Review Article

COVID-19 vaccine-induced immune thrombotic thrombocytopenia: A review

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Abstract:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible and pathogenic coronavirus responsible for the pandemic coronavirus disease 19 (COVID-19). It has significant impact on human health and public safety along with negative social and economic consequences. Vaccination against SARS-CoV-2 is likely the most effective approach to sustainably control the global COVID-19 pandemic. Vaccination is highly effective in reducing the risk of severe COVID-19 disease. Mass-scale vaccination will help us in attaining herd immunity and will lessen the negative impact of the disease on public health, social and economic conditions. The present pandemic stimulated the development of several effective vaccines based on different platforms. Although the vaccine is safe and efficacious, rare cases of thrombosis and thrombocytopenia following the use of vaccination with the ChAdOx1 CoV-19 vaccine (AstraZeneca, University of Oxford, and Serum Institute of India) or the Ad26.COV2.S vaccine (Janssen/Johnson & Johnson) have been reported globally. This review focussed on the definition, epidemiology, pathogenesis, clinical features, diagnosis, and management of vaccine associated thrombosis.

Keywords:

COVID-19 vaccine, heparin-induced thrombocytopenia, platelet factor-4, thrombosis, vaccine-induced immune thrombotic thrombocytopenia

There are various acronyms given to vaccine-associated thrombosis and thrombocytopenia such as vaccine-induced immune thrombotic thrombocytopenia (VITT), thrombosis with thrombocytopenia syndrome (TTS), vaccine-associated (immune) thrombotic thrombocytopenia (VATT) and vaccine-induced prothrombotic immune thrombocytopenia (VIPIT). Both the ChAdOx1 CoV-19 and Ad26.COV2.S vaccines used replication-incompetent adenovirus vectors. Although, the thrombosis and thrombocytopenia is associated with the two specific SARS-CoV-2 vaccines, however, we will use the term VITT, given the temporal relationship of disease with COVID-19 vaccination. These cases occurred within 5 to 30 days of vaccination, usually after the first dose.[1,2] The majority of cases occur in women and individuals younger than 60. The rate of COVID-19 vaccine-induced thrombotic events is around 0.61/million doses, compared to 4 cases / million doses reported by United Kingdom's (UK) regulator and 10 cases/million doses reported by Germany.[3] The risk of VITT occurrence varies substantially between the countries and even between individuals. It may be due to significant differences in the age and sex of those vaccinated and variability in data collection and reporting.[4] VITT is clinically characterised by the occurrence of thrombosis at unusual sites, such as severe cerebral venous sinus thrombosis (CVST), splanchnic venous thrombosis

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or arterial thrombosis. Laboratory examination reveals a consumptive coagulopathy characterised by thrombocytopenia, increased D-dimer levels, and a low fibrinogen levels along with the presence of antibodies against platelet factor 4 (PF4). It may progress rapidly and may portend a poor outcome

Definition of Vaccine-Induced Immune Thrombotic Thrombocytopenia

The Center for Disease Control (CDC) working case definition for TTS following COVID-19 vaccination has been divided into tier-1 and tier-2.[5,6]

Tier-1 TTS is defined as below:
- Presence of thrombosis in an unusual location, including cerebral venous sinuses, portal vein, splenic vein, and other rare venous and arterial thrombosis
- A Platelet count of less than 150,000 per microliter
- A positive (+) heparin anti-PF4 ELISA (supportive, not required).

Tier-1 patients may concurrently have thrombosis in usual locations.

Tier-2 TTS is defined as below:
- Thrombosis of common locations (e.g., venous thromboembolism (VTE), axillary vein thrombosis, deep vein thrombosis [DVT], and pulmonary embolism [PE])
- A platelet count of less than 150,000/μL
- A positive (+) heparin anti-PF4 ELISA (required).

The American Society of Hematology defined TTS by the presence of the following criteria:

Definitive diagnosis (must meet all five criteria):[7]
- COVID vaccine 4–42 days prior to symptom onset
- Any venous or arterial thrombosis (often cerebral or abdominal)
- Thrombocytopenia (platelet count <150 × 109/L)
- Positive PF4 heparin-induced thrombocytopenia ("HIT") ELISA
- Markedly elevated D-dimer (>4 times upper limit of normal).

Patients in the early stage of VITT may present with thrombosis and a normal platelet count. However, they require a continuous assessment for development of thrombocytopenia.

The Thrombosis and Haemostasis Society of Australia and New Zealand (THANZ) has advocated the following features of VITT/TTS (as of June 29, 2021) as below:[8]
- Onset 4–42 days after vaccination
- Thrombosis: Predominant sites – cerebral venous sinus or splanchnic. Other VTE and arterial ischemia also reported
- Thrombocytopenia or falling platelet count (platelets can be normal on presentation but drop within 4–6 h)
- High D-dimer (typically >5 times upper limit of normal)
- Some patients are refractory to standard anticoagulation
- Response to intravenous immunoglobulin (IVIG).

Epidemiology

To date, the following two adenoviral vector-based vaccines have been implicated in causing VITT: ChAdOx1 CoV-19 vaccine (AstraZeneca) and Ad26.COV2.S vaccine (Janssen; Johnson & Johnson). The ChAdOx1 CoV-19 vaccine uses a chimpanzee adenoviral vector and the Ad26.COV2.S vaccine uses a human adenoviral vector. The spike protein code between AstraZeneca and Johnson & Johnson vaccine is also different. However, there are other adenoviral-based COVID-19 vaccines in the market which do not show any association with VITT, e.g., Gam-COVID-Vac or Sputnik V (replication-incompetent adenovirus 26 and adenovirus 5 vector vaccine) and Ad5-based COVID-19 vaccine (CanSino Biologics).[9,10] It has not been reported with mRNA-based COVID-19 vaccine also. VITT may involve both genders but is predominantly a female dominant condition. VITT was first reported on March 2021 by the European Medicines Agency (EMA) in some of the patients vaccinated with ChAdOx1 CoV-19 vaccine (AstraZeneca). It led to the issuance of advisory by the health authorities of several European countries halting its use. However, after risk/benefit analysis, the vaccine was approved again by the EMA.[11] Similarly, the U. S. Food and Drug Administration (FDA) after analyzing the data on thrombotic events associated with the Ad26.COV2.S vaccine (Janssen; Johnson & Johnson) and the severity of the pandemic approved its use with the emergency use authorization clause on April 23, 2021.[12] VITT is a rare but life-threatening side effect of the adenoviral vector-based COVID-19 vaccine.

The *New England Journal of Medicine* published three different studies of vaccine-induced thrombosis from Germany and Austria, Norway, and the UK [Table 1]. A total of 39 cases of thrombosis were reported 5–24 days after administration of the ChAdOx1 nCoV-19 vaccine (AstraZeneca). The characteristic features of thrombosis were its atypical location, female predominance, younger age of involvement (<50 years), and thrombocytopenia. Other features include very high D-dimer levels and a low fibrinogen level. The thrombosis occurred in people with no significant clinical history and, in particular, previous thromboembolic

Table 1: Various studies of vaccination and vaccine-induced immune thrombotic thrombocytopenia development

	Germany and Austria[5]	Norway[6]	UK[7]	USA[8]
Vaccine	ChAdOx1 CoV-19 vaccine (AstraZeneca)	ChAdOx1 CoV-19 vaccine (AstraZeneca)	ChAdOx1 CoV-19 vaccine (AstraZeneca)	Ad26.COV2.S vaccine (Janssen; Johnson and Johnson)
Number of affected persons	11	5	23	12
Days of onset after vaccination	5-16 days	7-10 days	6–24 days	6-15 days
Median age (range)	36 years (22-49)	39 years (32-54)	46 years (21-77)	18-59 years, 11 females <50 years
Female: male	9:2	4:1	14:9	12:0
Thrombosis	CSVT: 9 (81%), PE: 3 (27%), SVT: 3 (27%), Others: 4	CSVT: 4 (80%), SVT: 1	CSVT: 13 (56%), PE: 5, Arterial: 4, SVT: 3, DVT: 3	CVST: 12, Intracerebral hemorrhage: 7, non-CVST thromboses: 8 ICU admission: 10 Intracerebral hemorrhage: 7 Died: 3
Platelet nadir×10^9/liter	8-107	10-70	7-113	9-127
D-dimer	>35 mg/L	1.8-142 mg/L		
Antibodies to PF4	All	All	Positive in 22 patients	11/12
Platelet-activating antibody	All 9	4 of 5 positive	5 of 7 tested	Negative in 8/9 cases
Low fibrinogen level	3/6 (50%)	3/5 (60%)	13/23 (57%)	8/12 (66%)
Fatal	5/11 died	3/5 died	30%	

PE=Pulmonary embolism, DVT=Deep vein thrombosis, SVT=Superficial vein thrombosis, CSVT=Cerebral venous sinus thrombosis, PF=Platelet factor

history. Few of the females were on estrogen replacement therapy. An important point of concern is a very high mortality (about 40% of cases), and it was caused by ischemic brain damage or hemorrhagic transformation of an initial ischemic event due to anticoagulation therapy.

Greinacher *et al*.[1] reported the occurrence of unusual thrombosis and thrombocytopenia after vaccination with the ChAdOx1 CoV-19 vaccine (AstraZeneca) in 11 patients in Germany and Austria. Majority of the patients were women (total 9 patients with a female-to-male ratio of 9:2), and the median age was 36 years (range, 22–49). The thrombotic events occurred 5–16 days postvaccination. Cerebral venous thrombosis (CVT) was the most common location seen in nine patients. Three patients each had splanchnic vein thrombosis and PE, respectively. Five patients also developed disseminated intravascular coagulation (DIC). Six patients had succumbed to death. A total of nine patients were tested strongly positive for antibody to PF4/heparin complex by immunoassay. The platelet activation assay showed strong positivity in the presence of PF4 and independently of heparin. Interestingly, platelet activation was inhibited by high concentrations of heparin, Fc receptor-blocking monoclonal antibody, and IVIG.

Five health-care workers (HCWs) (32–54 years of age) from Norway developed venous thrombosis and moderate-to-severe thrombocytopenia 7–10 days after receiving the first dose of the ChAdOx1 CoV-19 vaccine (AstraZeneca).[2] The five cases occurred in a population of more than 130,000 vaccinated persons (incidence of 1 in 26,000 vaccinated persons). Majority were females (four patients) and 60% died. Both the studies reported no previous use of heparin and the presence of antibodies against PF4. Four patients developed CVST and intracranial hemorrhage (ICH) also and three of them had a fatal disease. One had a splanchnic vein thrombosis. All patients had high levels of antibodies to PF4/polyanion complexes on immunoassays. The optical density (OD) was very high, ranging from 2.9 to 3.8.

Scully *et al*.[13] reported thrombosis and thrombocytopenia in 23 patients in the United Kingdom (UK), 6–24 days after receiving the ChAdOx1 nCoV-19 vaccine. Twenty-two patients developed acute thrombocytopenia and thrombosis and one patient presented with isolated thrombocytopenia and bleeding symptoms. The age range was 21–77, with a female-to-male ratio of 14:9. The main location of thrombosis was the CVT. Antibodies against PF4 were positive in 22 patients, and a fatal outcome was reported in 7 out of 23 patients.

See *et al*.[14] reported a case series of 12 patients with CVST with thrombocytopenia following vaccination with the Ad26.COV2.S vaccine (Janssen/Johnson & Johnson). These cases were identified through the Vaccine Adverse Event Reporting System which is a vaccine safety surveillance program of the CDC and the FDA, United States. All patients were white females with ages ranging from 18 to younger than 60 years. The time from vaccination to symptom onset ranged from 6 to 15 days. The nadir platelet counts were 9 to 127 × 103/μL. Out of the 12 patients with CVST, 7 also had an intracerebral hemorrhage and 8 patients had non-CVST thrombosis. Three patients had a fatal outcome. Eleven patients have tested positive for the heparin-PF4 antibody. A total of three deaths occurred in this cohort. Therefore, VITT is

a very rare and life-threatening side effect of COVID-19 vaccination.

Pottegård *et al*.[15] in a large population-based study from Denmark and Norway reported 59 cases of venous thromboembolic events among 281,264 people who received the ChAdOx1 CoV-19 vaccine compared with 30 events based on the incidence rates in the general population. The standardized morbidity ratio of VTE events was 1.97 (1.50–2.54). Eleven excess events of VTE per 100,000 vaccinations and 2.5 excess events of central venous thrombosis per 100 000 vaccinations were reported within 28 days of the first dose among the vaccine recipients. There was no increase in the rate of overall arterial events. Scully *et al*. reported thrombosis and thrombocytopenia in 23 patients from the UK 6–24 days after receiving the first dose of the ChAdOx1 CoV-19 vaccine. Their laboratory features showed low or normal fibrinogen levels and elevated D-dimer levels. Antibodies to PF4 were detected in 22 patients.

Schulz *et al*.[16] in a descriptive study from Germany estimated the incidence of CVT and other cerebrovascular events after vaccination with BNT162b2, ChAdOx1, and mRNA-1273. Data were collected online from the departments of neurology of university and nonuniversity hospitals in Germany regarding cases of cerebral sinus venous thrombosis, CVT, ischemic stroke, and hemorrhage within 1 month of a COVID-19 vaccination. A total of 62 cases of thrombosis were detected (CVT: 45, primary ischemic stroke: 9, primary ICH: 4, and other events: 4). A fatal outcome was reported in 18.3% of patients. Majority of the events occurred after vaccination with ChAdOx1 (85.5%). Nine events (14.5%) were reported after BNT162b2 vaccination and none after mRNA-1273 vaccination. The overall incidence rate of CVT within 1 month from first-dose administration was 6.5 (95% confidence interval [CI], 4.4–9.2)/100,000 person-years, whereas the incidence of CVT in the general population was 0.22–1.75/100,000 person-years. Therefore, the risk for CVT after ChAdOx1 vaccination is higher than the risk in the general population.

Smadja *et al*.[17] analyzed the data entered into the World Health Organization (WHO) Global Database for Individual Case Safety Reports (VigiBase) between December 13, 2020, and March 16, 2021, and reported the adverse effects following the Pfizer-BioNTech (BNT162b2), Moderna (mRNA-1273), and Oxford-AstraZeneca (ChAdOx1 nCoV-19) vaccine. They reported possible thrombotic complications of 0.21 (95% CI: 0.19–0.22) cases per million person vaccinated-days. This study has evaluated a largely vaccinated population. Moreover, rare reports of thrombotic complications have been observed following the mRNA-based Pfizer-BioNTech and Moderna vaccines.

Chan *et al*.[18] in a meta-analysis evaluated the risk of VITT following ChAdOx1-S recombinant COVID-19 vaccine (AstraZeneca, marketed as Vaxzevira™ and Covishield™) and reported VITT incidence of 0.73/100,000 persons (95% CI: 0.43, 1.23) receiving the first dose. The incidence in the age group of 65 and higher was 0.11/100,000 persons (95% CI: 0.05–0.26]. The incidence was significantly higher among those under age 55: 1.67/100,000 persons (95% CI: 1.30–2.14) in the UK and 5.06/100,000 persons in Norway (95% CI: 2.16, 11.86). The overall risk estimate for VITT following receipt of ChAdOx1-S recombinant COVID-19 vaccine is 1 in 139,000; for age ≥65, it is 1 in 1,000,000, and for age under 55, overall risk varies between 1 in 20,000 and 60,000.

Pathogenesis

VITT is an autoimmune condition mediated by antibodies that recognize platelet-bound PF-4 (also known as CXCL4). The anti-PF4 antibodies directly activate platelets and trigger thrombosis in the arterial and venous circulation.[4] VITT is similar to HIT but is distinct. To understand the pathogenesis of VITT, it is important to discuss HIT first. Both the conditions are characterized by the presence of thrombosis and thrombocytopenia. However, unlike the classic HIT, patients with VITT had no previous exposure to heparin treatment.[19] Moreover, unlike the classic HIT where antibody bound to PF4/heparin complex activates the platelets, it is the antibody to PF4/polyanion complex that stimulates platelets.[20] HIT is the most frequent drug-induced type of thrombocytopenia. It is an immune-mediated disease that occurs following treatment with heparin molecules. It is a prothrombotic type II hypersensitivity reaction and is classically characterized by the development of antibodies against PF4 and the heparin complex.[21] PF4 is a positively charged molecule and one of the most abundant chemokines that is released upon platelet activation from the α-granules of platelets. Heparin being a negatively charged molecule interacts with PF4 molecule in an exclusively charge-dependent manner.[22]

The large heparin–PF4 complexes cause potential conformational changes in the PF4 molecule. It exposes new antigens on the PF4 surface, thereby rendering the PF4 molecules immunogenic and promoting IgG antibody production.[23,24] The heparin/PF-4/antibody immune complex in turn activates the platelets by binding to the Fc receptor (FcRγIIa) present on the platelet surface. Subsequently, there occur intense intravascular platelet activation and aggregation, release of procoagulant factors leading to arterial-venous clotting, and platelet degradation.[25] The immune complexes also activate monocytes, which amplifies the hypercoagulable state in patients with HIT.[26] COVID-19 infection is also

associated with thrombocytopenia. There is a possibility of HIT being undiagnosed in COVID-19 patients. Thrombocytopenia ensues due to the consumption of platelets into thrombi and removal of platelets by the splenic macrophages. Moreover, the immune complex may also cause antibody-mediated endothelial injury and subsequent activation of the coagulation cascade.[25] There is a subset of HIT syndrome known as autoimmune or spontaneous HIT characterized by the development of platelet-activating PF4 antibodies in the absence of prior heparin exposure. It may be triggered by circulating glycosaminoglycans/polyanions. They often develop following infection or surgery. The DIC-like syndrome is more common in the autoimmune HIT. Clinical syndromes associated with autoimmune HIT include delayed-onset HIT, persistent/refractory HIT, and heparin "flush"-induced HIT. There are few nonheparin triggers such as fondaparinux-associated HIT, viral/bacterial infection, and following orthopedic surgery.[27] The course of autoimmune or spontaneous HIT may be prolonged and VITT resembles autoimmune HIT. Liu *et al*.[28] in a study of COVID-19 patients hospitalized in China reported a positive PF4/heparin ELISA in patients who had not been exposed to heparin. The most frequent types of thrombosis in HIT are DVT, PE, and arterial thrombosis.[29]

VITT is mediated by pathological IgG antibodies bound to PF4. The complex, in turn, causes uncontrolled platelet activation resulting in thrombosis and thrombocytopenia. The mechanisms of this antibody formation are not entirely clear. The pathophysiology of VITT is similar to HIT with few salient differences. Both conditions are characterized by high levels of anti-PF4 antibodies. Clinically, both HIT and VITT develop thrombosis and thrombocytopenia. However, unlike HIT, patients with VITT have not had previous exposure to heparin and their platelet reactivity is heparin independent [Table 2].[30] Additionally, platelet activation is also inhibited by heparin.[31] Huynh *et al*.,[30] using alanine-scanning mutagenesis, found that the anti-PF4 antibodies from patients with VITT were attached to eight surface amino acids that are located within the heparin-binding site. This binding is inhibited by heparin. The anti-PF4 antibodies in VITT also had a stronger binding response to PF4 and PF4–heparin complexes compared to anti-PF4 antibodies in HIT. Therefore, the anti-PF4 antibodies in VITT, by binding to a similar site on PF4, mimic the effect of heparin and cause the formation of PF4 tetramers which subsequently activate platelets in the absence of heparin. The IgG antibodies to PF4 subsequently lead to uncontrolled activation of platelets via the low-affinity FcγIIa receptor (FcRγIIa: these receptors bind with the Fc portion of IgG), release of prothrombotic materials from the activated platelets, and clot formation, often in unusual sites such as cerebral venous sinuses, splanchnic veins, and portal veins. VITT clinically mimics autoimmune HIT (autoimmune HIT) as the autoantibodies against PF4 can directly activate platelets in the absence of heparin.[13]

How the vaccines promote antibody formation is unknown. McGonagle *et al*.[32] hypothesized that the first step in VITT development is local tissue microtrauma, local microbleeding, and immune cell activity following vaccine inoculation that brings adenoviral DNA in contact with PF4. Subsequently, the complex is engulfed by the antigen-presenting cells and then memory B-cells in the regional lymph nodes, with substantially increased PF4 autoantibody production in susceptible subjects. Unlike the short-lived PF4 autoantibody responses in HIT, the antibody response in VITT is persistent and could be associated with T-cell response. The portal and cerebral vein circulation are preferentially involved as an extensive PF4 viruses/microbiota interaction already exists in these areas to ensure the normal immunity and antigen clearance. PF4 is secreted by the activated platelets in response to microbial invasion and acts as an antibacterial agent via the following mechanisms: released PF4 recruits neutrophils and facilitates neutrophil exocytosis to release myeloperoxidase and lysozyme.[33] Moreover, PF4 directly binds to bacteria, thus creating a neoantigen. The anti-PF4/heparin or anti-PF4/polyanion antibodies form immune complexes and help in bacterial clearance.[34]

Another mechanism involves interaction between platelets and SARS-CoV-2 spike proteins produced after vaccination. The SARS-CoV-2 can directly activate platelets via interactions between the spike protein and the angiotensin-converting enzyme 2 (ACE2) receptors expressed on platelet surfaces.[24] The possibility of cross-reactivity between antibodies to adenovirus components in the vaccine with PF4 has also been proposed that may trigger subsequent platelet activation and thrombus formations.[24,35]

Table 2: Features of heparin-induced thrombocytopenia and auto-immune heparin-induced thrombocytopenia

	HIT	Auto-immune HIT
Heparin exposure	Yes	No
Screening assay	Antibody to PF-4 by ELISA	Antibody to PF-4 by ELISA
Platelet activation	Enhanced by low levels of heparin, blocked by high levels of heparin	Independent of low levels of heparin, blocked by high levels of heparin
Confirmatory platelet activation assay	Heparin dependent	Heparin independent

ELISA=Enzyme-linked immunosorbent assay, PF=Platelet factor

Kowarz *et al*.[36] reported synthesis of secreted spike protein variants due to the alternative splice events and these spike protein variants initiate thrombosis by binding to ACE2-expressing endothelial cells in blood vessels. Normally, the spike proteins are membrane bound. The authors proposed the term "Vaccine-Induced COVID-19 Mimicry" syndrome (VIC19M syndrome) due to its similarity with SARS-CoV-2 virus pathogenicity mediated by the spike protein during natural infection. The binding of spike protein variants to ACE2-expressing endothelial cells may trigger cell damage via antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity (CDC). One hypothesis explaining the cerebral venous sinus predilection of thrombosis is the preferential accumulation of soluble spike protein variants in the cerebral venous sinuses with a nonunidirectional blood flow.

Greinacher *et al*.[37] proposed the following mechanisms for VITT. Initially, the ChAdOx1 nCoV-19 vaccine activates platelets by interaction with adenovirus, cell culture-derived unknown proteins, and ethylenediaminetetraacetic acid (EDTA), which is a vaccine constituent. The activated platelets subsequently release PF4. The released PF4 binds to virus proteins and other constituents of the vaccine. The EDTA molecules, by causing capillary leakage, promote blood dissemination of vaccine and human proteins. Moreover, the spike proteins may also cause endothelial damage and shedding of glycosaminoglycans.[38] The PF4/polyanionic complexes subsequently activate B-lymphocytes to secrete antibodies to the PF4/polyanionic complex and lead to thrombosis and thrombocytopenia. These antibodies, although triggered by complexes of the vaccine constituents and PF4, develop broader specificity and then act as autoantibodies. They no longer require the vaccine constituent for binding.[37] This is similar to autoimmune HIT developed after surgery where the antibodies do not require heparin to activate the platelets.[27]

Greinacher *et al*.[39] subsequently proposed a two-stage mechanism of VITT developments. Early reactions (days 1–2) are triggered by complexes formed by vaccine components and PF4 molecules, creating neoantigens. An inflammation-induced danger signal also sets in which amplify the anti-PF4 antibody production. Late reactions (days 5–20) involve PF4/vaccine-induced B-lymphocyte activation and subsequent secretion of high-titer anti-PF4 autoantibodies that bind to and activate platelets leading to thrombosis and thrombocytopenia. Moreover, the anti-PF4 antibodies also activate neutrophils to release procoagulant neutrophil extracellular traps (NETs) that also promote thrombosis.

Kadkhoda proposed a high-level adenoviremia following accidental injection of vaccine into the blood as the initial mechanism. Subsequently, replication-deficient adenoviruses infect the cells and secrete large amounts of soluble spike glycoproteins into the blood.[40] The spike glycoproteins can activate platelets and promote thrombosis.[41] Spike proteins, by binding to ACE2, cause intracellular trapping of ACE2. Subsequently, the membrane-bound ACE2 concentrations are reduced, leading to increased thrombotic events.[42] Greinacher *et al*.[43] recently noted that anti-PF4 antibodies from patients with VITT do not cross-react with the spike protein, indicating that the vaccine-induced immune response against SARS-CoV-2 spike protein is not the trigger of VITT and molecular mimicry between spike protein and PF4 epitopes is not possible. A report of the role of dysregulated activation of the complement system has also been proposed. However, it lacks enough supporting evidence at present.[44]

Clinical Presentation

Development of the following symptoms 5–30 days after vaccination should lead to the suspicion of VITT and necessary medical evaluation should be done immediately.[45] Patients with suggestive clinical symptoms of VITT should promptly undergo necessary investigations to rule out thrombotic events and the presence of thrombocytopenia. The triad in the diagnosis of VITT is the presence of thrombus often in unusual locations, thrombocytopenia, and confirmation of thrombus by imaging, surgical procedure, and pathology.

Following are the presenting symptoms:

- Headache: New onset, severe, or persistent. The headache is not relieved by usual painkillers or is getting worse. It worsens on lying down or bending over. It may be accompanied by blurred vision, nausea, and vomiting, visual changes, or any new neurological symptoms[46]
- Severe persistent abdominal pain, nausea, and vomiting
- Backache
- Chest pain and/or shortness of breath
- Leg pain or swelling
- Petechiae and easy bruising.

The VITT is predominantly venous and is characterized in majority cases by the unusual locations of the thrombosis such as cerebral venous sinus, splanchnic vein (splenic vein, portal vein, hepatic vein, and mesenteric veins), adrenal veins, and ophthalmic veins. Thrombosis may also occur in typical locations such as PE or DVT in the leg. Cerebral and peripheral arteries

may also be involved. Sudden death may occur due to PE, intracerebral hemorrhage, and coronary artery disease. A subset of patients developed the entity known as DIC. It is characterized by very high levels of D-dimer and low fibrinogen levels.

Differential Diagnosis

The various differential diagnosis of VITT is shown in Table 3.

1. SARS-CoV-2 infection: Fevers and other systemic features. Usually, they develop mild thrombocytopenia. Thrombosis has been reported in both venous and arterial systems and also in atypical locations such as CVT. PF4 antibody testing is negative
2. Thrombotic thrombocytopenic purpura: Microangiopathic hemolytic anemia (anemia, high lactate dehydrogenase, and bilirubin), high reticulocyte count, and schistocytes. Patients develop microvascular thrombosis. ADAMTS13 activity is reduced
3. Atypical hemolytic uremia syndrome: Renal failure, schistocytes, and normal ADAMTS13 activity
4. Antiphospholipid antibody syndrome: Antiphospholipid antibody, thrombosis, and thrombocytopenia
5. DIC: Systemic illness, high D-dimer, and decreased fibrinogen level
6. HIT: It occurs following heparin exposure. Anti-PF4/heparin antibodies are present. Thrombocytopenia and thrombosis occur.

Laboratory Investigations

Suspected patients of VITT should have platelet counts, D-dimer, and fibrinogen level estimation initially. Platelet counts of <150 000 platelets/μL and D-dimer >5 times the upper limit of normal ± low fibrinogen level should proceed for VITT testing and symptom-directed imaging. If the patients have a high D-dimer but normal platelet count initially, the platelet count should be repeated within 24 h or earlier as there can be rapid deterioration.[47] Diagnosis of VITT is based on typical clinical features in postvaccinated patients coupled with laboratory abnormalities. The diagnosis of VITT is confirmed if the PF4 ELISA test is positive in the appropriate clinical context, typically with an OD>2.00, or by a positive functional assay. However, the presence of thrombosis and/or thrombocytopenia in a post-COVID-19 vaccine recipient who has no prior history of proximate heparin exposure should be the ideal clinical context for considering VITT, even in the absence of laboratory confirmation. The PF4 ELISA test or the other assays may not be available in many centers or, if available, may take time for the results. In a suspected case of VITT, appropriate treatment should be initiated such as the use of the nonheparin agent for anticoagulation and IVIG till the laboratory report is available.[10] Complete blood count with platelet count should be done in all patients. Thrombocytopenia is a characteristic feature of VITT. It is defined as a platelet count below 150,000 platelets/μL or a decrease of 50% from a previous platelet count, provided that this value is known and measurement was reasonably recent (previous 3 months). In the event of

Table 3: Differential diagnosis of vaccine-induced immune thrombotic thrombocytopenia

	VITT	TTP	ITP
Thrombosis	Arterial, venous thrombosis often in an unusual location	Yes, microvascular thrombosis	Rare
Thrombocytopenia	8000-127,000/μL	Yes	Yes
Background history	Temporal relationship with adenovirus-based COVID-19 vaccine	Neurologic, kidney, and/or cardiac involvement may be seen.	Petechiae or purpura. Often an incidental finding
Laboratory abnormalities	D-dimer: often markedly raised, thrombocytopenia, normal or low Fibrinogen and a normal to slightly prolonged PT and aPTT	Normal PT and aPTT Normal fibrinogen and D-dimer	Normal PT and aPTT, normal fibrinogen and D-dimer. Normal hemoglobin (unless anemia from bleeding)
Microangiopathic hemolytic anemia and markers of hemolysis	No	Microangiopathic hemolytic anemia, schistocytes on the blood smear. Absence of schistocytes may argue against the diagnosis of TTP	No
Pathogenesis	Autoimmune, development of pathologic PF-4 antibody	Severe ADAMTS13 deficiency	Autoimmune mediated
Confirmation	Positive antibody to PF-4 ELISA or functional platelet assay	ADAMTS13 deficiency (activity <10%)	By exclusion
Management	Avoid heparin (UFH/LMWH) as they worsen disease. Avoid platelet and plasma transfusions. Use nonheparin anticoagulant, IVIG, and prednisolone	Therapeutic plasma exchange, glucocorticoids, rituximab, and caplacizumab in selected cases. Avoid platelet transfusions unless major bleeding	Platelet transfusions for critical bleeding. Glucocorticoids or IVIG for serious bleeding or severe thrombocytopenia. Rituximab, splenectomy

VITT=Vaccine-induced immune thrombotic thrombocytopenia, TTP=Thrombotic thrombocytopenic purpura, ITP=Immune thrombocytopenia, PT=Prothrombin time, aPTT=Activated partial thromboplastin time, ELISA=Enzyme-linked immunosorbent assay, UFH=Unfractionated heparin, LMWH=Low molecular weight heparin, IVIG=Intravenous immunoglobulin, PF=Platelet factor

thrombocytopenia, it is recommended to perform a blood smear to rule out pseudo-thrombocytopenia as a result of platelet clumping.[48] Coagulation abnormalities in VITT include a normal or mildly increased prothrombin time (PT), normal or mildly increased international normalized ratio (INR), and a normal or mildly increased activated partial thromboplastin time (aPTT). The D-dimer level is characteristically very high with the majority of the patients had a level above 4000 μg/L. A low fibrinogen level may be seen due to consumptive coagulopathy suggestive of DIC.

PF4 Antibody Assay

All patients with suspected VITT should be screened for PF4–heparin antibodies using the Enzyme-linked immunosorbent assay (ELISA). ELISA is the most sensitive method of detecting VITT antibodies. Before initiating any treatment, especially IVIG and danaparoid, samples should be collected into serum or plasma tubes based on the requirement of the testing facility for VITT testing as IVIG may cause a false-negative result.[49,50] OD is used to define ELISA reactivity and is graded as follows: strong (≥2.00), intermediate (1.00–1.99), and weak (0.50–0.99).[1] A negative PF4-binding assay rules out HIT or VITT. ELISA appears most sensitive in detecting PF4 antibody and other assays including chemiluminescence assays or particle centrifugation assays which are not reliable in detecting antibodies to PF4/heparin in VITT patients.[49,51] Different PF-4 conjugates have been used such as PF4/heparin, PF4/polyanion complex such as polyvinyl sulfate (PVS). Platton *et al*.[51] compared the sensitivity of four IgG-specific ELISA, two polyspecific ELISA, and four rapid assays performed on samples from 43 patients with suspected VITT from across the UK. They observed a poor sensitivity of rapid assays for detecting VITT compared to ELISA. Moreover, ELISA methods also failed to detect all possible/probable VITT cases. Therefore, in a patient with a negative ELISA test, a second ELISA or a platelet activation assay should be considered if the clinical suspicion is high. Schultz *et al*.[2] reported very high OD values in the range of 2.9–3.8, and in the absence of proximate heparin exposure, these high levels of OD are sufficient to confirm the diagnosis. About 4.3%–6.6% of blood donors have detectable PF4–heparin antibodies, however, the OD rarely goes above 1.6.[52]

Functional Platelet Activation Assay

If the PF4-binding assay is positive or the PF4-binding assay is not available, or the ELISA is negative or equivocal in a patient in whom VITT is strongly suspected, the sample should be subjected to one or multiple functional platelet activation assays.

- Serotonin release assay (SRA)
- Heparin-induced platelet activation assay
- Platelet aggregation test
- Heparin-induced multiple electrode aggregometry
- PF4-dependant P-selectin expression assay
- PF4-SRA
- PF4/heparin-SRA.

Serotonin Release Assays

Serotonin is one of the constituents of platelet dense granules, and measurement of the percentage release of serotonin can be used to quantitate the magnitude of platelet activation induced by the antibody to PF4.[53] SRA assays usually use 0.1 U/mL and 100 U/mL UFH concentration. The therapeutic concentrations (0.1 U/mL) support HIT immune complex and the supratherapeutic concentrations (0.1 U/mL) disrupt HIT immune complex. The overall interpretation is based on the response at both concentrations. The positive assay is defined by high percentage release (≥20%) in low heparin concentration and low percentage release (<20%) in high heparin concentration. The functional platelet activation assay may not be positive in all suspected VITT cases even if the PF4 ELISA is positive.[13] Adding exogenous PF4 enhances the detectability in the platelet activation assay, and recent use of IVIG may inhibit platelet activation by patients' antibodies.[1] Therefore, the sample for testing the antibody to PF4 or functional assay should be sent before starting treatment. Sørvoll *et al*.[54] evaluated the seroprevalence of thrombocytopenia and anti-PF4/polyanion antibodies in 492 HCWs recently vaccinated with the first dose of AZD1222 (ChAdOx nCoV-19) vaccine. The median age was 44 (21–69) and 76% were females. Six HCWs had anti-PF4/polyanion antibodies with OD values over cutoff ≥0.4 and all had normal platelet counts. A total of eight subjects had reduced platelet counts (all above 100×10^9/L). None of them had a clinically identified thrombosis. Therefore, the seroprevalence of anti-PF4/polyanion antibodies is low among vaccinated persons.

Imaging study

The presence of thrombosis at various sites or thromboembolism may need confirmation by the imaging studies. Imaging study should be signs and symptoms directed.

- Headache/vision changes or other neurological signs/symptoms: Magnetic resonance venography/magnetic resonance imaging or computed tomography (CT) venography
- Abdominal pain: CT with contrast/Doppler US
- Chest pain, shortness of breath, and tachycardia: CT pulmonary angiography, perfusion V/Q scan, and echocardiography
- Leg pain: Venous duplex US
- Leg ischemia: CT angiography.

Other Patterns of Laboratory Abnormalities

The patient may present with thrombocytopenia and bleeding, however, the coagulation parameters (PT, APTT, fibrinogen, and D-dimer levels) are normal. It is unlikely to be VITT and may indicate immunization-associated ITP. Management depends on the bleeding risk and may require administration of high-dose IVIG without anticoagulation. Patients may also present with thrombocytopenia and abnormal coagulation parameters (at least one of: PT, aPTT, fibrinogen, and D-dimer, especially with dynamic change) but without bleeding or thrombosis. This may occur in early VITT syndrome and management is similar to full-blown VITT syndrome. Patients may also have isolated thrombocytopenia without bleeding or thrombosis or abnormal coagulation parameters. It may be vaccine-associated isolated thrombocytopenia or primary ITP. Figure 1 shows the diagnostic modalities in VITT.

Treatment

A high degree of clinical suspicion should be kept in any patients who presented with symptoms of thrombosis (particularly in unusual locations) and thrombocytopenia after recent administration of the ChAdOx1 CoV-19 (AstraZeneca) and Ad26. COV2.S (Janssen; Johnson & Johnson) vaccine. For patients in whom the diagnosis of VITT is likely, therapy should be started without waiting for the laboratory results of PF4 antibody as the turnaround time of PF4 antibodies testing may be longer. Hematologist consultation should be taken wherever available. Occasionally, low platelet counts may lead to hemorrhagic transformation. Therefore, high-dose IVIG and/or corticosteroids should be used initially to rectify the low platelet counts before initiating the anticoagulation therapy. The patient should be admitted to an intensive care or high dependency unit for better monitoring.[55]

Anticoagulation

Confirmed cases of VITT should receive therapeutic dose anticoagulation. Anticoagulation with heparins should be avoided and alternative HIT compatible anticoagulants should be used until autoimmune HIT is ruled out as the cause of acute thrombocytopenia/thrombosis. Non-heparin anticoagulation such as parenteral direct thrombin inhibitor (Bivalirudin, Argatroban), direct oral anticoagulants (DOACs), fondaparinux or danaparoidshould be used.The DOACs include factor Xa inhibitor (Apixaban, Rivaroxaban etc) or direct thrombin inhibitor (Dabigatran) (Table-4).[56,57] The dose of the anticoagulant treatment should be adjusted based on the platelet counts. When fibrinogen level and platelet counts are more than 1.5 g/L and 50 × 10^9 /L respectively, consider starting non-heparin anticoagulation in patients without history of serious bleeding. If anticoagulation is needed before then, critical illness dose argatroban can be considered, initially without dose escalation and maintained at a low dose. The duration of anticoagulants should be a minimum of three months after normalization of platelets counts and no further thrombosis occurs. Patients without thrombosis should also receive anticoagulation 4–6 weeks after recovery of platelet counts.[10] Postdischarge, patients can be switched over to DOACs or warfarin only after recovery of platelet counts.[10] VITT-associated DIC may cause prolongation of aPTT known as "aPTT confounding." aPTT-adjusted anticoagulants such as argatroban and bivalirudin may show frequent treatment failure due to underdosing.[27]

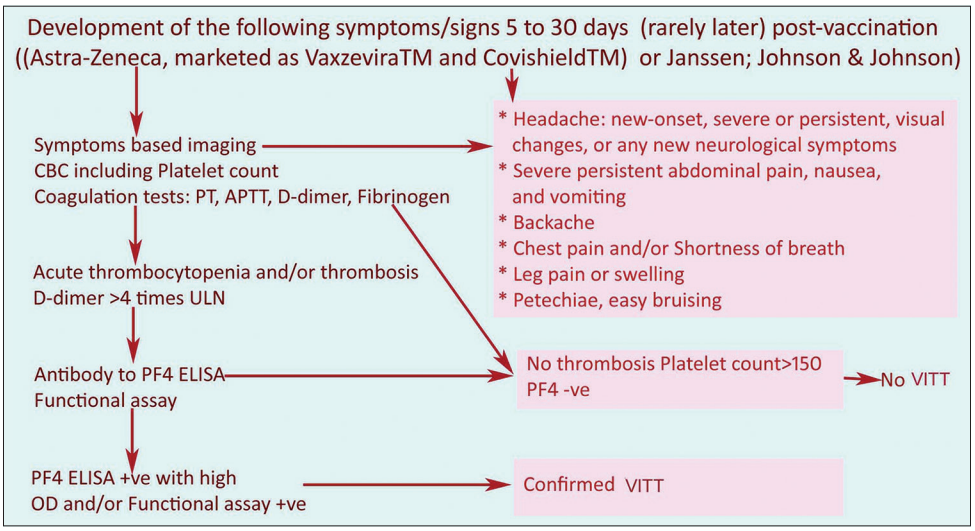

Figure 1: Showing diagnostic approach to VITT

Table 4: Various anticoagulants, their mechanisms of action and treatment duration

Drug	Dosage	Mechanism of actions	Treatment duration	Remarks
Rivaroxaban po	15 mg twice daily with food for three weeks; then 20 mg once daily with food	Direct factor Xa inhibitors	At least 3 months	May be considered in less severe patients, with no active bleeding and platelet count >50,000/μL
Apixaban po	10 mg twice daily for 1 week, then 5 mg twice daily	Direct factor Xa inhibitors	At least 3 months	To be considered in less severe patients, with no active bleeding and platelet count >50,000/μL
Dabigatran po	Parenteral anticoagulation for 5-10 days; then dabigatran 150 mg twice daily	Direct factor Xa inhibitors	At least 3 months	
Bivalirudin IV	0.75 mg/kg intravenously as a bolus followed by 1.75 mg/kg per hour during a procedure	Direct thrombin inhibitor and hirudin analog	Up to 3 months or switch to oral agents if stable at the time of discharge	aPTT with a target of 1.5-2.5 times the normal range
Argatroban IV	Adult patients: 2 μg/kg/min administered as a continuous intravenous infusion. Lower doses are used in critically ill patients	Direct thrombin inhibitor	≤14 days	aPTT: target range of 1.5-3 times of initial baseline, not to exceed 100 s
Fondaparinux SC	Weight-based dosing: Weighing <50 kg: 5 mg once daily Weighing 50-100 kg: 7.5 mg once daily Weighing >100 kg: 10 mg once daily subcutaneously	Selective Factor Xa Inhibitors	Up to 3 months or switch to oral agents if stable at the time of discharge	50% dose in case of platelet count <30,000/μL. Reduce dosing with severe renal impairment

aPTT=Activated partial thromboplastin time

Immunosuppression

IVIG should be used immediately at a dose of 0.5 to 1 g/kg daily for 2 days. IVIG competitively inhibits the interaction of anti-PF4 antibodies with the platelet FcγIIa receptors, thereby reducing platelet activation. Bourguignon *et al*.[58] published a series of three individuals with TTS and reported that administration of high-dose IVIG was associated with rapid increases in platelet counts, no new thrombosis, and reduced antibody-mediated platelet activation. Steroids such as prednisolone should be used at a dose of 1-2 mg/kg if the platelet count is less than 50,000/μl or if the IVIG is delayed.[7,45] The management of CVST requires anticoagulation even in the presence of ICH.[59] Stam *et al*.[60] in a meta-analysis that included data from two small trials involving 79 patients observed that anticoagulant treatment for cerebral sinus thrombosis appeared to be safe and was associated with a potentially important reduction in the risk of death or dependency, although statistically not significant. However, the therapeutic decision of administering anticoagulants must be individualized and a risk/benefit analysis should be done before advising anticoagulants.

Transfusions

The WHO recommends against the use of platelet transfusions in patients with VITT. However, platelet transfusions may be required in a situation such as emergent neurosurgery and the platelet count is <50,000/μL.[61] If the platelet count is <50,000/μL, treat thrombocytopenia first with IVIG and dexamethasone before starting anticoagulation therapy. Plasmapheresis or plasma exchange may be considered in patients with severe thrombocytopenia, unresponsive to the IVIG and steroids.[55]

Fibrinogen Correction

For patients who are actively bleeding and the plasma fibrinogen level is <1.5 g/L, correct fibrinogen levels with fibrinogen concentrate or cryoprecipitate to ensure a level above 1.5 g/L.[62] Fresh frozen plasma may be used in the early stages. The thrombopoietin receptor agonists and antiplatelet agents should be avoided. Patients should be monitored regularly during hospitalization and postdischarge, particularly to assess for the signs/symptoms of thrombosis and platelet counts. Platelets counts can drop once the effects of IVIG are gone and also need regular monitoring. Other coagulation parameters if abnormal should also be monitored.[7] Patients can be discharged once the platelet count is >50,000/μL and is improving steadily with no bleeding episode for at least 2–3 days and the patient is on stable anticoagulation with no new or progressive thrombosis. Bruton tyrosine kinase (Btk) inhibitor has a potential pleiotropic effect on FcγRIIA-mediated downstream signaling and thereby may cause inhibition of platelet aggregation, dense granule secretion, P-selectin expression, and platelet-neutrophil aggregate formation stimulated by FcγRIIA cross-linking. Moreover, Btk inhibitors may further inhibit C-type lectin-like receptor CLEC-2- and GPIb-mediated platelet activation, activation of monocytes, and the release of NETs. Smith *et al*.[63] had shown that platelet aggregation in serum of VITT patients was inhibited by Btk inhibitors. However, this potential medicine needs further study.

Finally, individuals who had developed VITT following adenoviral vector vaccinations should avoid subsequent adenoviral vector-based vaccination. They should use COVID-19 vaccine based on mRNA technology for the second dose or booster dose. The second dose should only be considered once the platelet count and D-dimer concentration are stable and within normal range, and the patient is fully anticoagulated.[4] There is no evidence supporting any screening laboratory or imaging evaluations in asymptomatic individuals before or after vaccination.[64] Underlying thrombotic risk factors including inherited thrombophilia, history of prior thrombosis, or prior HIT is not a contraindication to vaccination with any type of vaccine. However, individuals are at liberty to decide a nonadenoviral COVID-19 vaccine out of caution.

Conclusion

VITT is a rare, life threatening prothrombotic syndrome that occurs following vaccination with adenoviral vector based vaccines. VITT is characterized by thrombosis, particularly in unusual locations and thrombocytopenia. It is a rare complication of COVID-19 vaccination. It is distinct from the HIT syndrome. Pathogenesis of VITT includes autoantibodies to PF4 which activate platelets and cause thrombosis. VITT is rare but severe, with rapid progress and a high mortality rate. Treatment includes alternative anticoagulants and IVIG. The benefits of COVID-19 vaccines outweigh the risk of VITT and should be offered to all eligible persons. However, it is of utmost importance that all HCWs should be trained for early detection and proper management of the rare side effects of VITT. Early recognition, appropriate testing, and proper management are essential for a better outcome. The pathogenesis and monitoring of VITT requires further study.

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Conflicts of interest
There are no conflicts of interest.

References

1. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med 2021;384:2092-101.
2. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, *et al*. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. N Engl J Med 2021;384:2124-30.
3. Available from: https://health.odisha.gov.in/pdf/Advisory-diagnosis-treatment-Thrombosi s-Thrombocytopenic-26052021.pdf. [Last accessed on 2021 Aug 03].
4. Klok FA, Pai M, Huisman MV, Makris M. Vaccine induced immune thrombotic thrombocytopenia. Lancet Haematol 2022 Jan;9(1):e73-e80.
5. Available from: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-04-23/03-COVID-Shimabukuro-508.pdf. [Last accessed on 2021 Aug 03].
6. CDC Update: Thrombosis with Thrombocytopenia Syndrome (TTS) following COVID 19 Vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021 Tom Shimabukuro, MD, MPH, MBA CDC COVID 19 Vaccine Task Force Vaccine Safety Team. https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf. [Last accessed on 2021 Aug 03].
7. Bussel JB, Connors JM, Cines DB, Dunbar CE, Michaelis LC, Kreuziger LB, *et a*l. Thrombosis with Thrombocytopenia Syndrome (Also Termed Vaccine Induced Thrombotic Thrombocytopenia) (Version 1.6). Last updated on 2021 Aug 12]. https://www.hematology.org/covid-19/vaccine-induced-immune-thrombotic-thrombocytopenia. [Last accessed on 2021 Aug 03].
8. Available from: https://www.health.gov.au/news/joint-statement-from-atagi-and-thanz-on-thrombosis-with-thrombocytopenia-syndrome-tts-and-the-use-of-covid-19-vaccine-astrazeneca. [Last accessed on 2021 Aug 03; Last updated on 2021 May 23].
9. Connors M, Graham BS, Lane HC, Fauci AS. SARS-CoV-2 vaccines: Much accomplished, much to learn. Ann Intern Med 2021;174:687-90.
10. Warkentin TE, Cuker A. COVID-19: Vaccine-Induced Immune Thrombotic Thrombocytopenia (VITT). Available from: https://www.uptodate.com/contents/COVID-19-vaccine-induced-immune-thrombotic-thrombocytopenia-vitt#:~: text=Definitions%20and%20pathophysiology %20%E2%80%93% 20Vaccine%2Dinduced, receipt%20of%20one%20of%20two. [Last accessed on 2021 Jun 28].
11. European Medicines Agency Pharmacovigilance Risk Assessment Committee (PRAC). Signal Assessment Report on Embolic and Thrombotic Events (SMQ) with COVID 19 Vaccine (ChAdOx1 S [Recombinant]) – Vaxzevria (Previously COVID 19 Vaccine AstraZeneca) (Other Viral Vaccines); 2021. Available form: https://www.ema.europa.eu/en/documents/ prac recommendation/signal assessment report embolic thrombotic events smq COVID 19 vaccin chadox1 s recombinant_en.pdf. [Last accessed on 2021 Aug 03].
12. U.S. Food and Drug Administration. Janssen COVID 19 Vaccine; April 23, 2021. Available form: https://www.fda. gov/emergency preparedness and response/coronavirus disease 2019 COVID 19/janssen COVID 19 vaccine. [Last accessed on 2021 Jun 28; Last accessed on 2021 May 02].
13. Scully M, Singh D, Lown R, Poles A, Solomon T, Levi M, *et al*. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. N Engl J Med 2021;384:2202-11.
14. See I, 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;325:2448-56.
15. Pottegård A, Lund LC, Karlstad Ø, Dahl J, Andersen M, Hallas J, *et al*. Arterial events, venous thromboembolism, thrombocytopenia, and bleeding after vaccination with Oxford-AstraZeneca ChAdOx1-S in Denmark and Norway: Population based cohort study. BMJ 2021;373:n1114.
16. Schulz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, *et al*. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. Ann Neurol 2021;90:627-39.
17. Smadja DM, Yue QY, Chocron R, Sanchez O, Lillo-Le Louet A. Vaccination against COVID-19: insight from arterial and venous thrombosis occurrence using data from VigiBase. Eur Respir J. 2021;58(1):2100956.
18. Chan B, Bobos P, Odutayo A, Pai M. Meta analysis of risk of vaccine induced immune thrombotic thrombocytopenia following ChAdOx1 S recombinant vaccine. medRxiv [doi:

10.1101/2021.05.04.21256613].
19. Franchini M, Liumbruno GM, Pezzo M. COVID-19 vaccine-associated immune thrombosis and thrombocytopenia (VITT): Diagnostic and therapeutic recommendations for a new syndrome. Eur J Haematol 2021;107:173-80.
20. Toschi V. Thrombosis secondary to COVID-19 vaccination: A real clinical risk or a lot of ado about nothing? G Ital Cardiol 2021;22:448-51.
21. Lee GM, Arepally GM. Heparin-induced thrombocytopenia. Hematology Am Soc Hematol Educ Program 2013;2013:668-74.
22. Bock PE, Luscombe M, Marshall SE, Pepper DS, Holbrook JJ. The multiple complexes formed by the interaction of platelet factor 4 with heparin. Biochem J 1980;191:769-76.
23. Greinacher A. Heparin-induced thrombocytopenia. N Engl J Med 2015;373:1883-4.
24. Chen PW, Tsai ZY, Chao TH, Li YH, Hou CJ, Liu PY. Addressing vaccine-induced immune thrombotic thrombocytopenia (VITT) following COVID-19 vaccination: A mini-review of practical strategies. Acta Cardiol Sin 2021;37:355-64.
25. Ahmed I, Majeed A, Powell R. Heparin induced thrombocytopenia: Diagnosis and management update. Postgrad Med J 2007;83:575-82.
26. 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.
27. Greinacher A, Selleng K, Warkentin TE. Autoimmune heparin-induced thrombocytopenia. J Thromb Haemost 2017;15:2099-114.
28. Liu X, Zhang X, Xiao Y, *et al*. Heparin induced thrombocytopenia is associated with a high risk of mortality in critical COVID 19 patients receiving heparin involved treatment. medRxiv 2020. Available from: https://www.medrxiv. org/content/10.1101/2020.04.23.20076851v1.full#F4. DOI: 10.1101/2020.04.23.20076851.
29. Watson H, Davidson S, Keeling D; Haemostasis and Thrombosis Task Force of the British Committee for Standards in Haematology. Guidelines on the diagnosis and management of heparin-induced thrombocytopenia: Second edition. Br J Haematol 2012;159:528-40.
30. Huynh A, Kelton JG, Arnold DM, Daka M, Nazy I. Antibody epitopes in vaccine-induced immune thrombotic thrombocytopaenia. Nature 2021;596:565-9.
31. 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.
32. McGonagle D, De Marco G, Bridgewood C. Mechanisms of immunothrombosis in vaccine-induced thrombotic thrombocytopenia (VITT) compared to natural SARS-CoV-2 infection. J Autoimmun 2021;121:102662.
33. Cai Z, Greene MI, Zhu Z, Zhang H. Structural features and PF4 functions that occur in heparin-induced thrombocytopenia (HIT) complicated by COVID-19. Antibodies (Basel) 2020;9:E52.
34. Krauel K, Weber C, Brandt S, Zähringer U, Mamat U, Greinacher A, *et al*. Platelet factor 4 binding to lipid A of Gram-negative bacteria exposes PF4/heparin-like epitopes. Blood 2012;120:3345-52.
35. Rzymski P, Perek B, Flisiak R. Thrombotic thrombocytopenia after COVID-19 vaccination: In search of the underlying mechanism. Vaccines (Basel) 2021;9:559.
36. Kowarz E, Krutzke L, Reis J, Bracharz S, Kochanek S, Marschalek R. "Vaccine induced COVID 19 mimicry" syndrome: Splice reactions within the SARS CoV 2 Spike open reading frame result in Spike protein variants that may cause thromboembolic events in patients immunized with vector based vaccines. Res Sq 2021 [doi: 10. 21203/rs. 3.rs 558954/v1].
37. Greinacher A, Selleng K, Wesche J, Handtke S, Palankar R, Aurich K, *et al*. Towards understanding ChAdOx1 nCov-19 vaccine immune thrombotic thrombocytopenia (VITT). Preprint. Research Square. 10.21203/rs.3.rs440461/v1. Posted 20 April 2021.
38. Kantarcioglu B, Iqbal O, Walenga JM, Lewis B, Lewis J, Carter CA, *et al*. An update on the pathogenesis of COVID 19 and the reportedly rare thrombotic events following vaccination. Clin Appl Thromb Hemost 2021;27:1-14.
39. Greinacher A, Selleng K, Palankar R, Wesche J, Handtke S, Wolff M, *et al*. Insights in ChAdOx1 nCoV-19 vaccine-induced immune thrombotic thrombocytopenia. Blood 2021;138:2256-68.
40. Kadkhoda K. Post-adenoviral-based COVID-19 vaccines thrombosis: A proposed mechanism. J Thromb Haemost 2021;19:1831-2.
41. Bye AP, Hoepel W, Mitchell JL, Jégouic S, Loureiro S, Sage T, *et al*. Aberrant glycosylation of anti-SARS-CoV-2 spike IgG is a prothrombotic stimulus for platelets. Blood. 2021 Oct 21;138(16):1481-1489.
42. Zores F, Rebeaud ME. COVID and the renin-angiotensin system: Are hypertension or its treatments deleterious? Front Cardiovasc Med 2020;7:71.
43. Greinacher A, Selleng K, Mayerle J, Palankar R, Wesche J, Reiche S, *et al*. Anti-platelet factor 4 antibodies causing VITT do not cross-react with SARS-CoV-2 spike protein. Blood 2021;138:1269-77.
44. Mastellos DC, Skendros P, Lambris JD. Is complement the culprit behind COVID-19 vaccine-related adverse reactions? J Clin Invest 2021;131:151092.
45. Greinacher A, Langer F, Makris M, Pai M, Pavord S, Tran H, *et al*. Vaccine-induced immune thrombotic thrombocytopenia (VITT): Update on diagnosis and management considering different resources. J Thromb Haemost. 2022 Jan;20(1):149-156.
46. Public Health England. Guidance: COVID-19 Vaccination and Blood Clotting; 2021. Available form: https://www.gov.uk/government/publications/COVID-19-vaccination-and-blood-clotting/COVID-19-vaccination-and-blood-clotting. [Last accessed on 2021 Jun 28].
47. Suspected Vaccine Induced Immune Thrombotic Thrombocytopenia (VITT) THANZ Advisory Statement for Haematologists. Available from: https://www.thanz.org.au/documents/item/591. [Last accessed on 2021 Jun 28].
48. Diagnostic and treatment recommendations from the FACME ad hoc expert working group on the management of cerebral venous sinus thrombosis associated with COVID 19 vaccination. Neurologia 2021;36:451-61.
49. Nazy I, Sachs UJ, Arnold DM, McKenzie SE, Choi P, Althaus K, *et al*. Recommendations for the clinical and laboratory diagnosis of VITT against COVID-19: Communication from the ISTH SSC subcommittee on platelet immunology. J Thromb Haemost 2021;19:1585-8.
50. Oldenburg J, Klamroth R, Langer F, Albisetti M, von Auer C, Ay C, *et al*. Diagnosis and management of vaccinerelated thrombosis following AstraZeneca COVID-19 vaccination: Guidance statement from the GTH. Hamostaseologie 2021;41:184-9.
51. Platton S, Bartlett A, MacCallum P, Makris M, McDonald V, Singh D, *et al*. Evaluation of laboratory assays for anti-platelet factor 4 antibodies after ChAdOx1 nCOV-19 vaccination. J Thromb Haemost 2021;19:2007-13.
52. Hursting MJ, Pai PJ, McCracken JE, Hwang F, Suvarna S, Lokhnygina Y, *et al*. Platelet factor 4/heparin antibodies in blood bank donors. Am J Clin Pathol 2010;134:774-80.
53. Warkentin TE, Arnold DM, Nazi I, Kelton JG. The platelet serotonin-release assay. Am J Hematol 2015;90:564-72.
54. Sørvoll IH, Horvei KD, Ernstsen SL, Laegreid IJ, Lund S, Grønli RH, *et al*. An observational study to identify the prevalence of thrombocytopenia and anti-PF4/polyanion antibodies in Norwegian health care workers after COVID-19 vaccination. J Thromb Haemost 2021;19:1813-8.
55. Marcucci R, Marietta M. Vaccine-induced thrombotic thrombocytopenia: The elusive link between thrombosis and adenovirus-based SARS-CoV-2 vaccines. Intern Emerg Med

2021;16:1113-9.
56. Warkentin TE, Koster A. Bivalirudin: A review. Expert Opin Pharmacother 2005;6:1349-71.
57. Nadar SK, Goyal D, Shantsila E, Banerjee P, Lip GY. Fondaparinux: An overview. Expert Rev Cardiovasc Ther 2009;7:577-85.
58. Bourguignon A, Arnold DM, Warkentin TE, Smith JW, Pannu T, Shrum JM, *et al*. Adjunct immune globulin for vaccine-induced immune thrombotic thrombocytopenia. N Engl J Med 2021;385:720-8.
59. Fuentes B, Martínez-Sánchez P, Raya PG, Abenza MJ, Tejedor ED. Cerebral venous sinus thrombosis associated with cerebral hemorrhage: Is anticoagulant treatment safe? Neurologist 2011;17:208-10.
60. Stam J, De Bruijn SF, DeVeber G. Anticoagulation for cerebral sinus thrombosis. Cochrane Database Syst Rev. 2002;(4):CD002005.
61. Guidance for Clinical Case Management of Thrombosis with Thrombocytopenia Syndrome (TTS) following Vaccination to Prevent Coronavirus Disease (COVID 19) Interim Guidance; AQ13 July 19, 2021. Available from: https://www.who.int/publications detail redirect/WHO 2019 nCoV TTS 2021.1. [Last accessed on 2021 Aug 03].
62. Available from: https://b-s-h.org.uk/about-us/news/guidance- produced-by-the-expert-haematology-panel-ehp-focussed-on-vaccine -induced-thrombosis-and-thrombocytopenia-vitt/. [Last accessed on 2021 Jun 28].
63. Smith W, Kardeby C, Di Y, Lowe GC, Lester WA, Watson SP, *et al*. Platelet activation by vaccine induced immune thrombotic thrombocytopenia (TTS) patient serum is blocked by COX, P2Y12 and kinase inhibitors. medRxiv [doi: 10.1101/2021.04.24.21255655].
64. Elalamy I, Gerotziafas G, Alamowitch S, Laroche JP, Van Dreden P, Ageno W, *et al*. SARS-CoV-2 vaccine and thrombosis: An expert consensus on vaccine-induced immune thrombotic thrombocytopenia. Thromb Haemost 2021;121:982-91.