Brief Report - Most Anti-PF4 Antibodies in Vaccine-induced Immune Thrombotic Thrombocytopenia are transient

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Running title: Temporal aspects of VITT

SUPPLEMENTARY MATERIAL

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Supplementary Methods

Data collection

As described[1], we identified all patients referred to our laboratory with clinical suspicion of VITT in whom we had confirmed the diagnosis by a positive anti-PF4/heparin IgG (EIA) and by demonstrating antibodies inducing PF4-dependent platelet activation. We obtained informed consent via the treating physicians, who then periodically referred serum samples (obtained during routine follow-up visits) for anti-PF4 antibody testing.

Time of vaccination and onset of symptoms were retrieved from the laboratory documentation obtained during initial diagnosis. In case of missing data, patients were contacted via telephone or e-mail to complete the dataset. In addition, when a second dose of SARS-CoV-2 vaccine was given, we obtained information about the clinical course after vaccination and obtained a serum sample one to six weeks after vaccination.

Assays for PF4-dependent antibodies

Anti-PF4 IgG antibodies were tested by anti-PF4/heparin IgG EIA.[2] The cut-off of the EIA was defined as optical density (OD) 0.5; samples testing ≥0.5 - 1.0 were defined as positive, between >1.0 - 2.0 as strongly–positive; greater 2.0 OD were defined as very strongly positive. As anti-PF4/heparin antibody titers in VITT sera are very high, the OD in the anti-PF4/heparin EIA remains in the upper range, even when antibody titers decline (ceiling effect). To differentiate between truly persisting and declining antibody titers, we diluted the sera of 4 patients with persistently high ODs with saline.

Platelet activation by the antibodies was tested in a PF4-dependent washed platelet assay.[3] A positive result was defined as PF4-dependent activation of platelets (lag time, <30 min) of at least two of three different donors. A negative result was defined if the serum did not activate platelets (lag time, <30 min) of at least two of three different donors tested on two consecutive days (total, six donors tested).

Statistical analysis

Statistical analysis was performed in R, version 4.1.0.[4] As follow-up times are censored and differ between study participants, anti-PF4/heparin IgG EIAs and functional tests were analyzed using the Kaplan-Meier method. OD-thresholds of 2.0, 1.5, 1.0, and 0.5 were used to dichotomize the anti-PF4/heparin IgG antigen assay after linear interpolation between measurement time points. Patients that achieved negative and then relapsed to positive functional test results were considered positive at all times. Confidence intervals for Kaplan-Meier estimates were computed using Greenwood's method, confidence intervals for the binomial distribution were computed using Wilson's approximation. A one-sided, paired Wilcoxon's signed rank test was used to compare optical densities of the first and last anti-PF4/heparin IgG EIA measurement per patient.

Ethics and data protection rules

The study was approved by the ethics board of the University Medicine Greifswald (BB 052/21a) and registered at the German Clinical Trials Register (DRKS00025738).

Data sharing statement

Original data are available for research purposes on reasonable request. No individual patient data will be released which may allow identification of a patient for privacy protection. Please contact the corresponding author andreas.greinacher@med.uni-greifswald.de for patient characteristics and lars.kaderali@med.uni-greifswald.de for biostatistics questions.

References

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3. 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.
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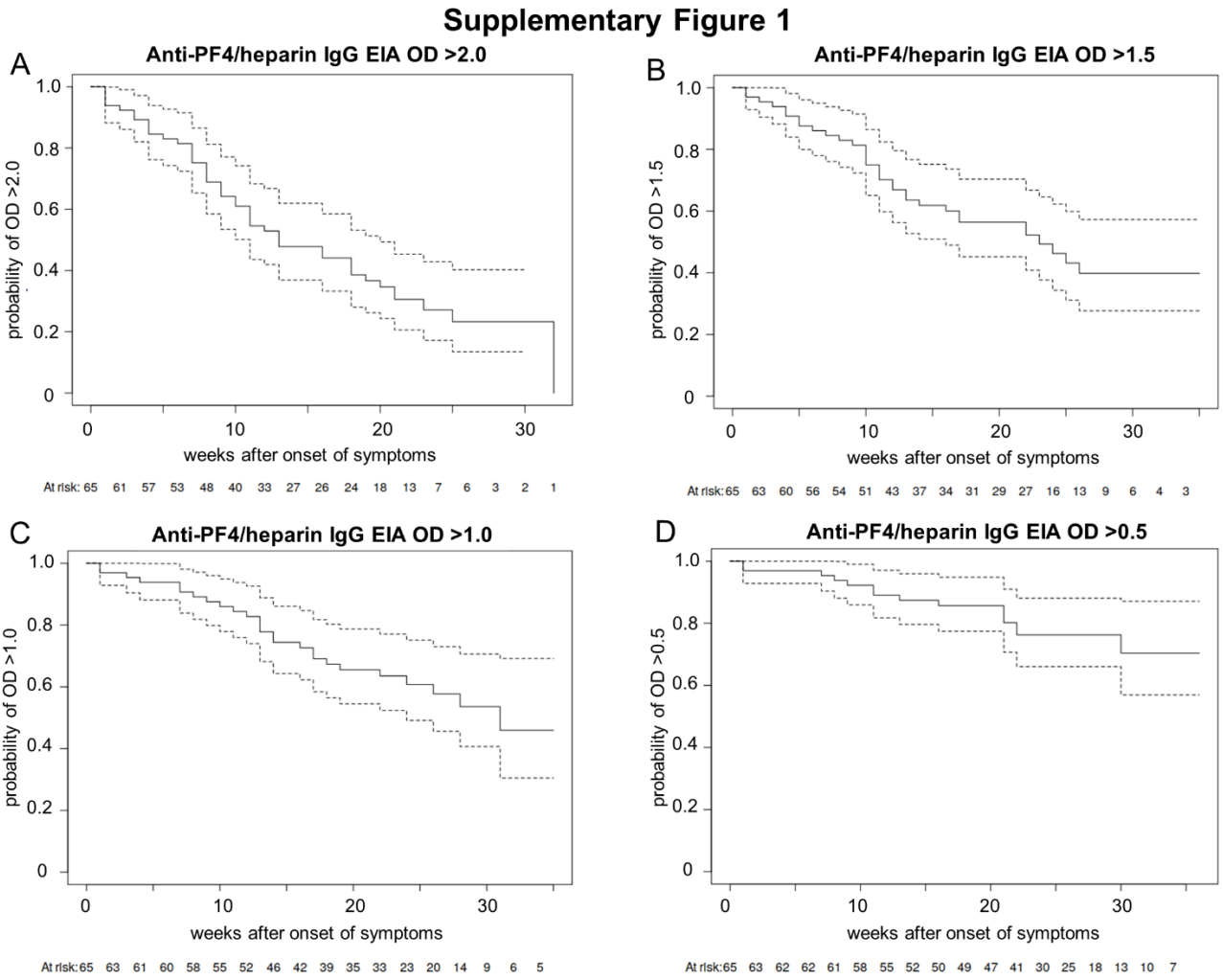
Supplementary Figures



Supplementary Figure 1: Kaplan-Meier Analysis of the proportion of patients with an anti-PF4/heparin EIA OD >2.0 (A), >1.5 (B), >1.0 (C), and >0.5 (D) (n=65)

Most patients initially showed high reactivity in the anti-PF4/heparin IgG EIA with ODs >2.0 at time of diagnosis of VITT. Antibody levels decreased over the time of follow-up. However, a negative EIA result (< 0.5 OD units) was only seen in 14 patients. Patients at risk are shown under each graph.