



Anti-PF4-antibodies in VITT the Greifswald findings

Andreas Greinacher

Institut für Transfusionsmedizin
Universitätsmedizin Greifswald, Germany



Disclosures for Andreas Greinacher

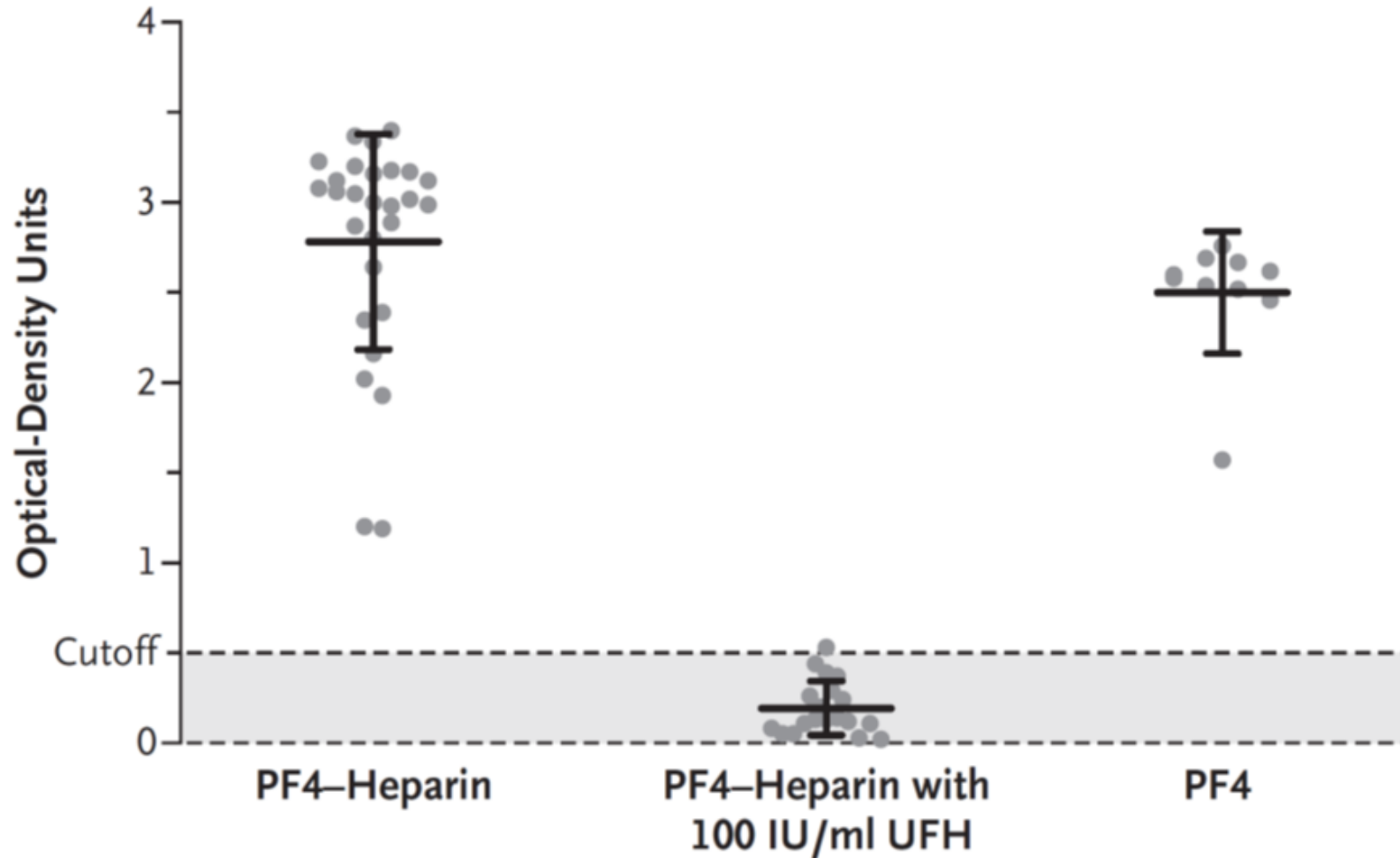
Research Support/P.I.	ASPEN
Employee	No relevant conflicts of interest to declare
Consultant	Instrumentation Laboratory; ASPEN; BioKit; MacoPharma; Chromatec
Major Stockholder	No relevant conflicts of interest to declare
Speakers Bureau	No relevant conflicts of interest to declare
Honoraria	No relevant conflicts of interest to declare
Scientific Advisory Board	Instrumentation Laboratory; ASPEN; BioKit

Structure

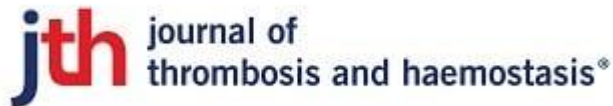
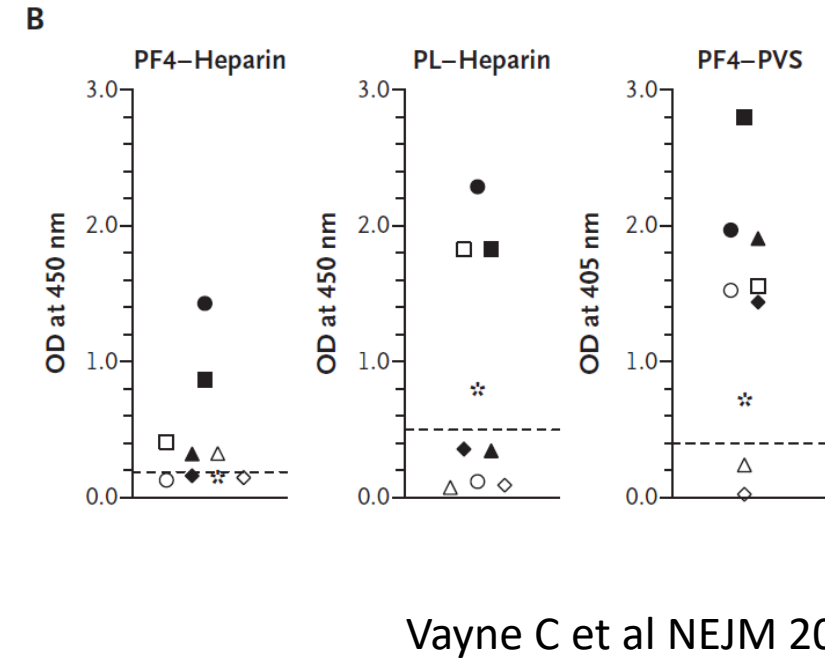
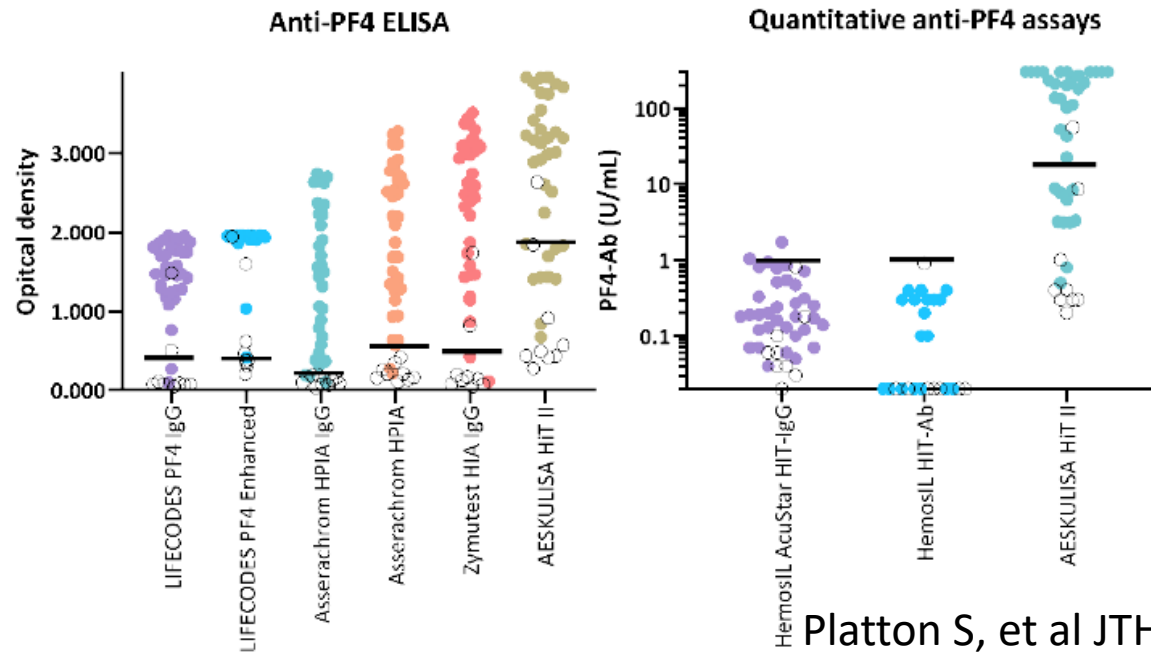
- **PF4 antibodies in VITT**
- **PF4 binding partners and VITT**
- **VITT-like abs independent of Covid-19 vaccination**

PF4/polyanion IgG ELISA VITT-patients

C ELISA Results for Combined Serum Samples from 28 Patients with VITT



PF4/polyanion antibody ELISA VITT - Patients



Anti-PF4 testing for vaccine-induced immune thrombocytopenia and thrombosis (VITT): Results from a NEQAS, ECAT and SSC collaborative exercise in 385 centers worldwide

Christopher Reilly-Stitt, Ian Jennings, Steve Kitchen, Mike Makris, Piet Meijer, Moniek de Maat, Marie Scully, Tamam Bakchoul, Isobel D. Walker

First published: 23 May 2022

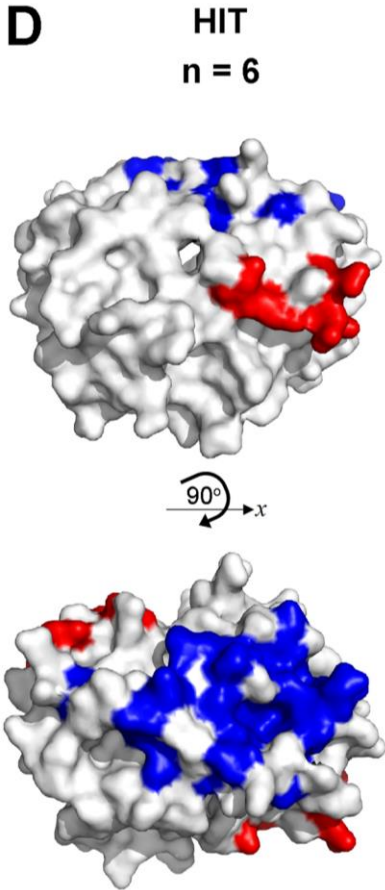
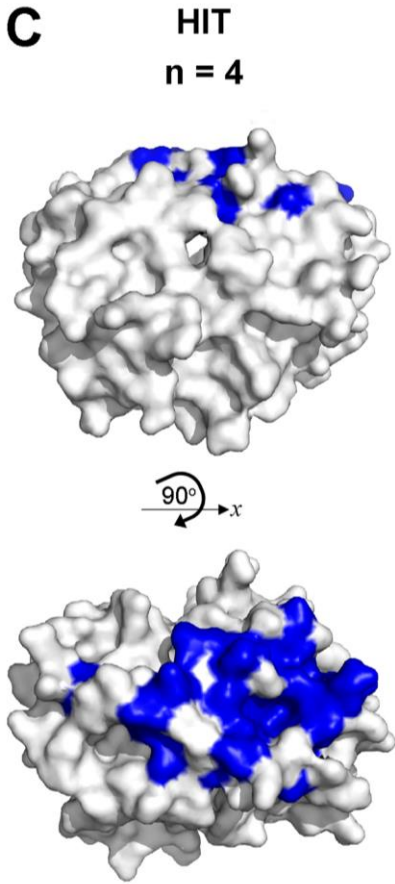
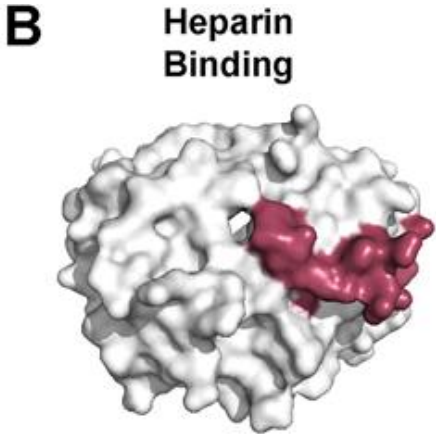
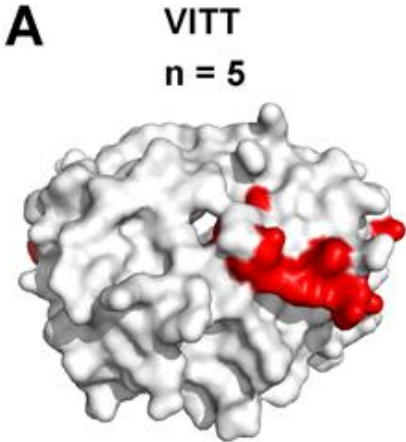
Summary PF4/polyanion lab tests for VITT:

1. ELISA plate based anti-PF4/polyanion assays work but with different sensitivities
2. Rapid tests, antigen coated on beads are insensitive (false neg)

Antibody epitopes in vaccine-induced immune thrombotic thrombocytopenia

<https://doi.org/10.1038/s41586-021-03744-4> Angela Huynh¹, John G Kelton^{1,2}, Donald M Arnold^{1,2}, Mercy Daka³ & Ishac Nazy^{1,2}✉

Nature, prepublished July 07 2021

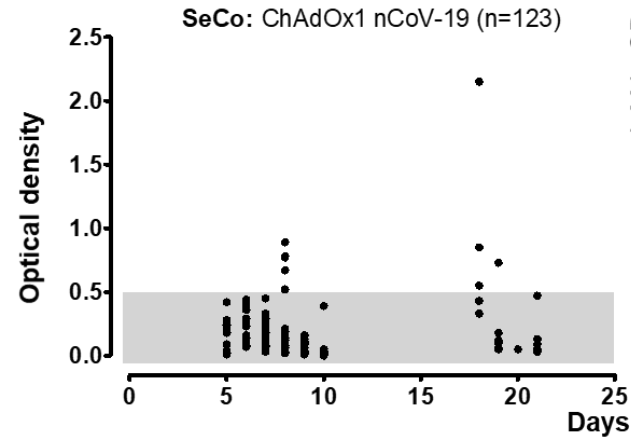
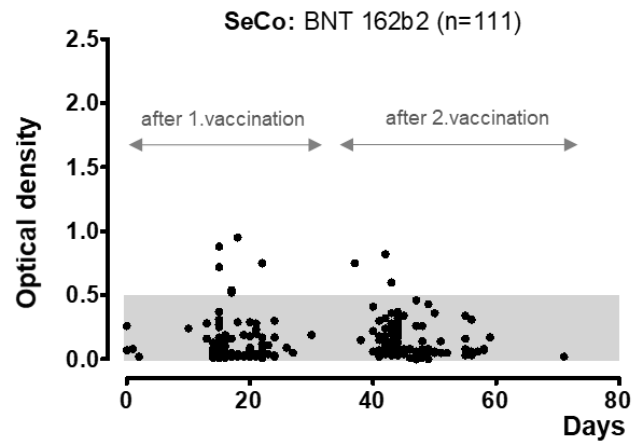


PF4-ELISA reactive antibodies are rather frequent

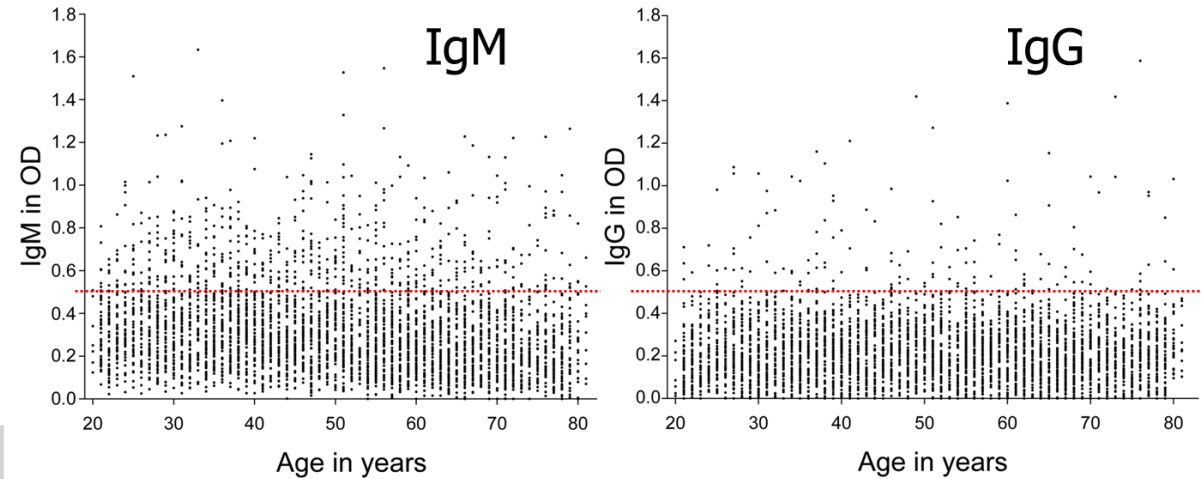
Vaccine	BNT162b2	ChAdOx1 nCoV-19	Total
Total (n)	143	138	281
PF4/polyanion EIA positive n (%)	8 (5.5)	11 (8.0)	19 (6.8)
PF4 – platelet activation assay	0	0	0

Anti-PF4/heparin Antibodies in the General Population

The Greifswald cross sectional population SHIP study
n=4,029



Thiele T et al, BLOOD 2021



Krauel K, et al. Blood 2011

The first VITT patients, March 17, 2021



170321 PF4-Charge 0

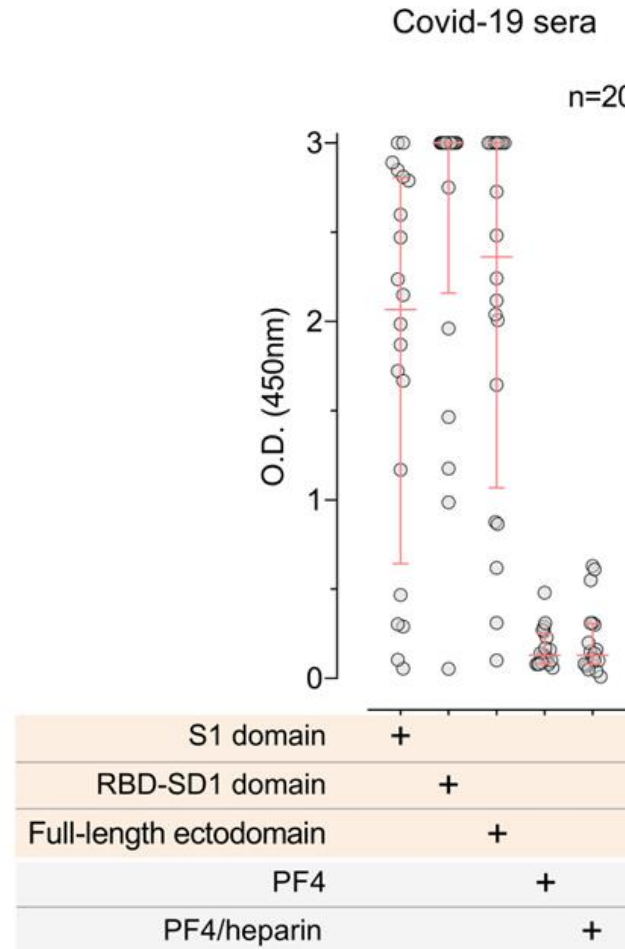
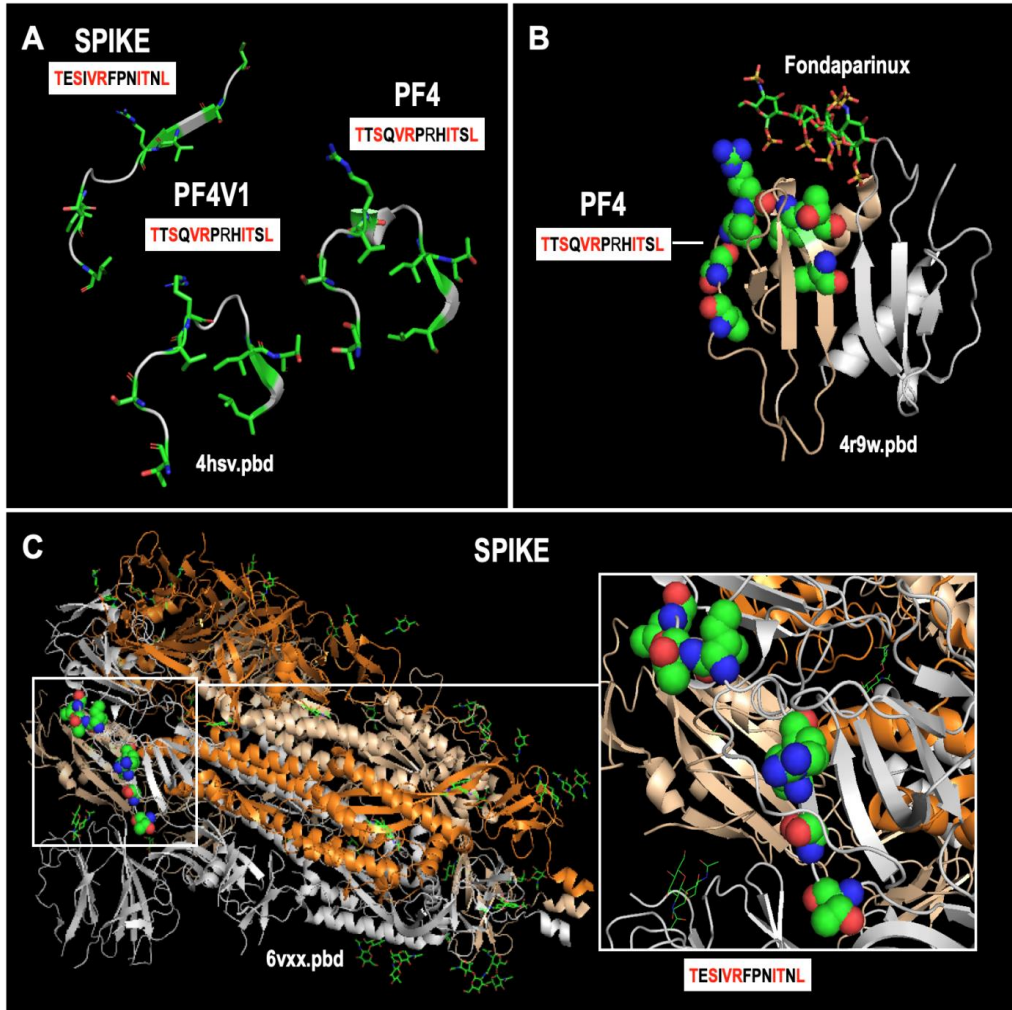
75ulTRc+20ul Serum

patient 1	1	2	3	Wenn 3 neg.dann Revi dazu		IV 3
final conc.	buffer	reviparin 0,2aFXa/ ml + 5U hirudin	+ PF4 10ug/ml End.k	+ PF4 10ug/ml End.k		PF4
donor 1	2	2	2			⊖
donor 2	20	5	2			⊖
donor 3	20	5	2			⊖

patient 2						IV 3
final conc.	buffer	reviparin 0,2aFXa/ ml + 5U hirudin	+ PF4 10ug/ml End.k	+ PF4 10ug/ml End.k		PF4
donor 1	2	⊖	2			⊖
donor 2	35	5	2			⊖
donor 3	5	5	2			⊖

patient 3						IV ?
final conc.	buffer	reviparin 0,2aFXa/ ml + 5U hirudin	+ PF4 10ug/ml End.k	+ PF4 10ug/ml End.k		PF4
donor 1	5	35	2			⊖
donor 2	5	35	2			⊖
donor 3	5	35	2			⊖

~~Crossreactivity of anti-spike antibodies with PF4?~~



CORRESPONDENCE

SARS-CoV-2 Infection in Patients with a History of VITT

Schönborn L., Seck S, Thiele T., Warkentin TE, Greinacher A.
New Engl J Med 2022; June 27 epub

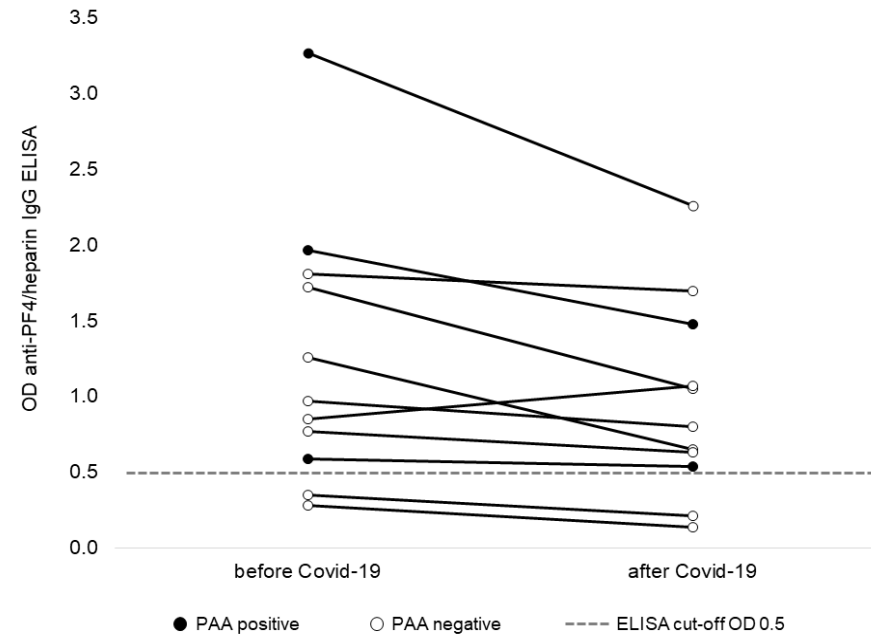


Observation in humans

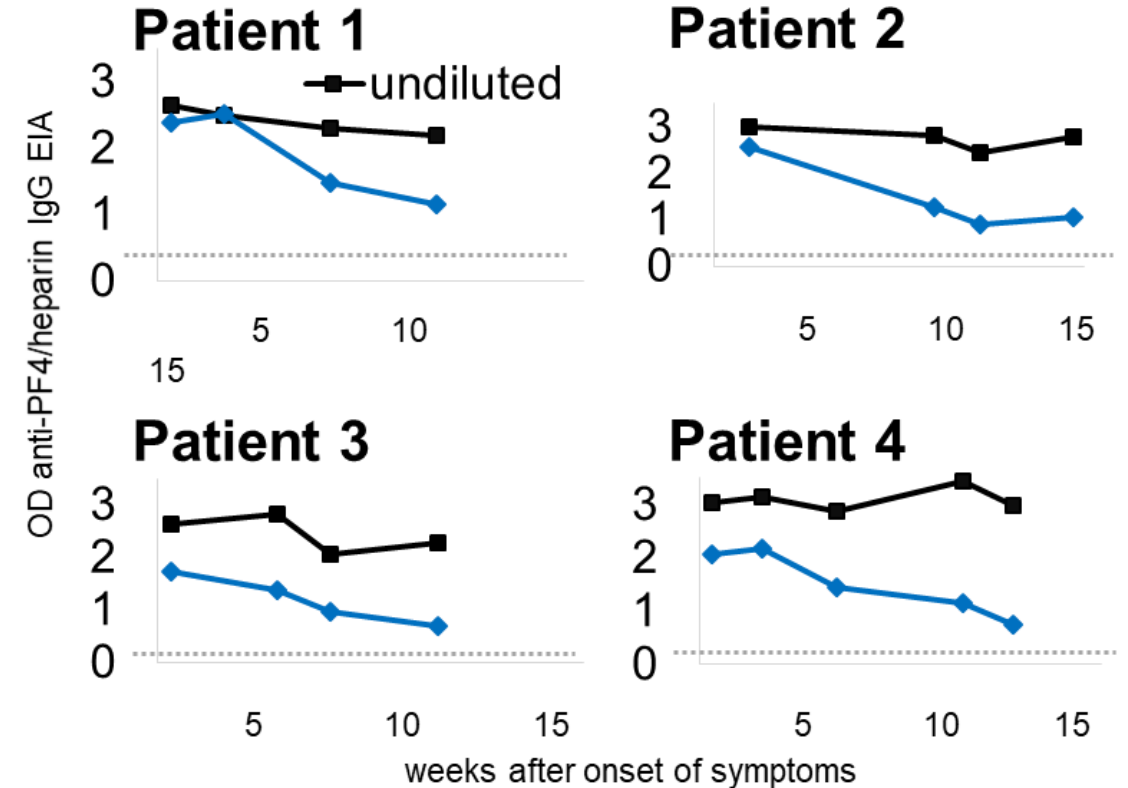
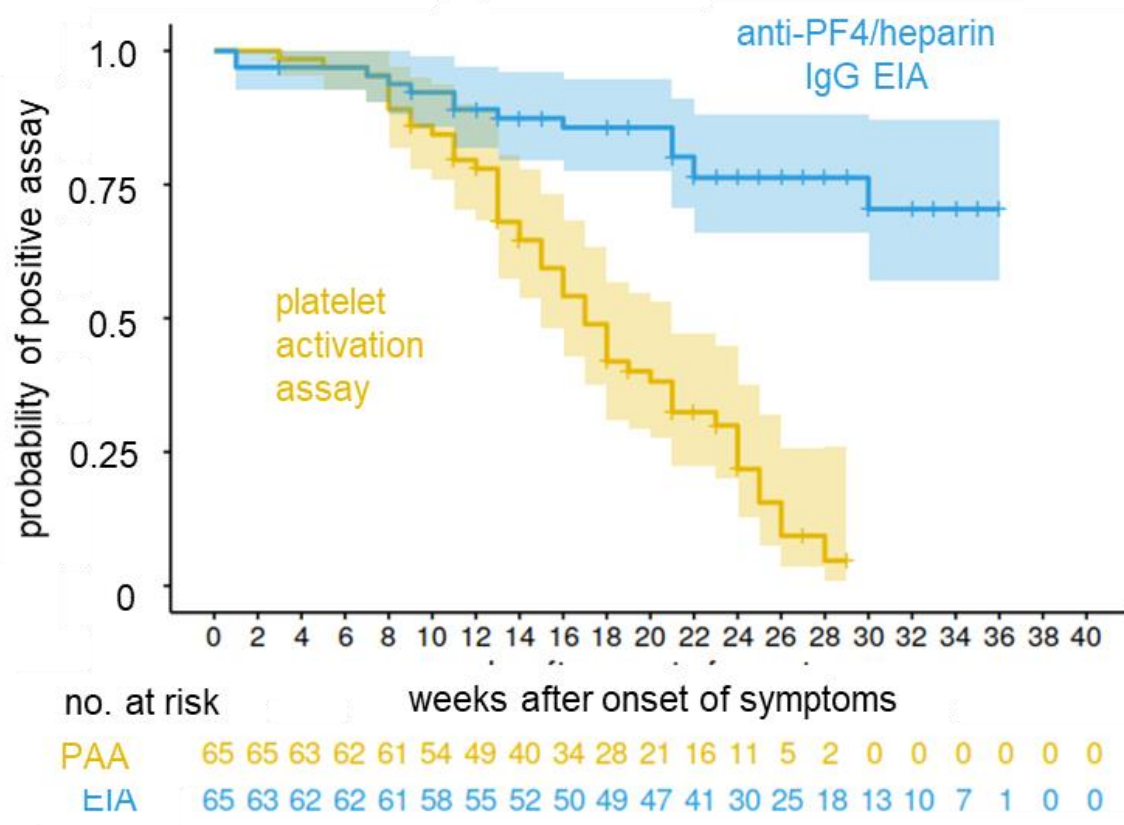
11 VITT patients developed Covid-19
anti-PF4 antibodies before and after Covid-19

Conclusion

Human in-vivo evidence that VITT is neither related to the spike protein nor to SARS-CoV2



VITT antibodies are transient

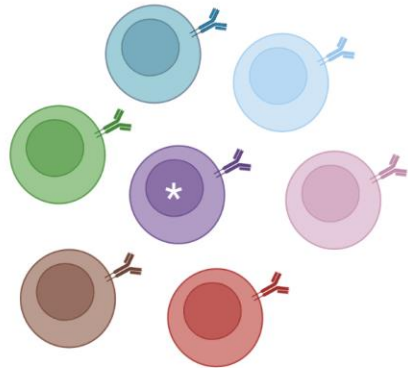


Schönborn L. et al. NEJM 2021; 385(19):1815-1816
 Schönborn et al. BLOOD 2022; ;139(12):1903-1907

Clonal restriction of VITT abs

A

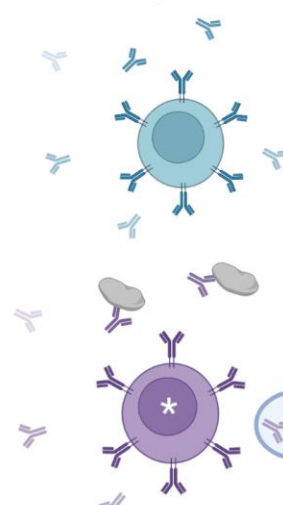
Individuals with V3-21+ λ -light chains



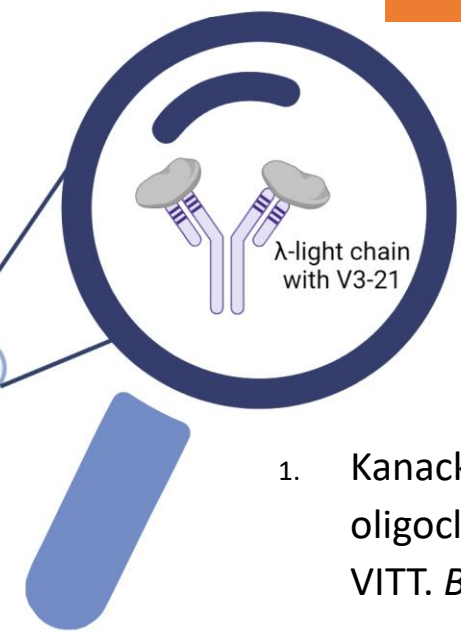
Viral vector based vaccination



Sars Cov2-specific IgG response



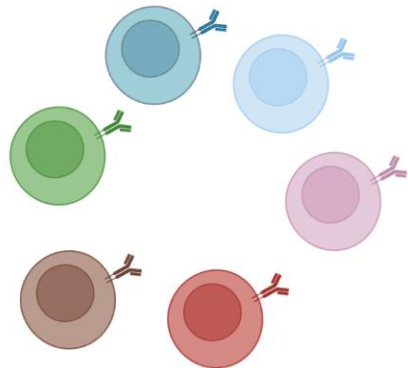
PF4-specific IgG response



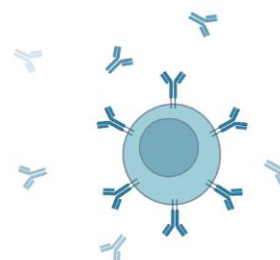
Genetic predisposition?

B

Individuals without V3-21+ λ -light chains

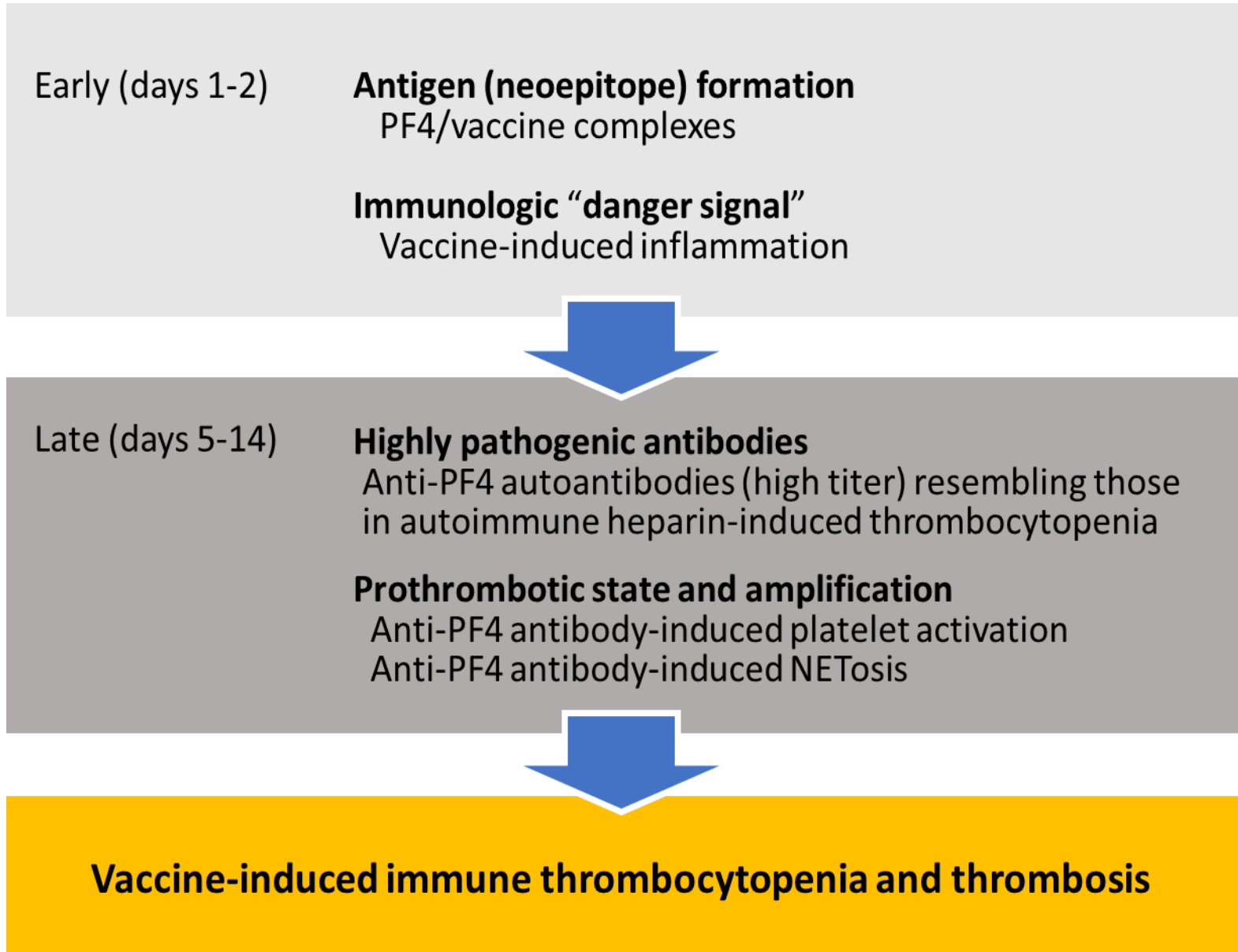


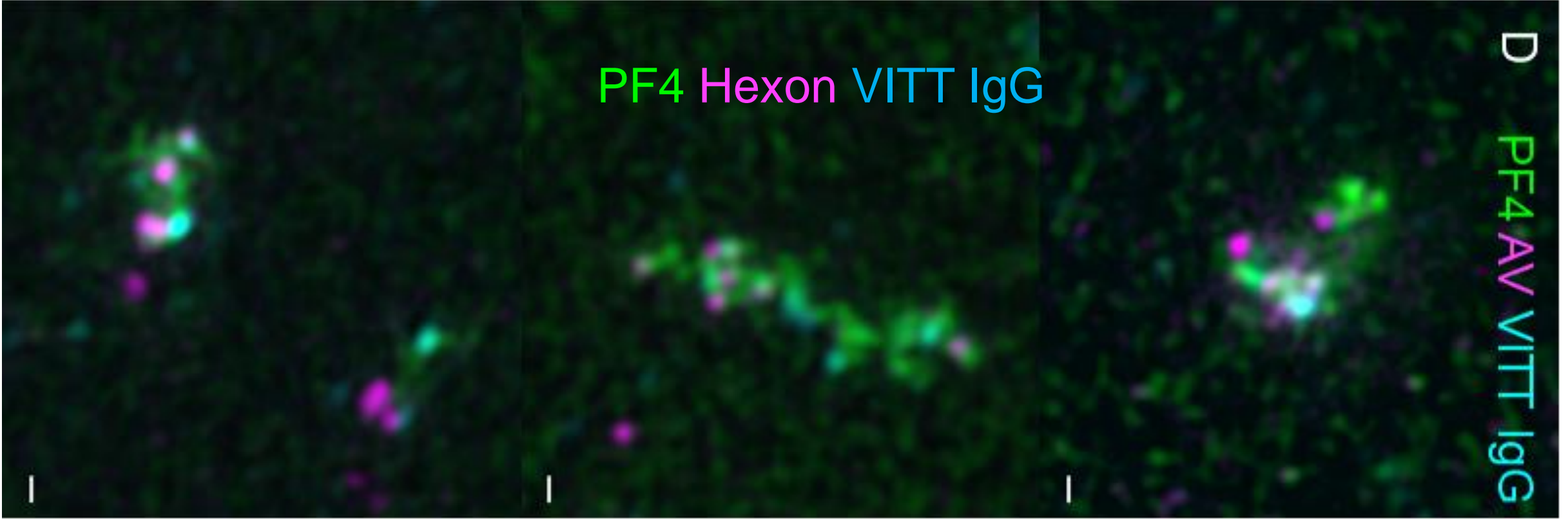
Viral vector based vaccination

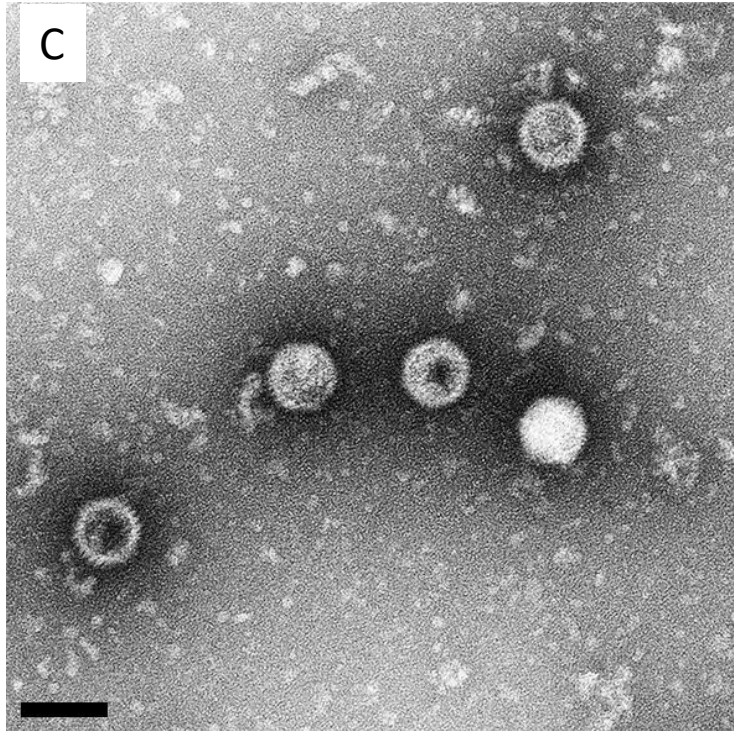


Sars Cov2-specific IgG response

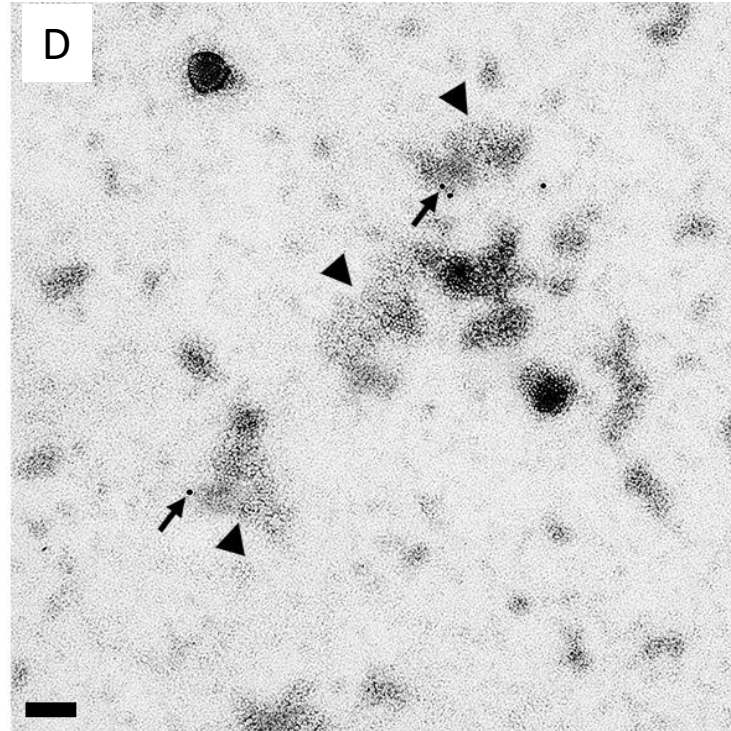
1. Kanack A, Bayas A, George G, et al. Monoclonal and oligoclonal anti-Platelet Factor 4 antibodies mediate VITT. *Blood*. 2022 epub.
2. Wang JJ, Armour BL, Ms, Chataway T, et al. Vaccine-induced immune thrombotic thrombocytopenia (VITT) is mediated by a stereotyped clonotypic antibody. *Blood*. 2022 epub
3. Greinacher A, Nimmerjahn F commentary *BLOOD* 2022, in press



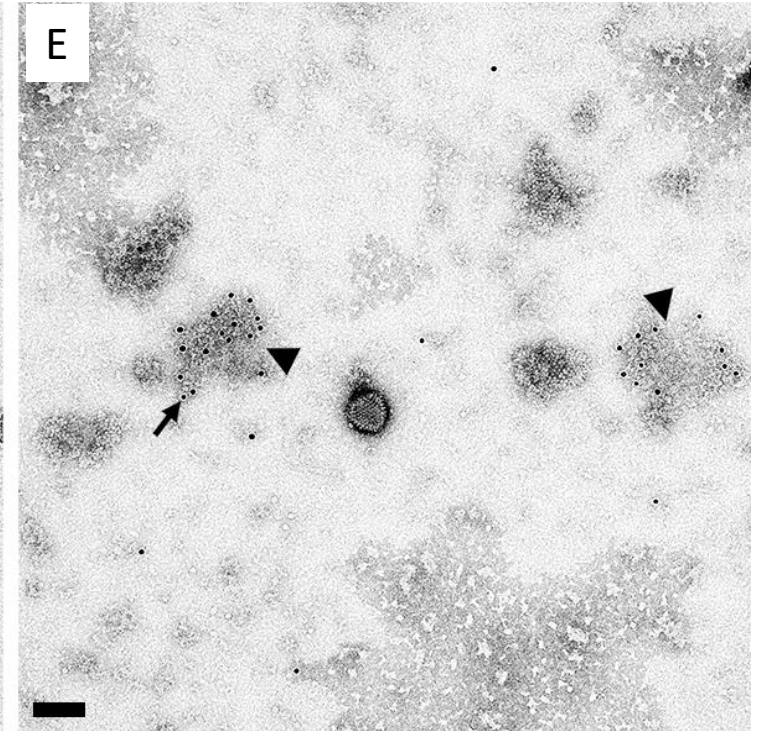




Vaccine

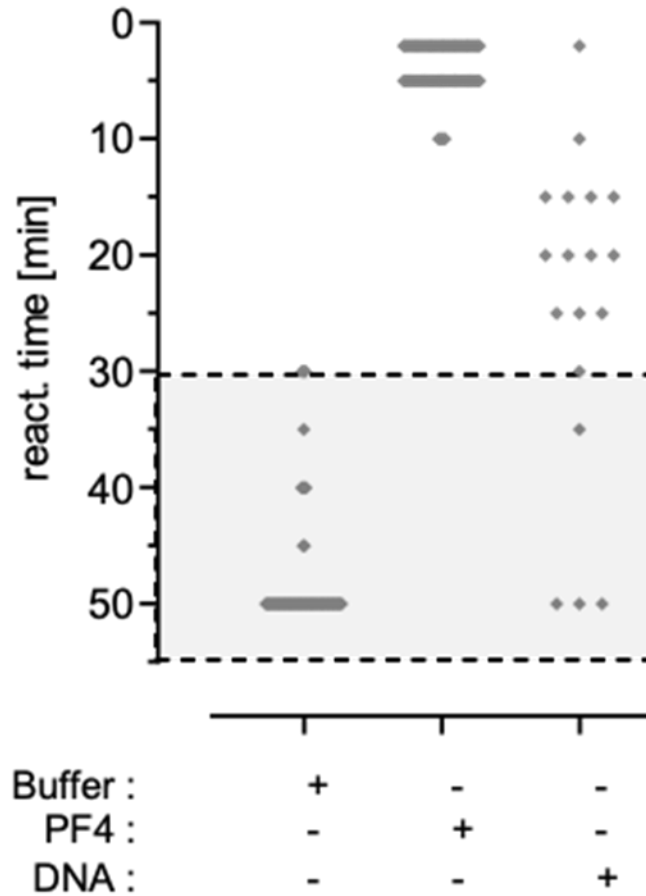


Vaccine + PF4
stained for adenovirus proteins

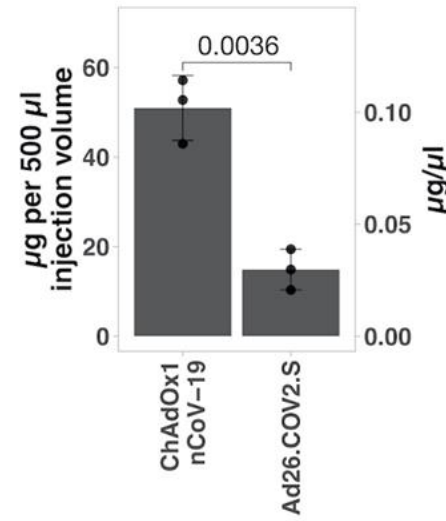


Vaccine + PF4
stained for PF4

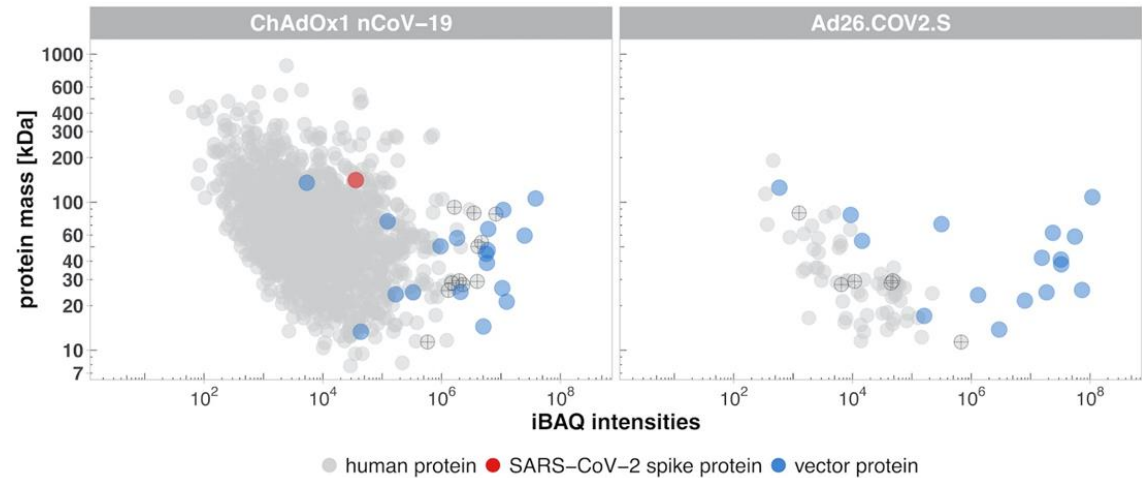
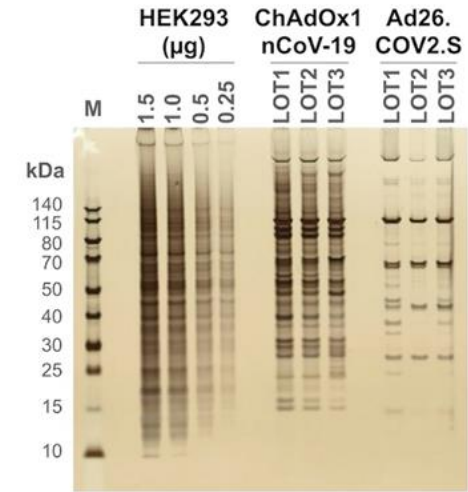
PF4 binding partners in VITT



A



B

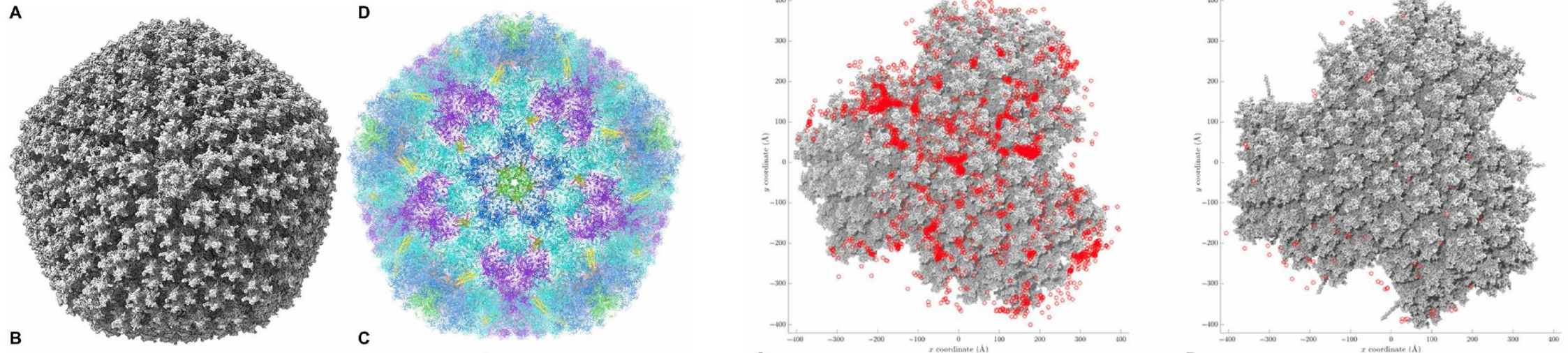


CORONAVIRUS

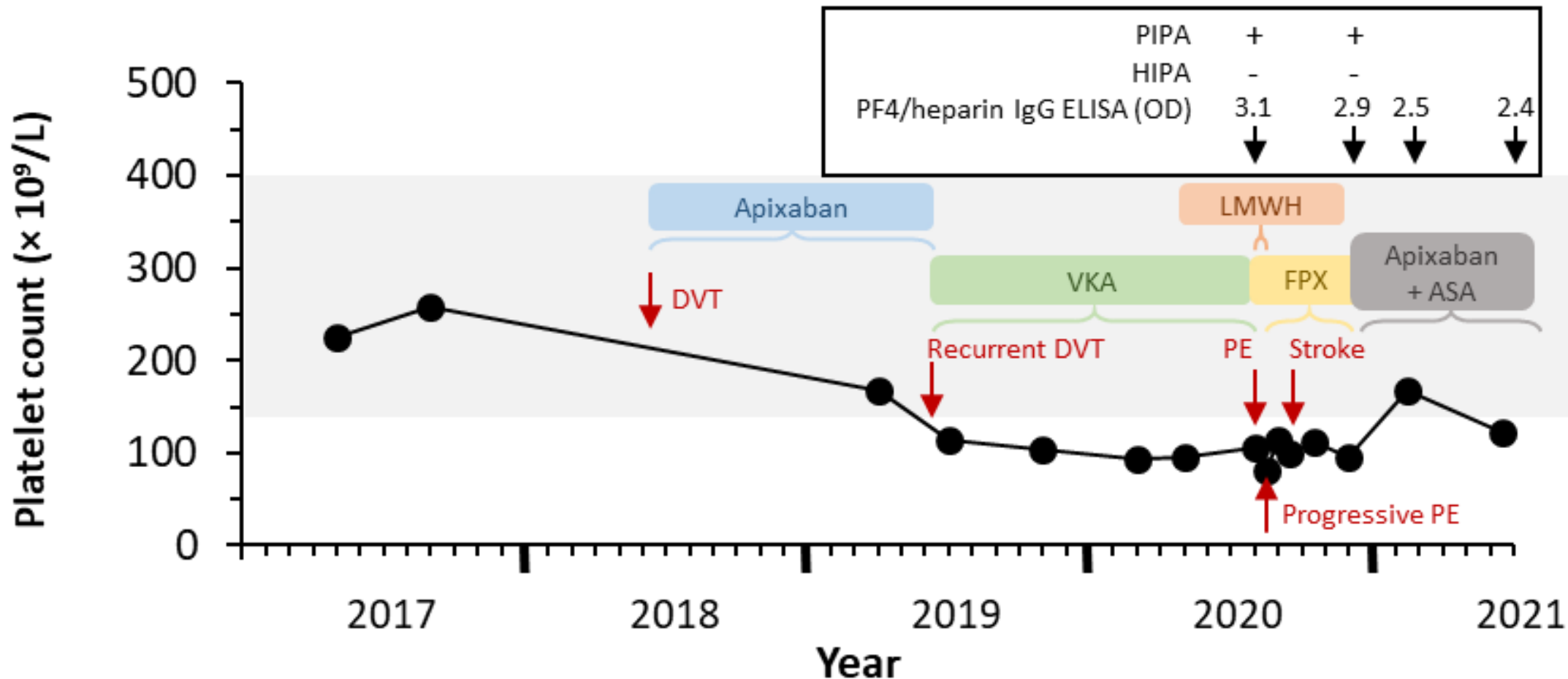
ChAdOx1 interacts with CAR and PF4 with implications for thrombosis with thrombocytopenia syndrome

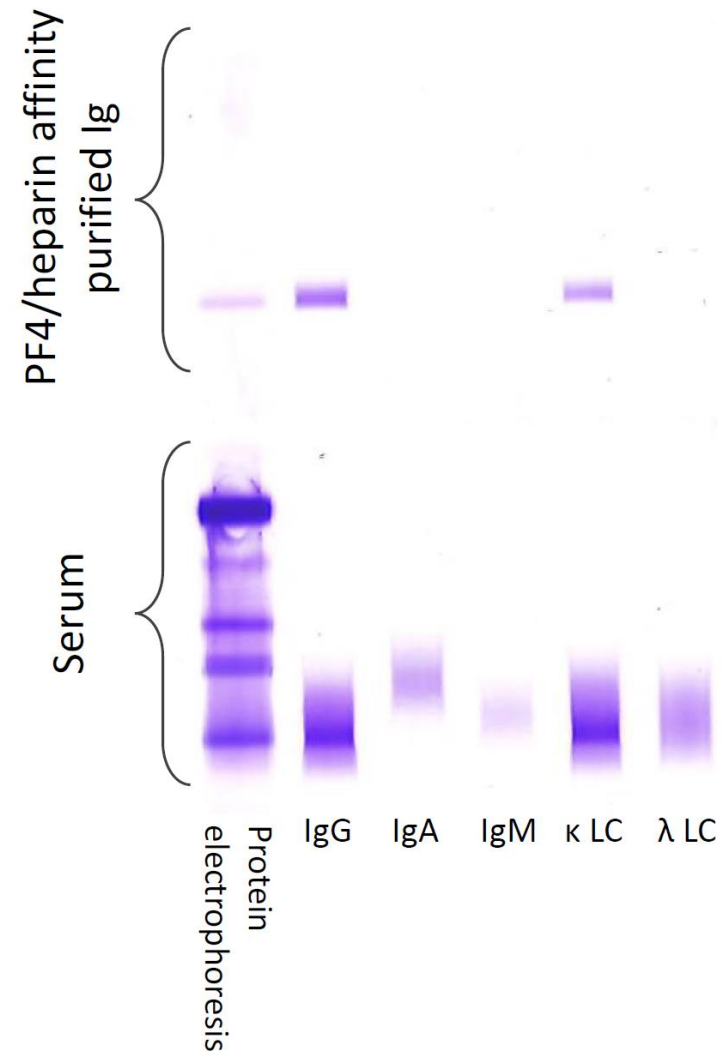
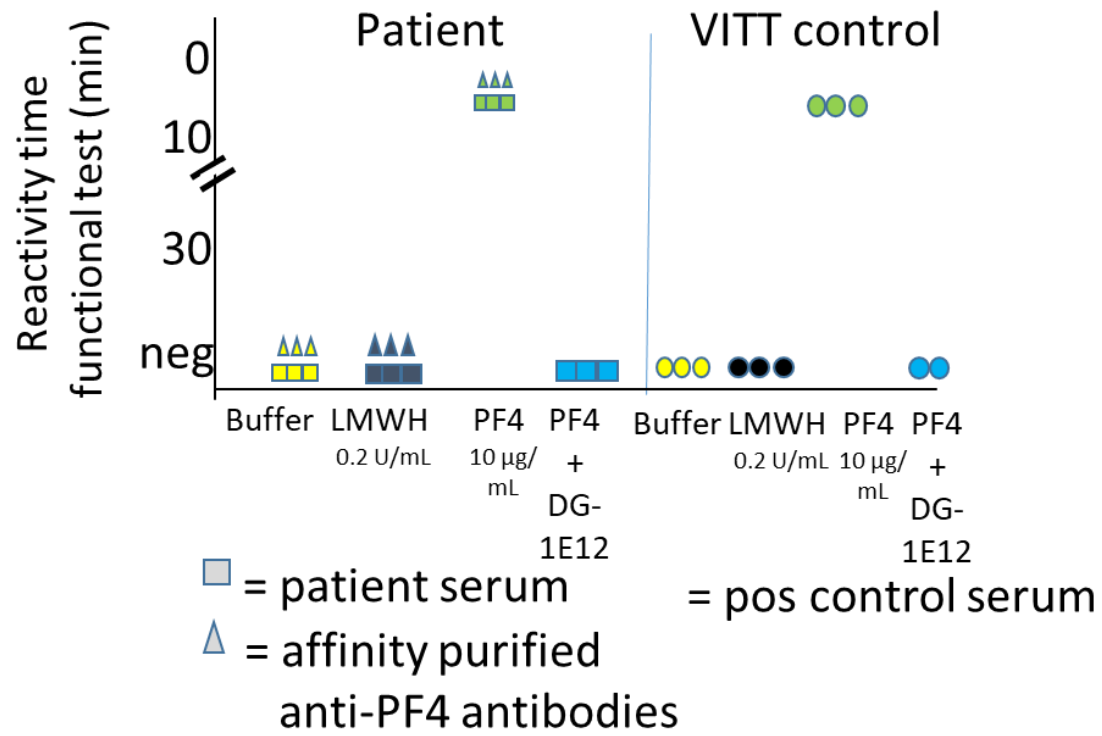
Alexander T. Baker^{1,2,3,4,5,6*†}, Ryan J. Boyd^{5,7†}, Daipayan Sarkar^{7,8,9‡}, Alicia Teijeira-Crespo^{6‡}, Chun Kit Chan^{10‡}, Emily Bates⁶, Kasim Waraich¹¹, John Vant^{5,7}, Eric Wilson^{5,7}, Chloe D. Truong^{5,7}, Magdalena Lipka-Lloyd¹², Petra Fromme^{5,7}, Josh Vermaas^{8,9}, Dewight Williams¹³, LeeAnn Machiesky¹⁴, Meike Heurich¹⁵, Bolni M. Nagalo^{1,2,4§}, Lynda Coughlan^{16,17}, Scott Umlauf¹⁴, Po-Lin Chiu^{5,7}, Pierre J. Rizkallah¹⁸, Taylor S. Cohen^{19*}, Alan L. Parker^{6*}, Abhishek Singharoy^{5,7*}, Mitesh J. Borad^{1,2,3,4*}

published 02.12.21



VITT beyond vaccination?





Summary

- VITT is caused by anti-PF4 antibodies
- VITT antibodies strongly activate platelets, no cofactor required
- The binding partner of PF4 triggering VITT antibodies is still unknown
- VITT antibodies are not triggered by SARS-CoV2 or spike protein
- VITT antibodies are transient
- VITT like antibodies can occur independent of Covid-19 vaccination

Kathleen Selleng
Jan Wesche
Stefan Handtke
Raghavendra Palankar
Konstanze Aurich
Michael Lalk
Karen Methling
Uwe Völker
Christian Hentschker
Stephan Michalik
Leif Steil
Linda Schönborn
Martin Beer
Kati Franzke
Chandini Rangaswamy
Reiner K. Mailer
Thomas Thiele
Stefan Kochanek
Lea Krutzke
Florian Siegerist
Nicole Endlich
Theodore E. Warkentin
Thomas Renne

Karin Weisser
Paul A. Kyrle
Sabine Eichinger
Martina Wolff
Carlo Zaninetti
Lena Ulm
Nils-Olaf Hübner
Karsten Becker
Julia Mayerle
Sven Reiche
Andrea Aebischer
Maximilian Muenchhoff
Johannes C. Hellmuth
Oliver T. Keppler
Daniel Duerschmied
Achim Lothar
Siegbert Rieg
Meinrad Paul Gawaz
Karin Anne Lydia Mueller
Christian S. Scheer
Matthias Napp
Klaus Hahnenkamp
Guglielmo Lucchese

Antje Vogelgesang
Agnes Flöel
Piero Lovreglio
Angela Stufano
Rolf Marschalek
Brigitte Keller-Stanislawski
Emil Reisinger
Markus Funk
Farid Salih
Siegfried Kohler
Christiana Franke
Martin Möckel
Thomas Dörner
Hans C. Bauknecht
Christian Pille
Jan A. Graw
Angelika Alonso
Johann Pelz
Hauke Schneider
Antonios Bayas
Monika Christ
Joji B. Kuramatsu
Matthias Endres



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH



DFG Deutsche
Forschungsgemeinschaft