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Vaccine-induced immune thrombotic thrombocytopenia

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Summary

In response to the COVID-19 pandemic, vaccines against coronavirus 2 (SARS-CoV-2) were developed, tested and introduced at remarkable speed. While the introduction of the vaccines had a major impact on the evolution of COVID-19, some potential rare side effects of the vaccines were observed. Within a short period, three groups from Norway, Germany and the UK reported the identification of cerebral venous sinus thrombosis with thrombocytopenia and anti-platelet factor 4 (anti-PF4) antibodies in patients following AstraZeneca-Oxford (AZ) vaccination and named this the new syndrome Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT). Later this was also reported in patients following the Johnson & Johnson vaccination. In this viewpoint, we discuss the epidemiology, pathophysiology as well as the optimal diagnostic and therapeutic management of VITT. The presentation of a patient with possible VITT should raise prompt testing for anti-PF4 antibodies and initiation of treatment targeting the autoimmune and prothrombotic processes with intravenous immunoglobulin and non-heparin anticoagulation, respectively.

Introduction

COVID-19 infection due to the SARS-CoV-2 virus has had an impact on people and economies throughout the world. As of 26th July 2021, 195 million infections and 4.2 million deaths due to COVID-19 have been reported.[1] The World Health Organisation declared it a pandemic on 11th March 2020 and less than nine months later the first vaccine against SARS-CoV-2 from Pfizer-BioNTech was first approved by the Medicines and Healthcare Products Regulatory Agency (MHRA), the UK regulator. As of 26th July 2021, 3.85 billion doses of vaccines against SARS-CoV-2 have been administered in 180 countries.[2]

Reports of thrombosis in relation to vaccination against SARS-CoV-2 started appearing in late February 2021 which led to an investigation by the MHRA and EMA regulators who announced on 11th March 2021 that no association was identified. In the following week, however, three groups from Norway, Germany and the UK reported in the press and on social media the identification of cerebral venous sinus thrombosis with thrombocytopenia and anti-platelet factor 4 (anti-PF4) antibodies in patients following AstraZeneca-Oxford (AZ) vaccination.[3-5] Although initially several terms were used to describe the syndrome such as Vaccine Associated Thrombosis with Thrombocytopenia (VATTS), and Vaccine-Induced Prothrombotic Immune Thrombocytopenia (VIPIT), the term that has gained widespread use is Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT).

As with COVID-19 infection social media has played a significant role in the dissemination of information about VITT. Between the announcement of VITT in a press conference and on social media on the 19th March 2021 and the on-line publication of the first two papers[3,4], social media played a key role in disseminating information about the disease (**Figure 1**). Three of us (FK: @Erik_Klok_MD, MP: @MPaiMD and MM: @ProfMakris) have been active on twitter in providing updates about VITT. Both COVID-19 and VITT have shown us that in the future social media rather than medical journals will be at the forefront of sharing information.

References for this review were identified through searches of PubMed, embase, Cochrane library, web of science, WHO Covid-19 database, Academic Search Premier, Google scholar, medRxiv

and bioRxiv, which were updated weekly until the date of acceptance of this manuscript for publication (full search string available online). The final reference list was generated on the basis of originality and relevance to the broad scope of this review.

Epidemiology

Incidence

The reported risk of VITT has varied enormously between countries even when just comparing individuals exposed to the AZ vaccine. This is partially because there are significant differences in the age and sex of those vaccinated as well as in the way data are collected and reported. In Norway, Schultz and colleagues reported 5 cases of VITT among 130,000 individuals who received the AZ vaccine giving an incidence of 1 in 26,000.[4] In the UK the MHRA reported 367 VITT cases after 24·7 million of the first and 44 after of the second AZ vaccination dose giving rates of 1 in 67,302 and 1 in 518,181 respectively.[6] See and colleagues from the USA reported 12 cases of VITT after the Johnson & Johnson vaccine after 7 million doses, suggesting a rate of 1 case per 583,000 vaccinations.[7]

SARS-CoV-2 vaccine type

VITT has been reported almost exclusively after the AZ and Johnson & Johnson adenoviral vaccines, with the majority after the first vaccination.[8,9] No formal case reports have been published after the mRNA vaccine from Pfizer-BioNTech, but the MHRA received 15 notifications of thrombosis and thrombocytopenia associated with this vaccine from health professionals and the public.[6] One probable VITT case was reported from the USA after Moderna vaccination.[10]

Age

There is an association between the risk for VITT and younger age. The MHRA gives the risk of VITT after the 1st dose of AZ vaccination as 1 in 100,000 for those over the age of 50 years and 1 in 50,000 for those aged 49 years or less.[6] Unfortunately, the MHRA do not provide information on the risk of

VITT by decade of age and the risk cannot be calculated because the data on persons vaccinated with AZ by decade of age has not being made public. In the UK VITT case series, the mean age at diagnosis was 48 years with 85% of the patients being <60 years old.[9]

Sex

Because four of the five (80%) patients from Norway (Schultz 2021) and nine of 11 (82%) from Germany (Greinacher 2021) were female it was initially thought that female sex was a risk factor for VITT.[3] Both these countries, however, used the AZ vaccine initially in front line healthcare workers who were primarily female. A more recent report from the UK suggested that the VITT sex bias may not be so extreme as only 119 (54%) of 220 patients with definite or probable VITT were female.[9]

Comorbidities and previous COVID-19 infection

There are no studies directly comparing the comorbidities of VITT patients with those of the general population. In the UK study of 220 patients with definite or probable VITT, 8% had a history of an autoimmune disorder, 2% had a history of previous venous thrombosis, 2% had a history of cancer and 19% had one or more risk factors for arterial disease (obesity, smoking, hypertension, or diabetes).[9] Three of 220 patients in the UK cohort reported COVID-19 infection in the previous three months and 10 had serological evidence of previous infection.[9]

Pathophysiology

VITT is an autoimmune phenomenon, characterized by antibodies that directly activate platelets, triggering thrombosis in the arterial and venous circulation. Patients with VITT develop a consumptive coagulopathy, with thrombocytopenia, hypofibrinogenemia, and an elevated D-dimer. The earliest papers describing VITT all demonstrated that patients had high titre IgG antibodies directed against platelet factor (PF4), a molecule stored in platelet alpha granules and released during platelet activation.[3-5,7] PF4 is likely part of our innate immune defence; this cationic molecule binds and

opsonizes polyanionic surfaces of pathogens, facilitating binding of anti-PF4 antibody produced by preformed B cells.[11,12] In VITT, anti-PF4 antibodies play a different role. They bind platelet FcγRIIa, causing platelet activation via intracellular signalling and release of procoagulant platelet microparticles. Released platelet microparticles can themselves carry PF4, and VITT antibodies cluster PF4 on the platelet surface as they bind.[13] Procoagulant microparticles can also express tissue factor, which may explain the propensity to cerebral venous sinus thrombosis (CVST) in VITT; tissue factor appears to play a key role in thrombogenesis in the cerebral venous system.[14]

The pathophysiology of VITT closely resembles that of heparin-induced thrombocytopenia (HIT), which is also caused by anti-PF4 antibodies (**Figure 2**). In classic HIT, these antibodies recognize the ionic complex of positively charged PF4 and negatively charged heparin.[15] HIT antibodies induce a pan-cellular response, causing Fcγ receptor-dependent activation of monocytes (promoting tissue factor expression, and thrombin generation) and of neutrophils (inducing NETosis).[16,17] Antibody binding to FcγRIIIA also contributes to platelet clearance and thrombocytopenia.[16] It is likely that VITT antibodies similarly amplify thrombosis and thrombocytopenia. In most cases, HIT is triggered by heparin exposure. However, in a rare subtype of HIT known as "autoimmune" HIT, heparin exposure is not implicated. In this condition, highly anionic molecules like pentosan polysulphate, chondroitin sulphate, and parts of the bacterial cell wall take the place of heparin, forming complexes with PF4 and triggering antibody formation.[15] The parallels between VITT and autoimmune HIT extend beyond pathophysiology; their clinical characteristics and treatment strategies are similar as well. A recent paper used alanine scanning mutagenesis to determine that the binding of VITT antibodies is however distinct from HIT antibodies.[18] The binding of anti-PF4 antibodies from 5 VITT patients was restricted to 8 surface amino acids, all located within PF4's heparin binding site. These findings may explain why, in most VITT patients, antibody binding in PF4 enzyme immunoassays is inhibited by heparin.[3]

The pathophysiology of VITT is still incompletely understood. A major area of uncertainty is how adenoviral vector vaccines trigger this condition. Adenoviral vector vaccines may interact directly with PF4, priming an anti-PF4 response. This hypothesis has been explored by Greinacher and

colleagues, who observed a direct interaction between ChAdOx1 and PF4 with transmission electron microscopy, and Baker and colleagues, who reported binding of PF4 to ChAdOx1 hexon proteins using computational simulations.[13,19] ChAdOx1's viral capsid may bind PF4, creating a novel antigen that is then taken up by monocytes and trafficked to lymph nodes, where it stimulates proliferation of anti-PF4 memory B cells.[13] Vaccine components, such as EDTA or other human proteins in the vaccine, may promote a proinflammatory milieu that potentiates the immune response.[13,20] This theory does not require heparin or any other anionic compound, and is supported by the existence of preformed anti-PF4 memory B cells.

The adenoviral vector itself is a hypothesized trigger. Thrombocytopenia is a well-recognized complication of many viral infections, and can be rapidly induced in animal studies by intravenous administration of adenovirus – resulting in nonspecific platelet destruction from deposition of circulating immune complexes, suppression of platelet production, and specific antiplatelet antibodies.[21] Some adenoviruses and adenoviral vectors are also capable of binding factor X, and adenoviruses can cause platelet aggregation and activation when added to platelet rich plasma.[22,23] The direct adenoviral trigger theory becomes less likely when we consider that *thrombosis* has not previously been associated with adenoviral infection, nor with other adenoviral vector vaccines (even when administered intravenously at very high doses in animal studies).[21] Moreover, platelet and coagulation factor binding of adenoviruses occurs rapidly, while VITT presents over 5 days post vaccination. Another hypothesis implicates trace amounts of spike splice variant transcripts (C-terminally deleted mRNAs) which could theoretically be created through alternative splicing, then translated.[24] The resultant alternative spike proteins could damage endothelial cells, triggering inflammation and platelet activation, and causing PF4 release and thrombosis. Yet at this time, variant transcripts and alternative spike proteins have not been detected following vaccination with adenoviral vector COVID-19 vaccines. Impurities in vaccine preparation are another theorized VITT trigger. ChAdOx1 contains both human and non-structural viral proteins, raising the concern that

functional autoantibodies can form against them.[25] Some jurisdictions have reported higher frequencies of VITT than others; varying levels of purity between vaccine batches could explain this.[26] VITT does not appear to be caused by SARS-CoV-2 infection. COVID-19 can induce a hypercoagulable state. Yet concomitant thrombosis and thrombocytopenia are uncommon in COVID-19, and active COVID-19 infection is not a feature of VITT.[27] VITT antibodies also do not cross react with the SARS-CoV-2 spike protein.[28] Anti-PF4 antibodies have been found in hospitalized patients with COVID-19, but they are no more frequent than in other hospitalized patients.[27,28]

Clinical presentation and diagnosis

Clinical presentation

The presentation of thrombocytopenia and thrombosis, occurring 5 to 30 days after a first vaccination with ChAdOx1 nCov-19 or Ad26.COV.2.S against SARS-CoV-2 is suggestive for VITT.[29] In the UK cohort of 220 definite or probable VITT cases, the median age was 48 years with a range of 18-78 years and 85% of patients were <60 years old. There was only a mild female preponderance at 54%. The median time from the first AZ vaccination to presentation was 14 days with 97% of the cases presenting between 5-30 days.[9] The median platelet count at presentation was $47x10^9$/L with only 11 patients having a count of $>150x10^9$/L; in all but two of these 11 the platelet count reduced to $<150x10^9$/L following admission to hospital. Venous thrombosis is often present at multiple sites and sometimes at unusual sites. While the initial literature described a preponderance of cerebral vein thrombosis (CVT), occurring predominantly in women under the age of 60[3,5], it became clear that patients also present with the more common presentations of deep-vein thrombosis (DVT) of the leg and pulmonary embolism (PE). Patients with splanchnic vein thrombosis (SVT), with localisation in the mesenterial, portal, hepatic or splenic veins, have also been described. Indeed, CVST was the most common thrombotic complication and was identified in 110 (50%) of the patients from the UK; in 40 of the 110 (36%) of these patients there was secondary intracerebral haemorrhage (ICH). DVT and PE were found

in 37%, splanchnic vein thrombosis (mostly portal vein) in 19%, arterial thrombosis in 21% and 29% of patients had thrombosis affecting multiple beds.[9]

Radiological diagnosis of thrombosis

Targeted urgent radiological investigation is required in all VITT patients. Standard imaging tests include CT or MR venography of the brain for suspected CVT[30], CT pulmonary angiography for suspected PE,[31] compression ultrasonography for suspected DVT[32], and ultrasound or CT venography of the splanchnic veins for suspected SVT.[33] Some experts have suggested that an abdominal ultrasound scan is recommended in all VITT patients to screen for asymptomatic portal vein thrombosis.[34]

Differential diagnosis

The main syndromes to be distinguished from VITT are immune thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP) and catastrophic antiphospholipid syndrome (CAPS). In ITP, there is no manifestation of thrombosis, while the D-dimer level and coagulation tests are normal.[35] In TTP, there is typically microvascular thrombosis, rather than VTE. Laboratory findings in TTP include a normal fibrinogen and D-dimer, microangiopathic haemolytic anaemia with laboratory findings of haemolysis and schistocytes on the blood smear. Also, there is a profound ADAMTS13 deficiency (< 10%).[36] CAPS is excluded by the absence of antiphospholipid antibodies.

Laboratory diagnosis

Initial laboratory evaluation should include, full blood count with blood film, PT, APTT, D-dimer, fibrinogen, liver and renal function.[29] In classical VITT, patients present with thrombocytopenia, very high D-dimer levels (often >5-10,000 ng/mL), moderately decreased fibrinogen, and normal or slightly elevated APTT and PT.

If a patient presents with thrombocytopenia and thrombosis, an anti-PF4 HIT enzyme-linked immunosorbent assay (ELISA) should be performed; if positive, this should ideally be confirmed by a functional heparin-induced platelet activation assay (HIPA) or serotonin-release assay (SRA).[37,38] ELISA HIT assays have the most appropriate sensitivity for anti-PF4 antibodies in VITT, while other HIT assay methods have shown a low sensitivity.[39,40] Thus, in the appropriate clinical setting, a VITT syndrome is strongly suggestive if both the HIT ELISA test and HIPA or SRA test are positive. VITT is not excluded by a negative, rapid immunoassay against PF4 such as particle centrifugation assay and chemiluminescence immunoassay, since these tests may reveal false-negative results.[40] **Figure 3** summarizes the main points of the diagnostic management of patients with suspected VITT.

Case definition

The UK Expert Haematology Panel considers five criteria in the definition of VITT: Onset of symptoms 5-30 days (or 5-42 days if isolated DVT/PE) following COVID-19 vaccination, presence of thrombosis, thrombocytopenia (platelet count $<150x10^9$/L), D-Dimer >4000 mcg/ml (FEU) and positive anti-PF4 ELISA assay. Patients with all five criteria are considered definite VITT, but when one criterion is missing it is considered probable.[9]

VITT without thrombosis

We and others have observed a number of patients who present 5-30 days after AZ vaccination with severe headache, thrombocytopenia, markedly raised D-Dimer levels, strongly positive anti-PF4 antibodies but no CVST on CVT/MRV imaging. In our view, these individuals form the spectrum of VITT diagnosis and should be treated as such. It is important to differentiate these patients from those with immune thrombocytopenia post COVID-19 vaccination who have normal levels of D-Dimer, are negative for anti-PF4 antibodies and where bleeding is common but thrombosis extremely rare.

Treatment

The therapeutic management of VITT is based on 3 pillars: 1) modulation of the autoimmune phenomenon, 2) anticoagulation, and 3) supportive care and management of (bleeding) complications (**Figure 4**).

Modulation of the autoimmune phenomenon

VITT consensus documents and guidelines have widely extrapolated the treatment of auto-immune HIT to VITT, and these recommendations have been supported by currently published VITT case reports and series.[9,37,38,41,42] In auto-immune HIT, intravenous immunoglobulin (IVIG) is important since heparin avoidance alone does not lead to a rapid de-escalation of HIT hypercoagulability; heparin independent antibodies can persist for days to weeks.[43,44] The recommended dosing of IVIG is 1g/kg/day. Further, plasma exchange may be considered in severe or therapy refractory disease, e.g. in settings of severe thrombocytopenia or extensive thrombosis. During plasma exchange, the IgG antibodies triggering VITT are removed. Four of five Norwegian cases were treated with IVIG (and prednisolone) early or later in the course of disease; all four showed an increase in platelet count, while prior frequent platelet transfusions did not have that beneficial effect.[45] The same effect of IVIG was observed in Canada and the UK.[9,46] Three patients with anticoagulation, IVIG and steroid refractory VITT received plasma exchange, and responded with ultimately increasing platelet counts and decreasing D-dimer levels; all three survived.[47] Avoiding heparin exposure and platelet transfusions are key aspects of the basic VITT treatment. Both may further provoke the autoimmune phenomenon and/or fuel the coagulopathy. Steroids and rituximab have also been used in the setting of severe or therapy refractory VITT, but experience is limited and treatment outcomes uncertain.[9,45-47]

Anticoagulation

Patients with VITT should receive therapeutic dose anticoagulants to prevent further thrombotic complications. Although it has not been fully established that heparin exacerbates the clinical

presentation, for now anticoagulation therapy should involve non-heparin-based therapies such as direct oral anticoagulants (DOACs), fondaparinux, danaparoid or argatroban. In patients with thrombocytopenia and markedly elevated D-dimer levels, but without thrombosis (who are deemed not to have TTP or ITP) non-heparin-based anticoagulation is the mainstay of treatment too.[48,49] Both for CVST and SVT, endovascular treatment is not the standard of care but may be considered in selected cases.[8] As for the choice of anticoagulant drug class, considering the lack of evidence for DOACs in CVST and SVT as well as the presence of thrombocytopenia and high risk of bleeding, initial treatment with a parenteral agent is preferred over apixaban or rivaroxaban in most cases; Edoxaban and dabigatran must be preceded by a lead-in with a parenteral agent. Treatment may be switched to a DOAC in the subacute and chronic phase of the disease. The treatment of acute PE should be based on formal risk stratification, with reperfusion therapy reserved for those patients with high risk PE.[31,50]

Supportive care and management of complications

The most feared complication of initial treatment in VITT is major bleeding. In patients with SVT or CVST, major bleeding may be the presenting symptom. The anticoagulant dosing strategy may require alteration in patients with active bleeding or with platelet counts below $30x10^9$/L. In this setting, anticoagulant therapy at a lower intensity or following platelet transfusions (preferably after IVIG is administered) may be considered, in addition to plasma exchange.[8,29] Transfusion to correct relevant hypofibrinogenemia is also recommended in patients with a fibrinogen concentration of <1.5g/l. Further, in addition to anticoagulant treatment, management of CVST may require decompressive craniectomy if intracranial pressure is raised, and anticonvulsant agents if there have been seizures.[48,49] For these reasons, patients with VITT associated CVST should be referred immediately to an expert centre for an optimal multidisciplinary treatment.[8,29] Also, for patients with SVT, surgical treatment may be indicated in patients presenting with shock, peritonitis, intestinal perforation or infarction, or acute gastrointestinal bleeding.[51,52] Other reported complications of VITT that require dedicated

management include amongst others adrenal haemorrhage, limb ischemia and acute coronary syndrome.

Outcomes

As VITT was first described less than 5 months ago, data on outcomes and natural history are very limited. In the initial publications on VITT from Norway and Germany/Austria the mortality associated with VITT was 60% and 55% respectively.[3,4] In the UK cohort of definite and probable cases 23% of the patients died at the time of reporting.[9] These early fatality rates fail to capture later mortality or morbidity such as secondary to cerebral bleeding or amputations secondary to leg arterial thrombosis. A particularly worrying group are patients with CVST with intracerebral haemorrhage and platelet count of $<30x10^9$/L where a mortality rate of 73% was reported.[9]

Follow-up post hospital discharge

Although the natural history of VITT is unknown, early experience suggests that the anti-PF4 antibodies persist at least for several months in the majority of patients. Some patients redeveloped thrombocytopenia after platelet count normalisation, possibly because the half-life of the IVIG has meant its effect was lost. Some patients have required retreatment with IVIG and rituximab. Post discharge it is suggested that patients are followed closely with measurement of platelet count, fibrinogen concentration and D-Dimer level every 2-3 days for 2 weeks. Anti-PF4 antibody measurements weekly for 4 weeks and then monthly for 6 months have been proposed.[29]

Future perspectives

The most urgent remaining questions on VITT involve the exact pathophysiological mechanism and the long-term management of VITT survivors. For now, it remains unknown which vaccine components trigger VITT, e.g. the adenovirus itself or contaminants. This knowledge will have important implication for the future manufacturing and application of adenovirus-based coronavirus vaccines. The other

major unanswered question relates to the natural history of VITT. Patients often have persistent high titres of antibodies to PF4 beyond the first months of treatment. Therefore, the authors treat VITT patients with anticoagulation for at least three months of anticoagulation, and continue this treatment thereafter until anti-PF4 Elisa tests turn negative.[29]

The realisation that VITT was a serious adverse event led to restrictions in the use of adenoviral vector vaccines in many jurisdictions. Where a first AZ dose had already been given, some countries (such as the UK and Canada) permitted a second AZ vaccine, whereas others offered a second dose with an mRNA vaccine. It appears that the use of mRNA vaccines in persons with previous AZ vaccination can lead to increased reactions[53], however the resulting heterologous prime boosting also appears to yield improved immunity.[54]

Patients who have experienced an episode of VITT should avoid further adenoviral vector vaccinations. VITT patients may have concerns about subsequent vaccination, but as they are unlikely to have achieved a high level of immunity after a single AZ vaccine, they should be counselled and supported to have a second dose employing mRNA technology. The safety of using mRNA vaccines in VITT patients is unproven but limited early unpublished experience is encouraging. It is prudent to proceed with a second dose vaccination once the platelet count and D-dimer are stable and within the normal range, and the patient is fully anticoagulated. Some physicians may elect to repeat VITT testing prior to vaccination, to ensure antibody levels are decreasing, and/or repeat a FBC in the week following vaccination, to ensure the platelet count has not again dropped. If the VITT episode followed J&J vaccination, further vaccination against SARS-CoV-2 is not required.

Conclusion

Our current understanding of VITT has progressed considerably in the four months since its initial recognition. Pre-clinical studies have shown directions to the underlying pathophysiology, diagnostic criteria have been widely endorsed and dedicated laboratory tests have been developed and made available. The experience gained in the few hundred cases published in the literature has confirmed

the initial thoughts that VITT should be managed as a HIT-like syndrome with modulation of the autoimmune phenomenon and non-heparin anticoagulation. Current research efforts should focus on determining the optimal long-term management of VITT survivors and further unravelling of its mechanism which will allow for the optimal use of adenovirus-based coronavirus vaccines in the future.

Declaration of interest

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Figure legends

Figure 1: Time line of development of adenovirus-based coronavirus vaccines and first recognition of VITT.

Note:

- 11.3.2020 The WHO declares infection with COVID-19 a pandemic
- 2.12.2020 First approval of the Pfizer-BioNTech vaccine (by the MHRA, in the UK)
- 30.12.2020 AstraZeneca-Oxford vaccine approved by the MHRA
- 29.1.2021 AstraZeneca-Oxford vaccine approved by the EMA in Europe
- 11.3.2021 Simultaneous press conferences by MHRA and EMA announce no increase in thrombotic risk following vaccination against SARS-CoV-2
- 19.3.2021 Scientists from Norway, Germany and the UK announce a new syndrome of thrombosis, thrombocytopenia and anti-PF4 antibodies after AZ Oxford vaccination
- 7.4.2021 The MHRA and EMA announce the recognition of the new syndrome of VITT. The UK advises avoiding the AZ vaccine to those <30 years
- 9.4.2021 The initial reports from Norway[4] and Germany[3] are published
- 16.4.2021 The initial report from the UK[5] is published
- 7.5.2021 The UK increases its age restriction for AZ vaccination to <40 years
- 12.8-2021 The full report from the first 294 UK cases published.[9]

Figure 2: Proposed pathophysiology of VITT

Figure 3: Overview of VITT diagnostic work-up

Figure 4: Overview of VITT therapeutic management