heparin, and the anti-PF4 IgG Abs in their sera could bind PF4 in the absence of heparin.

The induction of HIT by polyanionic factors other than heparin is known as autoimmune HIT, and cases of autoimmune HIT accompanied by CVST have been described (71). Clinically diverse examples of autoimmune HIT-like syndromes occurring without prior exposure to exogenous heparin have been reported following: (i) infection with pathogens such as influenza A virus, HIV-1, SARS-CoV and SARS-CoV-2 (71–77); (ii) treatment with polyanionic drugs such as pentosan polysulphate (78); or (iii) in response to exposure to joint cartilage-associated GAGs released as a consequence of tissue damage during orthopaedic surgery (79). As with heparin, polyanionic molecules produced in the above examples can similarly bind to PF4 and cause conformational changes to PF4 that expose epitopes that can induce the formation PF4-polyanion-specific Abs.

The ChAdOx-1 vaccine recipient VITT patient-derived anti-PF4 Abs did not recognise the coronavirus SARS-CoV-2 spike protein in *in vitro* assays, suggesting that it was unlikely that VITT was simply a consequence of cross-reactivity of the humoral immune response to the spike glycoprotein. A pre-print study from Greinacher and colleagues (50) has attempted to gain insight into the underlying mechanisms that may cause VITT. They used methods including 3D-super-resolution microscopy, transmission electron microscopy, mass spectrometry and nuclear magnetic resonance spectroscopy to investigate the interactions between components of the ChAdOx1 nCov-19 vaccine and the sera of VITT patients. From preliminary data obtained they proposed a sequence of post-vaccine inflammatory responses in the VITT patients reminiscent of autoimmune HIT that may have triggered the induction of anti-PF4 Abs that caused prothrombotic reactions and thrombocytopenia. Independent studies in mice have also shown that intravenous injection with a recombinant replication-deficient Ad5 adenovirus vector (similar to that used in the ChAdOx1 nCov-19 vaccine and used in the second dose of the Gam-COVID-Vac) can trigger platelet activation and aggregation (45, 80). The Ad5 vector also stimulated the release of ultra-large vWF from endothelial cells. These events could potentially cause thrombosis due to enhanced platelet aggregation, and thrombocytopenia due to the removal of platelets from the circulation by mononuclear phagocytes (45).
Greinacher and colleagues suggested that VITT is first initiated through interactions between the Ad5 vaccine vector and platelets, triggering their activation (50). The activated platelets then release PF4 which can complex with polyanionic vaccine components, in a similar manner to the PF4-polyanion complexes in autoimmune HIT patients (Fig. 1, green panel). The presence of these vaccine component-PF4 complexes in the circulation might then stimulate an acute pro-inflammatory response and the generation of anti-PF4 Abs, via a process analogous to HIT (57). Exacerbated prothrombotic reactions were then proposed to occur in the blood-stream of VITT patients when PF4 is recognised and clustered on the surface of platelets by high avidity anti-PF4 Abs (50). This, coupled with the opsonisation of the Ab-vaccine component-PF4 complexes by complement components, could then mediate the removal of platelets by mononuclear phagocytes, resulting in thrombocytopenia.

The polyanionic vaccine component/s that might bind platelets in certain individuals, and/or complex with PF4 to induce the generation of anti-PF4 Abs are not known. It is possible that vaccine constituent polyanionic protein(s) play a role. Proteomics analyses suggested that the vaccine preparation contained a mixture of >1,000 proteins including cell culture-derived human proteins and adenovirus vector proteins in addition to the SARS-CoV-2 spike glycoprotein (50). The negatively charged DNA within the adenovirus vector might be also a credible target, since free nucleic acid (DNA and RNA) can bind to PF4 and trigger a conformational change, exposing immunogenic epitopes that may be recognised by anti-PF4 Abs. Complexes containing DNA and PF4 can also trigger the generation of anti-PF4-heparin Abs in mice (67). However, the DNA in these vaccines is packaged within adenovirus vector particles, so it is uncertain whether sufficient free DNA would be available (for example due to virus particle lysis) in the serum to complex with PF4. Extracellular RNA is a possible alternative; while RNA is generally considered less stable than DNA, circular RNAs released in extracellular vesicles are highly stable and abundant in cell culture (81). Greinacher and colleagues also suggested that ingredients in the vaccine carrier solution such as EDTA might cause capillary leakage (50), enhancing endothelial cell activation and damage, and leading to amplification of the acute inflammatory response to vaccination at the injection site.

Excessive NETosis has been associated with the development of thrombosis after virus infections, including SARS-CoV-2 (82), but it is unknown whether NETosis contributes to VITT. As described above (Fig. 4), NETs can stimulate the production of anti-PF4 Abs and have pro-coagulant properties that can contribute to the development of thrombosis in HIT (66, 67). NETosis can also be triggered in neutrophils after in vitro treatment with PF4 and serum from VITT patients (50). Extracellular DNases can degrade the DNA within these NETs, and this may help regulate the balance between NETosis and NET clearance to prevent excessive inflammation and pathology (83). It was noteworthy that the sera of some VITT patients contained much lower levels of DNase activity when compared to healthy controls, and so it is possible that NET clearance was diminished in VITT patients (50). This raises the hypothesis that dysregulated NETosis may contribute to the development of VITT.
Why only some individuals?

In order to understand the events leading up to VITT, key information may be forthcoming from the identification of patient risk factors. Fortunately the incidence of VITT in recipients of adenovirus-vector based COVID-19 vaccinations has been extremely rare (28), and at the time of writing it was uncertain what characteristic/s in the VITT patients were responsible for their increased risk of developing these rare complications; there is no obvious group that has been disproportionately affected. The most comprehensive demographic data has been accrued by the UK ‘yellow card’ reporting system which catalogues the reported adverse reactions to the COVID-19 vaccines, although these are not independently verified (84). As of 16 Jun 2021, 24.5 million first doses and 19.6 million second doses of the ChAdOx-1 nCoV-19 vaccine had been administered in the UK. Following vaccination, a total of 389 cases of thrombotic thrombocytopenia had been reported (8.8 cases per million doses). Of these, 203 cases were in females and 183 were in males, and 3 cases did not have a gender assigned. The proportion of vaccinations given to each gender group was not described. Amongst these 389 cases, 31 occurred after a second dose. The number of reported cases of thromboembolic events accompanied by thrombocytopenia in a range of age groups is shown in Figure 5, however the total number of recipients in each age category was not provided. Of the affected patients, 68 had unfortunately died (17%), of whom 39 were female (19.2% of affected females) and 29 were male (15.8% of affected males). Additionally, CVST was reported in 140 vaccine recipients and other thromboembolic events were reported in 249 vaccine recipients.

If we make the assumption that the vaccinations were administered to approximately equal numbers of men and women, it would appear that the incidence of VITT is slightly higher in women than in men. This correlates with other non-vaccine-associated types of thromboses. There is an increased incidence of CVST in women of child-bearing age, during pregnancy and the post-partum period, and in those using oral contraceptives or oestrogen replacement therapies (85, 86). Additional risk factors for CVST and HIT include the presence of high titre and affinity anti-PF4 antibodies, age (below 50 years old), obesity, tissue trauma, certain infections and genetics (57, 71, 77, 79, 85-88). However, COVID-19 disease is itself associated with a higher risk of developing CVST (89) and HIT (90), and thrombocytopenia and thrombosis are relatively common in critically ill SARS and COVID-19 patients (74-76, 89-92).

Polymorphisms in specific genes involved in the HIT response can affect susceptibility to this outcome. For example, the risk of developing HIT is increased in patients that are homozygous for the FcyRIIIa-158VV allele, and these patients tend to have high levels of anti-PF4 Abs in their sera (93). A similar association has been reported for the FcyRIIa-131RR allele (94-97). In HIT patients these polymorphisms in FcγR-encoding genes might increase cell activation upon binding to Ab-PF4-heparin immune complexes (95). PECAM-1 is also expressed on the surfaces of platelets and neutrophils, and the PECAM-1 125VV polymorphism is similarly associated with an increased risk of HIT (98). A higher prevalence of the human platelet antigen (HPA)-1 a/b genotype amongst HIT patients has also been described, with the risk of developing thromboembolic complications increasing 8-fold in those carrying each of the FcγRIIA-131RR, and PECAM-1 VV125 polymorphisms.
and HPA-1 a/b genotype (98). Polymorphisms in other genes such as CXADR (encoding the coxsackie-adenovirus receptor) could also contribute, for example by enhancing the affinity of the adenovirus vector for platelets (45, 99). None of these genotypes have as yet been associated with VITT, or with the development of severe COVID-19 disease (100).

**Conclusions**

It is important to stress that at the time of writing, a definitive causal association between the use of adenovirus-based COVID-19 vaccines and extremely rare reports of thrombosis with thrombocytopenia has not been established. Nevertheless, safety statements have been updated to raise awareness of the potential occurrence of these very rare side-effects within 5-20 days after vaccination with adenovirus vector-based anti-SARS-CoV-2 vaccines. Importantly, due to the strong similarity of VITT with HIT, heparin should not be used to treat this syndrome. Since CVST is more prevalent in younger individuals, some countries including the UK have restricted the use of these vaccines to specific age groups as a countermeasure where alternative vaccine formulations are available.

Further research may identify possible mechanisms to modify these adenovirus vectors to reduce the occurrence of these rare side effects. For example, polyethylene glycol-modification of the Ad5 vector can prevent the induction of thrombocytopenia by reducing interactions between platelets and endothelial cells (101). However, this should be considered with caution as polyethylene glycol can cause anaphylactic reactions in some individuals (102), and its presence in the mRNA vaccines may have been a contributing factor to the rare but serious instances of anaphylaxis after injection with these vaccines (103). Modifications to the Ad5 capsid fibre protein might also reduce the induction of thrombocytopenia, as has been shown in mice infected with a replication-competent Ad5 strain (104). Similarly, thrombocytopenia after intravenous Ad5 vector injection was prevented by the inclusion of mutations in the adenovirus that block its ability to bind to CXADR on host cells and instead redirect cell entry via integrin α(v)β(6) (105). Reducing the EDTA concentration in the vaccine carrier solution could help reduce capillary leakage and endothelial cell damage around the injection site, and by doing so, reduce the acute inflammatory response encountered after vaccination (50). Finally, halving the dose of for the first inoculation has been put forward as a way to reduce the risk of clotting outcomes and also diminish other more common side effects (106).

These extremely rare cases of apparent VITT should not distract us from the overwhelming benefit of vaccination to both the recipient and the wider population by limiting SARS-CoV-2 infection rates, and reducing the incidence of serious COVID-19 disease, hospital admissions, and deaths. Current studies will also determine whether vaccination similarly reduces the incidence and impact of “long-COVID”. Due to their ability to be stored at standard refrigerator temperatures, these adenovirus-based vaccines will play important roles in vaccinating harder to reach communities and those in less developed nations across the globe. This is especially the case for the Ad26.COV2.S vaccine, as just one injection is required (8). These vector-based vaccines are also ideally suited for rapid modification in order to create adapted vaccines in response to novel SARS-CoV-2 variants of concern.
Funding
This study was supported by Institute Strategic Programme Grant funding from the BBSRC (BBS/E/D/20002172, BBS/E/D/20002173 & BBS/E/D/20002174). EG is also supported by a Wellcome Trust/Royal Society Sir Henry Dale Fellowship (211222_Z_18_Z).

Author contributions
The authors contributed to all aspects of the manuscript.

Conflict of interest
The authors declare no competing financial conflicts of interest.

Data availability
Data sharing is not applicable to this article as no new data were created or analysed in this study.

Reviewer acknowledgement
The authors (EG & NAM) and the Editor-in-Chief, Tim Elliott, would like to thank the Regional Editor, Tao Dong, and an anonymous reviewer for their contribution to the peer review of this article.
References

1. Remmel A. Communicating COVID vaccine safety poses a unique challenge. Nature. 2021;593:488-9.DOI:10.1038/d41586-021-01257-8
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. New Eng J Med. 2020;382:727-33.DOI:10.1056/NEJMoa2001017
3. Kreier F. ‘Unprecedented achievement’: who received the first billion COVID vaccinations? Nature. 2021;593:13.DOI:10.1038/d41586-021-01136-2
4. Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurman A, Lockart S, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature. 2020;586:589-93.DOI:10.1038/s41586-020-2639-4
5. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA vaccine against SARS-CoV—Preliminary Report. New Eng J Med. 2020;383:1920-31.DOI:10.1056/NEJMoa2022483
6. Sadoff J, Gray G, Vandebosch A, Cardenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. New Eng J Med. 2021;384:2187-201.DOI:10.1056/NEJMoa2101544
7. van Doremalen N, Haddock E, Feldmann F, Meade-White K, Bushmaker T, Fischer RJ, et al. A single dose of ChAdOx1 MERS provides protective immunity in rhesus macaques. Sci Adv. 2020;6:eaba8399.DOI:10.1126/sciadv.aba8399
8. Gaunt ER, Digard P. Compositional biases in RNA viruses: Causes, consequences and applications. WIREs RNA. 2021;https://doi.org/10.1002/wrna.1679
9. Almugrin A, Davidson AD, Kavanagh Williamson M, Lewis PA, Heesom KJ, Morris S, et al. SARS-CoV-2 vaccine ChAdOx1 nCoV-19 infection of human cell lines reveals low levels of viral backbone gene transcription alongside very high levels of SARS-CoV-2 S glycoprotein gene transcription. Genome Med. 2021;13:43.DOI:10.1186/s13073-021-00859-1
10. Voysey M, Costa Clemens SA, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2021;397:99-111.DOI:10.1016/S0140-6736(20)32661-1
17. Harris RJ, Hall JA, Zaisi A, Andrews NJ, Dunbar JK, Dabrera G. Effect of vaccination on household transmission of SARS-COV-2 in England. New Eng J Med. 2021;https://www.nejm.org/doi/full/10.1056/NEJMc2107717.DOI:10.1056/NEJMc2107717

18. Hunter PR, Brainard J. Estimating the effectiveness of the Pfizer COVID-19 BNT162b2 vaccine after a single dose. A reanalysis of a study of “real-world” vaccination outcomes from Israel. medRxiv. 2021;10.1101/2021.02.01.21250957.DOI:10.1101/2021.02.01.21250957

19. Dagan N, Barca N, Kepten E, Miron O, Perchik S, Katz MA, et al. BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. New Eng J Med. 2021;384:1412-23.DOI:10.1056/NEJMoa2101765

20. Public Health England. SARS-CoV-2 variants of concern and variants under investigation in England Technical briefing 152021 14 June 2021. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/993198/Variants_of_Concern_VOC_Technical_Briefing.pdf.

21. Sheikh A, McMenamin J, Taylor B, Robertson C. SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. Lancet. 2021;397:2461-2.DOI:10.1016/S0140-6736(21)01358-1

22. Public Health England. COVID-19 vaccine surveillance report Week 30. London, UK: Public Health England; 2021 29/7/21. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1007376/Vaccine_surveillance_report_-_week_30.pdf

23. Menni C, Klaser K, May AM, Polidori L, Capdevila J, Louca P, et al. Vaccine side-effects and SARS-CoV-2 infection after vaccination in users of the COVID Symptom Study app in the UK: a prospective observational study. Lancet Infect Dis. 2021;21:939-49.DOI:10.1016/S1473-3099(21)00224-3

24. Simpsons CR, Shi T, Vasileiou E, Katikireddi SV, Kerr S, Moore E, et al. First-dose ChAdOx1 and BNT162b2 COVID-19 vaccines and thrombocytopenic, thromboembolic and hemorrhagic events in Scotland. Nat Med. 2021;https://doi.org/10.1038/s41591-021-01408-4.DOI:10.1038/s41591-021-01408-4

25. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrik PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCoV-19 vaccination. New Eng J Med. 2021;384:2092-101.DOI:10.1056/NEJMoa2104840

26. Schultz NH, Sørøvoll JH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. New Eng J Med. 2021;384:2124-30.DOI:10.1056/NEJMoa2104882

27. See J, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;10.1001/jama.2021.7517.DOI:10.1001/jama.2021.7517

28. Public Health England. JCVI advises on COVID-19 vaccine for people aged under 40 2021 [Available from: https://www.gov.uk/government/news/jcvi-advises-on-covid-19-vaccine-for-people-aged-under-40.

29. Medicines & Healthcare products Regulatory Agency. Research and analysis Coronavirus vaccine - weekly summary of Yellow Card reporting Updated 30 July 2021 2021 [Available from: https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting.

30. Sadoff J, Davis K, Douchguih M. Thrombotic thrombocytopenia after Ad26.COV2.S aacination - response from the manufacturer. New Eng J Med. 2021;384:1965-6.DOI:10.1056/NEJMc2106075

31. Greinacher A, Selleng K, Warkentin TE. Autoimmune heparin-induced thrombocytopenia. J Thromb Haemost. 2017;15:2099-114.DOI:10.1111/jth.13813
32. Lee E-J, Cines DB, Gernsheimer T, Kessler C, Michel M, Tarantino MD, et al. Thrombocytopenia following Pfizer and Moderna SARS-CoV-2 vaccination. Am J Hematol. 2021;96:534-7.DOI:10.1002/ajh.26132
33. Centers for Disease Control and Prevention Newsroom. Transcript: CDC Update on COVID-19 [press release]. 2021. https://www.cdc.gov/media/releases/2021/t0106-cdc-update-covid-19.html
34. CDC COVID-19 Response Team and Food and Drug Administration. Allergic reactions including anaphylaxis after receipt of the first dose of moderna COVID-19 vaccine — United States, December 21, 2020–January 10, 2021. MMWR Morb Mortal Wkly Rep. 2021;70:125-9.DOI:10.15585/mmwr.mm7004e1
35. Pepe S, Gregory AT, Denniss AR. Myocarditis, pericarditis and cardiomyopathy after COVID-19 vaccination. Heart Lung Circ. 2021;DOI:10.1016/j.hlc.2021.07.011
36. Stam J. Thrombosis of the cerebral veins and sinuses. New Eng J Med. 2005;352:1791-8.DOI:10.1056/NEJMra042354
37. Bousser M-G, Ferro JM. Cerebral venous thrombosis: an update. Lancet Neurol. 2007;6:162-70.DOI:10.1016/S1474-4422(07)70029-7
38. Coutinho JM, Zuurbier SM, Aramideh M, Stam J. The incidence of cerebral venous thrombosis: a cross-sectional study. Stroke. 2012;43:3375-7.DOI:10.1161/STROKEAHA.112.671453
39. Devasagayam S, Wyatt B, Leyden J, Kleinig T. Cerebral venous sinus thrombosis incidence is higher than previously thought: a retrospective population-based study. Stroke. 2016;47:2180-2.DOI:10.1161/STROKEAHA.116.013617
40. Rand ML, Wright JF. Virus-associated idiopathic thrombocytopaenia purpura. Transfus Sci. 1998;19:253-9.DOI:10.1016/s0955-3886(98)00039-3
41. Battacharjee S, Benerjee M. Immune thrombocytopenia secondary to COVID-19: a systematic review. SN Compr Clin Med. 2020;2:2048-58.DOI:10.1007/s42399-020-00521-8
42. Black C, Kaye JA, Jick H. MMR vaccine and idiopathic thrombocytopaenia purpura. Br J Clin Pharmacol. 2003;55:107-11.DOI:10.1093/bja/14.1.31
43. Sauvé LJ, Scheifele D. Do childhood vaccines cause thrombocytopenia. Paediatr Child Health. 2009;14:31-2.DOI:10.1093/pch/14.1.31
44. Cecinati V, Principi N, Brescia L, Giordano C, Esposito S. Vaccine administration and the development of immune thrombocytopaenia purpura in children. Hum Vacin Immunother. 2013;9:1158-62.DOI:10.1016/j.hv.23601
45. Othman M, Labelle A, Mazzetti I, Elbatarny HS, Lillicrap D. Adenovirus-induced thrombocytopenia: the role of von Willebrand factor and P-selectin in mediating accelerated platelet clearance. Blood. 2007;109:2832-9.DOI:10.1182/blood-2006-06-032524
46. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020;191:145-7.DOI:10.1016/j.thromres.2020.04.013
47. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Muller MCA, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020;18:1995-2002.DOI:10.1111/jth.14888
48. UK Parliament. COVID-19 vaccines safety and blood clots 2021 [Available from: https://post.parliament.uk/covid-19-vaccines-safety-and-blood-clots/]
49. Periayah MH, Halim AS, Saad AZM. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. Int J Hematol Oncol Stem Cell Res. 2017;11:319-27
50. Greinacher A, Selleng K, Wescje S, Handtke S, Palankar R, Aurich K, et al. Towards understanding ChAdOx1 nCoV-19 vaccine-induced immune thrombotic thrombocytopenia (VITT). https://doi.org/1021203/rs3rs-440461/v1. 2021.DOI:10.21203/rs.3.rs-440461/v1
51. Mikhailov D, Young HC, Linhardt RJ, Mayo KH. Heparin dodecasaccharide binding to platelet factor-4 and growth-related protein-alpha. Induction of a partially folded state and implications for heparin-induced thrombocytopenia. J Biol Commun. 1999;274:25317-29.DOI:10.1074/jbc.274.36.25317
52. Muir K-L, Kallam A, Koespell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. New Eng J Med. 2021;384:1964-5.DOI:10.1056/NEJMc2105869
53. Linkins L-A. Heparin induced thrombocytopenia. Brit Med J. 2015;350:g7566.DOI:10.1136/bmj.g7566
54. Khandelwal S, Lee GM, Hester CG, Poncz M, McKenzie SE, Sachais BS, et al. The antigenic complex in HIT binds to B cells via complement and complement receptor 2 (CD21). Blood. 2016;128:1789-99.DOI:10.1182/blood-2016-04-709634
55. Newman PM, Chong BH. Heparin-induced thrombocytopenia: New evidence for the dynamic binding of purified anti-PF4-heparin antibodies to platelets and the resultant platelet activation. Blood. 2000;96:182-7.DOI:10.1182/blood.V96.1.182
56. Nguyen T-H, Medvedev N, Delcea M, Greinacher A. Anti-platelet factor 4/polyanion antibodies mediate a new mechanism of autoimmunity. Nat Commun. 2017;8:14945.DOI:10.1038/ncomms14945
57. Marchetti M, Zermatten MG, Calderara DB, Aliotta A, Alberio L. Heparin-induced thrombocytopenia: a review of new concepts in pathogenesis, diagnosis, and management. J Clin Med. 2021;10:683.DOI:10.3390/jcm10040683
58. Visentin GP, Ford SE, Scott JP, Aster RH. Antibodies from patients with heparin-induced thrombocytopenia/thrombosis are specific for platelet factor 4 complexed with heparin or bound to endothelial cells. J Clin Invest. 1994;93:81-8.DOI:10.1172/JCI116987
59. Blank M, Shoenfeld Y, Tavor S, Praprotnik S, Boffa MC, Weksler B, et al. Anti-platelet factor 4/heparin antibodies from patients with heparin-induced thrombocytopenia provoke direct activation of microvascular endothelial cells. Int Immunol. 2002;14:121-9.DOI:10.1093/intimm/14.2.121
60. Davidson SJ, Wadhamp P, Rogers L, Burman JF. Endothelial cell damage in heparin-induced thrombocytopenia. Blood Coag Fibrinolysis. 2007;18:317-20.DOI:10.1097/MBC.0b013e32806a8249
61. Arepally GM, Mayer IM. Antibodies from patients with heparin-induced thrombocytopenia stimulate monocytic cells to express tissue factor and secrete interleukin-8. Blood. 2001;98:1252-6.DOI:10.1182/blood.v98.4.1252
62. Rauova L, Hirsch JD, Greene TK, Zhai L, Hayes VM, Kowalska MA, et al. Monocyte-bound PF4 in the pathogenesis of heparin-induced thrombocytopenia. Blood. 2010;116:5021-31.DOI:10.1182/blood-2010-03-276964
63. Tutwiler V, Madeeva D, Ahn HS, Andrianova I, Hayes V, Zheng XL, et al. Platelet transactivation by monocytes promotes thrombosis in heparin-induced thrombocytopenia. Blood. 2016;127:464-72.DOI:10.1182/blood-2013-11-539262
64. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria: Science. 2004;303:1523-35.DOI:10.1126/science.1092385
65. Vorobjeva NV, Chernyak BV. NETosis: molecular mechanisms, role in physiology and pathology. Biochemistry. 2020;85:1178-90.DOI:10.1134/S0006297920100065
66. Perdomo J, Leung HH, Ahmadi Z, Yan F, Chong JJH, Passam FH, et al. Neutrophil activation and NETosis are the major drivers of thrombosis in heparin-induced thrombocytopenia. Nat Commun. 2019;10:1322.DOI:10.1038/s41467-019-09160-7
67. Jaax ME, Krauel K, Marschall T, Brandt S, Gansler J, Furll B, et al. Complex formation with nucleic acids and aptamers alters the antigenic properties of platelet factor 4. Blood. 2013;122:272-81.DOI:10.1182/blood-2013-01-478966
68. Krauel K, Schulze A, Jouni R, Hackbarth C, Hietkamp B, Selleng S, et al. Further insights into the anti-PF4/heparin IgM immune response. Thromb Haemost. 2016;115:752-61.DOI:10.1160/TH15-08-0654
69. Krauel K, Potschke C, Weber C, Kessler W, Furll B, Ittermann T, et al. Platelet factor 4 binds to bacteria, inducing antibodies cross-reacting with the major antigen in heparin-induced thrombocytopenia. Blood. 2011;117:1370-8.DOI:10.1182/blood-2010-08-301424
70. Prechel MM, Walenga JM. Emphasis on the role of PF4 in the incidence, pathophysiology and treatment of heparin induced thrombocytopenia. Thrombosis J. 2013;11:7.DOI:10.1186/1477-9560-11-7
71. Moores G, Warkentin TE, Farooqi MAM, Jevtic SD, Zeller MP, Perera KS. Spontaneous heparin-induced thrombocytopenia presenting as cerebral venous sinus thrombosis. Neurrol Clin Practice. 2020;DOI:10.1212/CNP.0000000000000805.
72. Thompson GR, Lawrence VA, Crawford GE. HIV infection increases the risk of heparin-induced thrombocytopenia. Clin Infect Dis. 2007;45:1393-6.DOI:doi.org/10.1086/522761
73. Ratzlaff RA, Ripoll JG, Kassab LL, Diaz-Gomez JL. Acute oxygenator failure: a new presentation of heparin-induced thrombocytopenia in a patient undergoing venovenous extracorporeal membrane oxygenation support. Case Rep. 2016;2016:bcr2016218179.DOI:10.1136/bcr-2016-218179
74. Nazy I, Jevtic SD, Moore JC, Huynh A, Smith JW, Kelton JG, et al. Platelet-activating immune complexes identified in critically ill COVID-19 patients suspected of heparin-induced thrombocytopenia. J Thromb Haemost. 2021;19:1342-7.DOI:10.1111/jth.15283
75. Yang M, Hon K-LE, Li CK. Thrombocytopenia in patients with severe acute respiratory syndrome (review). Hematology. 2005;10:101-5.DOI:10.1080/10245330400026170
76. Warkentin TE, Makris M, Jay RM, Kelton JG. A spontaneous prothrombotic disorder resembling heparin-induced thrombocytopenia. Am J Med. 2008;121:632-6.DOI:10.1016/j.amjmed.2008.03.012
77. Lasda E, Parker R. Circular RNAs co-precipitate with extracellular vesicles: a possible mechanism for circRNA clearance. PLoS ONE. 2016;11:e0148405.DOI:10.1371/journal.pone.0148407
78. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. J Exp Med. 2020;217:e202000652.DOI:10.1084/jem.20200652
79. Hakkim A, Furrrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. Proc Natl Acad Sci USA. 2010;107:9813-8.DOI:10.1073/pnas.0909927107
80. Medicines & Healthcare products Regulatory Agency. Research and analysis Coronavirus vaccine - weekly summary of Yellow Card reporting London, UK2021 [Available from: https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting.
81. McBane 2nd RD, Tafur A, Wysokiński WE. Acquired and congenital risk factors associated with cerebral venous sinus thrombosis. Thromb Res. 2010;126:81-7.DOI:10.1016/j.thromres.2010.04.015
82. Amoozegar F, Ronsley PE, Sauve R, Menon BK. Hormonal contraceptives and cerebral venous thrombosis risk: a systematic review and meta-analysis. Front Neurol. 2015;6:7.DOI:10.3389/fneur.2015.00007
87. Zuurbier SM, Arnold M, Middeldorp S, Broeg-Morvay A, Silvis SM, Heldner MR, et al. Risk of cerebral venous thrombosis in obese women. JAMA Neurol. 2016;73:579-84.DOI:10.1001/jamaneurol.2016.0001
88. Idiculla PS, Gurala D, Palanisamy M, Vijayakumar R, Dhandapani S, Nagarajan E. Cerebral venous thrombosis: a comprehensive review. Eur Neurol. 2020;83:369-79.DOI:10.1159/000509802
89. Taquet M, Husain M, Geddes JR, Luciano S, Harrison PJ. Cerebral venous thrombosis: a retrospective cohort study of 513,284 confirmed COVID-19 cases and a comparison with 489,871 people receiving a COVID-19 mRNA vaccine. Pre-print. 2021;https://osf.io/a9jdq/.DOI:10.17605/OSF.IO/H2MT7
90. Daviet F, Guerilly C, Baldesi O, Bernard-Guervilly F, Pilarczyk E, Genin A, et al. Heparin-induced thrombocytopenia in severe COVID-19. Circulation. 2020;142:1875-7.DOI:10.1161/CIRCULATIONAHA.120.049015
91. Alonso-Beato R, Morales-Ortega A, De la Hera Fernandez FJ, Moron AIP, Rios-Fernandez R, Rubio JL, et al. Immune thrombocytopenia and COVID-19: Case report and review of literature. Lupus. 2021;doi: 10.1177/09612033211021161.DOI:10.1177/09612033211021161
92. Gruel Y, Pouplard C, Lasne D, Magdelaine-Beuzelin C, Charroing C, Watier H. The homozygous FcgammaRIIIa-158V genotype is a risk factor for heparin-induced thrombocytopenia in patients with antibodies to heparin-platelet factor 4 complexes. Blod. 2004;104:2791-3.DOI:10.1182/blood-2004-01-0058
93. Brandt JT, Isenhart CE, Osborne JM, Ahmed A, Anderson CL. On the role of platelet Fc gamma RIIa phenotype in heparin-induced thrombocytopenia. Thromb Haemost. 1995;74:1564-72.
94. Rollin J, Pouplard C, Sung HC, Leroux D, Saada A, Gouilleux G, et al. Increased risk of thrombosis in FcγRIIA 131RR patients with HIT due to defective control of platelet activation by plasma IgG2. Blood. 2015;125:2397-404.DOI:10.1182/blood-2014-09-594515
95. Pouplard C, May M-A, Iochmann S, Amiral J, Vissac A-M, Marchand M, et al. Antibodies to platelet factor 4-heparin after cardiopulmonary bypass in patients anticoagulated with unfractionated heparin or a low-molecular-weight heparin. Circulation. 1999;99:2530-5.DOI:10.1161/01.CIR.99.19.2530Circulation
96. Garay RP, Labaune JP. Immunogenicity of polyethylene glycol (PEG). Open Conference Proc J. 2011;2:104-7.DOI:10.2174/2210289201102010104
97. Turk VE. Anaphylaxis associated with the mRNA COVID-19 vaccines: Approach to allergy investigation. Clin Immunol. 2021;227:108748.DOI:10.1016/j.clim.2021.108748
98. Raddi N, Vigant F, Wagner-Ballon O, Giraudier S, Custers J, Hemmi S, et al. Pseudotyping serotype 5 adenovirus with the fibre from other serotypes uncovers a key role of the fiber protein in adenovirus 5-induced thrombocytopenia. Hum Gene Ther. 2016;27:193-201.DOI:10.1089/hum.2015.154
105. Coughlan L, Vallath S, Gros A, Gimenez-Alejandre M, Van Rooijen N, Thomas GJ, et al. Combined fiber modifications both to target α(v)β(6) and detarget the coxsackievirus-adenovirus receptor improve virus toxicity profiles in vivo but fail to improve antitumoral efficacy relative to adenovirus serotype 5. Hum Gene Ther. 2012;23:960-79.DOI:10.1089/hum.2011.218
106. Kupferschmidt K, Vogel G. Hard choices emerge as link between AstraZeneca vaccine and rare clotting disorder becomes clearer 2021 [Available from: https://www.sciencemag.org/news/2021/04/hard-choices-emerge-link-between-astrazeneca-vaccine-and-rare-clotting-disorder-becomes.
107. European Medicines Agency. ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS. 2021. https://www.ema.europa.eu/en/documents/product-information/vaxzevria-previous-covid-19-vaccine-astrazeneca-epar-product-information_en.pdf
108. U.S. Department of Health and Human Services. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Pfizer-BioNTech COVID-19 Vaccine — United States. United States; 2021. in MMWR and Morbidity and Mortality Weekly Report. 2021: United States. p. 46-51. http://dx.doi.org/10.15585/mmwr.mm7002e1
109. Kadali RAK, Janagama R, Peruru S, Gajula V, Madathala RR, Chennaiahgari N, et al. Non-life-threatening adverse effects with COVID-19 mRNA-1273 vaccine: A randomized, cross-sectional study on healthcare workers with detailed self-reported symptoms. J Med Virol. 2021;93:4420-9.DOI:10.1002/jmv.26996
110. Xia S, Zhang Y, Wang Y, Wang H, Yang Y, Gao GF, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. Lancet. 2021;21:39-51.DOI:10.1016/S1473-3099(20)30831-8
111. FDA. FDA Briefing Document: Janssen Ad26.COV2.S Vaccine for the Prevention of COVID-19. In: Vaccines and related biological products advisory committee meeting February 26, editor. https://www.fda.gov/media/146217/download2021.
112. Bucci EM, Berkhoff J, Gillibert A, Gopakrishna G, Calogero RA, Bouter LM, et al. Data discrepancies and substandard reporting of interim data of Sputnik V phase 3 trial. Lancet. 2021;397:1881-3.DOI:10.1016/S0140-6736(21)00899-0
113. Wikipedia. List of COVID-19 vaccine authorizations 2021 [Available from: https://en.wikipedia.org/wiki/List_of_COVID-19_vaccine_authorizations#EpiVacCorona.
Figure 1. The central role of PF4 in heparin-induced thrombocytopenia (HIT) and autoimmune HIT. 1, Activated platelets release PF4. 2, PF4 is cationic and can bind to heparin or other polyanionic molecules (autoimmune HIT) and form ‘ultra-large’ complexes. 3, The binding of PF4 to heparin/polyanions exposes epitopes in PF4 that induce the formation PF4-heparin/polyanion-specific Abs. 4, Binding of PF4-heparin/polyanion-Ab immune complexes to Fcγ receptor IIa (FcγRIIa) on the platelet surface leads to their activation, creating a positive feedback-loop whereby PF4 released from activated platelets amplifies the production of anti-PF4-heparin-specific Abs. 5, A severe prothrombotic state is ultimately created. 6, Thrombocytopenia also occurs due to increased removal of platelets by mononuclear phagocytes. 7, Since PF4 can bind to certain pathogenic microorganisms it is plausible that this may play a role in protection against infection by aiding their clearance by mononuclear phagocytes.

Figure 2. The central role of PF4 in endothelial cell damage during HIT. 1, PF4 can activate local endothelial cells. 2, PF4 can complex with GAGs on the surfaces of activated endothelial cells. 3, This can cause further activation and damage to the endothelium and the release of vWF and procoagulants. 4, A positive feedback loop can be created whereby the enhanced formation of Ab-PF4-GAG-containing immune complexes leads to further activation and damage the endothelium.

Figure 3. The central role of PF4 in monocyte activation during HIT. 1, PF4 released from platelets during HIT can activate and recruit monocytes. 2, Complexes can form between PF4 and the GAG side-chains on monocyte surfaces. 3, PF4-GAG-Ab containing immune complexes can activate monocytes via FcγRIIa. 4, The pro-coagulant activity of the monocytes can trigger a positive feedback loop leading to further platelet activation and aggregation.

Figure 4. The central role of PF4 in NETosis during HIT. 1, Interactions between neutrophils and platelets, or 2, Ab-mediated stimulation via Fcγ receptors (including PF4-heparin-Ab complexes) can each activate neutrophils. 3, This can stimulate the release neutrophil extracellular traps (NETs), known as NETosis. NETs contain DNA and proteins that ‘trap’ extracellular pathogens and destine them for phagocytosis. 4, The negatively charged DNA within these NETs may then trap and complex with PF4, enhancing platelet activation and coagulation and the production of anti-PF4-heparin Abs, resulting in a positive feedback loop.

Figure 5. Numbers of suspected reports of thrombotic thrombocytopenia in the UK after immunization with the ChAdOx1 nCov-19 vaccine. Data presented by patient age group and include reported cases up to, and including, 16/6/21. Data derived from Ref. (84). Blue bars, non-fatal reports; Red bars, fatal reports.
| Vaccine Name(s)       | Manufacturer                | No. of countries with full (emergency) licensure | Date of first use in UK | Delivery system | Delivery details | Serious side effects (all very rare)                      | Refs       |
|----------------------|-----------------------------|-------------------------------------------------|-------------------------|----------------|----------------|----------------------------------------------------------|------------|
| ChAdOx1 nCov-19/ Vaxzevria/ Covishield/ AZD1222 | Astra Zeneca               | 2 (170)                                          | Jan 2021                | Vehicle        | Chimpanzee AdV  | Anaphylaxis, thrombosis with thrombocytopenia            | (107)      |
| BNT162b2             | Pfizer-BioNTech            | 5 (108)                                          | Dec 2020                | mRNA           | mRNA and lipid nanoparticles | Anaphylaxis, myocarditis or pericarditis                  | (35, 108)  |
| mRNA-1273            | Moderna                    | 2 (74)                                           | Apr 2021                | mRNA           | mRNA and lipid nanoparticles | Anaphylaxis, seizures, myocarditis or pericarditis        | (35, 109)  |
| BBIBP-CorV           | Sinopharm                  | 4 (73)                                           | Not expected            | Inactivated    | Chemically inactivated virus | None reported                                             | (110)      |
| Ad26.COV2.S          | Johnson and Johnson / Janssen | 2 (77)                                           | Pending; emergency approval May 2021 | Vehicle        | AdV 26 (human) | Hypersensitivity not classified as anaphylaxis, blood clots | (27, 111)  |
| Gam-COVID-Vac/ Sputnik V | Gamaleya            | 2 (72)                                           | Not expected            | Vehicle        | AdV 26 then AdV 5 (both human) | Deep vein thrombosis, haemorrhagic stroke, hypertension / insufficiently reported | (9, 112)   |

*Updated data on COVID-19 vaccinations available here (113).*
Figure 1

HIT

1. Platelet

2. Complements C3 & C4

3. Anti-PF4-heparin/polyanion Ab production

4. Platelet activation

5. Platelet aggregation (thrombosis)

6. Platelet clearance (thrombocytopenia)

7. Pathogen clearance?

Autoimmune HIT

1. Platelet

2. CR2

3. Anti-PF4-heparin/polyanion Ab production

4. FcγRIIa

5. Pro-coagulants

6. Mononuclear phagocytes

Pathogenic bacteria

GAGs

Ultra-large PF4-heparin complexes

PF4-heparin -Ab/complement immune complex

PF4-polyanion -Ab/complement immune complex

Downloaded from https://academic.oup.com/immunotherapy/advance-article/doi/10.1093/immadv/ltab019/6353340 by guest on 26 August 2021
Figure 2

1. Platelet activation
2. GAG-PF4-Ab immune-complex formation
3. Further endothelial cell activation/damage
4. PF4-Ab immune-complex formation

Microvascular endothelial cells

Endothelial cell activation/damage

PF4

Platelet aggregation

Pro-coagulants

von Willebrand factor

Complement
Figure 4

1. Stimulation via activated platelets

2. GAG-PF4-Ab immune-complex binding to FcγRIIa

3. Neutrophil activation and NETosis

4. PF4-DNA complex formation & platelet aggregation

- PF4
- Heparin
- Complement components
- ant-PF4-heparin Ab
- FcγRIIa
- P-selectin
- PSGL-1

pro-coagulant properties
Chromatin
Figure 5

No. suspected UK reports by 16/6/21

Age range (years)

Non-fatal reports
Fatal reports

18-29 30-39 40-49 50-59 60-69 70-79 80-89 90-99 Unknown