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BRIEF REPORT

Quantitative interpretation of PF4/heparin-EIA optical densities in predicting platelet-activating VITT antibodies

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Abstract

Background: Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a prothrombotic, heparin-induced thrombocytopenia (HIT)-mimicking, adverse reaction caused by platelet-activating anti-platelet factor 4 (PF4) antibodies that occurs rarely after adenovirus vector-based COVID-19 vaccination. Strength of PF4-dependent enzyme immunoassay (EIA) reactivity—judged by optical density (OD) measurements—strongly predicts platelet-activating properties of HIT antibodies in a functional test. Whether a similar relationship holds for VITT antibodies is unknown.

Objectives: To evaluate probability for positive platelet activation testing for VITT antibodies based upon EIA OD reactivity; and to investigate simple approaches to minimize false-negative platelet activation testing for VITT.

Methods: All samples referred for VITT testing were systematically evaluated by semiquantitative in-house PF4/heparin-EIA (OD readings) and PF4-induced platelet activation (PIPA) testing within a cohort study. EIA-positive sera testing PIPA-negative were retested following 1/4 to 1/10 dilution. Logistic regression was performed to predict the probability of a positive PIPA per magnitude of EIA reactivity.

Results: Greater EIA ODs in sera from patients with suspected VITT correlated strongly with greater likelihood of PIPA reactivity. Of 61 sera (with OD values >1.0) testing negative in the PIPA, a high proportion (27/61, 44.3%) became PIPA positive when tested at 1/4 to 1/10 dilution.

Conclusions: VITT serology resembles HIT in that greater EIA OD reactivity predicts higher probability of positive testing for platelet-activating antibodies. Unlike the situation with HIT antibodies, however, diluting putative VITT serum increases probability of a positive platelet activation assay, suggesting that optimal complex formation depends on the stoichiometric ratio of PF4 and anti-PF4 VITT antibodies.

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KEYWORDS
COVID-19, enzyme immunoassay, platelet factor 4, vaccination, vaccine-induced immune thrombotic thrombocytopenia

1 | 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] Diagnosis of VITT resembles the classic heparin-induced thrombocytopenia (HIT) paradigm of a "clinical-pathological" disorder, based on (a) clinical criteria (thrombosis and/or thrombocytopenia beginning at least 5 days post-vaccination),[5–7] and (b) pathological (serological) criteria (demonstration of anti-platelet factor 4 [PF4] antibodies by enzyme immunoassay [EIA] that activate platelets in the presence of PF4).[6] Platelet-activating anti-PF4 antibodies can be detected by PF4-enhanced serotonin-release assay, by whole blood platelet aggregation, or by PF4-enhanced flow cytometry, either with washed platelets or whole blood.[8] Our laboratory uses a PF4-enhanced washed platelet assay, the PF4-induced platelet activation test (PIPA).[2]

One explanation for why VITT was quickly identified in March 2021 was because the same screening immunoassay used for HIT—PF4-dependent EIAs—typically yield positive results in serum from VITT patients. However, whether strong reactivity by PF4-dependent EIAs predicts presence of platelet-activating antibodies (a feature well established in HIT) has not been formally assessed for VITT. In our present study, we examined whether greater reactivity by PF4-dependent EIA (judged by optical density [OD] values) predicted a higher likelihood of a positive functional test (PIPA) for detecting platelet-activating VITT antibodies. We encountered several putative VITT sera that tested EIA positive with an OD >1.0, in which a positive PIPA test could only be obtained if the sera were diluted prior to testing.

Essentials

- Vaccine-induced immune thrombotic thrombocytopenia (VITT) has many similarities to heparin-induced thrombocytopenia (HIT).
- In a large cohort of VITT patients we tested correlation of clinical presentation with laboratory test results.
- Higher enzyme immunoassay optical densities predict greater likelihood of platelet-activating VITT antibodies.
- False-negative platelet activation testing for VITT is reduced by diluting test serum 1/4 to 1/10.

2 | MATERIAL AND METHODS

2.1 | Data collection

We studied blood samples of consecutive patients referred to the platelet laboratory of the Institute of Transfusion Medicine Greifswald, Germany, between March 16, 2021 and February 17, 2022 for serological investigation of anti-PF4 VITT antibodies; only the first sample received was included in this analysis when multiple samples were received from an individual patient. Samples were excluded from analysis if the patient did not receive COVID-19 vaccination, or if neither thrombocytopenia nor thrombosis occurred.

Serum was prepared from whole blood by centrifugation, and tested by IgG-specific anti-PF4/heparin EIA and PIPA; remaining serum was stored at −20°C for use in follow-up assays. VITT diagnosis was considered confirmed by demonstrating anti-PF4 antibodies (EIA positive) inducing PF4-dependent platelet activation (PIPA positive).

Patient characteristics (age, sex, vaccination status) were obtained from the laboratory documentation provided by the referring physician.

2.2 | Anti-PF4/heparin IgG EIA

We used an in-house IgG-specific anti-PF4/heparin EIA[2] with cutoff 0.5 OD units. Samples testing 0.5 to <1.0 were defined as weakly positive, between 1.0 to <1.5 as moderately positive, between 1.5 to <2.0 as strongly positive, and ≥2.0 OD were defined as very strongly positive.

2.3 | PF4-dependent washed platelet assay

Presence of platelet-activating antibodies was detected by PIPA.[2,9] In brief, platelets were purified from acid citrate dextrose solution A anticoagulated whole blood obtained from healthy donors, washed, and diluted in Tyrode's buffer (300 000 platelets/μl). Heat-inactivated patient serum (20 μl) was incubated with the washed platelets (75 μl) in a microtiter plate (Greiner Bio-One) with either buffer or 10 μl PF4 (10 μg/ml [final], ChromaTec), with hirudin (5 U/ml) added to avoid effects of residual thrombin. Under stirring (1000 rpm) the transparency of the suspension was assessed using an indirect light source every 5 min. A positive result was defined as PF4-dependent activation of platelets (lag time, <30 min) of at least two of three different donors and inhibition at high heparin concentrations (100 IU/ml). Sera testing

negative were retested with a new panel of washed platelets. A serum that activated platelets at all conditions (buffer, low concentrations of PF4, and high concentrations of heparin) was classified as indeterminate. For sera with an OD >1.0 in the anti-PF4/heparin IgG EIA and a negative PIPA, the platelet activation assay was repeated with the serum diluted 1/4 (i.e., 50 µl serum and 150 µl saline); for 1/4-diluted sera that remained PIPA negative, PIPA was repeated with the serum diluted 1/10 (i.e., 20 µl serum and 180 µl saline).

2.4 | Statistical analysis

Statistical analysis was performed in R, version 4.2.1. Logistic regression was used to associate the ODs of the anti-PF4/heparin IgG EIA as independent variable with PIPA results as dependent variable. (For presentation in Figure 2, 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].) The probability of an EIA result to predict a positive PIPA test was calculated for five categories: OD <0.5; 0.5 to <1.0; 1.0 to <1.5; 1.5 to <2.0; and ≥2.0, using the binomial distribution with Wilson confidence intervals.

2.5 | Ethics and data protection rules

The study was approved by the ethics board of the University Medicine Greifswald (BB 052/21).

3 | RESULTS AND DISCUSSION

3.1 | Patient characteristics

In total, 975 patients suspected to have VITT were enrolled (Figure 1); 542 (55.6%) were female. Median age was 53 years (range, 13–91 years). Sex and age were unknown for 0.8% and 1.3% of the patients, respectively. Three hundred eighty-five patients (39.5%) were vaccinated with ChAdOx1 nCoV-19 (Vaxzevria, AstraZeneca), 188 patients (19.3%) with BNT1222 (Comirnaty, BioNTech/Pfizer), 29 patients (3.0%) with Ad26.COV2.S (Jcovden, Janssen-Cilag/Johnson & Johnson), 25 patients (2.6%) with mRNA-1273 (Spikevax, Moderna). Twenty patients (2.1%) were referred after second vaccination with an mRNA-vaccine after having first received a vector-based vaccine. For 328 patients (33.6%) no information regarding vaccine type was available.

3.2 | Results of EIA and PIPA testing

Six hundred seventy-five samples (69.2%) were negative in all assays; 107 samples (11.0%) showed a positive EIA but negative PIPA; and 175 samples (17.9%) showed the typical pattern of VITT, with positive results by both EIA and PIPA (Figure 1). Only one sample (0.1%) showed a negative EIA but positive PIPA. Seventeen sera were PIPA indeterminate.

3.3 | Strong association between OD levels in the EIA and a positive PIPA

We next determined the probability of the magnitude of a positive EIA result for the presence of PF4-dependent platelet-activating antibodies (Figure 2), as judged by the PIPA (17 PIPA indeterminate were not considered for calculation). Only one EIA-negative serum (OD <0.5) yielded a positive PIPA test result (1/676 sera [0.1%]); weakly positive sera (OD 0.5 to <1.0) showed a positive PIPA in 4.2% (3/72 sera); moderately positive sera (OD 1.0 to <1.5) yielded a positive PIPA in 21.9% (7/32 sera); those with a strong positive anti-PF4/heparin IgG EIA result (OD 1.5 to <2.0) showed a positive PIPA in 50.0% (9/18 sera); those sera that gave a very strong positive EIA result (OD ≥2.0) were positive by PIPA in 97.5% (156/160 sera). The probabilities for prespecified OD ranges to predict PIPA-positive status and the respective 95% confidence intervals are given at the top of Figure 2.

3.4 | Effect of antibody dilution on the PIPA

Initially, 64 samples with ODs >1.0 in the EIA tested PIPA negative. When the available 61 sera were diluted 1/4 and retested in the PIPA, 26 (42.6%) showed strong platelet activation, representing 14.8% of all PIPA-positive sera (for three patients no residual serum was available for retesting). In one serum, we observed a positive reaction in the PIPA when serum was diluted 1/10. Of the remaining 34 samples, which were still negative after dilution, we identified only one patient for whom treatment with intravenous immunoglobulin (IVIG) before the diagnostic blood sample was documented (strong potential for giving false-negative PIPA result due to interference of platelet activation by high-dose IVIG[10]). Please note: in Figure 1 only the final results of functional testing are given; that is, sera testing positive in the PIPA after dilution only are shown in the lowest boxes as EIA-positive and PIPA-positive samples.

Our study shows that the likelihood for a positive result in the PIPA strongly correlates with the OD measured in the EIA. Further, a negative PIPA despite a positive EIA (OD >1.0) should prompt 4-fold to 10-fold 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.[11,12] 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 we investigated activated platelets; this is a higher proportion than in most prospective HIT studies[11,13] and might be explained by the distinct clinical picture of VITT leading to a higher proportion of true VITT sera being referred for investigation. It is known that anti-PF4 antibodies

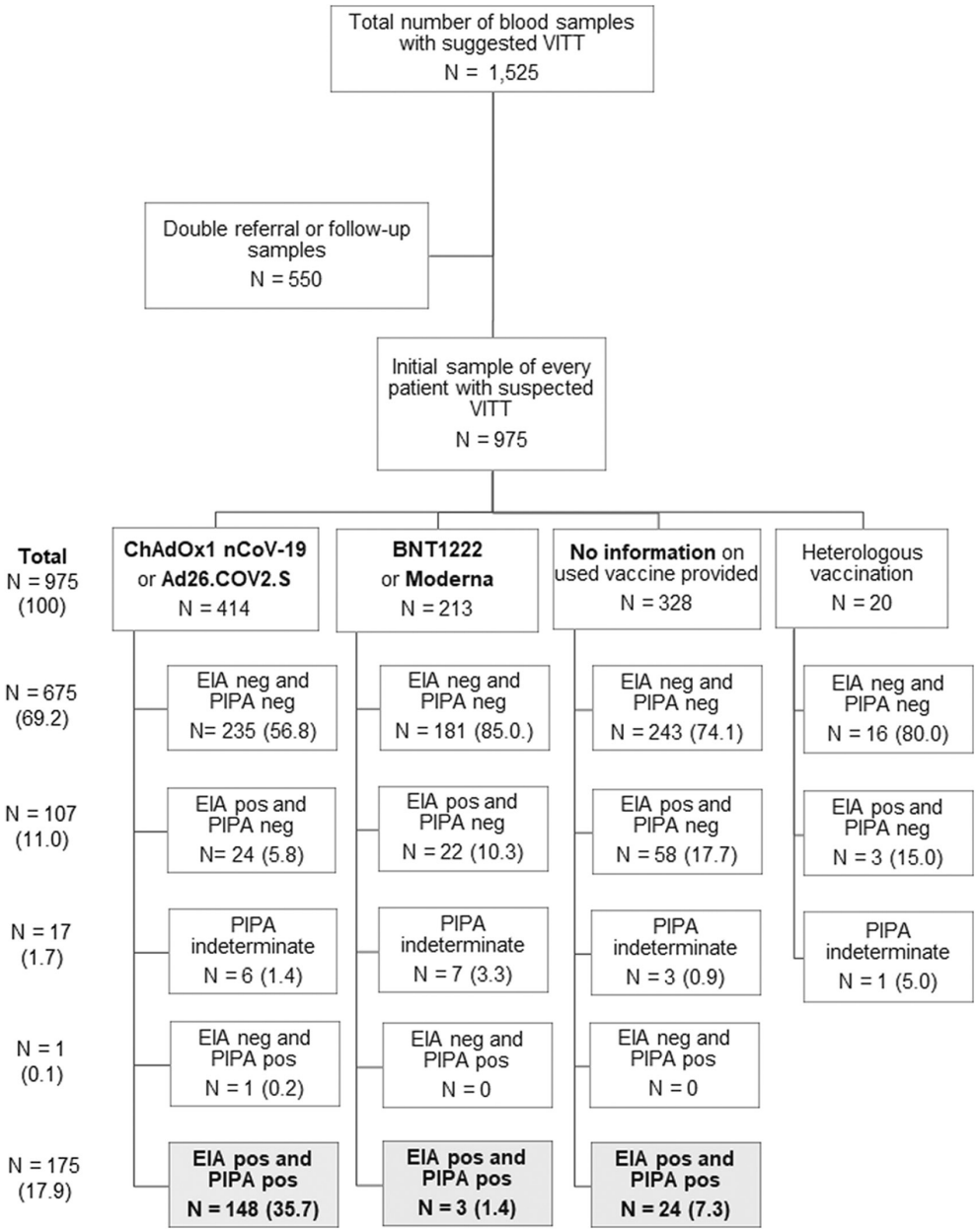

FIGURE 1 Overview on the patient cohort and results of the anti-PF4/heparin IgG EIA and the PF4-dependent washed platelet assay (=PIPA) depending on the type of COVID-19 vaccine. The percentages refer to the total number of each column. Please note: only the final results are given; that is, sera testing positive in the PIPA after dilution only are shown in the lowest boxes as EIA-positive and PIPA-positive samples. EIA, enzyme immunoassay; PF4, platelet factor 4; PIPA, platelet factor 4–induced platelet activation; VITT, vaccine-induced immune thrombotic thrombocytopenia.

occur in approximately 5% of individuals vaccinated with an adenovirus vector or mRNA vaccine.[14] Thus, some of the sera that 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 (data on file). This dilution dependency may also help explain the relatively low sensitivity of functional assays for VITT in a recent workshop.[6]

There are several potential explanations for this dilution effect. First, there could be an inhibitory factor in the serum which is outdiluted (e.g., fibronectin, which has been shown to interfere with platelet activation by anti-PF4/heparin antibodies in HIT).[15] Second, high concentrations of IgG after IVIG treatment can inhibit platelet activation in HIT.[16–19] 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[20] and VITT antibodies.[21] However, danaparoid treatment was

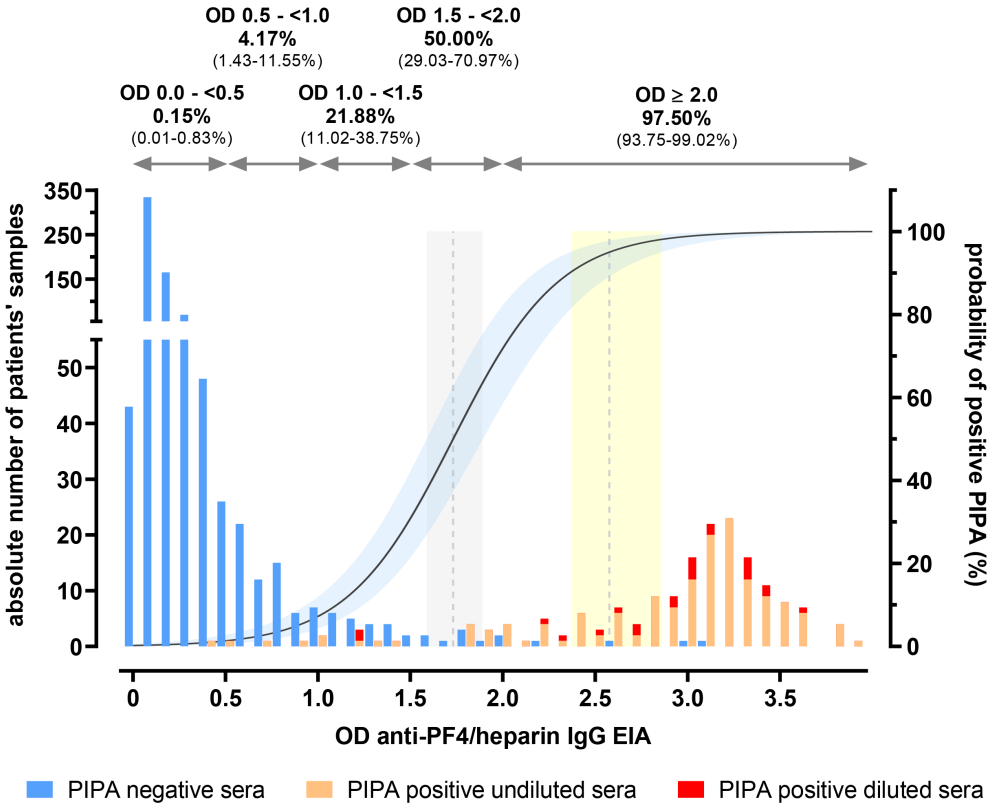

FIGURE 2 Correlation between reactivity in the anti-PF4/heparin IgG EIA and a positive PIPA. Absolute number of positive and negative results in the PF4-dependent washed platelet assay (PIPA) depending on the OD in the anti-PF4/heparin IgG EIA (blue bar = negative PIPA; orange bar = positive PIPA of 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 gray and yellow shades show the 50% and 95% probability (with CI) of a positive PIPA, respectively. 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 positive). The 17 sera that gave indeterminate results in the PIPA are not included into the figure and not included into the calculation of probability. CI, confidence interval; EIA, enzyme immunoassay; OD, optical density; PF4, platelet factor 4; PIPA, platelet factor 4–induced platelet activation.

excluded in the majority of patients assessed. We suggest that the most likely explanation is based on the fundamental findings of Huynh et al.,[22] 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.[23] It is well known from HIT that optimal complex formation depends on the stoichiometric ratio between PF4 and heparin.[24,25] 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 3). 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.[26] This concept is also consistent with the widely accepted observation that addition of PF4 strongly enhances reactivity of VITT antibodies.[2] Such a dependency of immune complex formation on antibody concentration is not observed in HIT, because the binding strengths between PF4 and heparin is so strong that large multimolecular PF4/heparin complexes will invariably be formed to which antibodies can subsequently bind.

Our findings show that the functional test (PIPA) and the antigen test (EIA) are complementary, and a strong positive result in the EIA may indicate a false-negative result in the PIPA, if the clinical presentation is otherwise suggestive for VITT.

Finally, we identified three patients vaccinated with BNT1222 who showed PF4-dependent platelet-activating antibodies. Isolated cases of anti-PF4 antibodies after mRNA-based COVID-19 vaccination were also described by other groups.[27,28] Whether these rare cases are caused by the mRNA vaccine or reflect association of two independent events requires further investigation.

Our data provide additional information on the characteristics of anti-PF4 antibodies in VITT and may help to improve diagnosis by including a 1/4 dilution step in functional assays for PF4-dependent

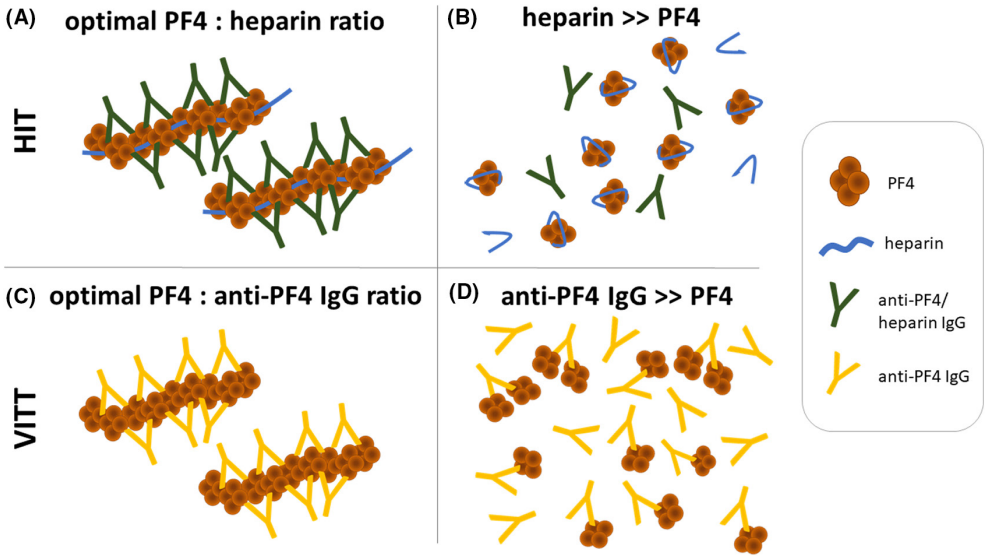

FIGURE 3 Schematic presentation of the dependency of immune complex formation in HIT and VITT on stoichiometric ratios. HIT (A) optimal ratio of PF4 (cationic) and heparin (anionic) results in formation of multimolecular complexes. Anti-PF4/heparin IgG antibodies bind to these complexes. The resulting 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 the 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 the 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. HIT, heparin-induced thrombocytopenia; PF4, platelet factor 4; VITT, vaccine-induced immune thrombotic thrombocytopenia.

platelet activating antibodies. Our findings may also be relevant for improving detection of other VITT-mimicking anti-PF4 disorders that occur independent of COVID-19 vaccination.[29]

AUTHOR CONTRIBUTIONS

A. Greinacher, L. Schönborn, T.E. Warkentin, and T. Thiele developed the concept; L. Schönborn, T. Thiele, K. El Debuch, S.E. Seck, and M. Esefeld characterized patients; K. El Debuch and J. Wesche performed experiments; L. Schönborn, J. Wesche, L. Kaderali, and M. Wolff created the figures; L. Kaderali performed the statistical analysis. All authors wrote the manuscript and critically revised and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

L. Schönborn reports that part of the results reported have been obtained in a study conducted by Universitätsmedizin Greifswald under service contract No. EMA/2021/17/TDA. T. Thiele reports grants from Deutsche Forschungsgemeinschaft, grants from European Medicines Agency, during the conduct of the study; personal fees, non-financial support, and other from Bristol Myers Squibb; personal fees, non-financial support, and other from Pfizer; personal fees from Bayer; personal fees, non-financial support, and other from Chugai Pharma; non-financial support and other from Novo Nordisk; personal fees from Novartis; non-financial support and other from Daichii Sankyo, all outside the submitted work. T.E. Warkentin has received lecture honoraria from Alexion and Instrumentation Laboratory, and royalties from Informa (Taylor & Francis) and UptoDate (Wolters Kluwer); has provided consulting services to Aspen Canada, Aspen Global, CSL Behring, Ergomed, Paradigm Pharmaceuticals, Octapharma, and Veralox Therapeutics; has received research funding from Instrumentation Laboratory; and has provided expert witness testimony relating to heparin-induced thrombocytopenia (HIT) and non-HIT thrombocytopenic and coagulopathic disorders. A. Greinacher reports personal fees from Aspen, grants from Ergomed, grants from Boehringer Ingelheim, personal fees from Bayer Vital, grants from Rovi, grants from Sagent, personal fees from ChromaTec, personal fees from Instrumentation Laboratory, grants and personal fees from Macopharma, grants from Portola, grants from Biokit, personal fees from Sanofi-Aventis, grants from Fa. Blau Farmaceutics, grants from Prosensa/Biomarin, grants and other from DRK-BSD NSTOB, grants from DRK-BSD Baden-Würtemberg/Hessen, personal fees from Roche, personal

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REFERENCES

1. 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.
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.
3. 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.
4. Salih F, Schönborn L, Kohler S, et al. Vaccine-induced thrombocytopenia with severe headache. *N Engl J Med*. 2021;385(22):2103-2105.
5. Pavord S, Scully M, Hunt BJ, et al. Clinical features of vaccine-induced immune thrombocytopenia and thrombosis. *N Engl J Med*. 2021;2021(385):1680-1689.
6. 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.
7. WHO. Guidance for clinical case management of thrombosis with thrombocytopenia syndrome (TTS) following vaccination to prevent coronavirus disease (COVID-19): World Health Organization; 2021 [updated July 19, 2021]. https://www.who.int/publications/i/item/WHO-2019-nCoV-TTS-2021.1
8. Warkentin TE, Greinacher A. Laboratory testing for VITT antibodies. *Sem Hemat*. 2022;59(2):80-88.
9. Handtke S, Wolff M, Zaninetti C, et al. A flow cytometric assay to detect platelet-activating antibodies in VITT after ChAdOx1 nCov-19 vaccination. *Blood*. 2021;137(26):3656-3659.
10. Bourguignon A, Arnold DM, Warkentin TE, et al. Adjunct immune globulin for vaccine-induced immune thrombotic thrombocytopenia. *N Engl J Med*. 2021;385(8):720-728.
11. Greinacher A, Ittermann T, Bagemühl J, et al. Heparin-induced thrombocytopenia: towards standardization of platelet factor 4/heparin antigen tests. *J Thromb Haemost*. 2010;8(9):2025-2031.
12. Warkentin TE, Sheppard JI, Moore JC, Sigouin CS, Kelton JG. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. *J Thromb Haemost*. 2008;6(8):1304-1312.
13. 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. *Thromb Res*. 2000;97(6):387-393.
14. 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.
15. 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.
16. Greinacher A, Liebenhoff U, Kiefel V, Presek P, Mueller-Eckhardt C. Heparin-associated thrombocytopenia: the effects of various intravenous IgG preparations on antibody mediated platelet activation - a possible new indication for high dose i.v. IgG. *Thromb Haemost*. 1994;71(5):641-645.
17. Padmanabhan A, Jones CG, Pechauer SM, et al. IVIg for treatment of severe refractory heparin-induced thrombocytopenia. *Chest*. 2017;152(3):478-485.
18. Chong B. Heparin-induced thrombocytopenia. *Aust NZ J Med*. 1992;22(2):145-152.
19. Warkentin TE. High-dose intravenous immunoglobulin for the treatment and prevention of heparin-induced thrombocytopenia: a review. *Expert Rev Hematol*. 2019;12(8):685-698.
20. 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. *J Thromb Haemost*. 2008;6(12):2160-2167.
21. 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.
22. Huynh A, Kelton JG, Arnold DM, Daka M, Nazy I. Antibody epitopes in vaccine-induced immune thrombotic thrombocytopenia. *Nature*. 2021;596:565-569.
23. Nguyen T-H, Medvedev N, Delcea M, Greinacher A. Anti-platelet factor 4/polyanion antibodies mediate a new mechanism of autoimmunity. *Nat Commun*. 2017;8(1):14945.
24. 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. *Thromb Res*. 2008;122(2):211-220.

25. Arepally GM, Chudasama S, Espinasse B, et al. Heparin modifies the immunogenicity of positively-charged proteins. *Blood*. 2010;116(21):1435.
26. 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. *J Exp Med*. 1935;61(4):563-591.
27. Su PH, Yu YC, Chen WH, et al. Case report: vaccine-induced immune thrombotic thrombocytopenia in a pancreatic cancer patient after vaccination with messenger RNA-1273. *Front Med (Lausanne)*. 2021;8:772424.
28. Sangli S, Virani A, Cheronis N, et al. Thrombosis with thrombocytopenia after the messenger RNA–1273 vaccine. *Ann Intern Med*. 2021;174(10):1480-1482.
29. Greinacher A, Langer F, Schönborn L, et al. Platelet-activating anti-PF4 antibodies mimicking VITT antibodies in an unvaccinated patient with monoclonal gammopathy. *Haematologica*. 2022;107(5):1219-1221.

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