



# Vaccine-induced Immune Thrombotic Thrombocytopenia

Andreas Greinacher

Institut für Immunologie und Transfusionsmedizin  
Universitätsmedizin Greifswald, Germany



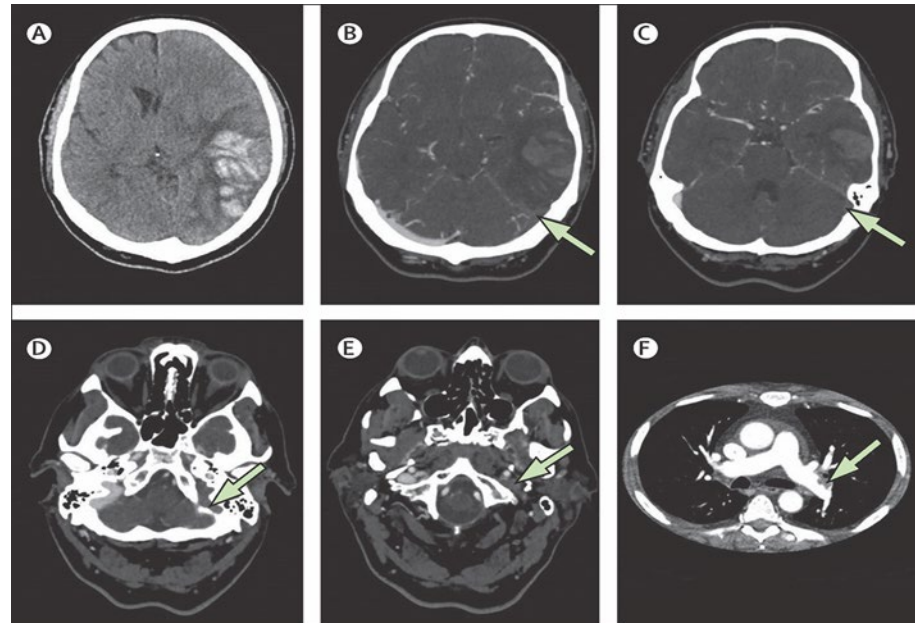
# Disclosures for Andreas Greinacher

---

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

# Clinical Observation

- since February 2021, several cases of unusual thrombotic events in combination with thrombocytopenia were observed in patients after vaccination with ChAdOx1 nCov-19 (AstraZeneca).
- Patients developed thrombocytopenia, cerebral vein thrombosis / splanchnic vein thrombosis 4-20 days after vaccination



BRIEF REPORT

## Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination

Nina H. Schultz, M.D., Ph.D., Ingvild H. Sørvoll, M.D.,  
Annika E. Michelsen, Ph.D., Ludvig A. Munthe, M.D., Ph.D.,  
Fridtjof Lund-Johansen, M.D., Ph.D., Maria T. Ahlen, Ph.D.,  
Markus Wiedmann, M.D., Ph.D., Anne-Hege Aamodt, M.D., Ph.D.,  
Thor H. Skattør, M.D., Geir E. Tjønnfjord, M.D., Ph.D.,  
and Pål A. Holme, M.D., Ph.D.

N Engl J Med. 2021 Jun 3;384(22):2124-2130.  
doi: 10.1056/NEJMoa2104882. Epub 2021 Apr 9.

ORIGINAL ARTICLE

## Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination

Marie Scully, M.D., Deepak Singh, B.Sc., Robert Lown, M.D.,  
Anthony Poles, M.D., Tom Solomon, M.D., Marcel Levi, M.D.,  
David Goldblatt, M.D., Ph.D., Pavel Kotoucek, M.D., William Thomas, M.D.,  
and William Lester, M.D.

N Engl J Med. 2021 Jun 10;384(23):2202-2211.  
doi: 10.1056/NEJMoa2105385. Epub 2021 Apr 16.

ORIGINAL ARTICLE

## Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination

Andreas Greinacher, M.D., Thomas Thiele, M.D., Theodore E. Warkentin, M.D.,  
Karin Weisser, Ph.D., Paul A. Kyrle, M.D., and Sabine Eichinger, M.D.

N Engl J Med. 2021 Jun 3;384(22):2092-2101.  
doi: 10.1056/NEJMoa2104840. Epub 2021 Apr 9.

ORIGINAL ARTICLE

## Clinical Features of Vaccine-Induced Immune Thrombocytopenia and Thrombosis

Sue Pavord, F.R.C.Path., Marie Scully, M.D., Beverley J. Hunt, M.D.,  
William Lester, M.D., Catherine Bagot, M.D., Brian Craven, M.B., B.Ch.,  
Alex Rampotas, M.R.C.P., Gareth Ambler, Ph.D., and Mike Makris, M.D.

N Engl J Med. 2021 doi: 10.1056/NEJMoa2109908.  
Epub 2021 Aug 11.

# Summary of 12 patients developing VITT after Ad26.COVS Vaccine

- 18 to < 60 years;
- All White women
- 6 to 15 days after vaccination
- Thrombocytopenia: 9 – 127,000/ $\mu$ L.
- Thrombosis: 12 CVST, 7 also had intracerebral hemorrhage;
- 8 non-CVST thromboses.
- 11/11 tested PF4/polyanion ELISA positive
- 8/9 SRA (washed platelet assay) negative

See I. et al. 2021 JAMA. doi:10.1001/jama.2021.7517

The NEW ENGLAND JOURNAL of MEDICINE

CORRESPONDENCE

## Vaccine-Induced Immune Thrombocytopenia and Thrombosis after the Sputnik V Vaccine

Herrera-Comoglio R, NEJM 2022, Sept 14

# VITT Prevalence

Germany:

1.49 per 100,000 for ChAdOx1 nCoV-19 (n=12,692,700 doses)

0.56 per 100,000 for Ad26.COVS.2 (n= 3,186,297 doses)

Paul-Ehrlich-Institute: [https://www.pei.de/SharedDocs/Downloads/DE/newsroom/dossiers/sicherheitsberichte/sicherheitsbericht-27-12-20-bis-30-09-21.pdf?\\_\\_blob=publicationFile&v=9](https://www.pei.de/SharedDocs/Downloads/DE/newsroom/dossiers/sicherheitsberichte/sicherheitsbericht-27-12-20-bis-30-09-21.pdf?__blob=publicationFile&v=9).

Nearly all VITT cases occurred after the first vaccination dose

## VITT in LMICs

2 reports from India

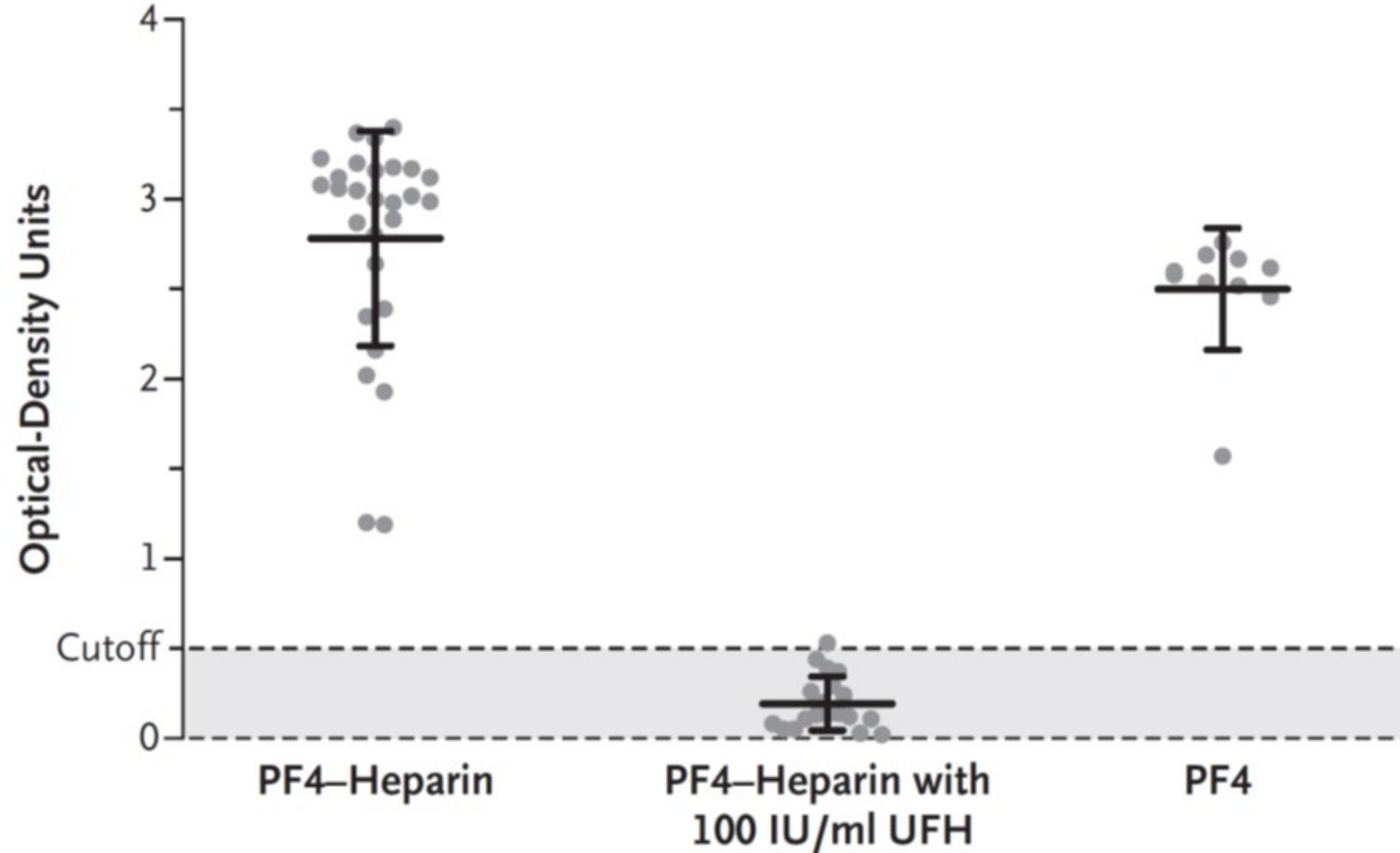
10 reports from Brasil

No reports from Africa

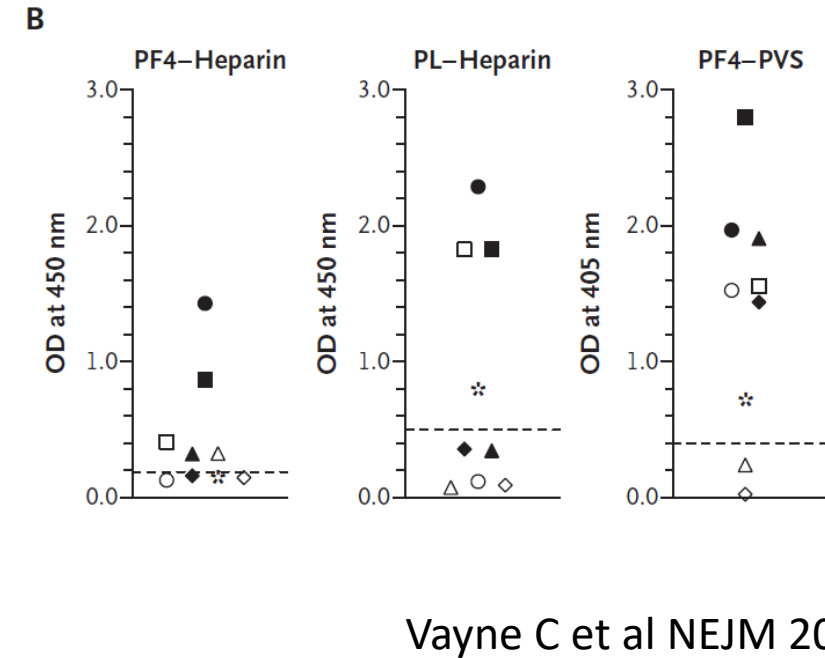
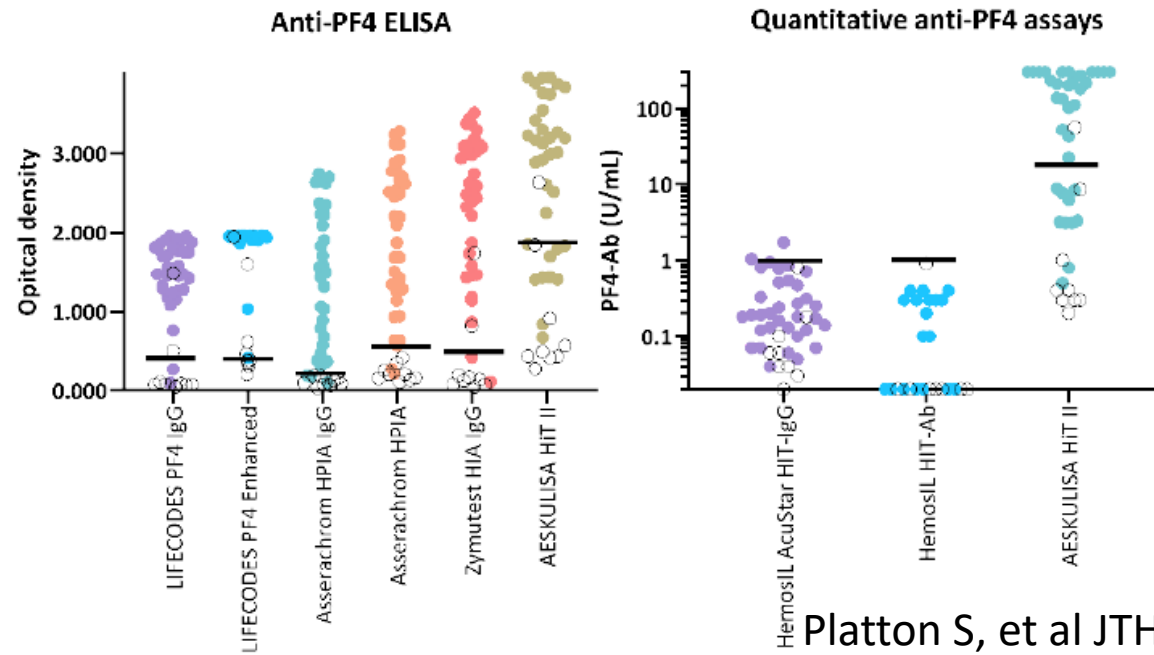
Underreporting or truly low prevalence due to protecting factors?

# 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:

VITT after AZ vaccination

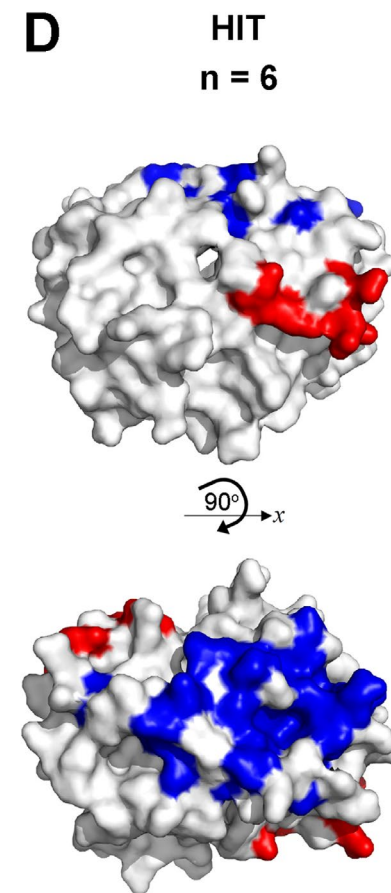
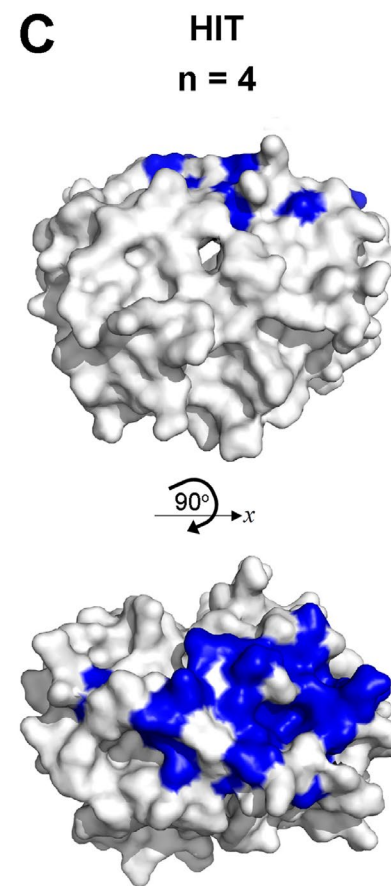
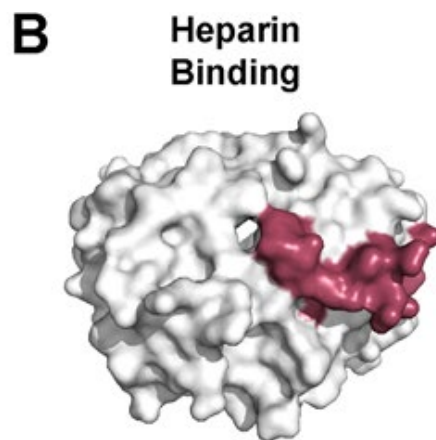
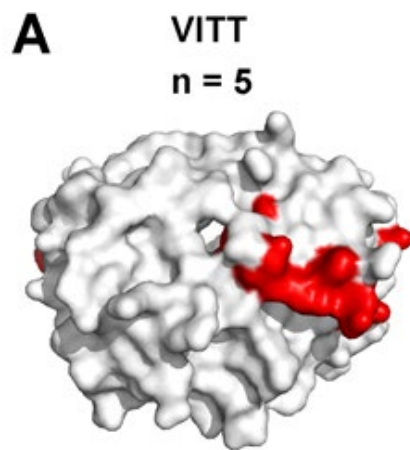
1. ELISA plate based anti-PF4/polyanion assays work but with different sensitivities
2. Rapid tests, antigen coated on beads are insensitive (false neg)



# Antibody epitopes in vaccine-induced immune thrombotic thrombocytopenia

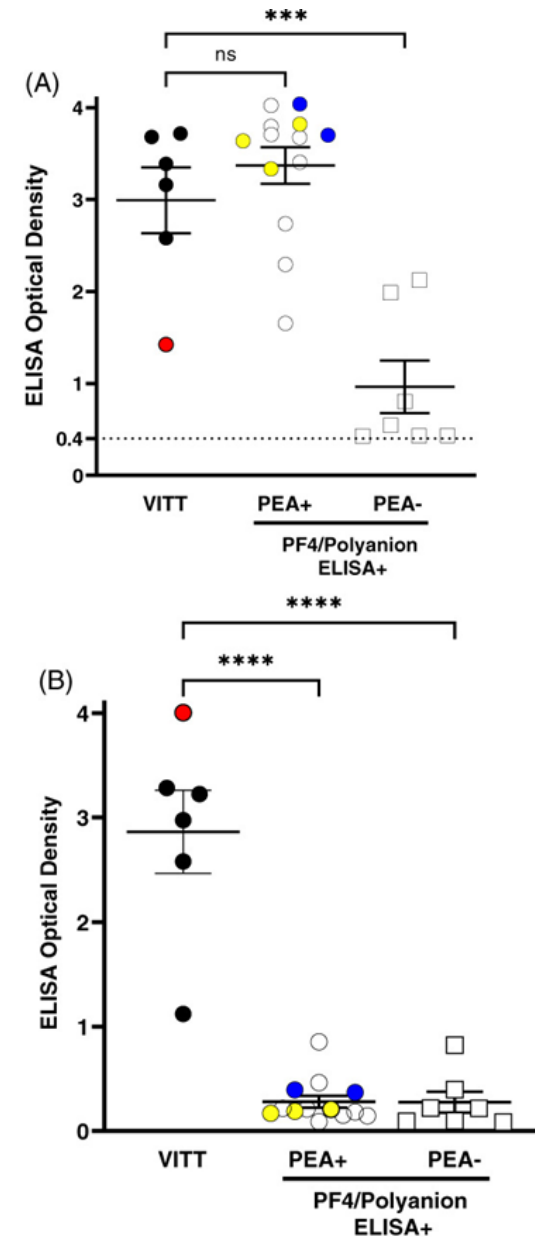
<https://doi.org/10.1038/s41586-021-03744-4> Angela Huynh<sup>1</sup>, John G Kelton<sup>1,2</sup>, Donald M Arnold<sup>1,2</sup>, Mercy Daka<sup>3</sup> & Ishac Nazy<sup>1,2</sup>✉

Nature, prepublished July 07 2021



PF4 and NAP2 share several epitopes, (some)  
VITT abs bind NAP-2  
Laboratory Morty Poncz, Oral Communication  
ISTH 2022 July 09

**There is no antigen assay available differentiating between HIT and VITT antibodies**

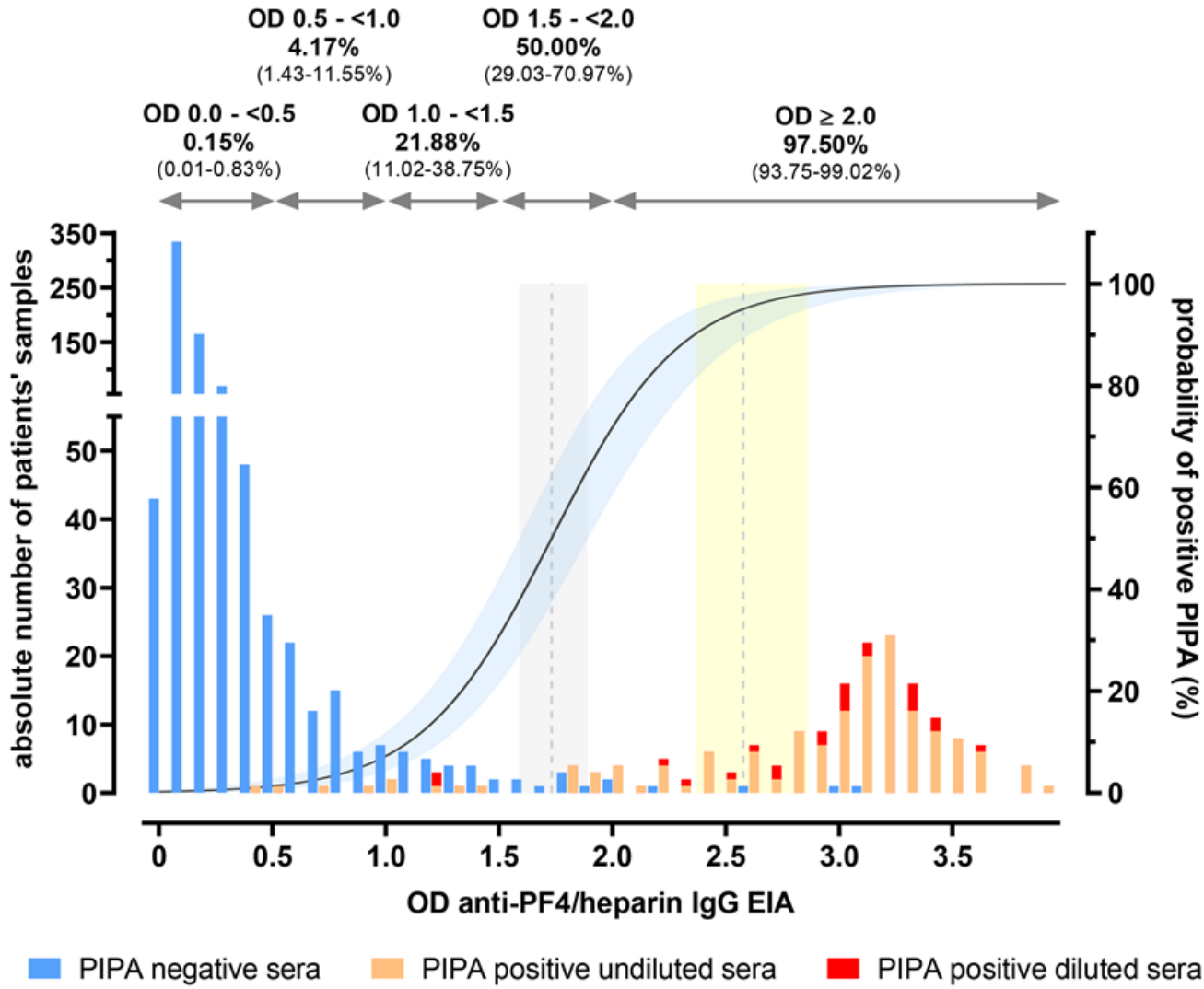
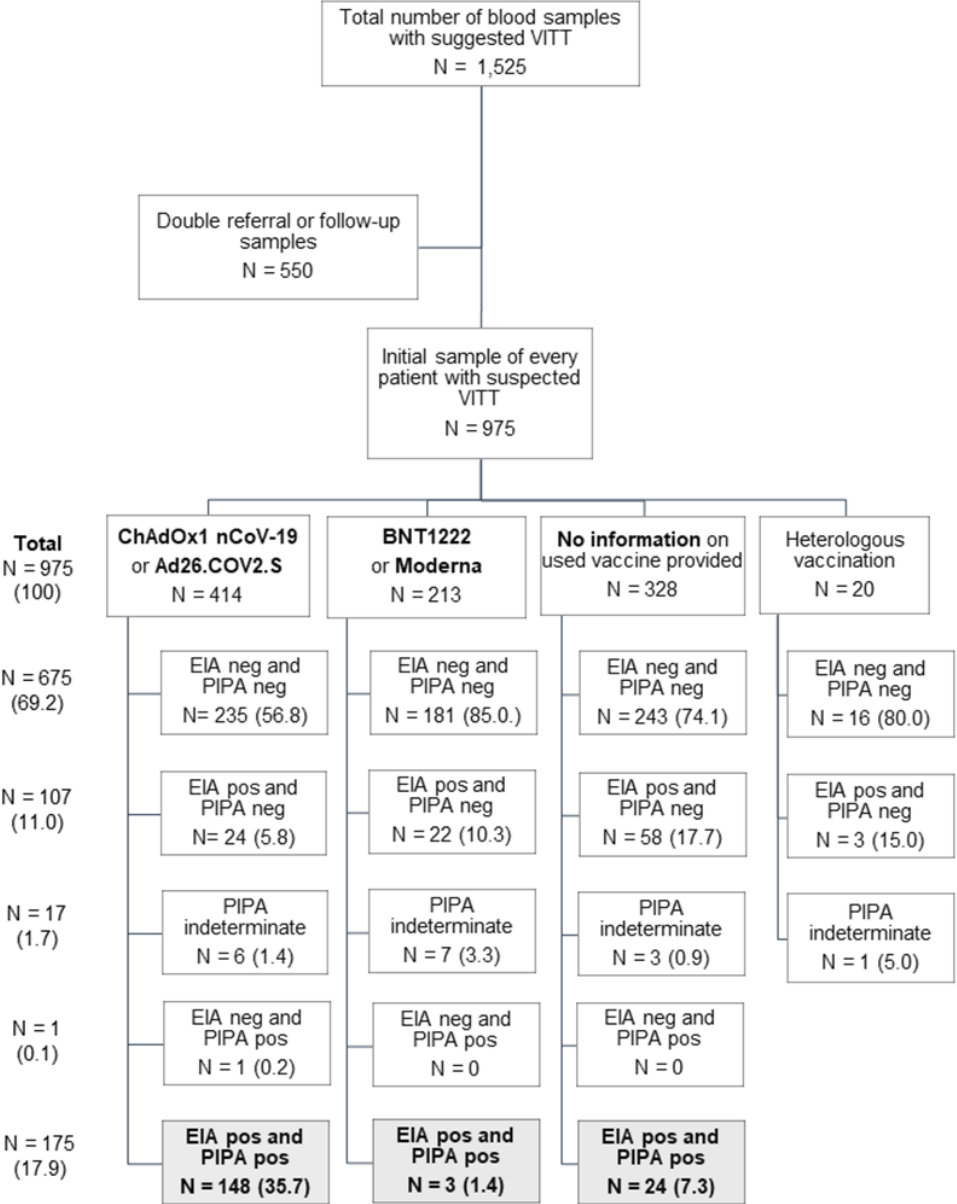




# Functional Assays for VITT

Assay	Comment
<b><i>Washed platelets</i></b>	
<b>SRA, PF4-SRA</b>	PF4-SRA more sensitive than SRA for detecting VITT antibodies
<b>PF4/heparin-SRA</b>	PF4/heparin-SRA more sensitive than SRA for detecting VITT antibodies
<b>HIPA, PIPA</b>	PIPA more sensitive than HIPA for detecting VITT antibodies
<b>PF4-PEA</b>	PF4-PEA more sensitive than SRA for detecting VITT antibodies
<b><i>Whole blood</i></b>	
<b>PIFPA</b>	PIFPA has high sensitivity and specificity for VITT
<b>Multiplate</b>	Minimal experience reported to date for diagnosis of VITT
<b><i>Platelet-rich plasma (citrated)</i></b>	
<b>HitAlert</b>	Minimal experience reported to date for diagnosis of VITT

# Characteristics of the PIPA

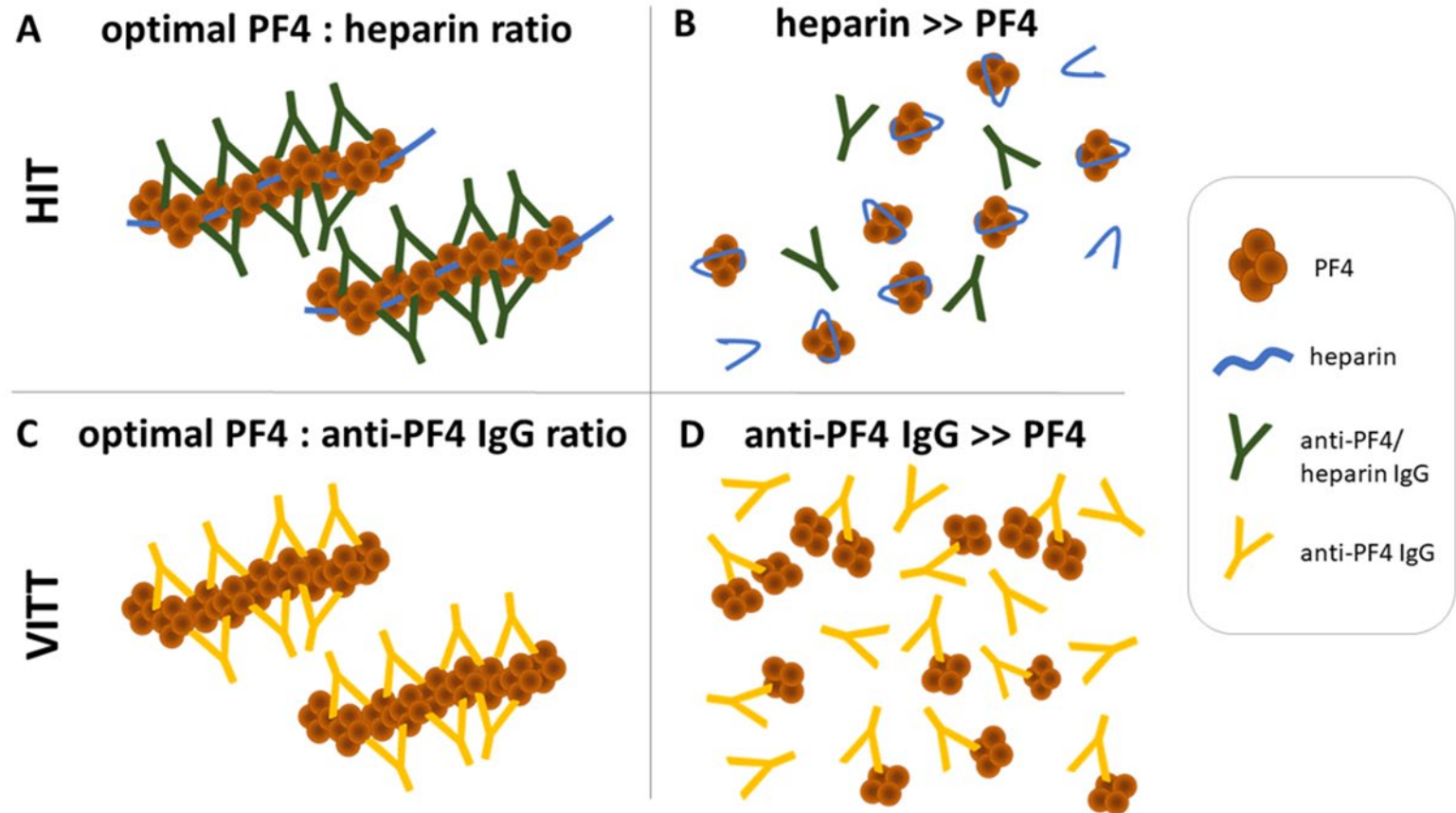


# Ratio of anti-PF4 antibodies : PF4 is important for functional tests

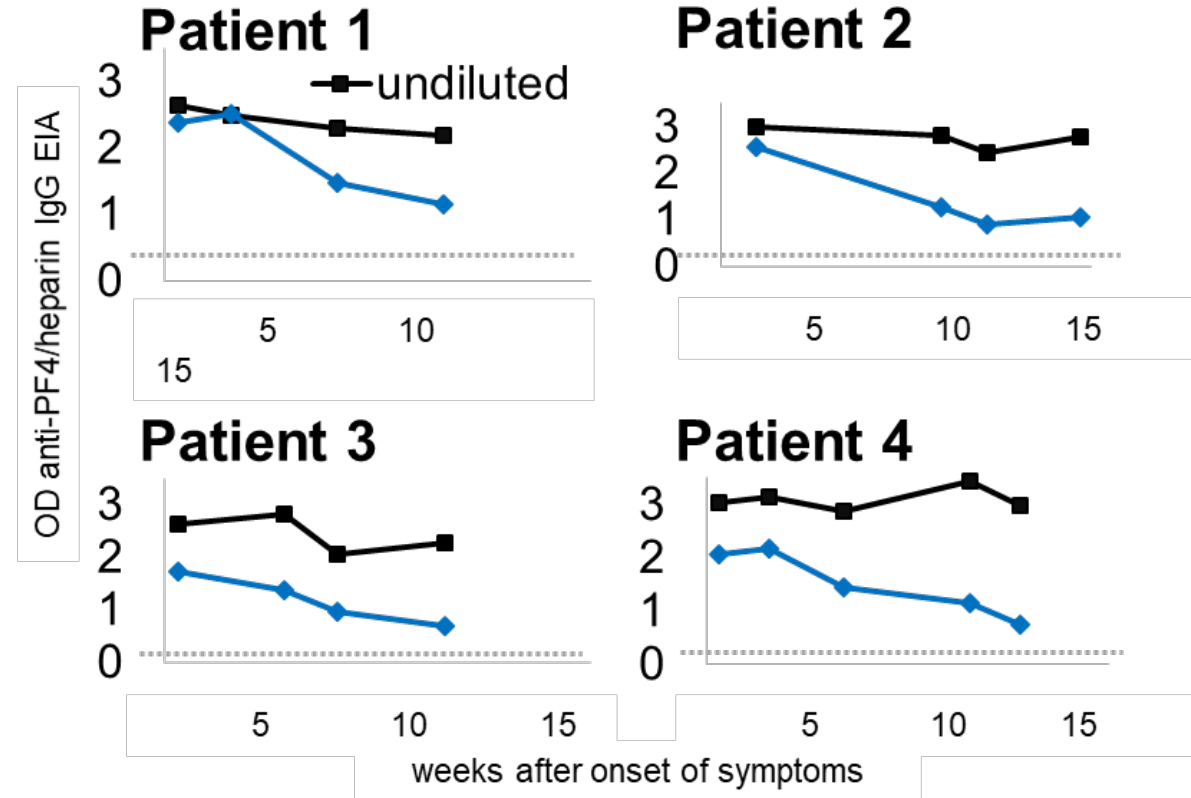
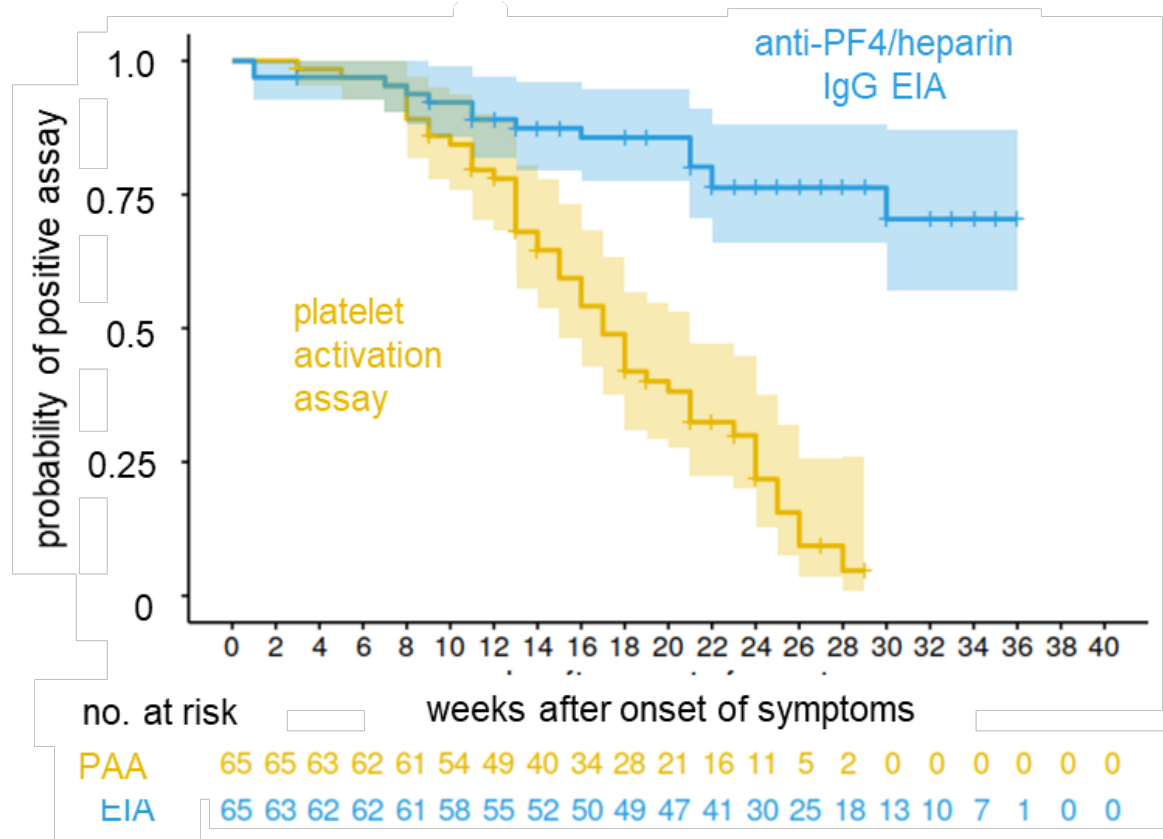
64 samples PF4 EIA pos but PIPA neg

diluted 1 in 4 or 1 in 10

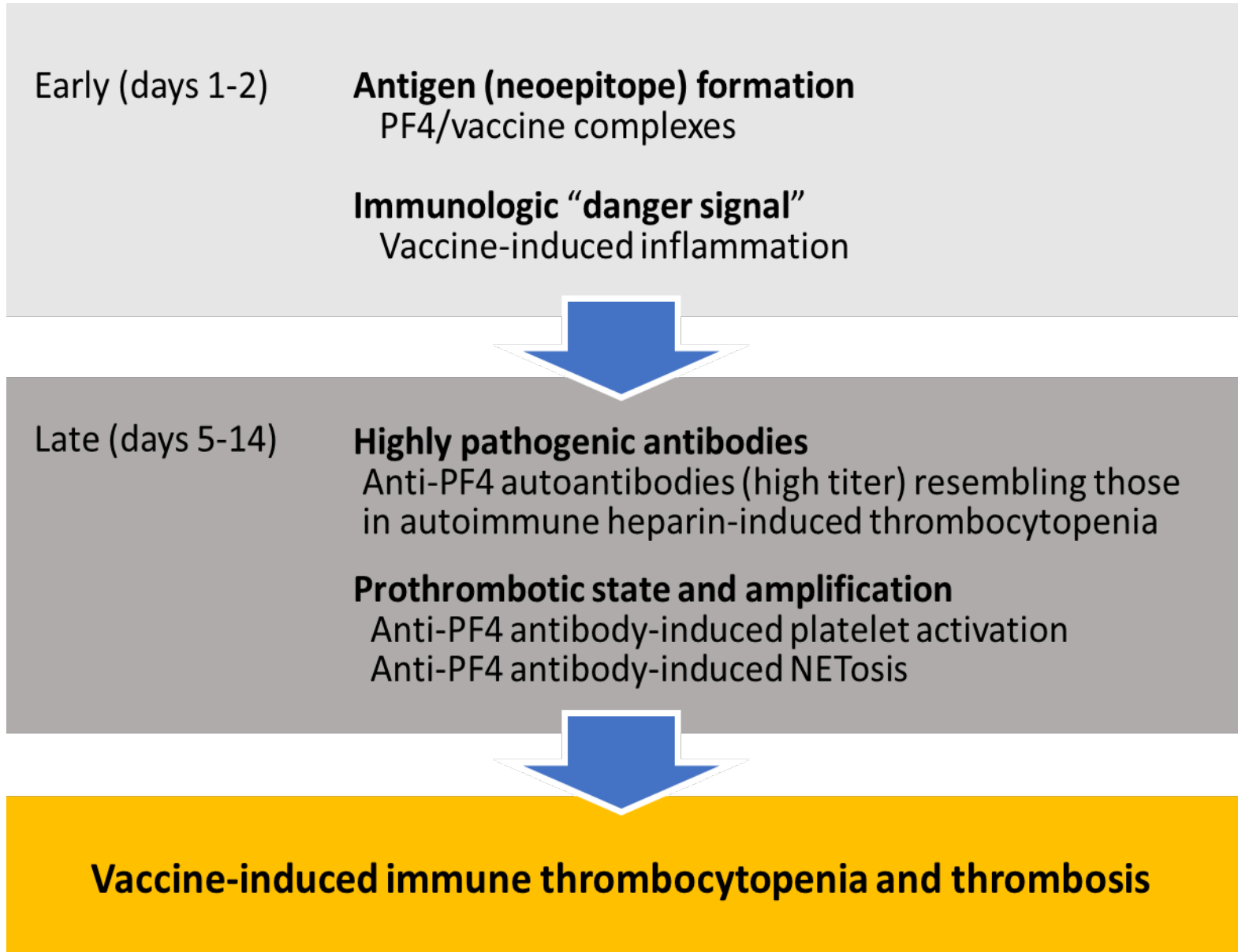
26 sera became PIPA pos



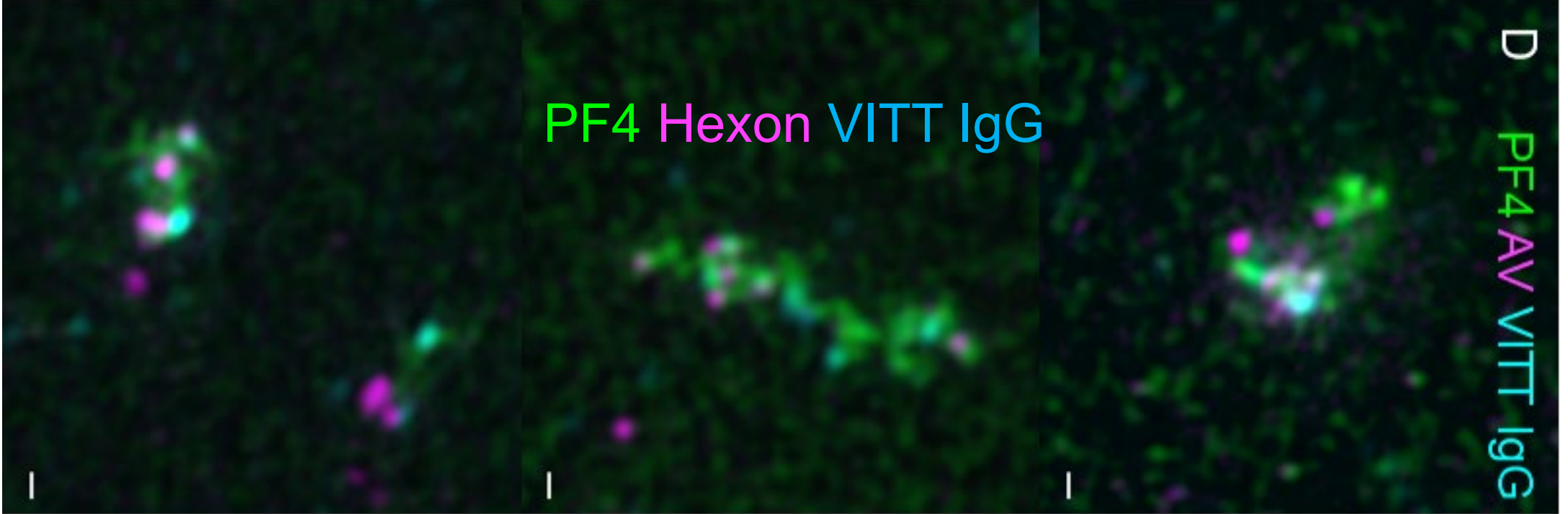
# VITT antibodies are transient



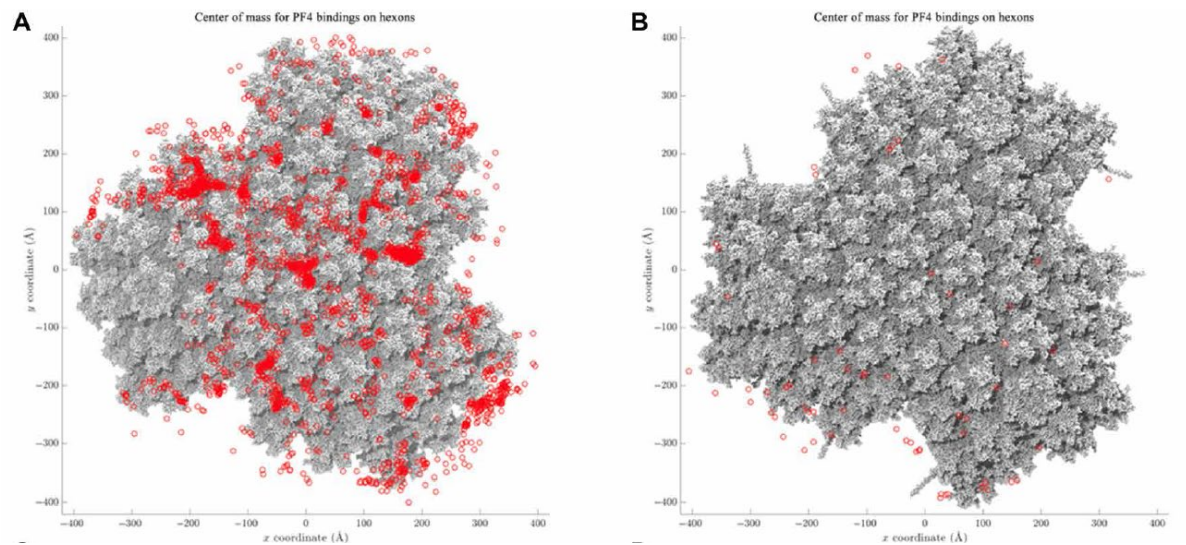
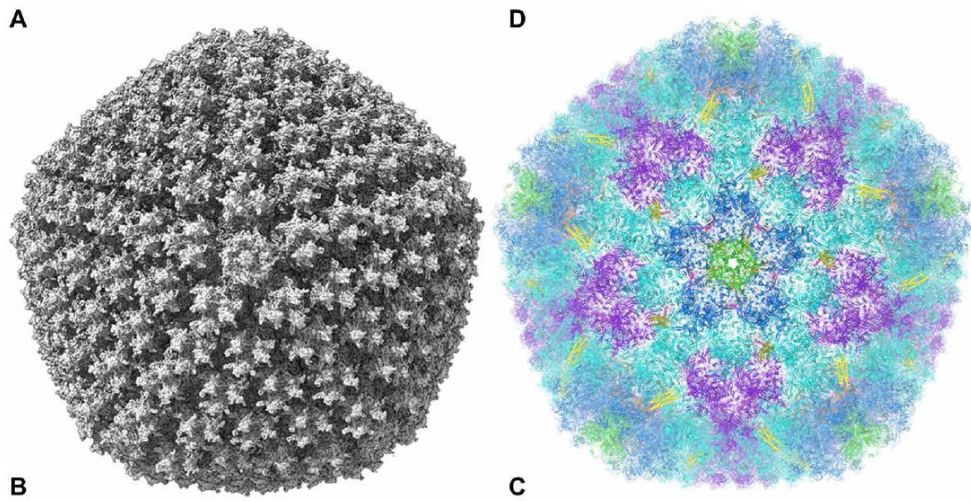
Schönborn L. et al. NEJM 2021; 385(19):1815-1816  
 Schönborn et al. BLOOD 2022; ;139(12):1903-1907



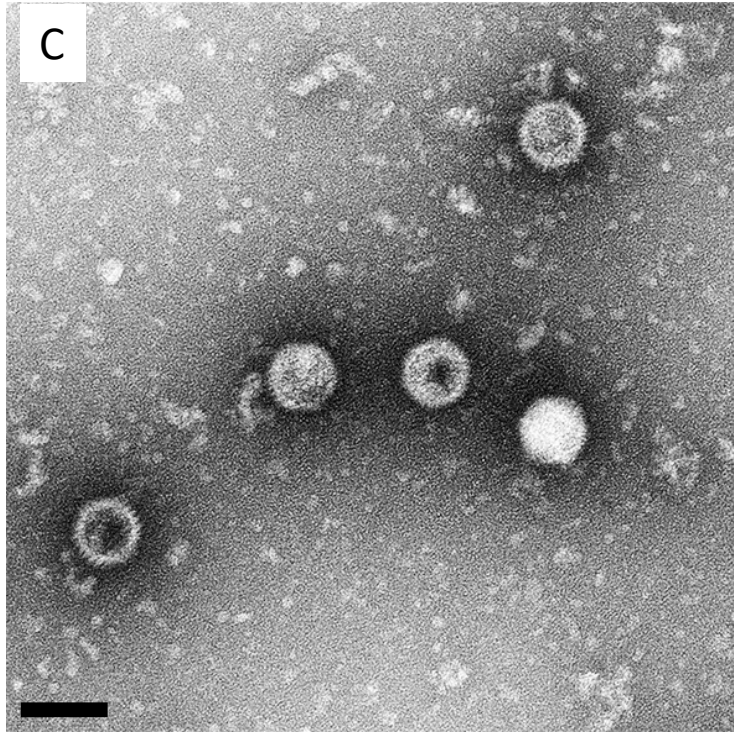




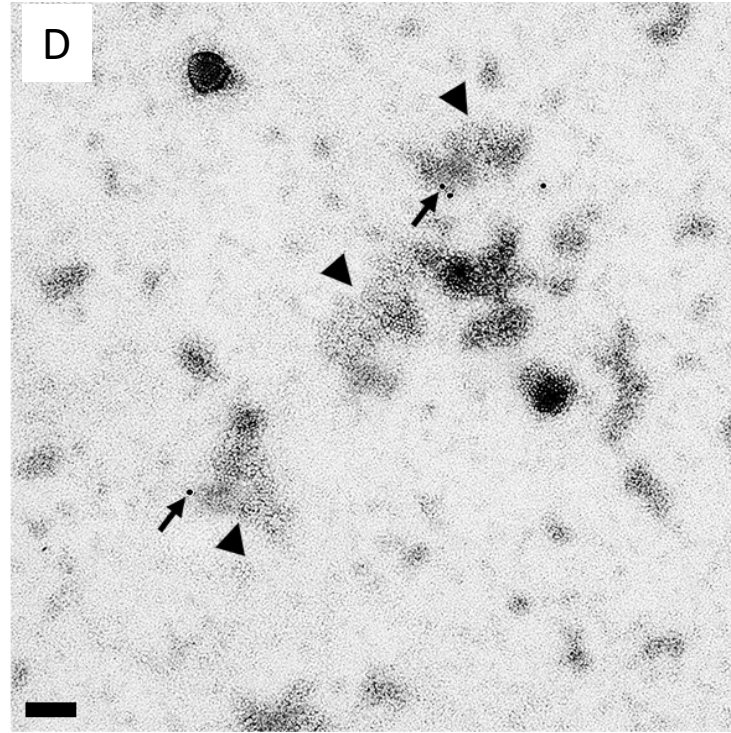
Greinacher A et al. BLOOD 09/2021



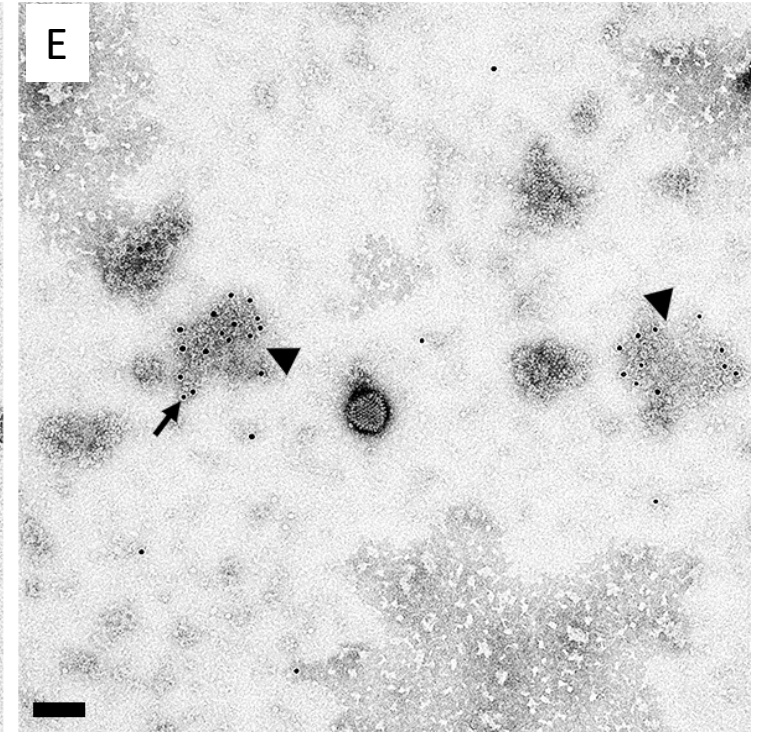
Baker AT et al. Sci Adv. 20213;7(49):eabl8213.



Vaccine

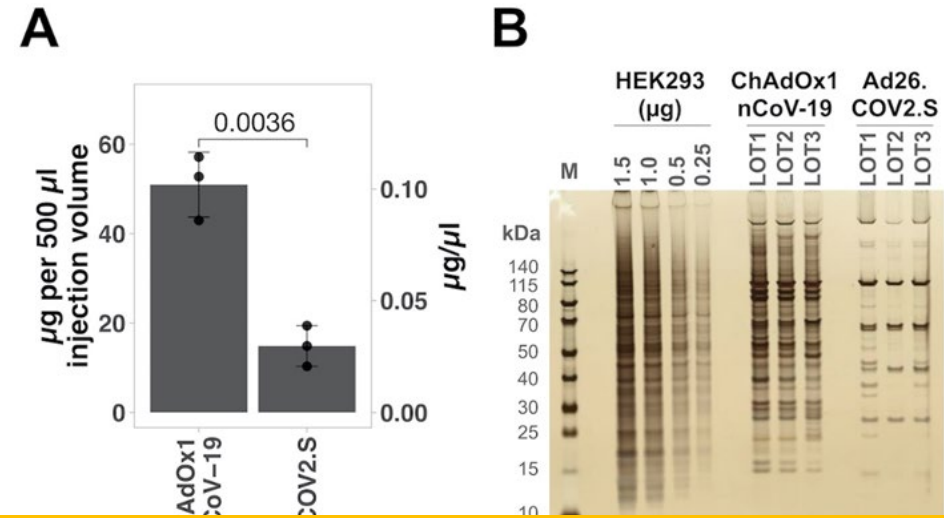
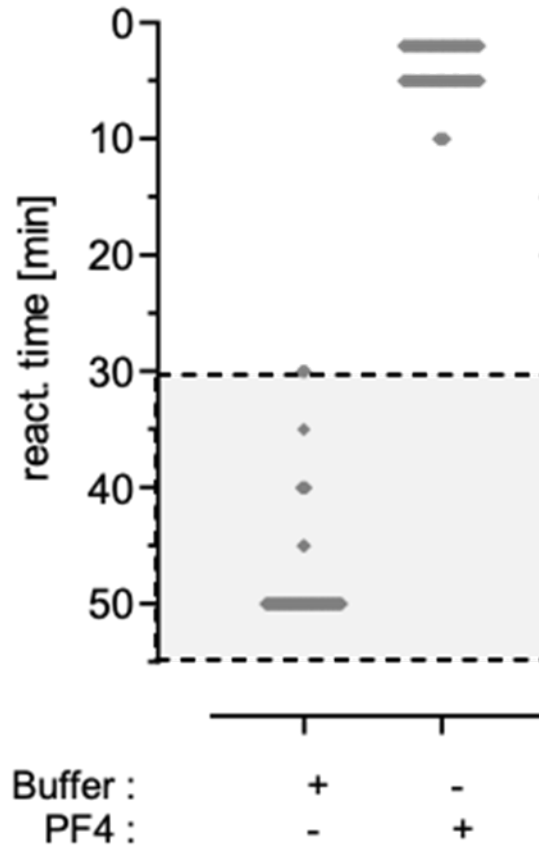


Vaccine + PF4  
stained for adenovirus proteins

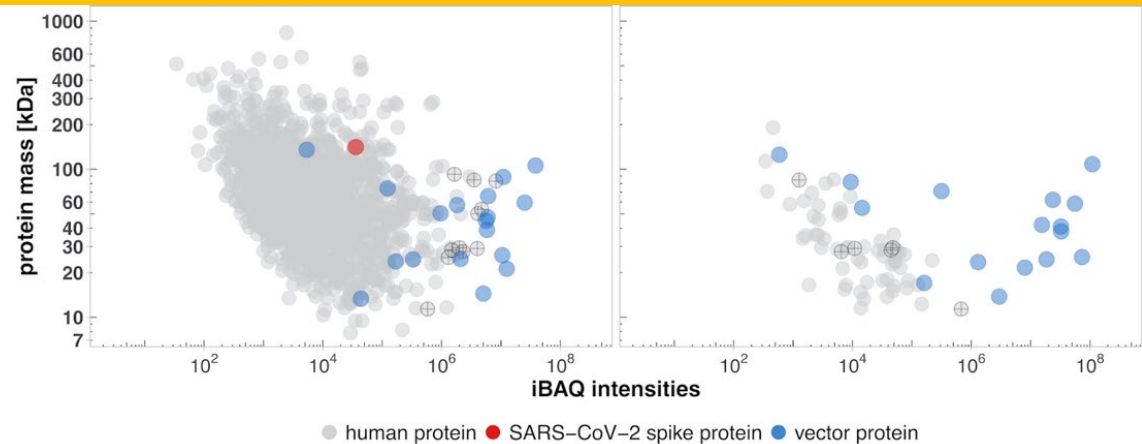


Vaccine + PF4  
stained for PF4

# PF4 binding partners in VITT



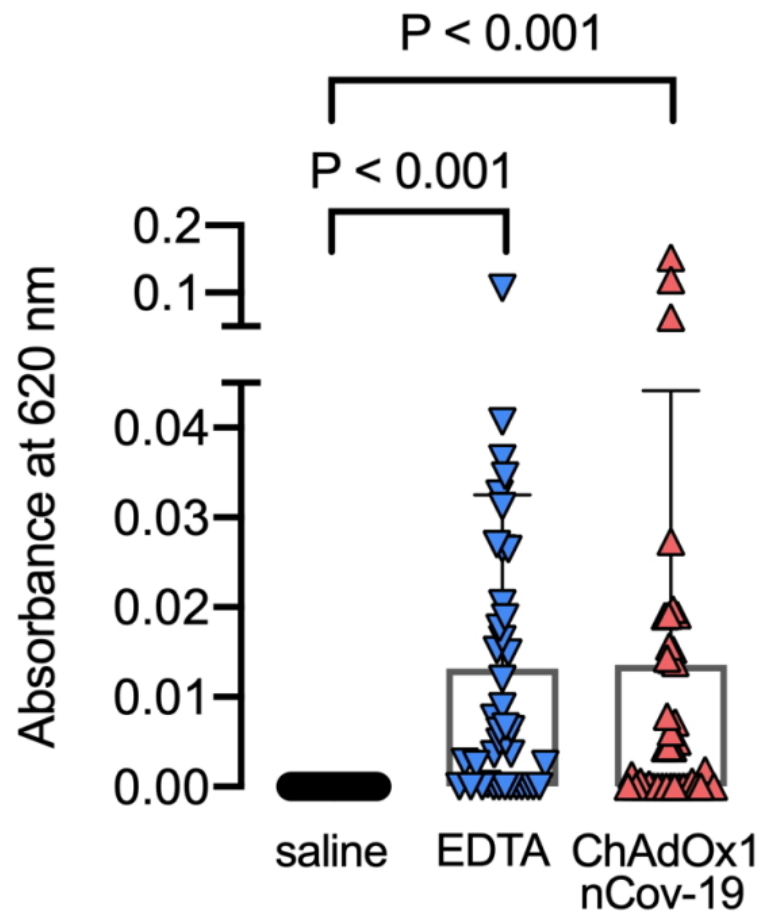
**Danger signal**



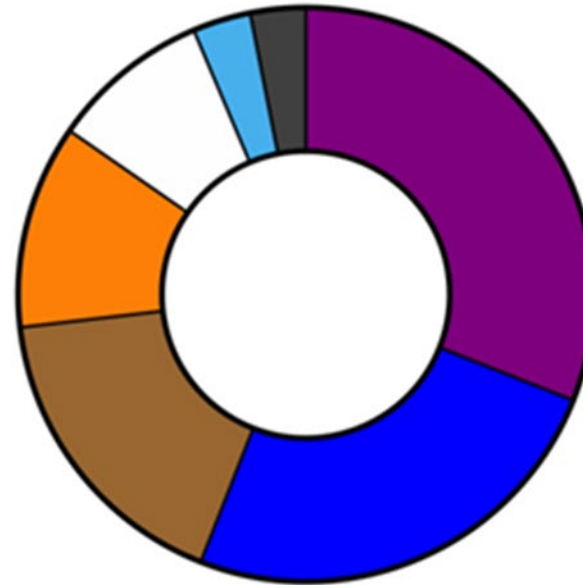
# EDTA in the vaccine ~ 0.1 mM

## Leakage

Mouse model intradermal injection



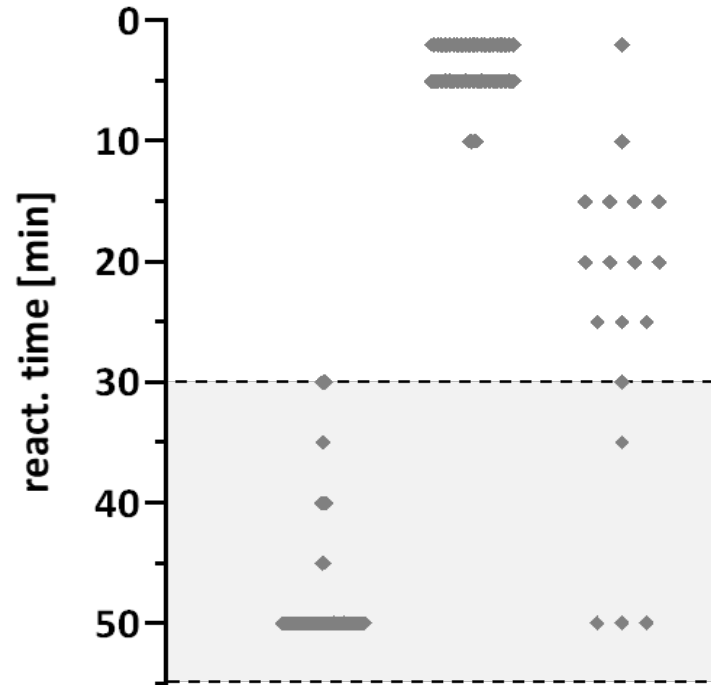
PCR chimpanzee DNA



Day 2 (6<sup>th</sup> March 2021 22:53)

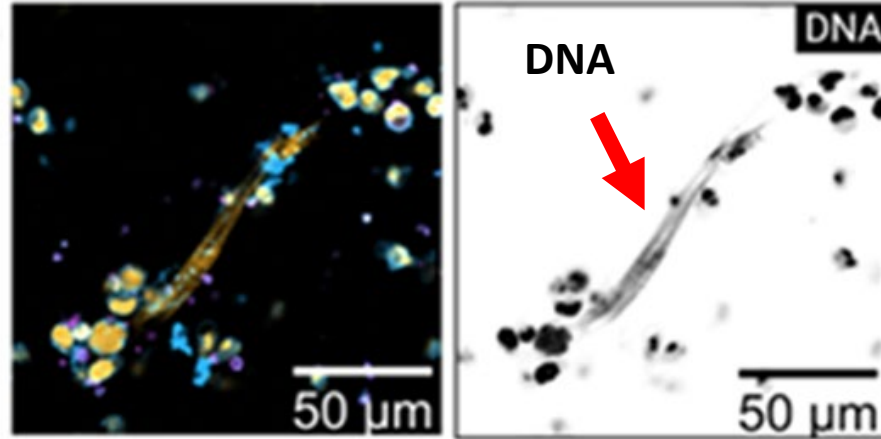
Day 14 (18<sup>th</sup> March 2021 20:07)

# High Titer Anti-PF4 Antibodies

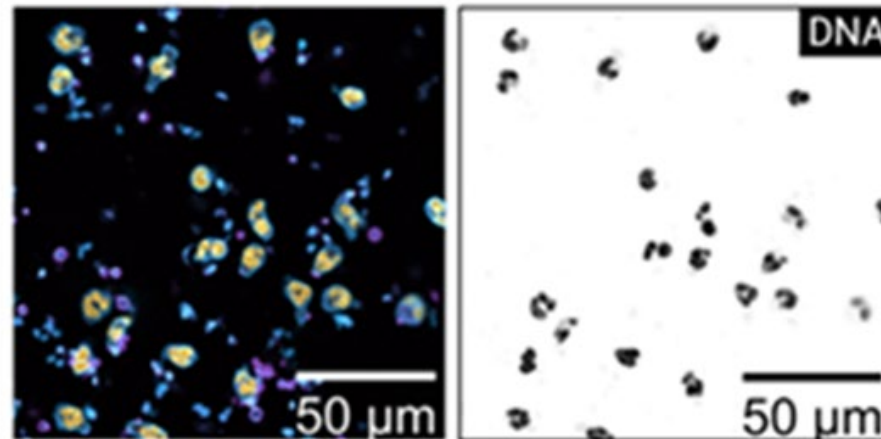


buffer:	+	-	-
10 $\mu\text{g/mL}$ PF4:	-	+	-
1 $\mu\text{g/mL}$ DNA:	-	-	+

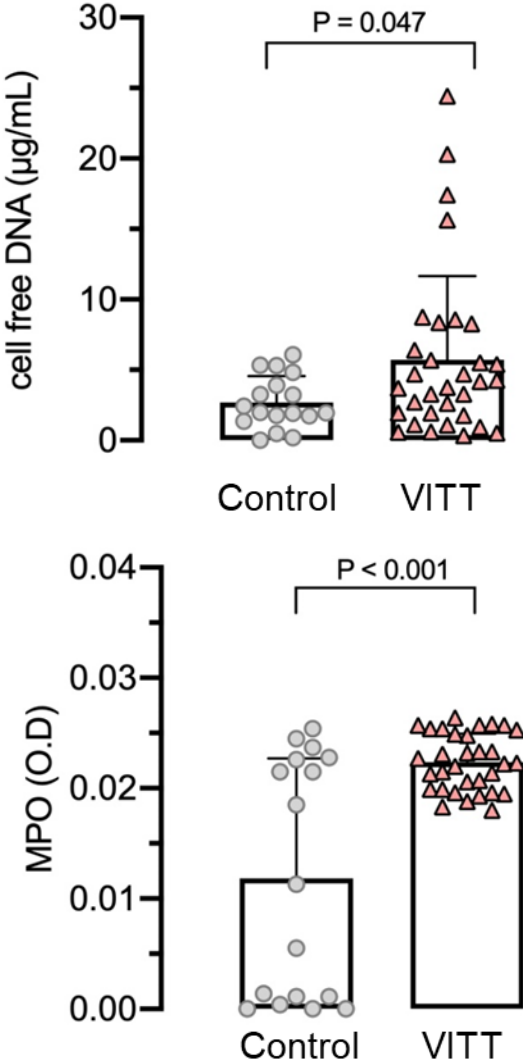
**A** Neutrophils + platelets + serum VITT patient



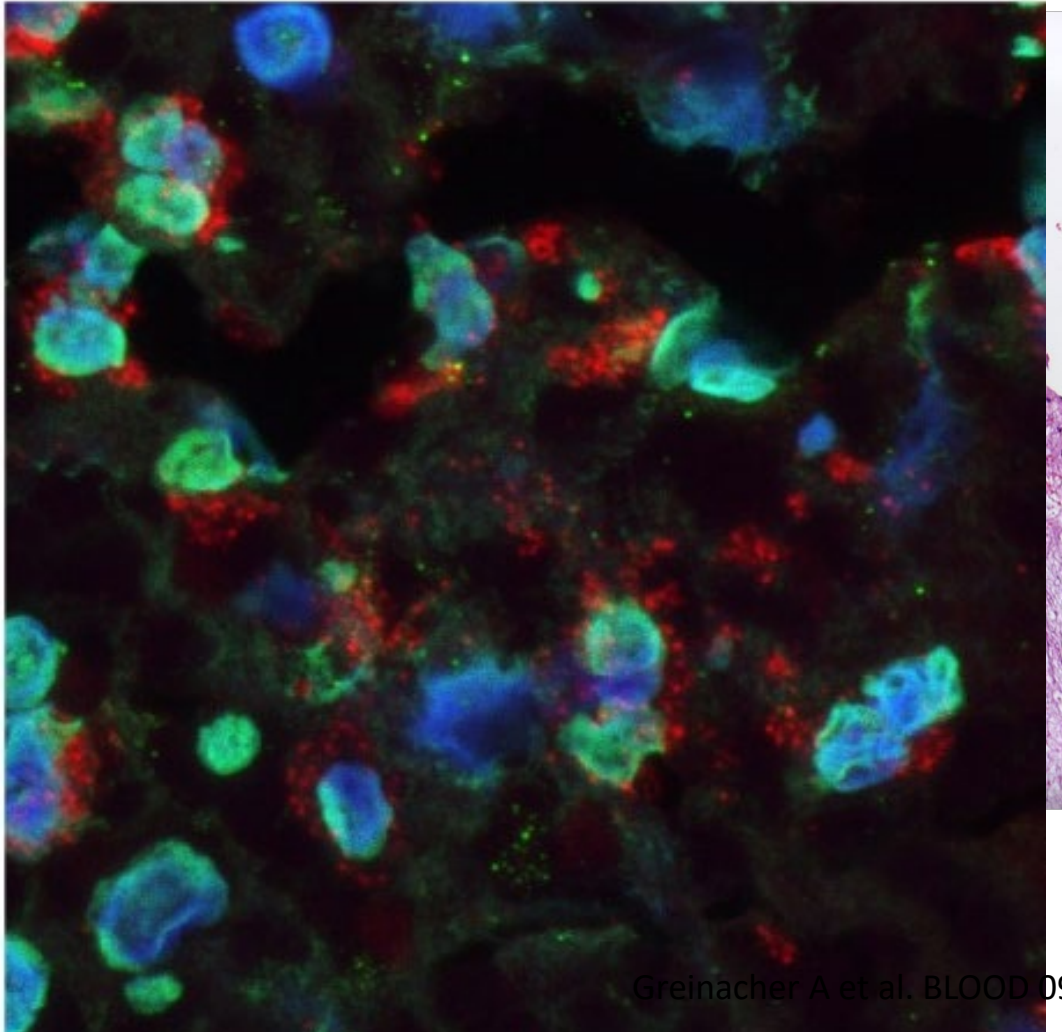
**B** Neutrophils + platelets + control



# NETosis, Amplification, Loss of control



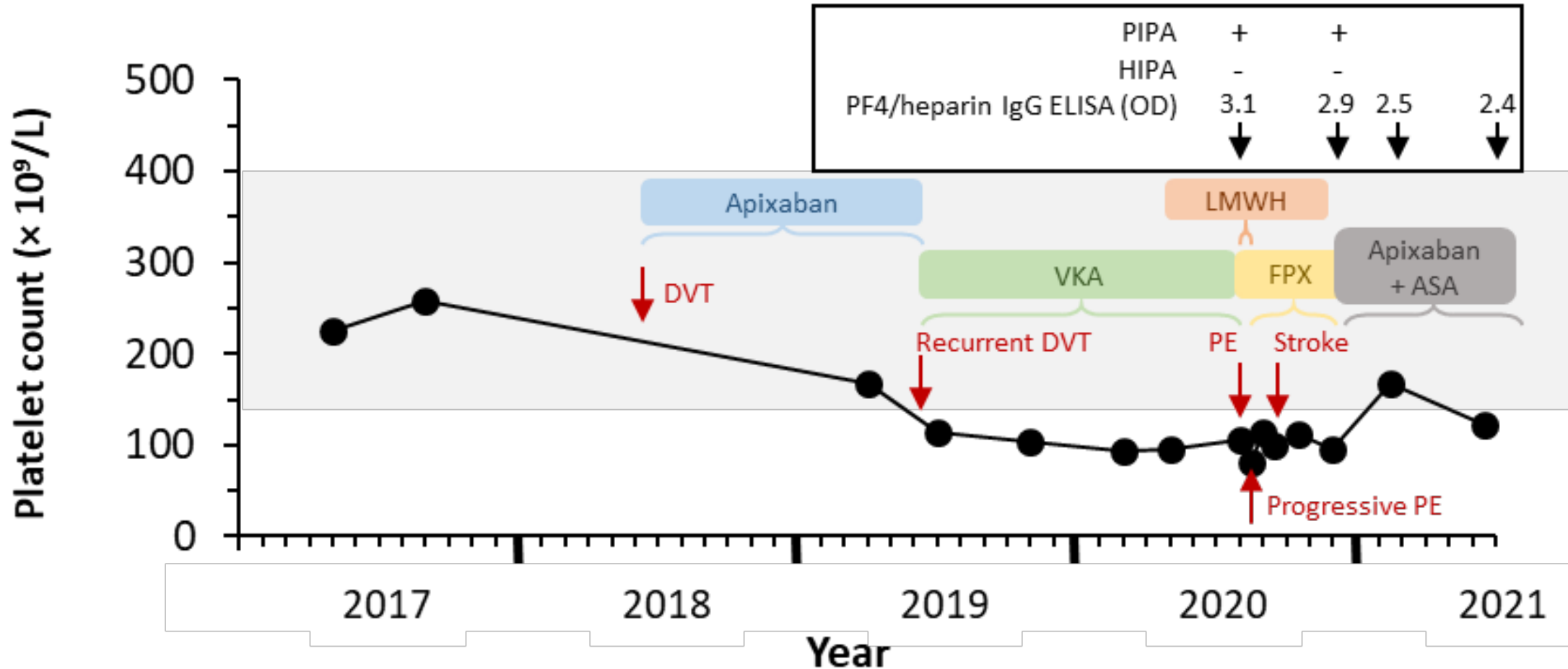
DAPI Chr NE



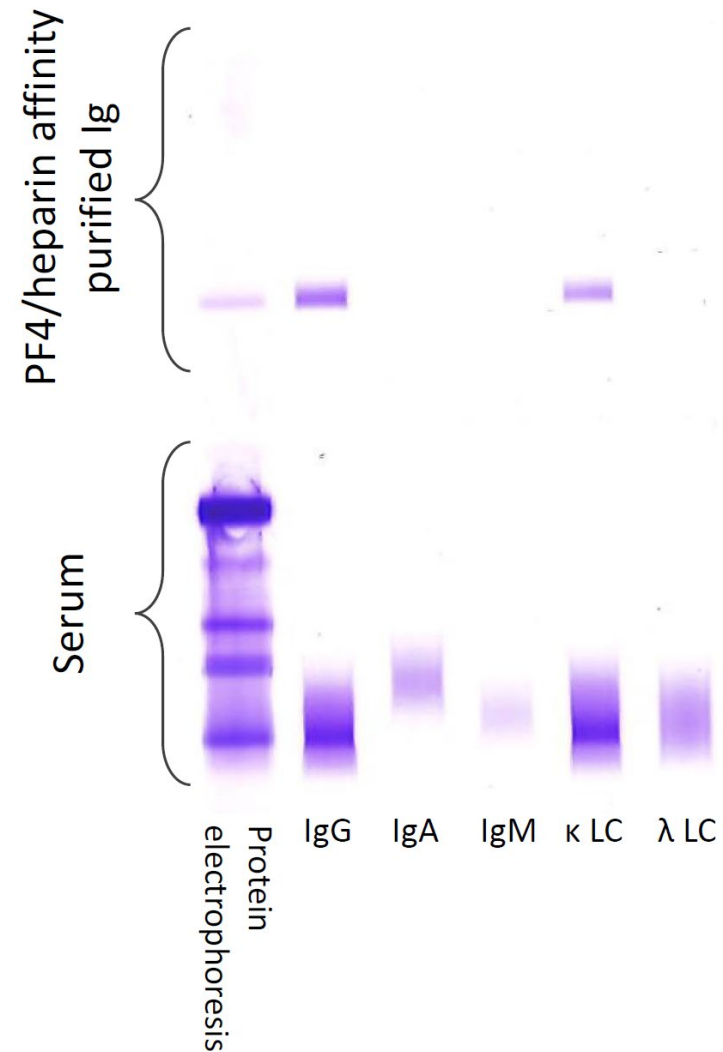
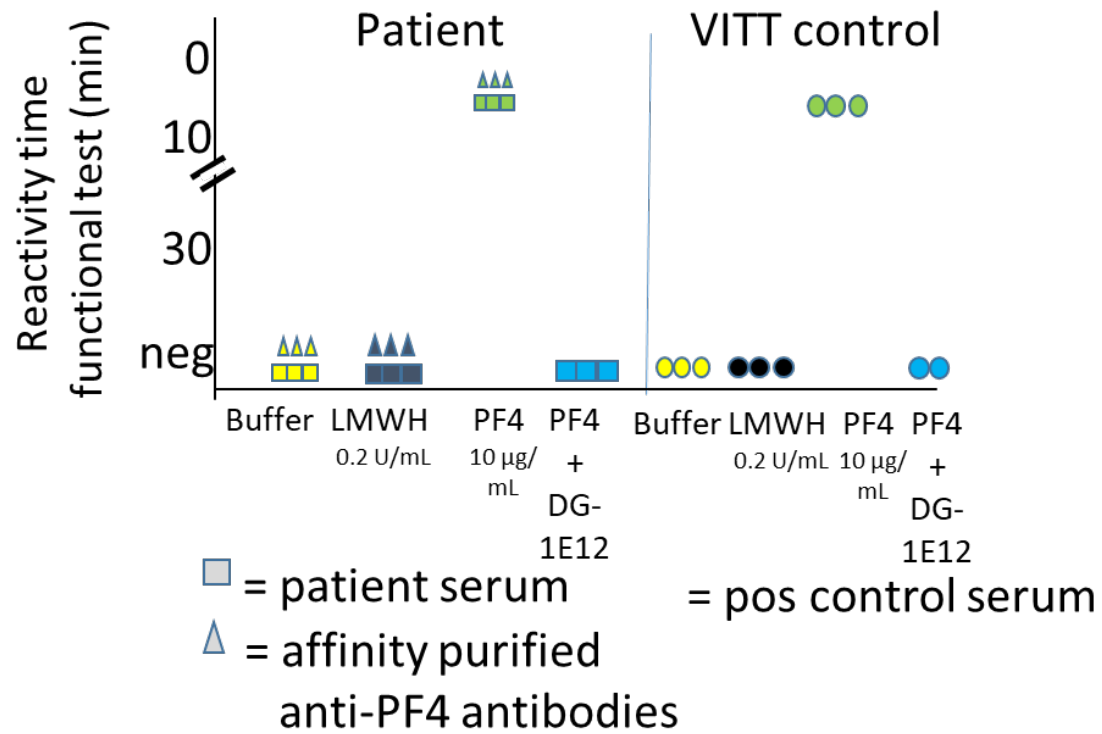
# Summary

- VITT is caused by anti-PF4 antibodies
- Human proteins and EDTA in the vaccine likely contribute to trigger the immune response to PF4
- Anti-PF4 autoantibodies activate platelets and activated platelets recruit granulocytes
- NETosis amplifies the reaction and causes a thrombin burst
- Unresolved: predisposition for CVST

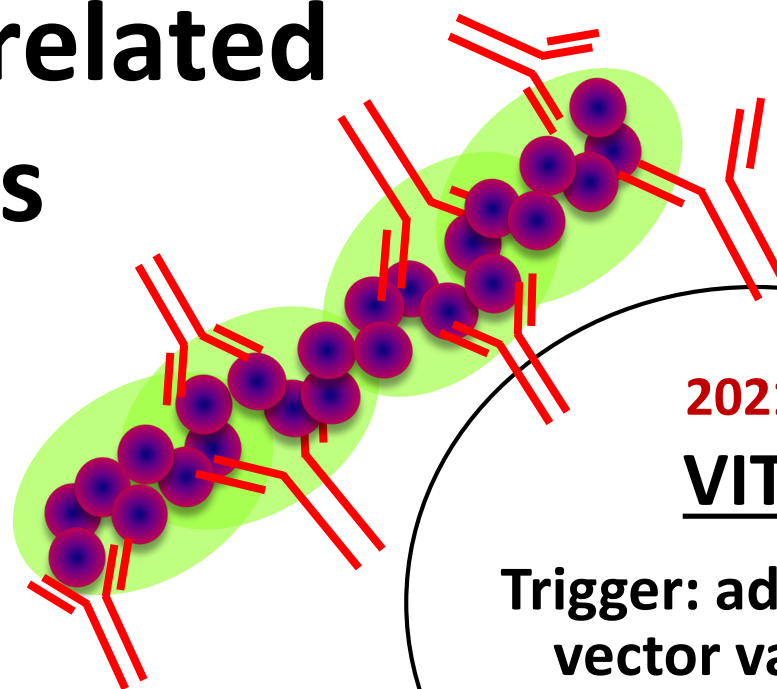
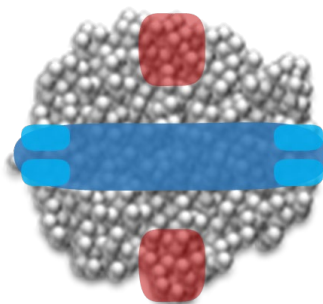
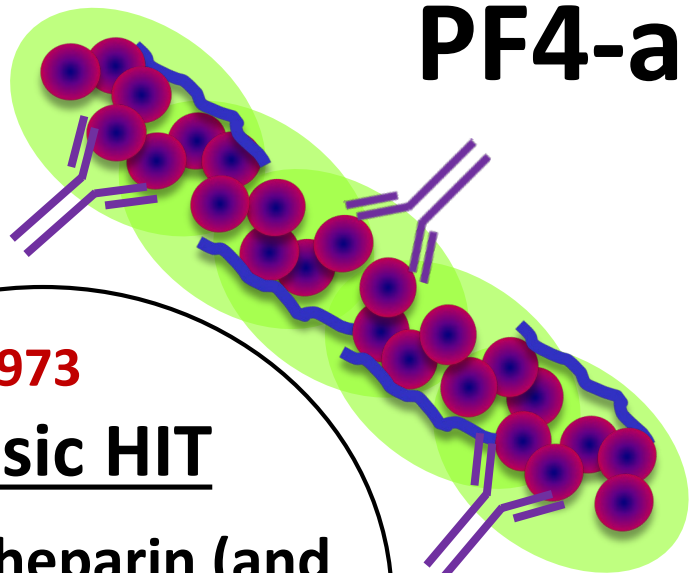
# VITT beyond vaccination?







# PF4-antibody related disorders



1973

## Classic HIT

Triggers: heparin (and certain other polyanions)

*heparin-dependent antibodies*

2021

## VITT

Trigger: adenovirus vector vaccines

*heparin-independent antibodies*

2001

## Autoimmune HIT

Trigger: heparin

*heparin-dependent and heparin-independent antibodies*

2008

## Spontaneous HIT

Triggers: total knee arthroplasty, infection

*heparin-independent antibodies*

**Heparin (or polyanion) triggered**

**Non-heparin triggered**