Final report

Vaccine-induced immune thrombotic thrombocytopenia and thrombosis syndrome (VITT/TTS) after vaccination against SARS-CoV-2

Protocol Number:	EMA/2021/17/TDA
Study type:	Patient registry
Indication:	Vaccine-induced immune thrombotic thrombocytopenia and thrombosis syndrome (VITT/TTS) after vaccination against SARS-CoV-2
Phase:	IV
Sponsor:	Universitätsmedizin Greifswald
Principal/Coordinating Investigator	Prof. Dr. med. Andreas Greinacher
Protocol Version and Date:	Version: 2.0; Date: March 03, 2022

Table of content

1 ABSTRACT / EXECUTIVE SUMMARY

Background: Rapid diagnosis and treatment improved outcome of patients with Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT). However, after the acute episode many questions on long term management of VITT remained. This prompted the European Medicines Agency to initiate a prospective patient registry to be performed by the Institute for Transfusion Medicine of the Universitätsmedizin Greifswald.

Methods: 71 patients with serologically confirmed VITT in Germany were enrolled into this prospective registry and followed by median 18 months. The course of anti-PF4 antibodies was analyzed by consecutive anti-PF4/heparin IgG enzyme immunoassay (EIA) and PF4-enhanced platelet activation assay. Patients and their treating physicians were regularly contacted by letter, email and telephone to obtain information on new events and changes in health. Informed consent was obtained to perform genome wide analysis to identify potential genetic risk factors to develop VITT.

Findings: Platelet activating anti-PF4-antibodies were transient in 87.3% of patients. In six patients platelet activating anti-PF4-antibodies persisted >18 months. Five of 71 patients (7.0%) showed recurrent episodes of thrombocytopenia and/or thrombosis. In four of them alternative explanations beside VITT were present. After further COVID-19 vaccination with an mRNA vaccine no reactivation of platelet activating anti-PF4-antibodies or new thrombosis was observed. No adverse events occurred in patients with a history of VITT vaccinated against influenza, tick-borne encephalitis, varicella, tetanus, diphtheria, pertussis, and polio. No new thrombosis occurred in 24 patients with a history of VITT developing COVID-19. We further identified the characteristics of anti-PF4 antibodies in VITT patients. There was a strong correlation between antibody reactivity in the PF4/heparin EIA and the probability that these antibodies also activate platelets. Most important, a subset of these antibodies only activates platelets in the confirmatory functional assay, if the serum is further diluted. The explanation for this is that these antibodies form aggregates of PF4 only if the molar ratio between antibodies and PF4 are in an optimal range. Because antibody titers are very high in VITT patients, sensitivity of the confirmatory assay increases if antibodies are diluted. The genetic studies are still ongoing. Analyses up to now did not reveal any candidate gene

polymorphism. As the numbers are limited, we plan to combine the genetic data with the data of similar studies performed in other countries.
Interpretation: Once the acute episode of VITT is over, patients seem to have a low risk for recurrent thrombosis. VITT Patients tolerate further vaccination. The immune response against PF4 is not boosted by a new SARS-CoV2 infection in VITT patients.

2 INTRODUCTION

Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare but serious side effect of COVID-19 vaccination associated with adenovirus vector-based vaccines.[1-3] VITT patients typically develop thrombocytopenia, hypercoagulability, and a high frequency of thrombosis, often in multiple and unusual locations (e.g., cerebral venous sinus thrombosis; splanchnic vein thrombosis).[4]

Early recognition and treatment with alternative anticoagulants and intravenous IgG reduced mortality of VITT.[5] While in the first case series nearly half of the patients died,[2-4] mortality was reduced to about 5-6% in Australia where the vaccination campaign was combined with an educational program informing the public and especially physicians about symptoms of VITT and appropriate treatment.[6]

Many open issues remained related to further management after the acute phase of VITT (**Table 1**). These questions were of high relevance for the individual patient as well as for treating physicians. While some of these questions had been answered by systematic and regular blood sampling, others were solved by careful observation of the outcomes after interventions.

This report summarizes the outcomes and results of our prospective patient registry involving 71 VITT patients in Germany from March 2021 to January 2023 initiated by the European Medicines Agency (EMA; EMA/2021/17/TDA).

Remaining questions after the acute phase of VITT	**Main findings of the study**
What is the natural course of the VITT immune response?	In most patients platelet activating anti-PF4 antibodies are transient. Antibody reactivity in the anti-PF4/heparin IgG EIA also declines, but persists much longer.
Is VITT an acute or chronic disease?	VITT is an acute disease. Chronic VITT occurs in very few patients only.

How many patients develop persistent thrombocytopenia or thrombotic complications after the acute episode?	Five of 71 patients (7.0%) showed recurrent episodes of thrombocytopenia and/or thrombosis. In four of them alternative explanations beside VITT were present.
Can patients with a history of VITT receive further COVID-19-vaccination?	Further COVID-19 vaccination with an mRNA vaccine is safe.
Can patients with a history of VITT receive any other vaccination (e.g. against influenza)?	No adverse events occurred in patients vaccinated against influenza, tick-borne encephalitis, varicella, tetanus, diphtheria, pertussis, and polio.
Do patients with a history of VITT have an increased risk for thrombotic complications in case of subsequent COVID-19?	No new thrombosis occurred in 24 patients with a history of VITT developing COVID-19.

Table 1. Remaining questions of patients and treating physicians after the acute episode of VITT (left column) and main study findings (right column) of this prospective longitudinal study.

3 PROTOCOL SYNOPSIS

The key topics of the study are summarized in the protocol synopsis.

<table>
<tr><td>Title:</td><td colspan="2">Vaccine-induced immune thrombotic thrombocytopenia and thrombosis syndrome (VITT/TTS) after vaccination against SARS-CoV-2</td></tr>
<tr><td>Protocol Number:</td><td colspan="2">EMA/2021/17/TDA</td></tr>
<tr><td>Indication:</td><td colspan="2">VITT/TTS after vaccination against SARS-CoV-2</td></tr>
<tr><td>Phase:</td><td colspan="2">IV</td></tr>
<tr><td>Type of Study:</td><td colspan="2">Patient registry</td></tr>
<tr><td>Sponsor:</td><td colspan="2">Universitätsmedizin Greifswald</td></tr>
<tr><td>Principal Investigator:</td><td colspan="2">Prof. Dr. med. Andreas Greinacher</td></tr>
<tr><td>Objectives:</td><td>Objectives</td><td>Endpoints</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td></td><td>What are the characteristics of the immune response against PF4 induced by SARS-CoV-2 vaccine?
Time of onset;
persistence of antibodies

Exploratory endpoints:</td><td>- First day of clinical symptoms of VITT/TTS
- Last day of positive anti-PF4 antibody test by EIA
- Last date of a positive functional test for PF4 dependent platelet activating antibodies
- Boostability of PF4-antibodies: to assess anti-PF4 antibody response to second or third dose of Covid-19 vaccine, vaccination with any other vaccine, or surgical interventions or heparin application.</td></tr>
<tr><td></td><td>Evaluation of the Brighton Collaboration interim case definition of TTS</td><td>- Reevaluation of TTS patients according to the Brighton collaboration case definition with special emphasis on occurrence of VITT/TTS in the absence of thrombosis.</td></tr>
<tr><td></td><td>Genome wide analysis of patients with VITT/TTS</td><td>- Sequencing the genome of VITT/TTS patients and comparison with the genomes of the matched probands of the normal population obtained by the Study of Health in Pomerania (SHIP)</td></tr>
<tr><td></td><td>Characterization of the anti-PF4 antibodies in VITT/TTS</td><td>- Assessment of the IgG subclasses of the anti-PF4 antibodies
- Determination of anti-PF4 antibody amount required to activate platelets to differentiate between differences in</td></tr>
</table>

		qualitative or quantitative characteristics of the anti-PF4 antibodies
Design:	This is a patient registry in which patients with laboratory confirmed VITT/TTS are followed prospectively, sequential serum samples are obtained and analysed by tests to detect anti-PF4 antibodies and platelet activating anti-PF4 antibodies. Anti-PF4 antibodies are further investigated for the subclasses and the glycosylation pattern. In addition, the study aims to identify potential genetic characteristics predisposing to VITT/TTS. The genome wide analysis is a cross-sectional study enrolling all VITT/TTS patients identified in the Greifswald laboratory independent whether they are enrolled into the follow-up study or not.	
Study Population:	All subjects with clinical diagnosis of VITT/TTS in whom the Greifswald reference laboratory has identified anti-PF4 antibodies and platelet activating PF4-dependent antibodies.	
Inclusion Criteria:	Prospective patient registry (Cohort 1): At the time of enrolment subjects are eligible to be included in the study only if all of the following criteria apply: 1. Signed written informed consent by the subject who is able to assess the nature, significance and scope of the patient registry. If the subject is temporarily incapable of consent, the consent of a legal representative or authorized representative will be accepted if permitted under applicable local regulations/ethics committee recommendations. 2. Males or females 3. Subjects with clinically suspected VITT/TTS between day 4 and 30 after vaccination with an adenoviral vector-based COVID-19-vaccine in whom VITT is confirmed by a positive anti-PF4/heparin IgG EIA and a positive PF4-enhanced platelet activation assay. Genome wide analysis (Cohort 2): All subjects with clinically suspected VITT/TTS after vaccination with an adenoviral vector-based COVID-19-vaccine in whom VITT is confirmed by a positive anti-PF4/heparin IgG EIA and a positive PF4-enhanced platelet activation assay	
Exclusion Criteria:	None	
Number of Subjects:	The patient registry aims to include a total number of 66 patients for the follow-up study. For the genome wide analysis all patients of whom DNA is available will be investigated.	
Countries/Number of Sites:	Germany, Greifswald laboratory	
Concomitant Medications:	All concomitant medications will be allowed according to the discretion of the treating physician.	
Duration of Participation:	Patients will be followed for 12 months after the anti-PF4 test becomes negative with a maximal preplanned follow up until 12-2022.	

Criteria for Evaluation:	
Efficacy Assessments:	The patient registry data will be analysed descriptively. The genome wide analysis will assess statistically significant differences between the VITT/TTS patient population and the general population (SHIP study population).
Safety Assessments:	Safety will be assessed by reported AEs and SAEs following second or third dose of Covid-19 vaccine, vaccination with any other vaccine, or surgical interventions or heparin application.
Statistical Methods:	Anti-PF4 antibody persistence: Statistical analysis will be performed in R. As follow-up times are right-censored and differ between study participants, anti-PF4/heparin IgG EIAs and functional tests will be analysed using the Kaplan-Meier method. OD-thresholds of 2.0, 1.5, 1.0, and 0.5 will be used to dichotomize the anti-PF4/heparin IgG antigen assay after linear interpolation between measurement time points to handle interval-censoring. Patients that achieved negative and then relapsed to positive functional test results will be considered positive at all times. Confidence intervals for Kaplan-Meier estimates will be computed using Greenwood's method, confidence intervals for the binomial distribution will be computed using Wilson's approximation. A one-sided, paired Wilcoxon's signed rank test will be used to compare optical densities of the first and last anti-PF4/heparin IgG EIA measurement per patient. Genome wide analysis: Whole genome sequencing will be performed at 30x coverage using paired end sequencing on a NoveSeq instrument. Raw data will be bioinformatically analyzed using FASTQC and BWA-Mem for mapping, and Platypus/GATK HaplotypeCaller for variant calling. Assembled genome sequences of VITT patients will be compared to age and sex matched probands of the population-based SHIP study. Chi squared / likelihood ratio tests will be used for association analysis; logistic regression will be further employed controlling for confounders. Correction for multiple testing will be done using a Bonferrone threshold of $5x10^{-8}$.

4 PATIENT REGISTRY

4.1 Characterization of VITT patients enrolled in the patient registry

Until January 23, 2023, 71 VITT patients consented and were recruited. One enrolled patient died 29 weeks after acute VITT due to sequelae of the initial episode, six patients were lost during follow up, and one patient rejected to provide further blood samples but will still be available for further clinical information. Forty-three females (61%) and 28 male patients (39%) are enrolled; median age at onset of symptoms was 48 years (range 18-80 years). All patients developed VITT after their first COVID-19 vaccination. Sixty-four patients after ChAdOx1 nCoV-19 COVID-19 vaccine, and 7 patients after the Ad26.COV.S COVID-19 Vaccine Janssen. During acute VITT, 35 patients (49.3%) developed cerebral venous sinus thrombosis (CVST), 12 (16.9%) splanchnic vein thrombosis, 21 (29.6%) pulmonary embolism, 14 (19.7%) deep vein thrombosis, one thrombophlebitis (1.4%, in Figure 1 was also counted as deep vein thrombosis), and four patients arterial thrombosis (5.6%). 25 patients (35.2%) showed multiple locations of thrombosis, 8 patients (11.3%) had typical pre-VITT with headache and thrombocytopenia but no thrombosis (Salih et al. NEJM 2021[5]) (**Figure 1**).
Median follow-up was 79 weeks (range 13-99 weeks). All 71 patients had serologically confirmed VITT and in all at least 2 blood samples were available. Median time between day of vaccination and onset of VITT-associated symptoms was 9 days (range, 4-30 days). Until January 23, 2023, 57 patients had been followed for over one year (≥52 weeks). Among the 13 remaining patients with a follow-up time of less than a year, one patient died 29 weeks after acute VITT; seven patients already showed negative results in the EIA and the functional assay before end of follow up and no additional blood samples were taken; six patients rejected further participation before their antibody tests became negative.

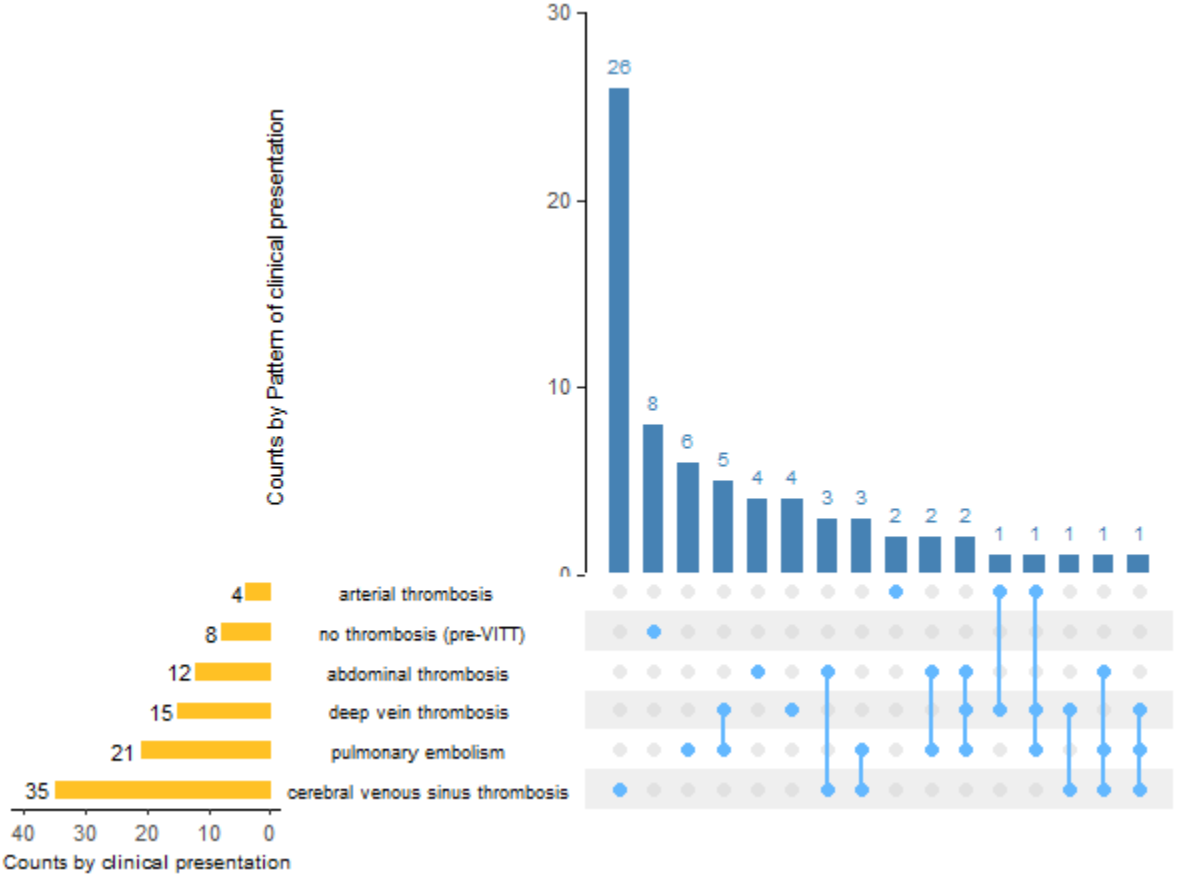

Figure 1 – Absolute number of patients with different clinical presentations of VITT. Multiple thrombotic complications are indicated by connected blue dots.

4.2 Characteristics and persistence of the anti-PF4-IgG response

4.2.1 Platelet-activating antibodies

The first follow-up data were reported in Schönborn et al. 2021[7]. The second follow-up data of this cohort of VITT patients has been published in Schönborn et al. *Blood* 2022

[8]. Additional data are published in Schönborn L and Greinacher A. Seminars in Hematology, 2022[9,10] and in Greinacher et al. Haematologica 2022[11].

Over the follow-up period, the PF4-dependent platelet activation assay became negative in 87.3% of patients (62/71 patients, median time to negative test 17.5 weeks [range, 5-79 weeks]). 57/71 patients had a follow-up time of more than one year after onset of VITT, seven of the patients with a follow-up time less than a year already showed negative assay results in both assays and blood sampling was regularly discontinued.

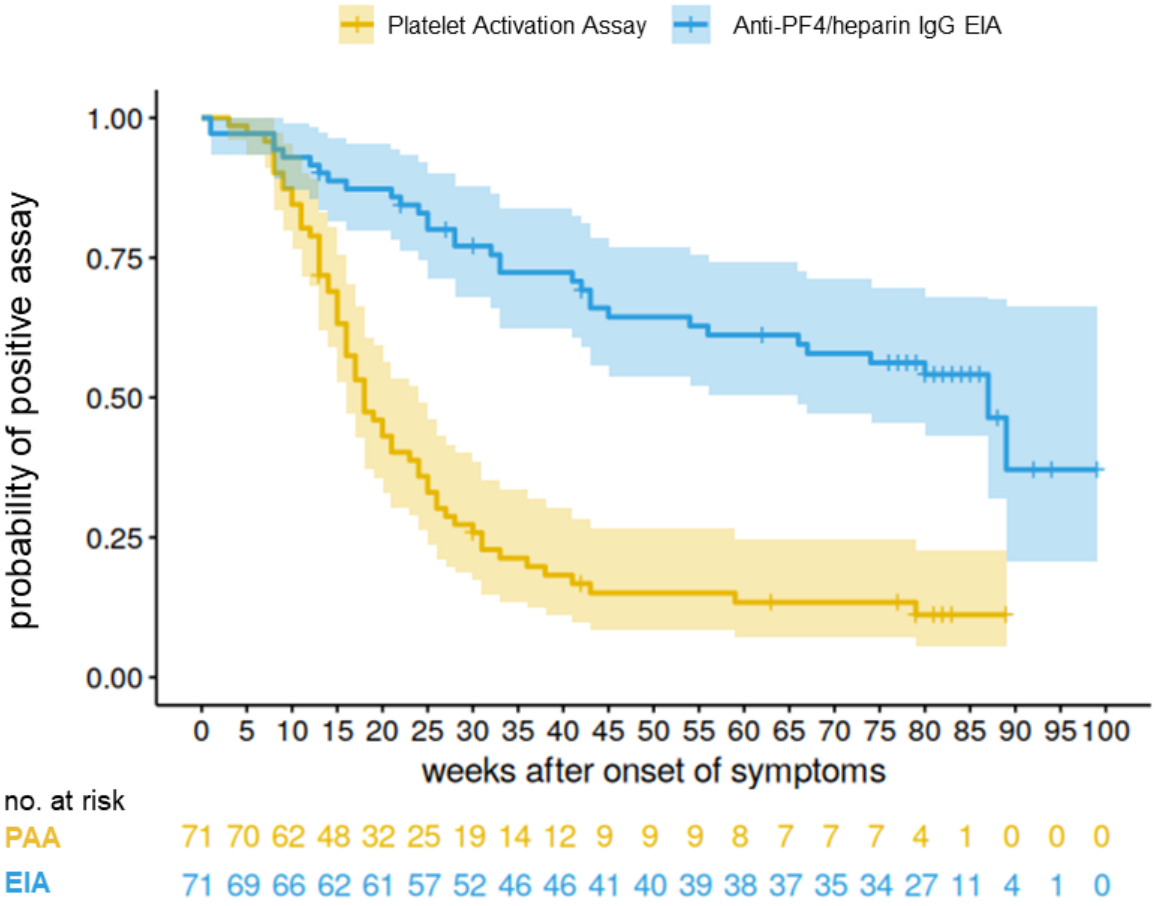

Figure 2: Decline of anti-PF4 antibody reactivity by enzyme-immunoassay (EIA) and platelet activation assay (PAA) (last update January 2023).

4.2.2 Anti-PF4/heparin IgG enzyme-linked immunosorbent assay (EIA)

Typically, VITT patients show very high anti-PF4/heparin IgG optical densities (ODs) at time of diagnosis (samples testing ≥0.5 - 1.0 defined as positive, >1.0 - 2.0 strongly–positive; >2.0 OD very strongly positive).[1-3,12] In our patient registry cohort, the initial OD was >2.0 in 65/71 patients at diagnosis. Only two patients presented with a weakly-positive IgG EIA. Anti-PF4 IgG antibody levels as measured by OD rapidly declined in most patients. However, only 32/71 patients (45.1%) reached a negative EIA result (<0.5 OD units) within the follow-up period (**Figure 2**).

The longer persistence of anti-PF4 antibodies in the EIA test in comparison to the functional assay are explained by two facts. On the one hand not all anti-PF4 antibodies giving a positive result in the EIA are platelet activating, and on the other hand the platelet activating capacity of antibodies depends on their titre. Thus, the discrepancy between the two tests can either be explained by qualitative but also by quantitative characteristics of the anti-PF4 antibodies. This is nicely reflected by the fact that most platelet activating antibodies show very high reactivity in the EIA (quantitative effect) but also a few antibodies which show moderate to weak reactivity in the EIA still activate platelets (qualitative effect).

In conclusion, our study confirms that anti-PF4 IgG antibody detectability by EIA persists longer than antibody detectability by platelet activation assay. The antibodies also persist much longer compared to anti-PF4 antibodies in heparin-induced thrombocytopenia, which typically disappear within 100 days.[13]

4.2.3 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. 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. We observed three patients with recurrent episodes of thrombocytopenia despite anticoagulation, one with new thrombosis[8], and two patients with recurrent thrombosis without thrombocytopenia with no or insufficient anticoagulation (please see below). This indicates that therapeutic-dose anticoagulation is sufficient to prevent subsequent thrombus formation in most of the patients after the acute episode of VITT.

For 52 of 71 patients, information on subsequent care after the acute in-hospital treatment was available. Twenty-two of those 52 patients (42.3%) received follow-up treatment in a rehabilitation centre, one of them is still living in an intensive care nursing home due to the severe sequelae of the cerebral venous sinus thrombosis. Thirty of the 52 patients (57.7%) did not require further inpatient hospitalization.

Up to now, we have not identified any common characteristics of individuals with persisting platelet activating antibodies. We found no evidence for an association with age, sex, concomitant underlying diseases, intake of drugs including hormonal contraceptives, presence or history of autoimmune disease, or history of thrombosis.

4.2.4 Persisting and recurrent VITT

Definitions:

Persisting VITT: Patients with persisting platelet-activating anti-PF4 antibodies and ongoing recurrent episodes of thrombocytopenia and/or thrombosis.

Recurrence of VITT: Patients who have clinically recovered from VITT (normal platelet counts) and suffer from recurrence of thrombocytopenia and/or thrombosis accompanied by recurrence of platelet-activating anti-PF4 antibodies.

Patients with ongoing anticoagulation and recurrent thrombocytopenia

None of the patients described in the following with persisting or recurrent VITT received further COVID-19 vaccinations.

We observed three patients with recurrent episodes of thrombocytopenia despite anticoagulation, one of them with new thrombosis[8].

In a patient, in whom VITT initially occurred as cerebral venous sinus thrombosis, we observed a new decrease of platelet count (nadir 115 Gpt/L) 14 weeks after the initial episode. The patient still received therapeutic dose anticoagulation and still had platelet-activating anti-PF4-antibodies. Platelet count recovered without any further treatment. Platelet-activating antibodies disappeared 22 weeks after the acute episode, but EIA remained still positive.

A second patient suffered from pulmonary embolism and severe portal and mesenteric vein thrombosis with consecutive mesenteric ischaemia in the acute episode of VITT. A large part of the small bowel had to be removed surgically. Until the last available blood sample 83 weeks after acute VITT, platelet-activating antibodies were still detectable and the platelet count was always below 100 Gpt/L. The patient is still receiving therapeutic dose anticoagulation. Whether the low platelet count is due to the anti-PF4 antibodies or splanchnic vein thrombosis-related liver damage is unclear.

A third male patient suffered from recurrent thrombosis and thrombocytopenia. The third and last thrombosis occurred 6 weeks after onset of VITT. Until the end of this study the patient still received therapeutic dose anticoagulation. Within the last 18 months the patient showed variable platelet counts with recurrent episodes of thrombocytopenia and variable D-Dimer levels (despite therapeutic dose anticoagulation), which required treatment with ivIgG. In this patient, platelet-activating antibodies were still detectable until the last available blood sample, which was taken 18 months after initial onset of VITT. This is probably a case of persisting VITT.

Two cases of recurrent thrombosis without thrombocytopenia:

1) A VITT-patient (<50 years) had a second episode of recurrent pulmonary embolism 4 months after the acute phase of VITT (also with pulmonary embolism). Before VITT the patient was healthy beside elevated blood pressure. During the initial episode of VITT the patient suffered from central pulmonary embolism and a thrombus in the right ventricle, which was removed by cardiac surgery. The postoperative course was uneventful. One week after discharge, the patient was readmitted with sepsis. The patient recovered and was anticoagulated with vitamin K antagonists. Two months later, during an episode of gastrointestinal infection, the patient developed a new pulmonary embolism. At that time the international normalized ratio (INR) was 1.8 and the platelet

count 377 Gpt/L. This was 49.9% lower than the last platelet count (755 Gpt/L) determined one month before pulmonary embolism occurred. Whether the significant decrease in platelet count has been caused by the still circulating anti-PF4 antibodies, or by pulmonary embolism itself cannot be differentiated.

2) A VITT-patient (>60 years) suffered from stroke of the stem ganglion, 12 months after the initial episode of VITT (VITT was complicated by cerebral sinus venous thrombosis). Anticoagulation had been discontinued 6 months before. At admission blood pressure was strongly elevated (190/80 mmHg). The platelet count at hospital admission was normal. The patient received aspirin as secondary prophylaxis.
The patient is unusual as the antibodies only reacted in the functional assay, but not in three different anti-PF4/heparin EIAs (in-house anti-PF4/heparin IgG EIA; Hyphen Biomed Zymutest HIA IgG; and Immucor PF4 IgG Test).
Beside hypertension, smoking (30 pack years) was a risk factor for stroke in this patient and the role of the persisting platelet-activating anti-PF4 antibodies remains unclear, especially as platelet counts remained stable during the acute episode of stroke.

In summary, both cases of recurrent thrombosis, one venous, one arterial, could have occurred independent of the presence of platelet activating anti-PF4 antibodies due to the presence of well-established risk factors.
All complications occurred while platelet activating antibodies were still present.
Although it is nearly impossible to distinguish between causality and association, patients with a negative functional assay seem to have a very low risk of recurrence of thrombosis or thrombocytopenia.

4.3 Consecutive vaccination against COVID-19

Of the 71 VITT patients, 48 patients (67.6%) received a second vaccine shot with the mRNA vaccine Comirnaty (n=44; BioNTech/Pfizer) or Moderna (n=4), administered at a median time of 22 weeks (range 4-74 weeks) after the onset of symptoms after their first shot with ChAdOx1 nCoV-19 (n=45) or Ad26.COV.S COVID-19 (n=3). At least 35 of them still received therapeutic dose and one received prophylactic dose anticoagulation at the time of consecutive vaccination. Twenty-five patients already received a third shot with an mRNA vaccine (Comirnaty n=18, Moderna n=5, unknown: n=2) and four

patients already received their fourth vaccination with Comirnaty (**Figure 3**). None of them developed symptomatic new thrombotic complications. After 63/77 vaccinations (second, third, and fourth COVID-19-vaccination) the platelet count was monitored. Platelet counts remained stable in 60 vaccination episodes in a total of 48 patients (= platelet count increased or the decrease was less than 20%). Three patients showed a decrease of 23-27%, but all showed decreasing anti-PF4 IgG EIA OD values and no recurrence of platelet-activating antibodies.

In 30 patients a negative platelet activation assay was documented before the second vaccination shot, while in 18 patients it was still positive. In eight patients, only the initial sample of VITT diagnosis was available before the second vaccination shot. In most of the patients OD values remained stable (OD +-0.2) or decreased, four patients showed a slight increase in OD of the anti-PF4/heparin IgG EIA after second vaccination without any signs of thrombosis or thrombocytopenia.

These follow-up data confirm that mRNA vaccines are very well tolerated regardless whether PF4-dependent platelet activating antibodies are still circulating or not. 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, or for boosting the immune response against PF4.

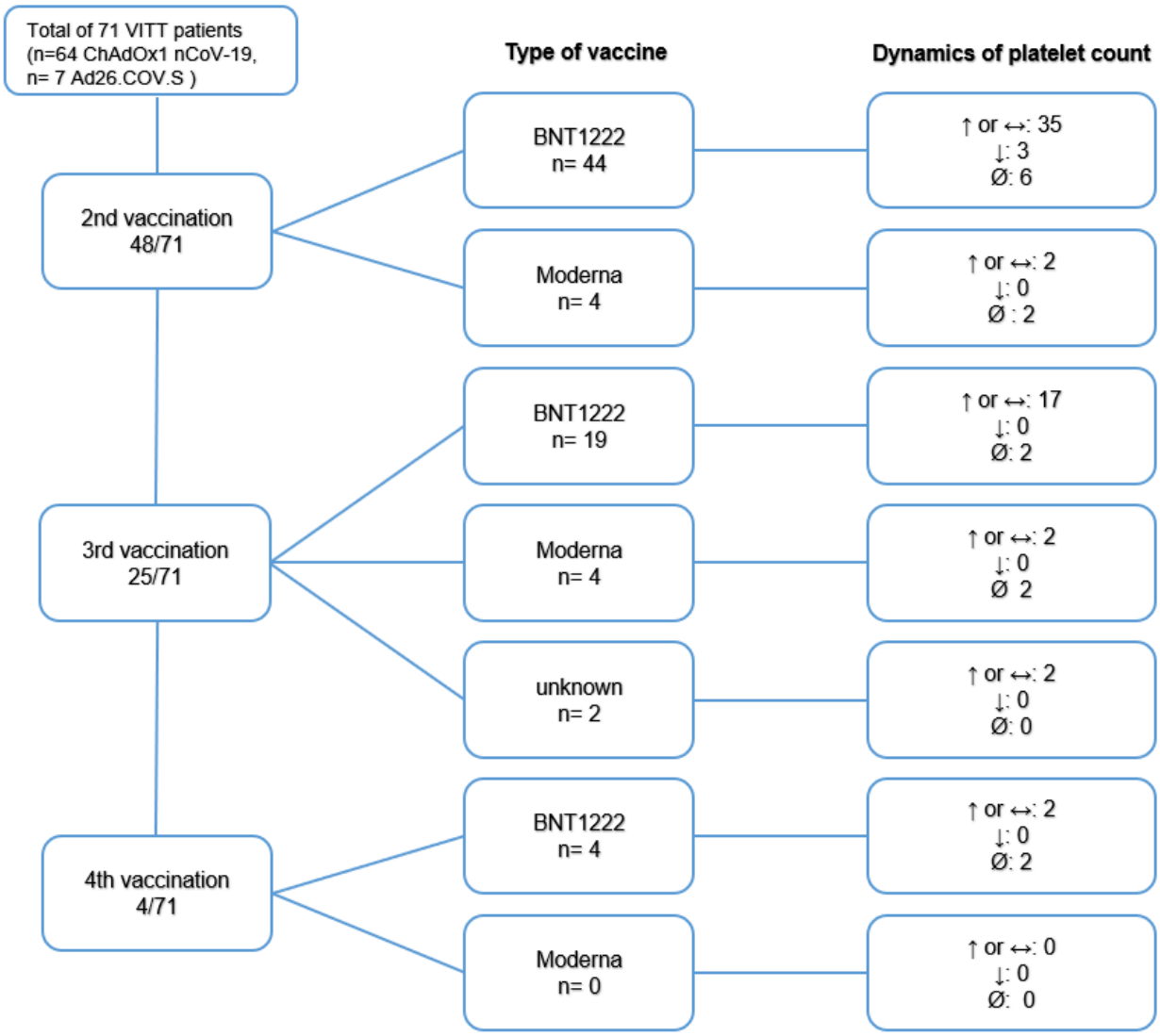

Figure 3. Further COVID-19 vaccination of patients with a history of VITT. ↑ = increase, ↔ = stable, ↓ = decrease, Ø = not monitored.

4.4 Vaccinations other than COVID-19

Until January 23, 2023, we became aware of 7 vaccinations against influenza (6 in winter 2021/2022, one in winter 2022/23) in patients with a history of VITT. Two patients were vaccinated with Vaxigrip Tetra, three patients with Influvac, and one patient was vaccinated with Influsplit Tetra, in one patient the vaccine was not reported. None of them reported any complications, no reactivation of platelet-activating antibodies was observed.

One patient was vaccinated two times against tick-borne encephalitis, no complication, or reactivation of platelet-activating antibodies occurred.
One patient was vaccinated against varicella with Shingrix, no complication or reactivation of platelet-activating antibodies occurred.

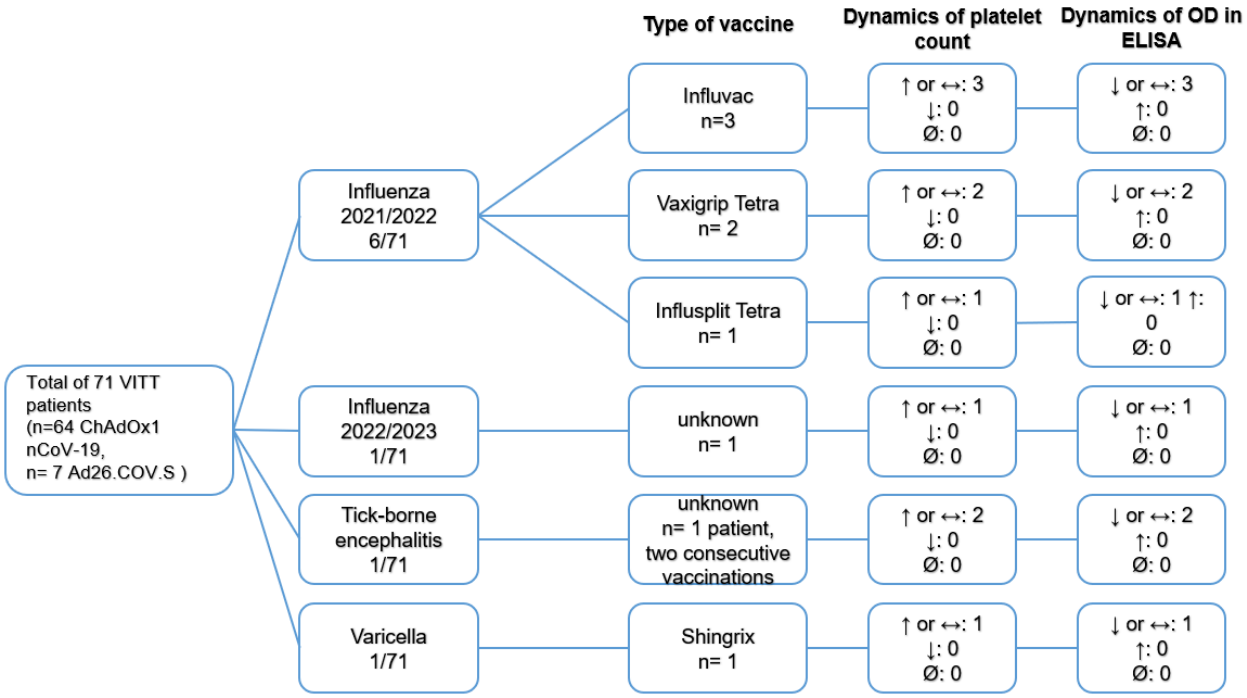

Figure 4. Consecutive vaccinations others than against COVID-19 in patients with a history of VITT. ↑ = increase, ↔ = stable, ↓ = decrease, Ø = not monitored.

4.5 COVID-19 in patients after VITT

Until January 23, 2023 we identified 24 patients of our follow-up cohort with confirmed SARS-CoV-2 infection with one patient reporting a second confirmed SARS-CoV-2 infection. One patient reported a SARS-CoV2 infection in December 2020 prior to VITT diagnosis. All patients presented with mild, respiratory symptoms, one of them also had headache for a few days and another patient reported additional loss of smell. The patient with confirmed second SARS-CoV2 infection stated that symptoms were milder during the second episode. In none of these patients, reactivation of PF4-dependent platelet-activating antibodies was observed. Eleven of these patients did not receive any

further vaccination against SARS-CoV-2 after onset of VITT, eight patients received a second vaccination with an mRNA-based vaccine, four patients received 2 further shots of SARS-CoV-2 vaccination, and one patient received a fourth SARS-CoV-2 vaccination (**Figure 5**). In 20 patients the anti-PF4-antibody ODs in the EIA decreased or remained on a similar level. Only two patients showed a slight OD increase by 0.22 (0.85 to 1.07) and 0.15 (2.1 to 2.25). Blood samples of two patients after infection were not available. Platelet counts remained stable in 16 patients (= platelet count increased or the decrease was less than 20%). Three patients showed a decrease of 32%, 22.1% and 20.7%, respectively. Platelet levels were not available for three additional patients. However, there was no recurrence of platelet-activating antibodies in any patient. 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.[14] Our cohort study provides solid in-vivo evidence that SARS-CoV2 exposure does not boost the VITT anti-PF4 immune response.

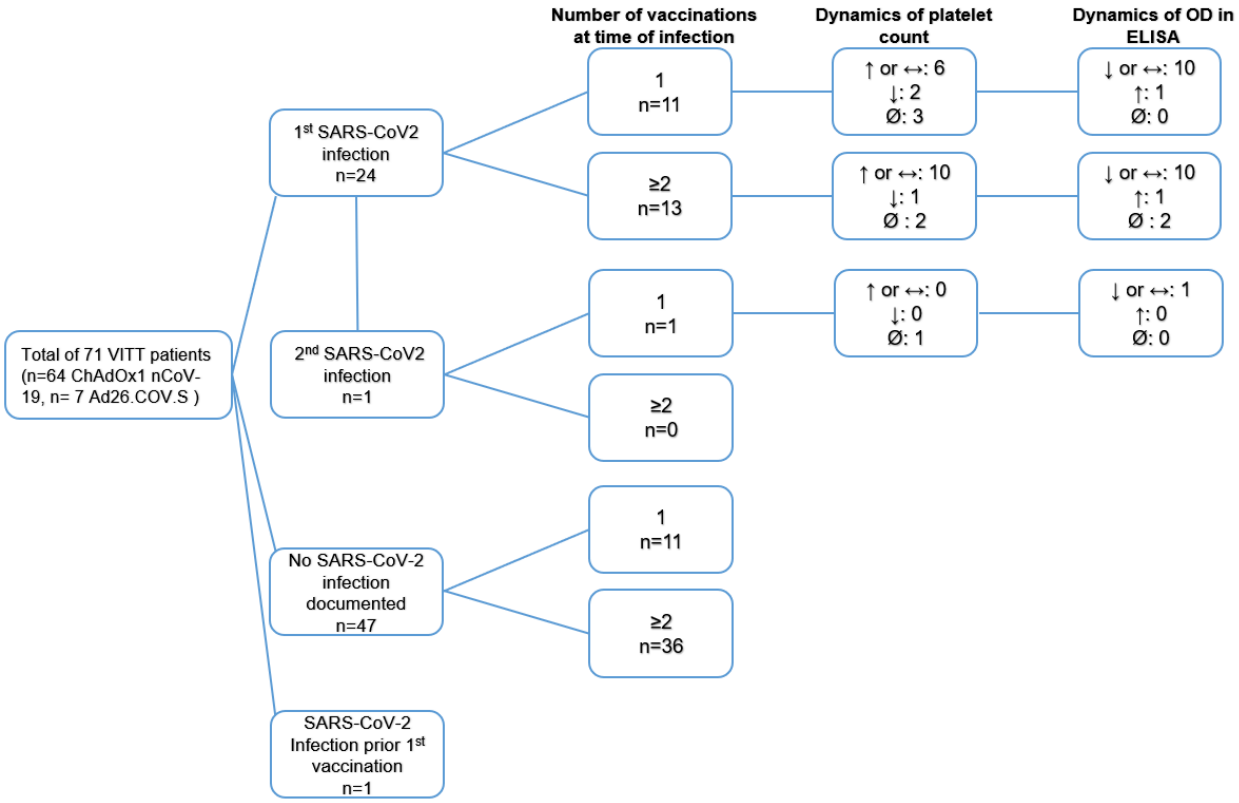

Figure 5. SARS-CoV-2 infection in patients with a history of VITT. ↑ = increase, ↔ = stable, ↓ = decrease, Ø = not monitored.

5 GENOME WIDE ANALYSIS

We identified 104 patients (median age 51 years, 60.6% females) with an adenoviral vector-based COVID-19-vaccine in whom VITT was confirmed by a positive anti-PF4/heparin IgG EIA and a positive PF4-enhanced platelet activation assay, and of whom buffy-coats for DNA-extraction were available.

We plan to combine the results of these 104 patients with the results of other cohorts currently assessed in other countries for a joint analysis of data. In order to enable such integrated data analyses, the methods for DNA extraction, library preparation and DNA-sequencing have been harmonized. DNA preparation from buffy coats and quality control have been optimized at the Interfaculty Institute of Genetics and Functional Genomics. Of the 104 patients, DNA-extraction in sufficient quantity was possible for 101 patients. Of those, DNA quality of 95 samples was sufficient for genome sequencing (performed at the Competence Center for Genomic Analysis in Kiel). The analysis of these data is currently ongoing at the Institute of Bioinformatics of the Universitätsmedizin Greifswald. Due to the large amount of data, the bioinformatics studies will continue for the next months. In addition, the joint analysis with other groups depends on the availability of their data.

First important results:

Recently, Wang et al.[15] demonstrated that VITT is caused by structurally similar antibodies by a single or only a few B-cell clones that undergo rapid clonal expansion, resulting in the production of just a few closely related antibodies. They also showed that the gene encoding the variable region of the immunoglobulin G (IgG) light chain seems to show the same polymorphism (IGLV3-21∗02) in all investigated patients.

The whole genome sequencing of 95 evaluable VITT patients revealed that the haplotype IGLV3-21∗02 is present in 79/95 patients (83.2%), 16 patients (16.8%) did not have this polymorphism. Together with colleagues from Australia (Tom Gordon et al.) we are currently performing further specification of these antibodies of patients with and without the IGLV3-21∗02 haplotype. Although, at the time of this report the final results are not available yet, the samples have already been transferred to Australia and the analysis is ongoing.

Our preliminary analyses showed no association of VITT antibodies with the AB0 blood

group. This is important, because we have recently shown that the presence of platelet activating antibodies in HIT is higher in blood group 0 individuals.[16] We also performed a preliminary analysis of the HLA type of the patients and found no preponderance of a specific HLA subtype. We are planning to match these findings with other cohorts. Since their data analysis is not finished yet, pooling of the data was not possible so far. As soon as findings emerge from further analysis, we will inform EMA of new results.
We also performed the preliminary analysis, for which we mapped the genome by data to the same genome edition version as for the Study of Health In Pomerania (SHIP) cohort and compared the 95 VITT patients with 500 controls. Thereby no obvious signal had been obtained. However, this could be due to the relatively low number of VITT patients and we will repeat the analysis once the data of other cohorts are available.
In summary the genome wide analysis ruled out a similar association with the AB0 blood group in VITT as it has been found in HIT. It also demonstrated that the assumption in the literature of the restriction of VITT antibodies to the IGLV3-21∗02 haplotype is incorrect, which opened a new field for research to assess the structure of the antibodies which are encoded by individuals who do not have this haplotype gene. The other preliminary data gave no indication for a specific genetic predisposition for VITT, but the overall numbers are rather small and the planned combined analysis with other cohorts may provide further information.

6 EVALUATION OF THE BRIGHTON COLLABORATION INTERIM CASE DEFINITION OF TTS

We are actively sharing our findings with the Brighton Collaboration and the WHO. The WHO is updating their guideline on TTS and includes the Greifswald data. We are in contact with WHO but the process is still ongoing due to administrative processes within WHO. We will inform the EMA after the end of the study as soon as there are any new results.

7 CHARACTERIZATION OF THE ANTI-PF4 ANTIBODIES IN VITT/TTS

A functional test is very important to confirm that anti-PF4 antibodies can activate platelets and are therefore clinically relevant. Most VITT patients, who are not enrolled into the present study, are only tested by an enzyme-immunoassay (EIA). The large number of patients enrolled into our analysis allows to provide an estimate, whether the reactivity strength in the anti-PF4/heparin enzyme immunoassay has predictivity for a positive platelet activation testing. All samples referred for VITT testing to our laboratory were systematically evaluated by semiquantitative in-house PF4/heparin-EIA (OD readings) and PF4-induced platelet activation (PIPA) testing (total 975 samples; the initial samples of the 71 patients of the prospective patient registry are included). EIA-positive sera testing PIPA-negative were retested following 1:4 and 1:10 dilution. Logistic regression was performed to predict the probability of a positive PIPA.

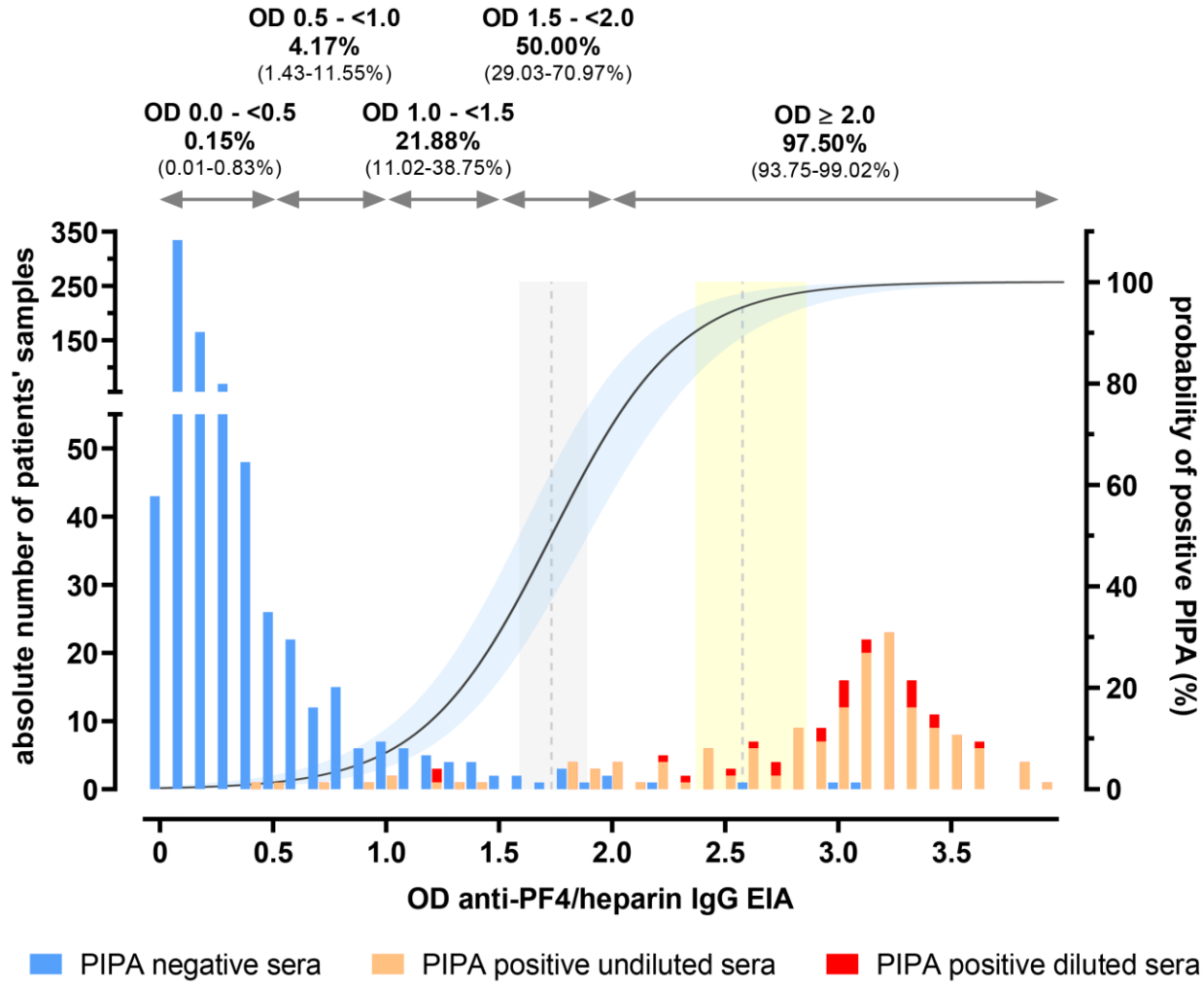

Figure 6. Absolute number of positive and negative results in the PF4-dependent washed platelet assay (PIPA) depending on the OD in the anti-PF4/heparin IgG EIA (blue bar = negative PIPA; orange bar = positive PIPA of undiluted sera, red bar = positive PIPA of diluted sera only). The right y-axis and the blue curve show the probability of a positive PIPA depending on the anti-PF4/heparin IgG EIA OD. The blue shade around the blue curve shows the 95% confidence interval of the blue probability curve. The grey and yellow shades show the 50% and 95% probability (with CI) of a positive PIPA respectively. For presentation in Figure 6 the OD values were rounded to the first decimal number (e.g. OD 0.94 became OD 0.9, and OD 0.95 became OD 1.0). For calculation of probability of a positive PIPA for OD intervals, shown above the graph, only the final results were used (e.g., positive PIPA after dilution of the serum is considered as positive). The 17 sera which gave indeterminate results in the PIPA are not included into the figure and not included into the calculation of probability.

Our study shows that the likelihood for a positive result in the PIPA strongly correlates with the OD measured in the EIA (**Figure 6**). Further, a negative PIPA despite a positive

EIA (OD >1.0) should prompt fourfold to tenfold serum dilution, which leads to a positive PIPA in about half of the samples. The correlation of a positive functional test with the magnitude of the OD in the anti-PF4/heparin IgG EIA is well known in HIT.[17,18] Also, as in HIT, a considerable percentage of anti-PF4 antibodies are not platelet-activating (approximately 35-40%). Overall, 176 of 282 (62.4%) EIA-positive sera activated platelets. This is a higher proportion than in most prospective HIT studies.[17,19] This might be explained by the distinct clinical picture of VITT. In contrast to HIT, clinical presentation of VITT is rather unique, while HIT is often suspected in severely sick patients who have many other explanations for thrombocytopenia. The better preselection of patients, based on clinical characteristics, leads to a higher proportion of true VITT sera being referred for investigation. It is known that anti-PF4 antibodies occur in approximately 5% of individuals vaccinated with an adenovirus vector- or an mRNA-vaccine.[20] Thus, some of the sera which are only weakly to moderately positive by EIA might reflect these clinically-irrelevant antibodies.

The most important finding of our study is a fundamental difference between the reactivity profiles of the anti-PF4 antibodies in HIT and VITT. We found that approximately half of the antibodies showing an OD>1.0 by EIA yielded a positive result by PIPA only following 1/4 to 1/10 serum dilution. Such dilution-dependency has never been reported with HIT sera, nor have we observed this phenomenon with HIT sera. This dilution dependency may also help explain the relatively low sensitivity of functional assays for VITT in a recent workshop.[21] These findings are of major importance for interpretation of assays for TTS and VITT and for improving patient safety.

There are several potential explanations for this dilution effect. First, there could be an inhibitory factor in the serum which is out-diluted (e.g., fibronectin, which has been shown to interfere with platelet activation by anti-PF4/heparin antibodies in HIT).[22] Second, high concentrations of IgG after IVIG treatment can inhibit platelet activation in HIT.[23-26] However, we observed the dilution effect also in sera obtained before IVIG treatment was started. Third, danaparoid in high concentrations can inhibit platelet activation in HIT[27] and VITT antibodies[28]. However, danaparoid treatment was excluded in the majority of patients assessed. We suggest that the most likely explanation is

based on the fundamental findings of Huynh et al. 2021[29], who showed that VITT antibodies have very high avidity to PF4. This feature is very similar to autoimmune HIT antibodies, which can form complexes with PF4 independent of any cofactor.[30] It is well known from HIT that optimal complex formation depends on the stoichiometric ratio between PF4 and heparin.[31,32] If either heparin or PF4 are present in too high concentration, the complexes do not form or are disrupted. When only PF4 and the anti-PF4 antibodies are the binding partners in the complex, our data indicate that optimal complex formation also depends on their stoichiometric ratio. If there are too many anti-PF4 antibodies, not enough binding sites are available on PF4 to form complexes (**Figure 7**). Instead of aggregating into large complexes, which requires binding of both Fab arms of several VITT IgG antibodies, each antibody binds to only one PF4 molecule according to the paradigmatic Heidelberger-Kendall precipitation curve, describing that the size of soluble immune-complexes depends on the antigen to antibody ratio.[33] This concept is also consistent with the widely-accepted observation that addition of PF4 strongly enhances reactivity of VITT antibodies.[2]

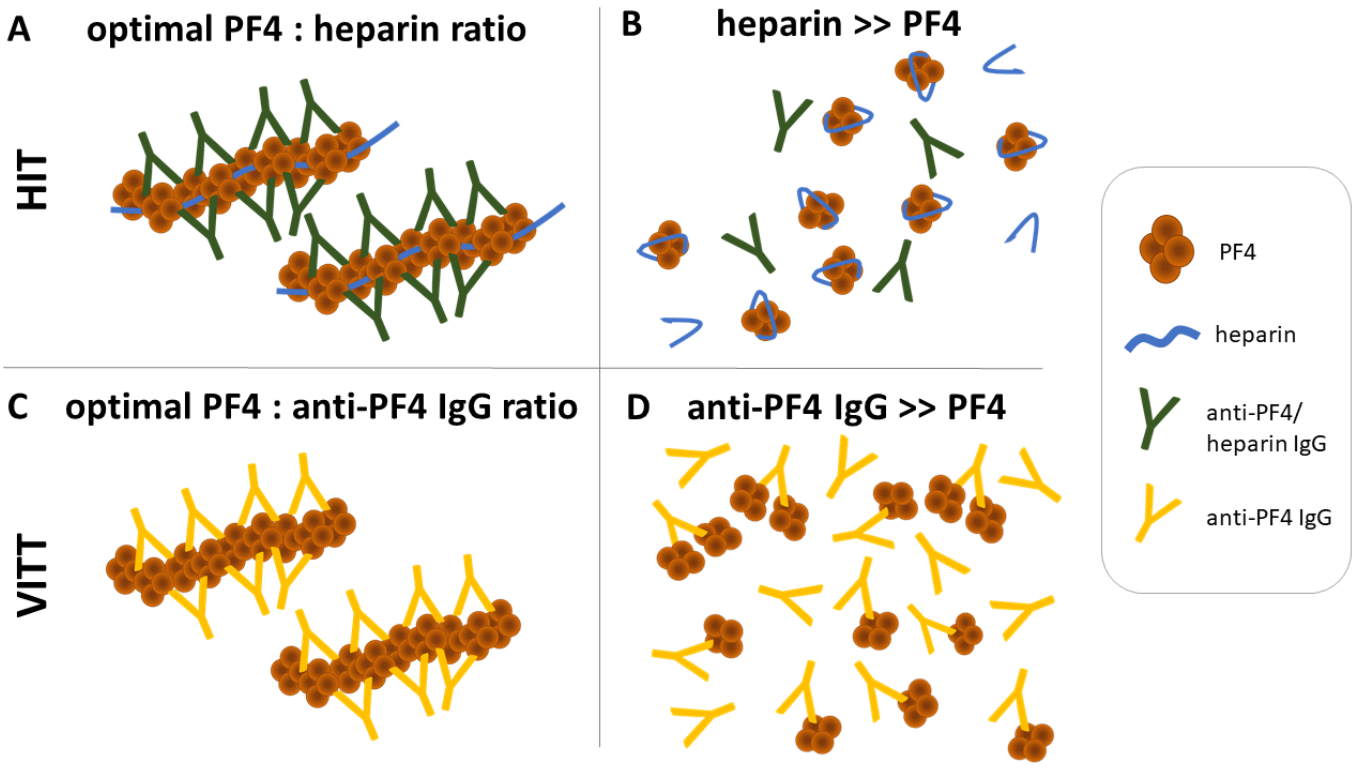

Figure 7. **Schematic presentation of the dependency of immune complex formation in HIT and VITT on stoichiometric ratios.**

HIT (A) Optimal ratio of PF4 (cationic) and heparin (anionic) results in formation of multimolecular complexes. Anti-PF4/heparin IgG antibodies bind to these complexes. The resulting immune complexes activate platelets in functional assays. Even if anti-PF4/heparin antibodies are present in excess, interaction of PF4 and heparin is so strong that the state of lowest energy is always formation of multimolecular PF4/heparin complexes, to which subsequently HIT antibodies bind. **(B)** In case of heparin excess, the long heparin molecules wrap around the PF4 tetramer (or several shorter heparin chains bind along the rim of positive charge) and no complexes are formed. Accordingly, anti-PF4/heparin IgG antibodies do not bind to PF4 and no platelet activation is observed in the functional assay.

VITT (C) Anti-PF4 antibodies bind to PF4 alone and form multimolecular complexes without addition of a polyanion. **(D)** In case of very high concentrations of anti-PF4 VITT antibodies, there are not enough PF4 tetramers for all Fab-parts of the IgG. Overall, the state of lowest energy is reached if each antibody can bind to one PF4. A situation in which few antibodies form complexes with PF4 while others have no binding partner would be thermodynamically unfavorable. Dilution of these sera lowers anti-PF4 IgG concentration and subsequent formation of immune complexes causing platelet activation in the PIPA.

Together with colleagues at Werfen, Barcelona (Spain) we now developed a prototype automatable rapid assay for VITT antibodies, which does not recognize HIT antibodies.

For the first time this allows to distinguish between both entities by EIA. The first results had been presented as oral presentation at the Congress of the German Society of Thrombosis and Hemostasis (GTH) 2023. The assay is currently a prototype. Further studies on test characteristics are ongoing, test performance metrics such as sensitivity, specificity, positive predictive value, etc. cannot yet be quantified.

8 DISCUSSION

Two years after the first cases of patients with VITT were described[1-3], this prospective patient registry allows to answer many of the questions of VITT patients and treating physicians about the long-term management of VITT (**Table 1**).

Of course, only patients who survived the acute phase of VITT could be enrolled into this registry. Only one of the 71 enrolled VITT patients died during the study period. This indicates that once the acute phase of VITT has been survived, the risk for new thrombotic complications is low. However, several of the 71 patients are still severely impaired by the sequalae of the initial thrombotic complications during the acute phase of VITT. Also, the one deceased patient died because of long-term VITT sequalae.

VITT resembles heparin-induced thrombocytopenia in several aspects. It is caused by antibodies against PF4, although these antibodies bind to a different epitope on PF4, which may be an important reason for the different clinical manifestations.[29] Both anti-PF4 responses are transient but the VITT immune response lasts much longer then antibody persistence in heparin-induced thrombocytopenia.[13] Whether this is caused by the primarily oligoclonal nature of the VITT antibody response in comparison to a polyclonal immune response in heparin-induced thrombocytopenia is still unresolved.[15,34] Nevertheless, after one year, in the vast majority of VITT patients platelet activating anti-PF4 antibodies have disappeared. In our cohort of 71 patients, in only 6 patients (8.5%) platelet activating anti-PF4 antibody persisted for more than 18 months. Only in one patient VITT seems to be persistent with recurrent thrombocytopenia and thrombotic complications.

An interesting observation of our registry is that recurrent thrombocytopenia and/or new thrombotic complications only occurred in patients in whom platelet activating anti-PF4 antibodies were still present. Whether this is an association or causality remains unresolved. At least in one patient with recurrent thrombocytopenia severe liver impairment due to VITT related massive splanchnic thrombosis is an alternative explanation. Also, in the two patients with recurrent thrombosis without thrombocytopenia, alternative explanations for thrombosis are likely. In the first patient with recurrent pulmonary embolism four months after the acute phase of VITT, new

pulmonary embolism occurred during a gastrointestinal infection with related subtherapeutic INR. The other patient who developed stroke had a history of smoking and uncontrolled hypertension when stroke occurred.

Neither additional vaccinations against COVID-19, nor any of the other vaccines caused boosting of anti-PF4 antibodies in patients with a history of VITT. This is an important information for patient management and indicates that anti-PF4 antibodies in VITT are likely triggered by a class effect of adenovirus vector-based vaccines. None of the consecutive vaccines applied to these patients with a history of VITT during the study were adenovirus vector-based vaccines. However, in the UK cohort five VITT patients had received a second shot of an adenovirus vector-based vaccine.[35] Also in them no complications occurred. In contrast to the present study, anti-PF4 antibodies were not measured in these patients before and after second vaccination. Thus, the question remains unresolved whether the anti-PF4 antibodies might have been increased.

As a note of caution, the number of patients who received further non-COVID-19 vaccines within our registry is still small. Since it is still not clear, which vaccine constituent triggered VITT, VITT patients should be monitored after each new vaccination to recognize a platelet count decrease or new thrombosis in time. This is relevant, because one patient has been reported who developed a VITT-like clinical presentation after vaccination with the human papilloma virus vaccine, which is not based on an adenovirus vector.[36]

24 of the 71 VITT patients developed COVID-19 within the study period. In none of them infection with SARS-CoV-2 induced an increase in anti-PF4 antibodies, thrombocytopenia, or thrombosis. 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. Our study provides solid in-vivo evidence that SARS-CoV-2 exposure does not boost the VITT anti-PF4 immune response.

Rapid development of vaccines against COVID-19 is unprecedented and was a game changer in fighting the pandemic. VITT was an unexpected and rare, but life-threatening complication. Joint efforts of physicians, researchers and regulatory agencies identified

the mechanism of VITT[29,37,38] and provided rapid tools for diagnosis and treatment[39]. This decreased mortality of VITT by nearly 90%.[6,40]

The strength of our study is the large number of individuals enrolled and the very low number of individuals lost during follow up. A further unique strength of this study is the availability of consecutive laboratory samples over time, which allowed definite conclusions on the safety of further vaccinations and/or SARS-CoV-2 infections regarding boosting of the anti-PF4 antibodies. The combination of careful clinical observation of the patients with cutting edge laboratory testing provides data which are not available in any other patient cohort. The biomaterial saved within this study bears the perspective to address further research questions in VITT in the future.

Limitation of this study is that only patients had been enrolled who survived the acute phase of VITT. Thus, characteristics of the most severely affected individuals cannot be deducted from this registry. We also have no RNA samples of these patients or individual leukocyte subpopulations collected. This causes major limitations for unraveling the potential underlying immunological mechanisms leading to the immune response.

General Conclusion

The EMA initiated the present registry to solve the many open issues and questions which remained after the acute phase of VITT. This registry provides evidence on how to manage VITT patients after the acute episode based on the largest prospective longitudinally observed patient cohort. In the meantime, first case reports occurred indicating that a VITT-like immune response also underlies recurrent thrombotic complications in other patients independent of vaccination.[11,41] The information provided in this report may have the potential to contribute to manage these patients in future, beyond its relevance for the safety and management of the individuals affected by the rare adverse vaccination event VITT.

9 PUBLICATIONS CONNECTED TO CONTRACT EMA/2021/17/TDA

Publications resulting from the prospective cohort study on VITT patients supported by the European Medicines Agency under contract EMA/2021/17/TDA:

[1] Schönborn L, Thiele T, Kaderali L, et al. Most anti-PF4 antibodies in vaccine-induced immune thrombotic thrombocytopenia are transient. Blood. 2022;139(12):1903-1907. doi:10.1182/blood.2021014214.

[2] Greinacher A, Schönborn L, Siegerist F, et al. Pathogenesis of vaccine-induced immune thrombotic thrombocytopenia (VITT). Semin Hematol. 2022;59(2):97-107. doi:10.1053/j.seminhematol.2022.02.004.

[3] Schönborn L, Greinacher A. Longitudinal Aspects of VITT. Semin Hematol. 2022;59(2):108-114. doi:10.1053/j.seminhematol.2022.03.

[4] Schönborn L, Seck SE, Thiele T, Warkentin TE, Greinacher A. SARS-CoV-2 Infection in Patients with a History of VITT. New England Journal of Medicine 2022;387(1):88-90. DOI: 10.1056/NEJMc2206601.

[5] Schönborn L, Thiele T, Esefeld M, et al. Quantitative interpretation of PF4/heparin-EIA optical densities in predicting platelet-activating VITT antibodies. J Thromb Haemost. 2022;20(11):2579-2586. doi:10.1111/jth.

10 PRESENTATION OF STUDY RESULTS FOR STUDY PATIENTS

This study was only possible thanks to the continuous support of VITT patients and their treating physicians. As part of this study, we analyzed more than 700 blood samples (about 10 blood samples per patient!). In addition, we were immediately provided with clinical information, especially after follow-up vaccinations and SARS-CoV-2 infections. This, in turn, has enabled us to advise other VITT patients on further vaccinations. We regularly informed all study participants about ongoing study results in update letters (see Appendix 1-3), so that patients and their treating physicians could benefit directly from our findings. In addition, we were available for individual consultations by telephone for patients and treating physicians during the entire follow-up period.

Appendix 1 – Update letter September 2021

Appendix 2 – Update letter April 2022

Appendix 3 – Update letter December 2022

11 INTERNATIONAL RECOGNITION AND AWARDS

The results of the study were not only published in high-ranking international journals, they had been also recognized by the international scientific community by the following awards:

2021	Virchow Award of the Aktionsbündnis Thrombose (Andreas Greinacher)
2022	Award of the German Medical Universities (Linda Schönborn, Andreas Greinacher, Thomas Thiele, and others)
2022	Award for Analytical Biochemistry (Andreas Greinacher)
2022	Award and Recognition by the Physicians Association of the state Mecklenburg-Western Pomerania (Linda Schönborn, Andreas Greinacher, and others)
2023	Bayer Thrombosis Research Award (Linda Schönborn)
2023	Wallace H. Coulter distinguished lecture and award (Andreas Greinacher)

REFERENCES

1. Scully M, Singh D, Lown R, et al. Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination. N Engl J Med 2021;384(23):2202-2211. DOI: 10.1056/NEJMoa2105385.
2. 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(22):2092-2101. DOI: 10.1056/NEJMoa2104840.
3. Schultz NH, Sorvoll IH, Michelsen AE, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med 2021;384(22):2124-2130. DOI: 10.1056/NEJMoa2104882.
4. Pavord S, Scully M, Hunt BJ, et al. Clinical Features of Vaccine-Induced Immune Thrombocytopenia and Thrombosis. N Engl J Med 2021. DOI: 10.1056/NEJMoa2109908.
5. Salih F, Schonborn L, Kohler S, et al. Vaccine-Induced Thrombocytopenia with Severe Headache. N Engl J Med 2021;385(22):2103-2105. DOI: 10.1056/NEJMc2112974.
6. TGA. COVID-19 vaccine safety report - 23-09-2022. In: Care AG-DoHaA, ed.2022.
7. Schönborn L, Thiele T, Kaderali L, Greinacher A. Decline in Pathogenic Antibodies over Time in VITT. N Engl J Med 2021. DOI: 10.1056/NEJMc2112760.
8. Schönborn L, Thiele T, Kaderali L, et al. Most Anti-PF4 Antibodies in Vaccine-induced Immune Thrombotic Thrombocytopenia are transient. Blood 2022. DOI: 10.1182/blood.2021014214.
9. Greinacher A, Schonborn L, Siegerist F, et al. Pathogenesis of vaccine-induced immune thrombotic thrombocytopenia (VITT). Semin Hematol 2022;59(2):97-107. DOI: 10.1053/j.seminhematol.2022.02.004.
10. Schonborn L, Greinacher A. Longitudinal Aspects of VITT. Semin Hematol 2022;59(2):108-114. DOI: 10.1053/j.seminhematol.2022.03.001.
11. Greinacher A, Langer F, Schönborn L, et al. Platelet-activating anti-PF4 antibodies mimicking VITT antibodies in an unvaccinated patient with monoclonal gammopathy Haematologica 2021:online ahead of print.
12. Platton S, Bartlett A, MacCallum P, et al. Evaluation of laboratory assays for anti-platelet factor 4 antibodies after ChAdOx1 nCOV-19 vaccination. J Thromb Haemost 2021. DOI: 10.1111/jth.15362.
13. Warkentin TE, Kelton JG. Temporal aspects of heparin-induced thrombocytopenia. N Engl J Med 2001;344(17):1286-92. DOI: 10.1056/NEJM200104263441704.
14. Greinacher A, Selleng K, Mayerle J, et al. Anti–platelet factor 4 antibodies causing VITT do not cross-react with SARS-CoV-2 spike protein. Blood 2021;138(14):1269-1277. DOI: 10.1182/blood.2021012938.
15. Wang JJ, Armour BL, Chataway T, et al. Vaccine-induced immune thrombotic thrombocytopenia (VITT) is mediated by a stereotyped clonotypic antibody. Blood 2022 (In eng). DOI: 10.1182/blood.2022016474.
16. Karnes JH, Rollin J, Giles JB, et al. ABO O blood group as a risk factor for platelet reactivity in heparin-induced thrombocytopenia. Blood 2022;140(3):274-284. (In eng). DOI: 10.1182/blood.2021014240.
17. Greinacher A, Ittermann T, Bagemühl J, et al. Heparin-induced thrombocytopenia: towards standardization of platelet factor 4/heparin antigen tests. Journal of Thrombosis and Haemostasis 2010;8(9):2025-2031. DOI: https://doi.org/10.1111/j.1538-7836.2010.03974.x.
18. Warkentin TE, Sheppard JI, Moore JC, Sigouin CS, Kelton JG. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. Journal of Thrombosis and Haemostasis 2008;6(8):1304-1312. DOI: https://doi.org/10.1111/j.1538-7836.2008.03025.x.

19. Lindhoff-Last E, Eichler P, Stein M, et al. A Prospective Study on the Incidence and Clinical Relevance of Heparin-Induced Antibodies in Patients after Vascular Surgery. Thrombosis Research 2000;97(6):387-393. DOI: https://doi.org/10.1016/S0049-3848(99)00198-X.
20. Thiele T, Ulm L, Holtfreter S, et al. Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2. Blood 2021;138(4):299-303. DOI: 10.1182/blood.2021012217.
21. Nazy I, Sachs UJ, Arnold DM, 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(6):1585-1588. DOI: 10.1111/jth.15341.
22. Krauel K, Preusse P, Warkentin TE, et al. Fibronectin modulates formation of PF4/heparin complexes and is a potential factor for reducing risk of developing HIT. Blood 2019;133(9):978-989. DOI: 10.1182/blood-2018-05-850370.
23. Greinacher A, Liebenhoff U, Kiefel V, Presek P, Mueller-Eckhardt C. Heparin-Associated Thrombocytopenia: The Effects of Various Intravenous lgG Preparations on Antibody Mediated Platelet Activation - A Possible New Indication for High Dose i.v. IgG. Thromb Haemost 1994;71(05):641-645. (In En).
24. Padmanabhan A, Jones CG, Pechauer SM, et al. IVIg for Treatment of Severe Refractory Heparin-Induced Thrombocytopenia. Chest 2017;152(3):478-485. DOI: https://doi.org/10.1016/j.chest.2017.03.050.
25. Chong B. Heparin-induced thrombocytopenia. Australian and New Zealand journal of medicine 1992;22(2):145-152.
26. Warkentin TE. High-dose intravenous immunoglobulin for the treatment and prevention of heparin-induced thrombocytopenia: a review. Expert Review of Hematology 2019;12(8):685-698. DOI: 10.1080/17474086.2019.1636645.
27. Krauel K, FÜRll B, Warkentin TE, et al. Heparin-induced thrombocytopenia – therapeutic concentrations of danaparoid, unlike fondaparinux and direct thrombin inhibitors, inhibit formation of platelet factor 4–heparin complexes. Journal of Thrombosis and Haemostasis 2008;6(12):2160-2167. (https://doi.org/10.1111/j.1538-7836.2008.03171.x). DOI: https://doi.org/10.1111/j.1538-7836.2008.03171.x.
28. Singh A, Toma F, Uzun G, et al. The interaction between anti-PF4 antibodies and anticoagulants in vaccine-induced thrombotic thrombocytopenia. Blood 2022;139(23):3430-3438. DOI: 10.1182/blood.2021013839.
29. Huynh A, Kelton JG, Arnold DM, Daka M, Nazy I. Antibody epitopes in vaccine-induced immune thrombotic thrombocytopenia. Nature 2021. DOI: 10.1038/s41586-021-03744-4.
30. Nguyen T-H, Medvedev N, Delcea M, Greinacher A. Anti-platelet factor 4/polyanion antibodies mediate a new mechanism of autoimmunity. Nature Communications 2017;8(1):14945. DOI: 10.1038/ncomms14945.
31. Greinacher A, Alban S, Omer-Adam MA, Weitschies W, Warkentin TE. Heparin-induced thrombocytopenia: A stoichiometry-based model to explain the differing immunogenicities of unfractionated heparin, low-molecular-weight heparin, and fondaparinux in different clinical settings. Thrombosis Research 2008;122(2):211-220. DOI: https://doi.org/10.1016/j.thromres.2007.11.007.
32. Arepally GM, Chudasama S, Espinasse B, et al. Heparin Modifies the Immunogenicity of Positively-Charged Proteins. Blood 2010;116(21):1435. DOI: https://doi.org/10.1182/blood.V116.21.1435.1435.
33. Heidelberger M, Kendall FE. The precipitin reaction between type III pneumococcus polysaccharide and homologous antibody: III. A quantitative study and a theory of the reaction mechanism. Journal of Experimental Medicine 1935;61(4):563-591. DOI: 10.1084/jem.61.4.563.

34. Kanack AJ, Bayas A, George G, et al. Monoclonal and oligoclonal anti-platelet factor 4 antibodies mediate VITT. Blood 2022;140(1):73-77. DOI: 10.1182/blood.2021014588.
35. Lacy J, Pavord S, Brown KE. VITT and Second Doses of Covid-19 Vaccine. N Engl J Med 2021. DOI: 10.1056/NEJMc2118507.
36. Johansen S, Lægreid IJ, Ernstsen SL, et al. Thrombosis and thrombocytopenia after HPV vaccination. J Thromb Haemost 2021. DOI: https://doi.org/10.1111/jth.15604.
37. Greinacher A, Selleng K, Palankar R, et al. Insights in ChAdOx1 nCoV-19 vaccine-induced immune thrombotic thrombocytopenia. Blood 2021;138(22):2256-2268. DOI: 10.1182/blood.2021013231.
38. Selvadurai MV, Favaloro EJ, Chen VM. Mechanisms of Thrombosis in Heparin-Induced Thrombocytopenia and Vaccine-Induced Immune Thrombotic Thrombocytopenia. Semin Thromb Hemost 2023(EFirst) (In En). DOI: 10.1055/s-0043-1761269.
39. Oldenburg J, Klamroth R, Langer F, et al. Diagnosis and Management of Vaccine-Related Thrombosis following AstraZeneca COVID-19 Vaccination: Guidance Statement from the GTH. Hamostaseologie 2021;41(3):184-189. (In eng). DOI: 10.1055/a-1469-7481.
40. Cines DB, Greinacher A. Spotlight on vaccine-induced thrombosis and thrombocytopenia (VITT). Blood 2023. DOI: 10.1182/blood.2022017696.
41. Lindhoff-Last E. Platelet factor 4 IgG antibodies - a novel autoimmune marker for acquired thrombophilia. Annual Congress of the Society of Thrombosis and Hemostasis Research. Frankfurt a. M.2023.