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# Thrombosis after SARS-CoV2 infection or COVID-19 vaccination: will a nonpathologic anti-PF4 antibody be a solution?—A narrative review

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**Abstract**

The coronavirus disease 2019 (COVID-19) pandemic was triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a previously unknown strain of coronavirus. To fully understand the consequences and complications of SARS-CoV-2 infections, we have reviewed current literature on coagulation dysfunctions that are related to the disease and vaccination. While COVID-19 is more commonly considered as a respiratory illness, studies indicate that, in addition to respiratory illness, a coagulation dysfunction may develop in individuals after the initial infection, placing them at the risk of developing thrombotic events. Patients who died of COVID-19 had higher levels of D-dimer, a biomarker for blood clot formation and breakdown. Effective treatments for coagulation dysfunctions are critically needed to improve patient survival. On the other hand, antibodies against platelet factor 4 (PF4)/heparin may be found in patients with rare instances of vaccine-induced immunological thrombotic thrombocytopenia (VITT) following vaccination with adenovirus-based vaccines. VITT is characterized by atypical thrombosis and thrombocytopenia, similar to immune-mediated heparin-induced thrombocytopenia (HIT), but with no need for heparin to trigger the immune response. Although both adenovirus-based and mRNA-based vaccines express the Spike protein of SARS-CoV-2, VITT is exclusively related to adenovirus-based vaccines. Due to the resemblance with HIT, the use of heparin is highly discouraged against treating patients with thrombotic thrombocytopenia after SARS-CoV-2 infection or with VITT after vaccination. Intravenous immunoglobulin therapy coupled with anticoagulation is recommended instead. The well-studied anti-PF4 monoclonal antibody RTO, which does not induce pathologic immune complexes in the presence of heparin and has been humanized for a potential treatment modality for HIT, may provide a nonanticoagulant HIT-specific solution to the problem of increased blood coagulation after SARS-CoV-2 infection or the VITT after immunization.

**Keywords:** antibody, coagulation dysfunctions, COVID-19, PF4, vaccine-induced immunological thrombotic thrombocytopenia

## Introduction

Coronavirus disease 2019 (COVID-19) has evolved into a global epidemic with far-reaching consequences for society, culture, and the global economy. This disease has become a pandemic since 2019 and is caused by the infection of a coronavirus, dubbed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Over 2 years after its initial discovery and identification, COVID-19 continues to cause significant morbidity and mortality on a global scale, devastating millions. While respiratory sickness is a significant aspect of the disease, studies have suggested that a distinct coagulation dysfunction may occur after the initial infection, putting patients at risk for thrombotic events.[1] To understand the consequences of SARS-CoV-2 infections, we have reviewed current literature for coagulation dysfunctions that are related to both the disease as well as the COVID-19 vaccines.

## Database search strategy

We performed a literature search using PubMed and Google Scholar. The following combinations of keywords were used to identify articles to be evaluated in detail: COVID-19, clot, heparin, platelet, PF4, and VITT. Most of the cited studies (80% of all references) were published between 2012 and 2022. Ten publications before 2012 were included in consideration of their relevance to the mechanism of coagulation and function of platelet factor 4 (PF4).

## Dysfunction of coagulation after SARS-CoV-2 infection

Elevated levels of the D-dimer, which is a fragment of fibrin degradation and a biomarker of blood clot formation and breakdown, are associated with mortality of patients infected by COVID-19. Higher D-dimer levels are often associated with thrombosis or other thrombotic events, and widespread microthrombi in multiple organs following infection by SARS-CoV-2 are positively correlated with elevated D-dimer levels.[2] In addition to elevated circulating D-dimer, a prolonged prothrombin time has also been associated with decreased patient survival and increased need for critical care.[3] Patients who succumbed

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to severe novel coronavirus pneumonia showed abnormal coagulation results, with >70% of deceased patients meeting the criteria of disseminated intravascular coagulation, which is a serious condition that causes blood clotting throughout the body's blood vessels.[4] Preliminary reports on infected patients in other studies have shown similar results, with >40% displaying elevated D-dimer levels and >30% developing thrombocytopenia, a condition that is associated with deficiency of platelets and leads to slow blood clotting and bleeding. These rates are even higher in patients with severe COVID-19 infection, with >50% developing thrombocytopenia and >59% displaying elevated D-dimer levels.[5]

Both elevated D-dimer levels and thrombocytopenia can be attributed to the extravagant activation of the coagulation cascade and platelets following viral infections. The development of intra-alveolar or systemic fibrin clots is notable in COVID-19 infections and can be found in both human and animal models.[2] Overt clot formation is due to the prothrombotic response, which attempts to dissolve alveolar hemorrhage, but may instead have a detrimental effect on patient recovery and survival. Diseases caused by other coronavirus strains, such as the SARS-CoV-1 and the Middle East respiratory syndrome coronavirus, are also associated with thrombotic complications and hematologic manifestations.[6] Researchers are inspired to study coagulation dysfunctions after SARS-CoV-2 infection, hoping for insights to improve patient survival.

Platelets are key players in the coagulation process. Platelets express the angiotensin-converting enzyme 2 (ACE2), which is a protein that the Spike protein of SARS-CoV-2 can bind to, along with the transmembrane protease serine 2 (also known as TMPRSS2), which is a serine protease that cleaves and prepares the Spike protein to mediate SARS-CoV-2 virus-cell membrane fusions (Fig. 1). It is reported that the Spike protein directly enhanced platelet activation via the MAPK pathway upon binding to ACE2.[7] The Spike protein was also shown to enhance thrombosis formation in vivo when mice were transfused with hACE2 transgenic platelets, but not with wild-type platelets. As a result, COVID-19 patients may be at significant risk of platelet activation and thus risk of thrombotic events, particularly those with high viral levels.

## Current treatment for thrombosis related to SARS-CoV-2 infection

The American Society of Hematology (ASH) has issued guidelines on the use of anticoagulation in patients with COVID-19.[8] If a critically sick COVID-19 patient does not have suspected or confirmed venous thromboembolism, the ASH recommends prophylactic-intensity anticoagulation. Initially, therapeutic-dose anticoagulation with heparin was tested to treat critically ill COVID-19, but improvement of clinical outcomes, including survival, the average time of hospital stay, and the number of

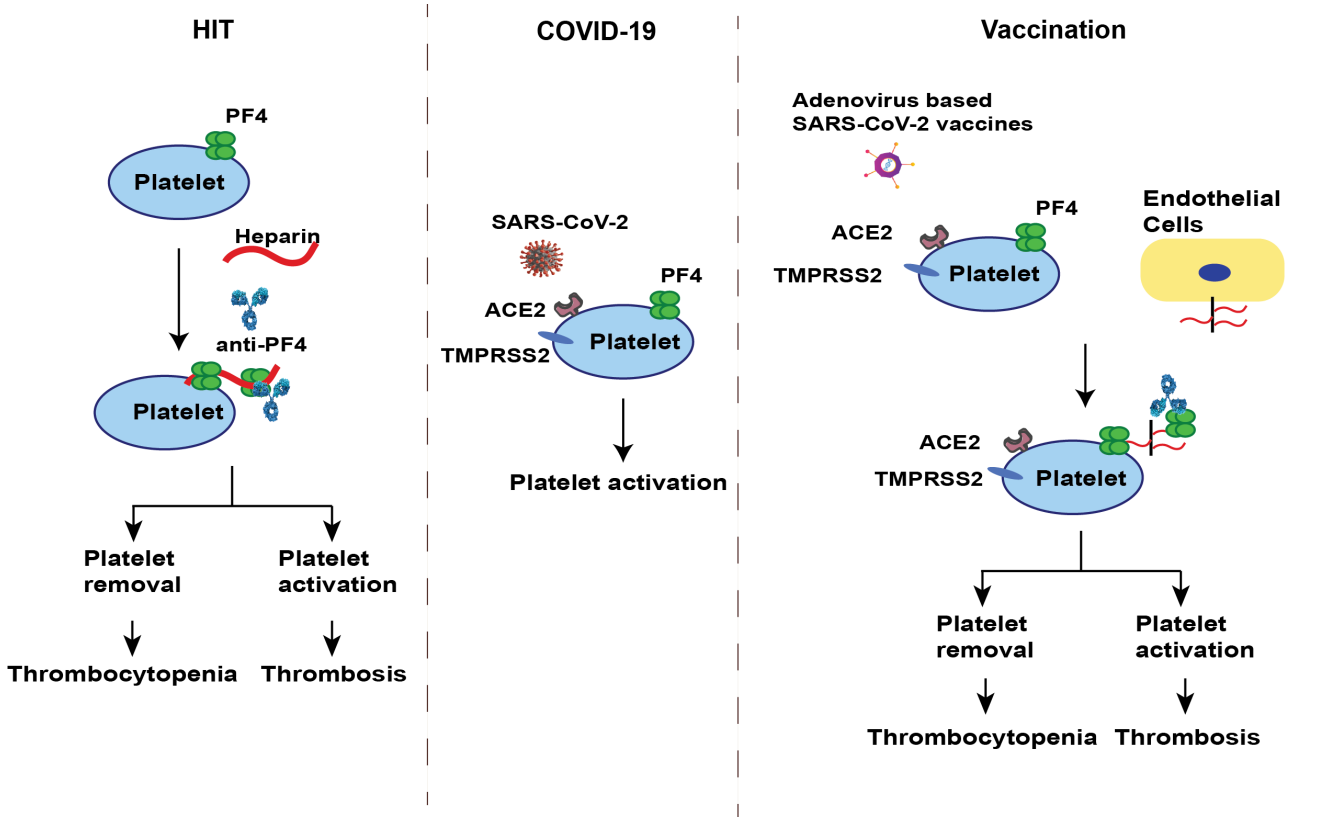


**Figure 1.** Abnormal coagulation in HIT, COVID-19, and vaccination with adenovirus based SARS-CoV-2 vaccines. In the presence of heparin, pathological PF4 antibodies bind to PF4 on platelets and promote the formation of immune complexes that activate human platelets and result in thrombosis. Meanwhile, platelets are depleted (thrombocytopenia). SARS-CoV-2 infection could also activate platelets and cause coagulation dysfunction. After vaccination with adenovirus-based vaccine, PF4 (released by activated platelets) form complexes with endogenous polyanionic PG (released by damaged endothelial cells), stimulating B cells to produce pathological anti-PF4 antibodies to induce VITT. ACE2=angiotensin-converting enzyme 2, COVID-19=coronavirus disease 2019, HIT=heparin-induced thrombocytopenia, PF4=platelet factor 4, SARS-CoV-2=severe acute respiratory syndrome coronavirus 2, TMPRSS2=transmembrane protease serine 2, VITT=vaccine-induced immunological thrombotic thrombocytopenia.

days free of cardiovascular or respiratory organ support, was not observed when compared with patients treated with prophylactic anticoagulation.[9] The median time for remaining organ support-free in a total of 1098 patients was 1 day for the therapeutic-dose group (*n*=534) but 4 days for the prophylactic dose group (*n*=564). Even though the therapeutic-dose group appeared to have worse outcomes, there was no statistically significant difference between the two groups. Both groups had a similar percentage of patients who recovered enough to be discharged from the hospital (62.7% vs 64.5). Major bleeding occurred in 3.8% of the patients in the therapeutic-dose group, while only 2.3% of patients in the prophylactic dose group experienced major bleeding. Again, there is no statistically significant difference between the two treatment groups.

In the INSPIRATION trial, both standard-dose (lower dose) and intermediate-dose of enoxaparin (Lovenox) had similar critical or lethal outcomes (45.7% vs 44.1%) within 30 days for ICU patients. However, the intermediate-dose (1 mg/kg) led to higher rates of major bleeding events (2.5% vs 1.4%) and severe thrombocytopenia (2.2% vs 0%, *P*=0.01).[10] Based on results from the INSPIRATION trial, which was terminated prematurely due to futility, routine use of intermediate-dose prophylactic anticoagulation in COVID-19 patients in ICU was riskier and should be avoided in practice.

For acutely ill COVID-19 patients who are not experiencing venous thromboembolism, the ASH guideline also suggests prophylactic-intensity over intermediate-intensity or therapeutic-intensity anticoagulation. In a VA study, early anticoagulation within 1 day of admission of COVID-19 patients reduced the risk of 30-day mortality as compared with no anticoagulation use (14.3% vs 18.7%, hazard ratio [HR] 0.73, 95% confidence interval [CI] 0.66–0.81).[11] The mortality advantage of anticoagulation appeared to be reduced for those who were transferred to ICU within 1 day of hospital admission. Low-dose preventive heparin use appeared to reduce the risk of mortality much further (HR 0.69, 95% CI 0.61–0.77) compared to the use of therapeutic anticoagulation (HR 0.81, 95% CI 0.73–0.90). Since no advantage was observed for high-dose therapeutic heparin, it is reasonable to use prophylactic-intensity anticoagulation to avoid a higher risk of bleeding for high-dose heparin.[11]

However, while therapeutic-dose anticoagulation failed to produce better outcomes in critically ill COVID patients, the strategy generated positive outcomes in noncritical but hospitalized COVID-19 patients. In an open-label and multicenter trial,[12] among 2219 patients with moderate disease, when compared with prophylactic anticoagulation, therapeutic-dose anticoagulation slightly increased the chance of organ support-free survival within 21 days (80.0% vs 76.4%). The superiority of therapeutic-dose anticoagulation over usual-care thromboprophylaxis was more significant in patients with high D-dimer levels (77.9% vs 72.2%, adjusted odds ratio [OR], 1.31; 95% CI, 1.00–1.76) than those with low D-dimer levels (81.2% vs 79.8%, adjusted OR, 1.22; 95% CI, 0.93–1.57). The higher dose of anticoagulation is consistently associated with bleeding risk, with a rate of 1.9% for major bleeding in the therapeutic-dose group as compared with only 0.9% in the lower dose group.

In another small trial named HESACOVID, a therapeutic dose of enoxaparin improved respiratory outcomes in severe COVID-19. In the therapeutic group of 10 COVID-19 patients, the $PaO_2/FiO_2$ ratio improved significantly from 163 at baseline to 209 at day 7 and 261 at day 14. In comparison, the gas exchange rate was not much changed in the control group that was treated with prophylactic doses. Patients in the therapeutic-dose group also came off mechanical ventilation faster.[13] However, numerically more minor bleeding was observed in the therapeutic-dose group.

For discharged COVID-19 patients who have not experienced venous thromboembolism, the ASH guideline suggests not using prophylactic-intensity anticoagulation. However, the outcomes of additional clinical trials may change these guidelines in the future.

## Thrombocytopenia and thromboembolism after COVID-19 vaccination

Vaccine-induced immune thrombotic thrombocytopenia (VITT) was observed in rare cases following vaccinations using the ChAdOx1 nCoV-19 vaccine[14] and the Ad26.COV2.S vaccine,[15] both of which are based on replication-incompetent adenoviral vectors. VITT is characterized by simultaneous thrombosis (at atypical sites) and thrombocytopenia, similar to immune-mediated heparin-induced thrombocytopenia (HIT), but with a lack of heparin to induce the immune-mediated response.

In 23 patients with no history of prothrombotic issues, thrombosis and thrombocytopenia were reported at 6 to 24 days after the first dose of ChAdOx1 nCoV-19.[14] All the patients had low or normal fibrinogen levels but elevated D-dimer levels. No evidence of thrombophilia or causative precipitous was identified. Notably, 22 of 23 patients were positive for anti-PF4 antibody.

In a prospective study of patients in the United Kingdom with suspected VITT, of 294 patients evaluated, 170 were identified as definite cases of VITT, and an additional 50 were identified as probable cases.[16] All these patients were hospitalized 5 to 48 days (median time: 14 days) after they received the first dose of ChAdOx1. The overall mortality of these VITT patients was 22%. The risk factors for death after VITT were cerebral venous sinus thrombosis (OR 2.7, 95% CI, 1.4–5.2), decrease in the baseline platelet count (OR 1.7 for every 50% decrease, 95% CI, 1.3–2.3), higher baseline D-dimer levels, and decrease in the baseline fibrinogen levels (OR 1.7 for every 50% decrease, 95% CI, 1.1–2.5). In patients with low platelet counts (<30,000/μL) and intracranial hemorrhage, the observed mortality was 73%.

VITT was also reported in people vaccinated with the Ad26.COV2.S vaccine. Muir et al[15] first describes thrombosis associated with severe thrombocytopenia and disseminated intravascular coagulation in one case. Following this report, the Phase 3 clinical trial for Ad26.COV2.S was paused to review adverse events. Out of approximately 50,000 vaccinated people, one was identified to have cerebral venous sinus thrombosis with thrombocytopenia.[17] According to the primary analyses of results from the single-dose Ad26.COV2.S vaccine trial, the vaccine group had slightly higher rates of deep vein thrombosis, pulmonary embolism, and transverse sinus thrombosis, but the occurrence rates were low in both groups and no absolute conclusion could be drawn.

The *BMJ* includes several case reports[18–20] of this rare condition, including one report involving the development of VITT in the venosplanchnic and pulmonary arterial circulation from a hospital in the United Kingdom. Ten days after receiving the Ad26.Cov2.S, the female patient had a headache, a significantly low platelet count, and elevated D-dimer levels, along with mildly deranged liver function tests.[21] Further investigation confirmed the presence of pulmonary embolism and acute portal vein thrombosis. The patient had no prior or familial history of thromboembolic disease, and after treatment with intravenous immunoglobulins (IVIG), the patient was discharged 12 days later.

The incidence rate of VITT after ChAdOx1 is estimated to be between one in 125,000 and one in one million.[22] With such a low incidence rate, there is currently little to no evidence suggesting that individuals that have suspected or confirmed venous thromboembolism or a history of prior thrombosis are at increased risk for developing VITT. However, the majority of VITT patients were young females (20–55 years old), suggesting a possible relationship between gender and susceptibility to VITT.[22]

Notably, vaccine-induced thrombosis was observed to occur frequently at cerebral vessels and splanchnic circulation, though the reason as to why thrombosis occurred in these areas is yet unknown. Antibodies against PF4 were also observed in VITT through enzyme-linked immunosorbent assays (ELISA),[23] again similar to HIT. Studies have indicated that PF4 ELISAs are reliable screening assays for identifying VITT, but not particle gel immunoassay, lateral flow assay, or automated chemiluminescence immunoassay.[23,24] A confirmatory functional assay is preferred but not available in most situations.

Antiplatelet antibodies formed post-vaccination through the immune stimulation process were thought to potentially promote excessive platelet activation, thus leading to immune thrombotic thrombocytopenia. It has been proposed that anti-Spike antibodies could cross-react with PF4 and induce VITT. However, although both adenovirus-based and mRNA-base SARS-CoV-2 vaccines express the Spike protein in the host, VITT is mostly seen in people vaccinated with adenovirus-based vaccines.[26] VITT cases after mRNA vaccine administration are extremely rare.[25] In addition, it has been shown that anti-PF4 antibodies responsible for causing VITT do not cross-react with the SARS-CoV-2 spike protein.[27] On the other hand, adenovirus is known to bind to and activate platelets,[28] promoting the idea that, in certain people, excess activation of platelets by this type of vector may induce the production of pathological PF4 antibodies that are responsible for VITT.

Fortunately, the presence of anti-PF4 antibodies in most VITT patients is transient. In a follow-up study of 35 VITT patients, the PF4-dependent platelet activation assay results turned negative for 23 patients (medium follow-up time: 11 weeks). For those who were followed up for >12 weeks, 14 of 15 patients turned negative for the platelet activation assay. Additionally, levels of anti-PF4–heparin antibodies for all 35 patients had declined by 53% by the end of follow-up. However, a full sero-reversion to a negative ELISA result, which is classified as having an optical density of <0.5, was only observed in three patients.[29]

For people vaccinated with ChAdOx1nCoV-19, a second shot is needed to achieve full protection.[30] After their initial first vaccination, five of the 35 patients mentioned in the study above received BNT162b2 at 10 to 18 weeks later. Of these patients, four had shown a negative result in the platelet activation assay before receiving the second shot. In these patients, levels of anti-PF4–heparin antibodies were normal, and there were no further signs of thrombotic complications. This study indicates that, once the platelet activation assay becomes negative, patients may be safe enough to receive an mRNA vaccine as the second shot.[29]

## Current management of VITT

For patients with VITT, ELISA assays are used to detect high levels of antibodies against PF4–polyanion complexes. In addition, a platelet activation assay is suggested to identify abnormal activity in the presence of PF4.[31] The ASH recommends treatments in patients of VITT similar to that of patients with severe HIT.[32]

Usage of high-dose IVIG therapy for 2 days with additional anticoagulation therapy is recommended for VITT treatment.[33,34] To avoid potential interference of IVIG with anti-PF4 ELISA and platelet activation assays, it is recommended to evaluate patients for HIT/VITT before the administration of IVIG.[34]

Because of the similarity between VITT and HIT, the usage of heparin is highly discouraged as it has the potential to increase the risk of VITT. Instead, preferred treatments are non-heparin anticoagulants, including but not limited to thrombin inhibitors, antifactor Xa inhibitors that do not contain a heparin bridge, and selective factor Xa inhibitors such as Fondaparinux, etc. Platelet transfusions are also recommended unless otherwise advised by a hematologist.[32,34]

In one report from Canada, three VITT patients were identified and treated. Two patients were diagnosed with limb-artery thrombosis, and the third was diagnosed with cerebral venous and arterial thrombosis. Following initiation of IVIG therapy, all three patients showed reduced levels of antibody-induced platelet activation in serum.[34] In another report, five VITT patients with confirmed anti-PF4 antibodies were treated with IVIG for 2 to 5 days.[35] Absolute platelet increment was observed within 48 hours. Four patients achieved complete platelet response with platelet count rising to $\geq 100 \times 10^9$/L within 96 hours. In addition to IVIG, all patients also received parenteral anticoagulation.[35]

## Involvement of PF4 in thrombosis and VITT

PF4, also known as chemokine ligand four (CXCL4), is a CXC chemokine released from α-granules during platelet activation.[36] This chemokine is associated with promoting blood coagulation through the neutralization of heparin-like molecules on the endothelial cell surface of blood vessels. PF4 is known to bind with high affinity to heparin, a drug widely used for thromboprophylaxis and dubbed as "blood thinner," and negatively charged glycosaminoglycans (GAGs), such as heparan sulfates and dermatan sulfates found on cell surface membranes. The formation of PF4-heparin complexes promotes the production of anti-PF4-heparin IgG antibodies,[37] which then bind to FcRγIIA receptors to induce platelet activation. Covalently, GAGs are associated with core proteins to form proteoglycans (PG). The high affinity between PF4 and GAGs/PGs allows PF4 to gather at sites of vascular injury at high concentrations and enhance clot formation.[38]

Studies have shown that as PF4 concentrations increase, thrombus formation also increases, but following a bell-shaped curve.[39,40] The positively charged PF4 binds to the negatively charged GAGs to neutralize the charge on the endothelial cell surface and allow for easy approximation by platelets, consequently promoting thrombus formation. When there is either an excess or lack of PF4, cell surfaces then become positively or negatively charged, respectively, and thus do not allow for optimal thrombosis. Therefore, excess PF4 displays anticoagulant effects rather than procoagulant effects. However, in the presence of excess PF4, when heparin is added, the excess charge on the cell surface is neutralized,[40] creating an optimal environment for thrombosis. It is also reported that PF4 enhances the cofactor activity of thrombomodulin, a transmembrane glycoprotein located on endothelial cells. Thrombomodulin can bind

to thrombin and promote the production of activated protein C, a potent anticoagulant, through the cleavage of protein C by thrombin.[41] As such, we can see that PF4 is a key agent in regulating thrombosis and displays both procoagulant and anticoagulant effects.

HIT is a condition in which patients develop thrombocytopenia following exposure to heparin due to the emergence of anti-PF4-heparin IgG antibodies. Despite the thrombocytopenia, rather than bleeding into tissues and slow blood clotting, HIT leads to the development of serious arterial and venule thrombi.[42] There are two different types of HIT: Type I, which is nonimmune and caused by the agglutinating effects of heparin, and shows transient thrombocytopenia that is typically resolved once heparin is withdrawn from the patient; and Type II, which refers to immune-mediated heparin-induced thrombocytopenia, is associated with more serious thrombosis. The immune-mediated Type II HIT represents a greater concern to most patients, and is the focus of our study.

When PF4 binds to heparin, the complex undergoes a conformational change and becomes immunogenic. PF4 and unfractionated heparin form intermediate (44–120 kDa) or ultra-large (>650 kDa) size of complexes.[43] The ultra-large complexes are highly immunogenic, leading to the generation of IgG antibodies against these PF4-heparin complexes. The antibody binding site that leads to HIT is located within PF4, so heparin alone is unable to induce the immune-mediated adverse reaction. As such, PF4 is also capable of forming antigenic complexes independent of heparin by binding to GAGs on the surface of platelets.[44] Following the use of heparin, a subset of patients who have high surface PF4 levels can form a significant number of antigenic complexes on the platelet and are at high risk of HIT.

The resulting anti-PF4-heparin IgG antibodies are then able to activate platelets through interaction with FcγIIa receptors. This activation releases microparticles from platelets to promote thrombosis. In addition, the PF4-heparin-IgG immune complexes can interact with monocytes, leading to the production of tissue factors and potential endothelial injury. These multimolecular immune complexes, also known as prothrombotic microparticles, can contribute to the activation of the coagulation cascade and the generation of the procoagulant thrombin, which results in thrombus formation.[45]

A murine monoclonal $IgG_{2b}$ kappa antibody (KKO), which specifically binds to PF4-heparin complexes, was identified as an antibody to cause heparin-induced thrombosis and thrombocytopenia.[46] In the presence of GAGs, KKO binds to PF4 on platelets and promotes the formation of pathogenic immune complexes (PF4-heparin-KKO) that shared serologic and functional properties with naturally occurring PF4-heparin antibodies found in patients with HIT.[47] KKO also demonstrated the ability to activate human platelets through a PF4- and heparin-dependent mechanism using FcγRIIA.[48]

The formation of PF4 tetramers is the key to forming such pathogenic antigenic complexes. When heparin is present, the PF4 tetramer is stabilized after monomers are clamped together through the closed end, thus producing an open end that functions as a binding site for KKO. Without a stable PF4 tetramer, there is a significant decrease in KKO binding due to a lack of display of binding sites, and PF4 is thus unable to induce HIT.[37] As such, KKO demonstrates heparin-dependent binding to PF4 but did not recognize heparin itself.

After SARS-CoV-2 infection or vaccination of adenovirus-based vaccine, PF4 could be released by activated platelets and circulate to form complexes with endogenous polyanionic PG released by damaged endothelial cells, stimulating B cells to produce anti-PF4 antibodies.[23] In VITT, in the absence of heparin, PGs function as the stabilizer for the required tetramers to induce pathologic PF4 antibodies (Fig. 1).

## The anti-PF4 antibody as a potential approach to prevent HIT and VITT

Since HIT and VITT are induced by the PF4-heparin-IgG multimolecular immune complexes and the interaction between IgG and Fc receptors,[48] one treatment strategy is to block the crosslinking of the FcγIIa receptors on the platelet surface. The use of IVIG provides a large quantity of immunoglobulins that compete against anti-PF4 antibodies for binding to FcγIIa receptors.[49]

However, not all PF4 antibodies are pathologic. RTO, a KKO-isotype-matched anti-PF4 antibody, does not generate pathologic immune complexes in the presence of heparin. This non-heparin-dependent and non-HIT inducing antibody binds to PF4 in such a way that the interface for tetramer is interrupted, thus preventing the tetramerization of PF4.[37] With the capability to inhibit the tetramerization of PF4, RTO also inhibits its KKO-induced heparin-dependent platelet activation and thrombosis in a dose-dependent manner.[37] Notably, while KKO required the presence of heparin to bind to PF4, RTO demonstrated heparin-independent binding to PF4, and binding was unaffected by the presence of heparin.

The key to avoiding HIT or VITT is preventing the formation of the PF4-heparin-IgG multimolecular immune complexes. While IVIGs provide an available regime for VITT, it is not specific and far away from an ideal solution. Due to the similarities between HIT and VITT,[50] RTO provides a potential nonanticoagulant HIT-specific solution to preventing excessive blood coagulation following infection or vaccination. Of course, although RTO has been humanized,[36] further research is needed to verify its clinical activity.

## Limitations

The study conducted in this literature review is limited by a few factors. The major limitation is the nature of the study. Although we identified literature reporting associations between the coagulation dysfunction seen in COVID-19 and the raised level of anti-PF4 antibodies in VITT patients after vaccination, it was difficult to determine what the causal factor is. There was no direct evidence showing that anti-PF4 antibodies were the pathological antibodies in VITT. Since the incidence rate of VITT was very low, it would be difficult to design an animal model for VITT to test whether RTO could prevent VITT. Nevertheless, we believe in vitro studies could be performed first to verify whether hRTO could prevent platelet activation induced by the sera from VITT patients.

## Conclusions

Due to the resemblance with HIT, the use of heparin is highly discouraged against treating patients with thrombotic thrombocytopenia after SARS-CoV-2 infection or with VITT after vaccination. Intravenous immunoglobulin therapy coupled with anticoagulation is recommended instead for VITT. The well-studied anti-PF4 monoclonal antibody RTO, which does not induce pathologic immune complexes in the presence of heparin and has been humanized for a potential treatment

modality for HIT, may provide a nonanticoagulant HIT-specific solution to the problem of increased blood coagulation after an infection or immunization.

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## Author contributions

ER participated in literature search, manuscript writing, and review. PG participated in manuscript writing and review. HZ participated in manuscript design, literature search, manuscript writing, and review. All authors approved the final version of the manuscript.

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## Conflicts of interest

There are no conflicts of interest.

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