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Longitudinal Aspects of VITT

Linda Schönborn, Andreas Greinacher*

Department of Transfusion Medicine, Institute of Immunology and Transfusion Medicine, Universitätsmedizin Greifswald, Greifswald, Germany

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ABSTRACT

In hundreds of patients worldwide, vaccination against COVID-19 with adenovirus vector vaccines (ChAdOx1 nCoV-19; Ad26.COV2.S) triggered platelet-activating anti-platelet factor 4 (PF4) antibodies inducing vaccine-induced immune thrombotic thrombocytopenia (VITT). In most VITT patients, platelet-activating anti-PF4-antibodies are transient and the disorder is discrete and non-recurring. However, in some patients platelet-activating antibodies persist, associated with recurrent thrombocytopenia and sometimes with relapse of thrombosis despite therapeutic-dose anticoagulation. Anti-PF4 IgG antibodies measured by enzyme-immunoassay (EIA) are usually detectable for longer than platelet-activating antibodies in functional assays, but duration of detectability is highly assay-dependent. As more than 1 vaccination dose against COVID-19 is required to achieve sufficient protection, at least 69 VITT patients have undergone subsequent vaccination with an mRNA vaccine, with no relevant subsequent increase in anti-PF4 antibody titers, thrombocytopenia, or thrombotic complications. Also, re-exposure to adenoviral vector-based vaccines in 5 VITT patients was not associated with adverse reactions. Although data are limited, vaccination against influenza also appears to be safe. SARS-CoV-2 infection reported in 1 patient with preceding VITT did not influence anti-PF4 antibody levels. We discuss how these temporal characteristics of VITT provide insights into pathogenesis.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the coronavirus disease 2019 (COVID-19) pandemic. Vaccination is key to controlling the pandemic. One of the most widely used vaccines against COVID-19 is the recombinant chimpanzee adenoviral (ChAdOx1-S) vector vaccine, ChAdOx1 nCoV-19 COVID-19 vaccine (Vaxzevria; Oxford/AstraZeneca) [1,2].

Adenovirus vector-based vaccines can rarely induce severe adverse effects. After vaccination with ChAdOx1 nCoV-19 [3–5] or the recombinant adenovirus type 26 vector COVID-19 Vaccine (Johnson & Johnson/Janssen) [6,7], patients were described presenting with thrombosis at unusual sites, associated with moderate to severe thrombocytopenia [8–10]. This post-vaccination adverse event is now called vaccine-induced immune thrombotic thrombocytopenia (VITT; synonym, thrombosis with thrombocytopenia syndrome, TTS). VITT occurs in the majority of individuals after the first vaccination shot. Despite its low incidence, hundreds of cases have been described worldwide because of the very high number of people vaccinated [5,11,12].

Platelet-activating anti-platelet factor 4 (PF4) IgG antibodies have been identified as the cause of VITT [8-10,13-15]. The presence of these anti-PF4 antibodies in sera of VITT patients can be confirmed by anti-PF4/polyanion enzyme-immunoassays (EIAs) and by PF4-enhanced platelet activation assays using washed platelets [8]. There are many similarities between VITT [5,8-10] and autoimmune heparin-induced thrombocytopenia (aHIT) [16–20]. Particularly the variant of aHIT known as “spontaneous HIT syndrome” shows strong similarities with VITT regarding the clinical presentation and the in vitro characteristics of the anti-PF4 antibodies. Observational studies of VITT patients indicate that the immune response after vaccination occurs in the same 5 to 20 day time window [5,8-10] as seen in HIT [18–20]. In contrast to classic HIT, however, in functional in vitro tests, platelet activation in VITT patients occurs in the presence of PF4 rather than low heparin concentrations [8,14,21]. Furthermore, the binding sites on PF4 differ between anti-PF4 antibodies in HIT and in VITT [15].

An unusual characteristic of the immune response in HIT (Warkentin et al. 2001 [19]) is the rapid decline of platelet-activating antibodies, often within weeks (median time to antibody non-detectability, ~7 weeks). In the majority of individuals antibodies are no longer detectable after 3 months. Antibodies reacting in the anti-PF4/heparin EIA are often detectable for a few more weeks (median time to non-detectability, ~15 weeks) [19]. However, in aHIT pathogenic antibodies can persist considerably longer, occasionally even for years [16,17].

* Corresponding author. Andreas Greinacher, Professor, Institut für Transfusionsmedizin, Universitätsmedizin Greifswald, Sauerbruchstraße, Greifswald, D-17489, Germany. Tel.: +49 3834 865482; Fax: +49 3834 865489.

E-mail address: andreas.greinacher@med.uni-greifswald.de (A. Greinacher).

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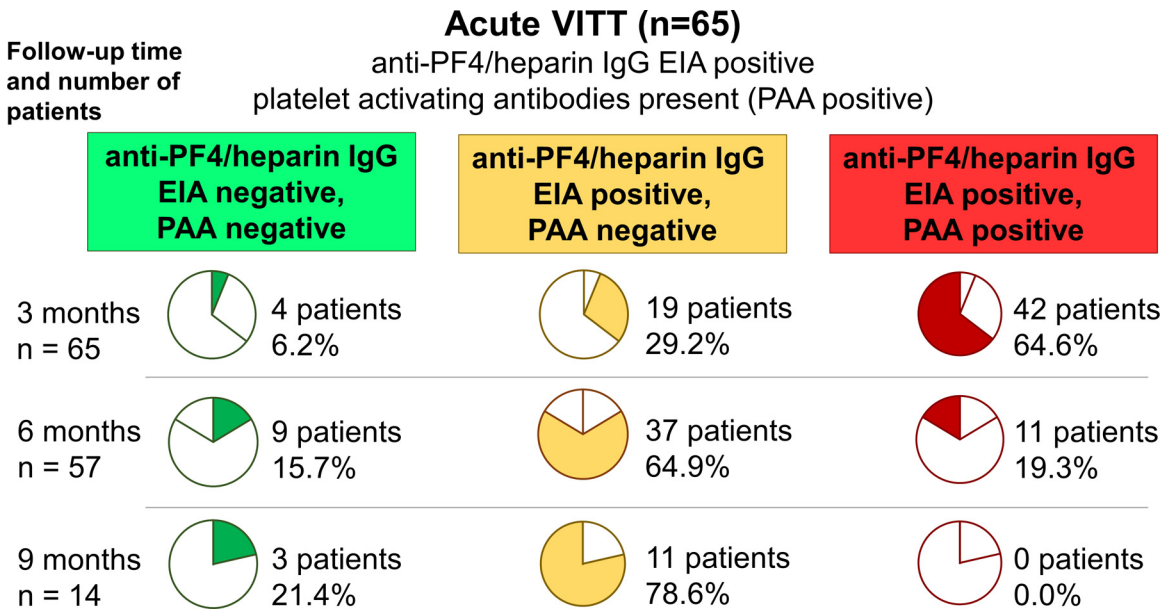

Fig. 1. Dynamic of the anti-PF4-response within the German follow-up cohort. In the acute phase of VITT all 65 patients showed a positive anti-PF4/heparin IgG EIA and platelet-activating antibodies were confirmed in the platelet activation assay (PAA). Platelet activating anti-PF4 antibodies are transient. First, the PAA becomes negative, and later the anti-PF4/heparin IgG EIA. The proportion of patients with the respective assay results is shown after a follow-up period of 3 months (65 patients), 6 months (57 patients), and 9 months (14 patients). As not all patients have reached a follow-up time of 6 and 9 months, respectively, the overall number of analyzed patients declined over time. After 9 months in all observed patients (n = 14) platelet-activating antibodies had disappeared. However, further follow-up analysis will be required to re-evaluate potential antibody persistence in a small group of patients.

The first follow-up analysis of VITT patients showed that platelet-activating antibodies are also transient in VITT [22,23]. In the context of the pandemic this is of high interest, especially with regard to whether further vaccination against SARS-CoV-2 is possible without inducing a relapse of VITT. To achieve sufficient protection against COVID-19, individuals require at least 2 vaccination shots [24–26], and additional booster vaccination shots were recently recommended [27]. Since VITT is a novel disorder first described only 9 months ago, few data exist on long-term follow-up including tolerance of further vaccinations. There is a high level of uncertainty regarding crucial issues such as duration of anticoagulation, or the safety of further vaccinations against COVID-19 after an episode of VITT.

In addition, it is likely that not all individuals who develop platelet-activating anti-PF4 antibodies post-vaccination experience a clinical breakthrough of VITT, similar to the well-known situation in HIT, where prospective trials showed the presence of platelet activating anti-PF4/heparin antibodies in asymptomatic patients [28]. It has been shown in HIT that these asymptomatic patients are at risk for rapid-onset HIT upon re-exposure with heparin, as long as the platelet-activating antibodies are present [29]. Thus, in an analogous fashion, application of a second vaccine shot might be dangerous if platelet activating anti-PF4 antibodies are still present. As long as the constituent of the vaccine is not identified, which is interacting with PF4, it remains unclear, whether the amount given with a 500 µL vaccine shot is sufficient to facilitate platelet activation by the anti-PF4 antibodies.

This review summarizes observations on the dynamics of the VITT-inducing antibody response, the clinical course of VITT patients, and tolerance of further vaccinations against SARS-CoV-2.

Dynamics of anti-PF4-antibodies during long-term follow-up of VITT patients

Few data exist on the long-term course of anti-PF4 antibodies after an episode of VITT. In Germany, we are monitoring a cohort of 65 VITT patients with a current follow-up period up to 9 months (Fig. 1). The first follow-up data were recently reported in Schönborn et al. 2021 [22]. In all patients, VITT was initially confirmed in the Greifswald laboratory with a positive in-house anti-PF4/heparin EIA [30] and a positive PF4-enhanced washed platelet activation assay [8]. Subsequently, serum samples were periodically obtained for anti-PF4 antibody testing over a follow-up period of up to 39 weeks (>350 follow-up samples analyzed).

Platelet-activating antibodies

Over the follow-up period, the PF4-dependent platelet activation assay became negative in 74% of patients (48/65 patients, median time to negative test, 15.5 weeks [range, 5-28 weeks]) [23]. The time period until disappearance of platelet activating antibodies seems to be slightly longer than anticipated based on preliminary data [22]. Fifty-three of 65 patients had a follow-up time of >20 weeks after onset of VITT. Among these 53 patients, in 33 patients (62.3%) the platelet-activation assay became negative within 20 weeks, in 11 patients (20.8%) it became negative within 20 to 30 weeks. In the remaining 9/53 (17.0%) patients, platelet-activating antibodies still persist. However, in none of the 14 patients followed for more than 9 months have platelet-activating anti-PF4 antibodies persisted (Fig. 1) [23].

Anti-PF4/heparin IgG EIA

Typically, VITT patients show very high anti-PF4/heparin IgG optical densities (ODs) at time of diagnosis [8-10,31]. In our follow-up cohort, the initial OD was >2.0 in 59/65 patients at diagnosis. Only 2 patients presented with a weakly-positive IgG EIA (OD 0.50-0.99). Anti-PF4 IgG antibody levels as measured by OD rapidly declined in most patients. However, only 14 patients reached a negative EIA result (<0.5 OD units) within the follow-up period (Fig. 1) [23].

In conclusion, anti-PF4 IgG antibody detectability by EIA persists for longer than antibody detectability by platelet activation

assay. This pattern is similar to the dynamics of antibody seroreversion observed in HIT [19]. However, VITT antibodies appear to persist for longer than typically observed in HIT. One reason could be that VITT antibodies resemble more the profile of antibodies observed in aHIT, that is, they do not need a cofactor (heparin) to bind to PF4 and are initially present in very high titers.

Patients with long lasting high anti-PF4 antibody titers

A few patients in our follow-up cohort show persistently high ODs in the anti-PF4/heparin EIA over the entire follow-up period [23]. Consistently high titers of anti-PF4 antibodies in VITT patients are also described by other groups [32]. This raises the issue of whether VITT can evolve into a chronic autoimmune disorder. However, the persistence of high-titer anti-PF4 antibodies may also reflect a misleading EIA "ceiling" effect. EIA ODs have a limited dynamic range, and once a certain antibody titer is reached, maximal ODs are measured, that is, even a fivefold higher antibody titer would yield the same OD result. We therefore titrated the sera of these patients to measure them within the dynamic range of the assay. This showed again a substantial decline in antibody levels over time. IgG has a half-life of 21 days [33]. Persistence of antibodies beyond 9 months therefore makes it likely that the antibody-producing B-cells persist, but probably they produce less antibodies over time.

The variability in duration of the anti-PF4 antibodies between patients is likely determined by a composite of antibodies produced at the time of acute VITT and persistence of anti-PF4 antibody-producing B-cells. In case of extremely high antibody titers produced during the acute phase by short-lasting B cells, therapeutic plasma exchange (TPE) or immunoadsorption would be a rational treatment, and appears effective in some patients [34,35]. On the other hand, immunosuppressive treatment might be needed in case of persisting anti-PF4 antibody-producing B cells. Differentiation between these 2 entities is not only relevant for treatment considerations but also for risk assessment. Patients with persisting antibody-producing B cells are likely at much higher risk to relapse compared to patients in whom these B cells produce anti-PF4 antibodies transiently.

The anti-PF4 antibody immune response in VITT is likely a secondary but transient immune response

In all VITT patients of our cohort, onset of clinical symptoms occurred in the typical time window between days 5 and 20 after vaccination, with very high anti-PF4/heparin IgG EIA ODs. High-titer anti-PF4 antibodies occurring as early as 5 days after vaccination cannot be explained by a primary immune response. As in HIT [18], the immune response in VITT shows features characteristic of a secondary immune response. Whether pathogens may trigger the primary anti-PF4 immune response, as in HIT [36], is currently unresolved. If so, PF4 bound to virus is the most likely cause for primary immunization. Adenovirus hexon protein is negatively charged [37] and binding of PF4 to adenovirus has been described before [38]. In addition we have recently demonstrated direct binding of anti-PF4 antibodies from VITT patients to multimolecular complexes containing PF4 and hexon protein of the ChAdOx-1 virus [21]. As the vaccine contains (besides free hexon proteins) more than 2000 proteins from the HEK cell line, in which the virus is propagated [39], we cannot currently exclude that other vaccine constituents are involved in the formation of PF4-containing complexes.

On the other hand, after a strong secondary immune response, antibodies typically persist for months and years, especially if induced as a secondary response to vaccination. This is obviously not the case in the majority of patients with VITT. The temporal features of anti-PF4 IgG antibody decline after development of VITT is similar to the antibody dynamics in HIT [19]. However, the time to seroreversion seems to take somewhat longer than in HIT, likely because antibody levels in VITT patients are unusually high during acute VITT [8,9]. The rapid decline of platelet-activating antibody levels indicates that the immune response in VITT, similar to that observed in HIT, does not follow the typical "classic" antibody response pattern. In a mouse model of HIT, the anti-PF4 immune response has been attributed to marginal zone B cells [40], a B-cell subpopulation known to provide a first-line of defense by rapidly producing IgM and class-switched IgG antibodies in response to infections by blood-borne viruses and encapsulated bacteria [41]. Our clinical observations suggest that marginal zone B-cells are also likely candidates for producing anti-PF4 antibodies in VITT. However, the observation of patients with persisting platelet activating anti-PF4 antibodies suggests that in a subgroup of VITT patients a longer lasting immune response is induced. Whether this persisting immune response involves B cells other than marginal zone B-cells is unresolved.

Clinical follow-up of VITT patients

The clinical course of VITT varies greatly among patients, and depends largely on the severity of complications caused by the prothrombotic state during acute VITT. Naturally, only patients who survived the initial VITT episode can be enrolled into a follow-up study. Therefore, we cannot exclude that patients with a fatal course of acute VITT may have had another type of B-cell antibody response. The vast majority of the follow-up cohort of VITT patients suffered no further thrombotic complication once therapeutic-dose anticoagulation was initiated, often together with additional treatments such as high-dose intravenous immunoglobulin (IVIG) or immunosuppression. In 26 patients in whom platelet-activating anti-PF4 antibodies persisted for at least 3 months, we observed only 2 patients with recurrent episodes of thrombocytopenia despite anticoagulation, 1 with new thrombosis [23]. Both patients are described below. This indicates that therapeutic- dose anticoagulation is sufficient to prevent subsequent thrombus formation in most patients. Although many patients recovered to a normal life, some remain in rehabilitation facilities due to severe neurological sequelae of the thrombotic complications during acute VITT.

Persisting and recurrent VITT

In HIT most patients show an increase in platelet counts within 1 week [42]. This is also the case for most VITT patients [43–47], although no comprehensive analysis of platelet recovery has yet been performed in a large cohort. One must differentiate between those VITT patients who show refractory thrombocytopenia or a relapse of thrombocytopenia within or immediately after the acute episode [48,49] (persisting VITT), and those who show decreasing platelet counts weeks or months after full recovery of the acute episode [50] (recurrent VITT). For each scenario a representative patient example is described below. In analogy to HIT [42], persistent VITT could be defined as persistent thrombocytopenia for more than 1 month.

Corica et al [49]. described a VITT patient with severe abdominal pain with onset 8 days after receiving ChAdOx1 nCoV-19, computed tomography revealed portal vein thrombosis, with partial thrombosis of the superior mesenteric vein. Laboratory results showed thrombocytopenia (15×10^9/L) and elevated D-Dimers (4.3 mg/L). Under treatment with fondaparinux, dexamethasone and IVIG, the splanchnic vein thrombosis progressed. Additionally, 6 courses of TPE were performed. Platelet count rose to $>150 \times 10^9$/L,

however, the authors observed a sudden decrease in platelet count after day 25 (nadir 95×10^9/L). The patient developed thrombosis of the right subclavian and axillary veins, associated with a central venous line in the vessel, which was then removed. Afterwards, the platelet count recovered, and during follow-up, no further relapses of VITT were observed. This patient resembles persistent VITT with a transient response to therapeutic interventions.

In the German follow-up cohort, we observed 2 patients with ongoing anticoagulation who presented with recurrent thrombocytopenia that required treatment long after the initial episode of VITT [23]. One patient suffered from rethrombosis despite ongoing anticoagulation. In this patient, platelet-activating antibodies were still detectable until the last available blood sample, which was taken 29 weeks after initial onset of VITT. EIA OD levels were consistently high but showed decreasing ODs when we diluted the sera as described above. Whether this is a case of persisting VITT (in analogy to aHIT [16]) or just a result of very high antibody titres, requires a longer follow-up observation.

Another patient originally described by Günther et al [50]. presented with severe headache, thrombocytopenia (platelet count 37×10^9/L) and elevated D-Dimers (>30 mg/L) 12 days after vaccination with ChAdOx1 nCoV-19. CVST was confirmed by cranial imaging, followed by intracranial hemorrhage within 24h after admission. Due to recurrent thrombocytopenia, the patient received 4 courses of IVIG on 2 consecutive days until day 30. Under treatment with prednisolone the platelet count remained $>150 \times 10^9$/L between days 40 to 90. However, after day 90, a new rapid decrease in platelet count of >50% was observed despite ongoing anticoagulation. The patient died after >20 weeks due to neurological sequelae caused by the initial VITT episode.

It is currently unclear why some patients develop a relapse of VITT despite ongoing anticoagulation, while other patients do not.

Antibody persistence in different VITT patient groups

Antibody persistence in patients with VITT-associated thrombosis

In patients with highly suspected or confirmed VITT, anticoagulation is one of the most important pillars of acute treatment [51–53]. Treatment of VITT is reviewed in detail in "*Treatment of VITT" (Chapter 5)*. The optimal duration of anticoagulation is likely similar to non-VITT thrombosis. However, as we observed a strong variation of the duration of persisting platelet-activating antibodies (5-28 weeks until the first negative platelet-activation assay) in the German follow-up cohort (Fig. 2) [23], we recommend to follow VITT patients for at least 20 weeks after acute VITT to recognize patients with a persisting prothrombotic state caused by PF4-dependent platelet activating antibodies. This is especially relevant when anticoagulation initiated for the VITT-associated thrombotic complication is stopped after several months. Patients in whom the platelet count remained (borderline) reduced might be at increased risk for recurrent thrombosis after stop of anticoagulation. Monitoring of the platelet count before, and D-Dimer levels after stop of anticoagulation may indicate patients at risk. Follow-up serological studies (EIA, platelet activation assay) can then confirm or rule out persisting platelet-activating reactivity. The optimal treatment in these patients is currently unclear. Restarting anticoagulation, immunosuppression, inhibition of FcγRIIa signaling [54,55] alone or in combination might be options.

The NICE guidelines [56] recommend continuing anticoagulation until anti-PF4 antibodies are no longer detectable by EIA. Especially where platelet activation assays are not available, this is a useful approach. However, Platton et al [57]. showed that detection of anti-PF4-antibodies over a follow-up period is assay-dependent. For instance, the Zymutest EIA detected anti-PF4-antibodies in 1 patient at the initial VITT episode, but not 14 weeks later; however, platelet-activating antibodies were still detectable by functional assay. As the authors recommend, monitoring of platelet counts and D-Dimer likely reveal patients with a remaining prothrombotic state.

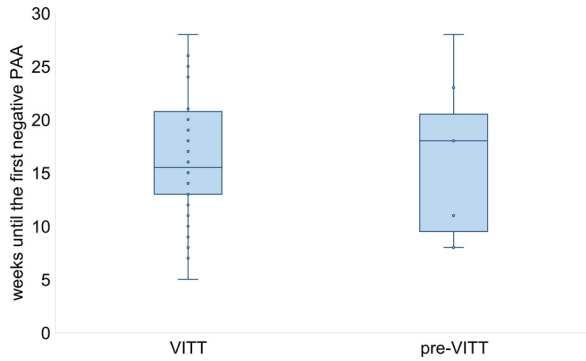

Fig. 2. Time in weeks until first negative platelet activation assay (PAA) in patients with VITT (platelet-activating antibodies + thrombocytopenia + thrombosis; n = 42) vs patients with pre-VITT (platelet-activating antibodies + thrombocytopenia without thrombosis; n = 7). There are no major differences in the persistence of platelet-activating antibodies between VITT and pre-VITT patients. However, we followed only a small number of pre-VITT patients and some patients have not yet attained a negative PAA (15 VITT patients, 1 pre-VITT patient).

Antibody persistence in VITT patients without thrombosis (pre-VITT)

In a few patients, VITT occurs with no thrombosis [43,58,59]. We enrolled 8 patients who fulfill the criteria of so-called "pre-VITT" in our longitudinal study. Their platelet-activating antibodies persisted for a median 10 weeks (range 4-24 weeks) after the acute episode of VITT. Two of them were already anticoagulated when VITT manifested due to atrial fibrillation.

Another pre-VITT patient (originally included in Salih et al [43].) is a 54-year-old female who presented with severe headache, thrombocytopenia of 66×10^9/L and D-Dimers of 4.3 mg/L 8 days after vaccination with ChAdOx1 nCoV-19. Cranial imaging did not reveal any thrombosis. The patient was treated with 5 mg apixaban but after 1 dose she developed a drug exanthema and anticoagulation was stopped. Platelet count recovered slowly without further treatment (Fig. 3). Platelet-activating PF4-dependent antibodies persisted for 24 weeks after the acute episode of VITT. However, her platelet counts remained stable, while D-Dimer was not measured after the acute episode. These anecdotal observations indicate that also in pre-VITT patients platelet-activating antibodies may persist for many weeks or even months. However, it is recommended for patients with pre-VITT that anticoagulation be given at least until platelet counts have normalized. As in classic HIT, reduced platelet counts indicate intravascular platelet activation and a prothrombotic state, and anticoagulation likely prevents further thrombus formation [43].

In summary, serial platelet counts and D-Dimer levels are likely more important parameters to guide clinical management of VITT and pre-VITT patients than anti-PF4 antibody titers or the presence of platelet-activating PF4-dependent antibodies

Further vaccination against SARS-CoV-2 in VITT patients

Individuals vaccinated with ChAdOx1 nCoV-19 COVID-19 vaccine [24,25] and Ad26.COV2.S [26] require at least 2 vaccination shots to achieve sufficient protection against COVID-19, including against new variants of the SARS-CoV-2 virus. In most patients, VITT occurred after the first vaccination shot [5,11], and thus immunization against SARS-CoV-2 is incomplete.

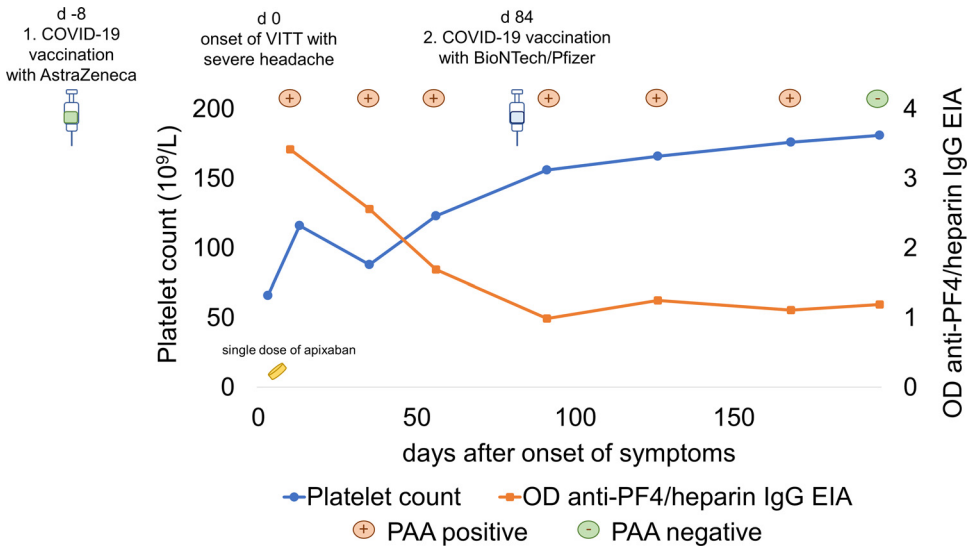

Fig. 3. Long-term follow-up of a patient with pre-VITT (originally described in Salih et al [43].). Platelet count and optical density of the anti-PF4/heparin IgG EIA over time are shown together with the results of the platelet activation assay (PAA; plus = positive result, minus = negative result). In this patient VITT appeared as severe headache 8 days after vaccination with ChAdOx1 nCoV-19; cranial imaging did not reveal any thrombosis. The patient was treated with 5 mg apixaban but after 1 dose she developed a drug exanthema and anticoagulation was stopped. Platelet count slowly recovered without further treatment. Second vaccination with the COVID-19 vaccine of BioNTech/Pfizer did not show any effect on the dynamics of platelet count and anti-PF4 antibodies.

VITT patients receiving mRNA vaccines

Further immunization against SARS-CoV-2 of VITT patients can be achieved safely by mRNA vaccines. To date, several VITT patients received their second vaccination shot with the mRNA vaccines, BNT1222 (BioNTech/Pfizer) or the Moderna vaccine, without signs of thrombosis or decreasing platelet counts [22,60,61].

At December 10, 2021, 34 patients in the German follow-up cohort had been vaccinated a second time 4 to 39 weeks after the acute episode of VITT [23]. We analyzed blood samples of 30 patients before and after second vaccination in the anti-PF4/heparin IgG EIA and the PF4-enhanced platelet activation assay. In most patients, EIA ODs continued to decline despite a second vaccination shot, and in those patients who showed no decline, the OD did not increase beyond inter-assay variability (±0.1). Only 1 patient showed a slight increase in OD of the anti-PF4/heparin IgG ELISA (2.47-2.82) after second vaccination. In 15 patients, in whom the functional test for platelet-activating antibodies became negative before second-dose vaccination, no reactivation of platelet-activating antibodies occurred. Ten patients received the second vaccination shot while still having circulating platelet-activating PF4 dependent antibodies; in none of them did thrombosis or thrombocytopenia recur.

This is corroborated by an independent cohort of 35 VITT patients in the United Kingdom who received a second vaccination dose with an mRNA vaccine without any complications [61].

In summary, further vaccination against SARS-CoV-2 with mRNA vaccines is safe in patients with VITT. Furthermore, this is strong in vivo evidence indicating that the mRNA vaccines do not contain the cofactor(s) required for anti-PF4 antibody-mediated prothrombotic activation of platelets.

VITT patients receiving a second dose of vector-based vaccines

It is known from HIT that a brief re-exposure to heparin is well tolerated in most patients, when platelet-activating anti-PF4-HIT antibodies are no longer present at time of re-exposure [62]. Re-vaccination with an adenovirus vector vaccine in patients with a history of VITT is a highly relevant question for low-income countries where only ChAdOx1 nCoV-19 is available. Lacy et al. 2021 [61] described 5 patients (1 with confirmed and 4 with possible VITT) who received a second vaccination dose with ChAdOx1 nCoV-19. These patients showed no relapse of VITT or any other adverse reaction. Whether platelet-activating antibodies were still detectable at the time of second vaccination was not analyzed. Therefore, repeat vaccination with an adenovirus vector vaccine in VITT patients seems to be feasible but more data are required before a definite recommendation can be given.

Beyond VITT the temporal data of the anti-PF4 immune response have implications for planning vaccination campaigns. To speed up the vaccination campaign the recommended 3-month time interval between the 2 vaccination shots of ChAdOx1 nCoV-19 has been shortened in some countries. This will increase the risk of applying the vaccine to still asymptomatic individuals with circulating PF4-dependent platelet activating antibodies developed after the first vaccination shot. Whether this increases the risk of adverse events is currently unresolved. A potential signal can be obtained by including the time period between first and second vaccination into reports of adverse vaccination events.

Vaccination of VITT patients against pathogens others than SARS-CoV-2

It is known from HIT [18] that HIT requires a polyanionic cofactor (ie, heparin) that complexes with PF4 to induce the immune response. To date, the analogous cofactor involved in the development of VITT has not been identified (but does not appear to be contaminating heparin-like glycosaminoglycans in the vaccine) [63]. As long as it is unclear whether any constituent of the vaccine beside the adenovirus and/or its proteins are key for triggering the immune response of VITT, it is not possible to predict, which other vaccines may increase risk of VITT recurrence.

VITT was discovered rapidly because the total number of cases was high, and because a widely available screening test for anti-PF4 antibodies (EIA) was strongly positive. Never before in medical history have so many people been simultaneously vaccinated with the same vaccines. This suggests that VITT may have occurred before with other vaccines, but not have been recognized. In this regard, a recently described case of VITT after vaccination against human papilloma virus (Gardasil) is especially interesting. The patient

developed the typical clinical picture of VITT with anti-PF4/heparin IgG antibodies, and PF4-dependent, platelet-activating antibodies [64]. This observation is the first hint that VITT may also occur after other (non-COVID-19) vaccines. Influenza vaccination is of special interest, as most patients usually receive it annually. In the German follow-up cohort so far we observed 4 VITT patients who were vaccinated with inactivated vaccines against influenza (Vaxigrip Tetra, n = 2; Influvac, n = 2) without recurrence of VITT or VITT antibodies. All 4 patients already had a negative platelet activation assay at the time point of influenza vaccination.

COVID-19 in VITT patients

VITT usually occurs after the first vaccination shot and so patients have insufficient protection against COVID-19. Since severe thrombosis can also complicate COVID-19, it was initially suspected that the spike protein may play a crucial role in both COVID-19 and VITT thrombosis. However, this appears unlikely as we have shown that anti-PF4 antibodies causing VITT do not cross-react with the SARS-CoV-2 spike protein [65]. In the German follow-up cohort, 1 VITT patient later acquired COVID-19. In her, VITT following vaccination with ChAdOx1 nCoV-19 manifested as stroke (anterior cerebral artery) presenting with aphasia and arm paresis. Eleven weeks later, she had a positive SARS-CoV-2 PCR test and mild symptoms of rhinosinusitis for 3 days. At the time of infection, the patient had a positive anti-PF4/heparin IgG ELISA with an OD of 1.70 and no detectable platelet-activating antibodies. In the follow-up blood sample obtained 4 weeks after SARS-CoV-2 infection, the OD had declined to 1.06 and the functional assay remained negative. This is the first indication that SARS-CoV2 exposure does not boost the VITT anti-PF4 immune response.

Conclusion

In the spring of 2021, the first cases of VITT appeared. Few data exist on the long-term course of VITT. In the majority of patients with VITT the anti-PF4 immune response is transient with a rapid decline of platelet-activating PF4-dependent antibodies, which disappear within 16 to 20 weeks. This seroreversion profile shows similarities to the HIT immune response, although antibody detectability seems to last somewhat longer in VITT. After the initial episode of VITT, most patients recover without further thrombotic complications. In most patients with persisting platelet-activating, PF4-dependent antibodies, therapeutic-dose anticoagulation seems to sufficiently control thrombin generation. However, few patients 2/65 (3%) show recurrent episodes of thrombocytopenia and/or thrombosis despite therapeutic-dose anticoagulation. In them recurrence was associated with persistence of platelet-activating antibodies. Whether these patients may benefit from immunosuppressive treatment is currently unresolved. The optimal duration of anticoagulation of VITT patients with and without thrombosis is unclear. Platelet count course and D-Dimer levels are likely more relevant to identify patients with a persisting prothrombotic state than anti-PF4 antibody monitoring. Careful monitoring is especially relevant when therapeutic-dose anticoagulation initiated because of the thrombotic complication of VITT is stopped, to identify patients who may require longer anticoagulation.

A second vaccination shot with the mRNA vaccines Comirnaty (BioNTech/Pfizer) or Moderna after an episode of VITT appears to be safe, independent of the persistence of platelet-activating anti-PF4 antibodies and preliminary observations indicate that even re-exposure with an adenovector based vaccine is also safe and efforts should be made to obtain this information given the relevance of adenovirus vector-based vaccines for low and middle income countries and the need of future vaccinations in VITT patients. Finally, influenza vaccination in VITT patients also seems to be safe although numbers are still very small.

Conflicts of interest

Dr Schönborn is the recipient of a young investigator grant of the medical faculty of the Universitätsmedizin Greifswald.

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Authors' contributions

LS and AG developed the concept, analyzed data, took care for patients and laboratory studies; LS and AG wrote the manuscript; LS created figures. Both authors have critically revised and approved the final version of the manuscript.

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