TTS / VITT after COVID Vaccination

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Outline / Objectives

- To review the clinical presentation of TTS/VITT and discuss the diagnostic criteria for TTS/VITT
- To explore the pathophysiology of TTS/VITT, including understanding of HIT and autoimmune HIT physiology
- To discuss the recommended evaluation of TTS/VITT, including the limitations of the laboratory assessment
- To discuss the treatment recommendations for TTS/VITT

TTS versus VITT

Thrombosis & Thrombocytopenia Syndrome

Vaccine induced Thrombosis & Thrombocytopenia

This is an official

CDC HEALTH ALERT

Distributed via the CDC Health Alert Network
April 13, 2021, 1:00 PM ET
CDCHAN-00442

Cases of Cerebral Venous Sinus Thrombosis with Thrombocytopenia after Receipt of the Johnson & Johnson COVID-19 Vaccine

Summary

As of April 12, 2021, approximately 6.85 million doses of the Johnson & Johnson (J&J) COVID-19 vaccine (Janssen) have been administered in the United States. The Centers for Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA) are reviewing data involving six U.S. cases of a rare type of blood clot in individuals after receiving the J&J COVID-19 vaccine that were reported to the Vaccine Adverse Events Reporting System (VAERS). In these cases, a type of blood clot called cerebral venous sinus thrombosis (CVST) was seen in combination with low levels of blood platelets (thrombocytopenia). All six cases occurred among women aged 18–48 years. The interval from vaccine receipt to symptom onset ranged from 6–13 days. One patient died. Providers should maintain a high index of suspension for symptoms that might represent serious thrombotic events or thrombocytopenia in patients who have recently received the J&J COVID-19 vaccine. When these specific type of blood clots are observed following J&J COVID-19 vaccination, treatment is different from the treatment that might typically be administered for blood clots. Based on studies conducted among the patients diagnosed with immune thrombotic thrombocytopenia after the AstraZeneca COVID-19 vaccine in Europe, the pathogenesis of these rare and unusual adverse events after vaccination may be associated with platelet-activating antibodies against platelet factor-4 (PF4), a type of protein. Usually, the anticoagulant drug called heparin is used to treat blood clots. In this setting, the use of heparin may be harmful, and alternative treatments need to be given.

CDC will convene an emergency meeting of the Advisory Committee on Immunization Practices (ACIP) on Wednesday, April 14, 2021, to further review these cases and assess potential implications on vaccine policy. FDA will review that analysis as it also investigates these cases. Until that process is complete, CDC and FDA are recommending a pause in the use of the J&J COVID-19 vaccine out of an abundance of caution. The purpose of this Health Alert is, in part, to ensure that the healthcare provider community is aware of the potential for these adverse events and can provide proper management due to the unique treatment required with this type of blood clot.

Background

VAERS is a national passive surveillance system jointly managed by CDC and FDA that monitors adverse events after vaccinations. The six patients (after 6.85 million vaccine doses administered) described in these VAERS reports came to attention in the latter half of March and early April of 2021 and developed symptoms a median of 9 days (range = 6–13 days) after receiving the J&J COVID-19 vaccine. Initial presenting symptoms were notable for headache in five of six patients, and back pain in the sixth who subsequently developed a headache. One patient also had abdominal pain, nausea, and vomiting. Four developed focal neurological symptoms (focal weakness, aphasia, visual disturbance) prompting presentation for emergency care. The median days from vaccination to hospital admission was 15 days (range = 10–17 days). All were eventually diagnosed with

https://emergency.cdc.gov/han/2021/han00442.asp

Clinical Presentation of TTS

New onset thrombosis and thrombocytopenia

- Thrombosis more commonly seen in rare and severe locations (e.g. cerebral venous sinus thrombosis, splanchnic venous thrombosis)
  - Typical thrombosis locations also occur
- Symptoms typically arise within 5-30 days of vaccination

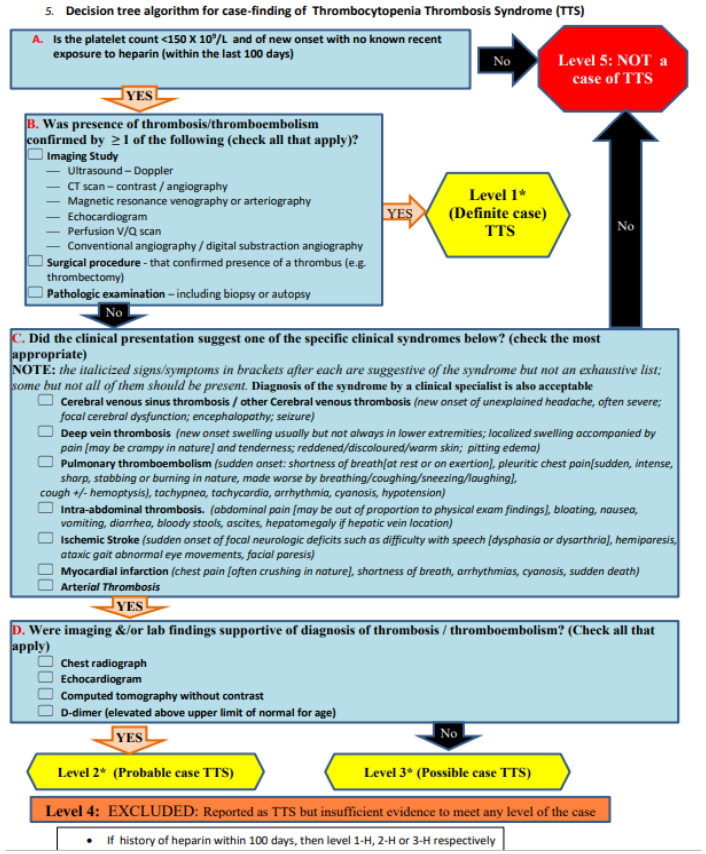
Associated laboratory findings

- Low fibrinogen
- Elevated D-dimer

Primarily reported after adenovirus based COVID-19 vaccination

- Astra-Zeneca (ChaAdOx1)
- Johnson & Johnson (Ad26.COV2.S)

Variety of Diagnostic Criteria



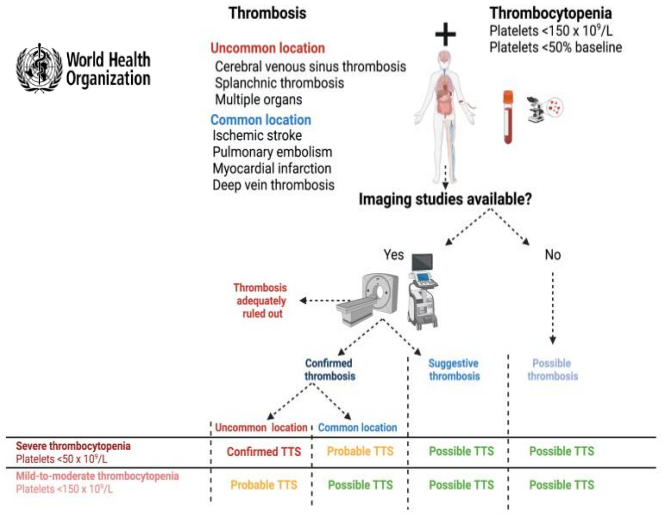
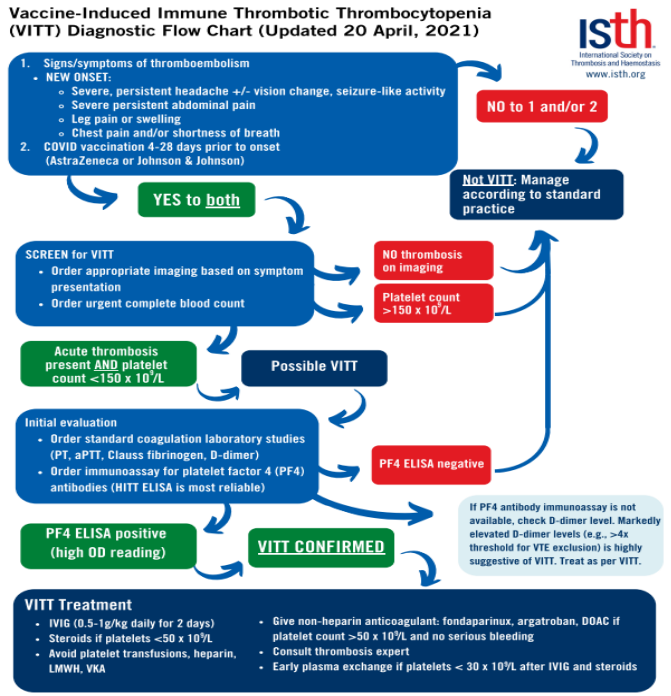


Figure 1: Algorithm for the clinical diagnosis of thrombosis thrombocytopenia syndrome

Figure created with BioRender.com



Thrombosis with Thrombocytopenia Syndrome (TTS)

- **Definitive Diagnosis** (must meet all five criteria):

1. COVID vaccine 4 to 42 days prior to symptom onset#
2. Any venous or arterial thrombosis (often cerebral or abdominal)
3. Thrombocytopenia (platelet count < 150 x 109/L)*
4. Positive PF4 "HIT" (heparin-induced thrombocytopenia) ELISA
5. Markedly elevated D-dimer (> 4 times upper limit of normal)

Diagnostic Criteria – UK Assessment of 294 Patients following ChAdOx1 nCoV-19 Vaccine

- Clinical Criteria for Diagnosis
  - Onset of symptoms
  - Presence of thrombosis
  - Presence of thrombocytopenia
  - D-dimer
  - Presence of antibodies to PF4

Table 1. Case Definition Criteria for Vaccine-Induced Immune Thrombocytopenia and Thrombosis (VITT), According to an Expert Hematology Panel.*

Type of VITT	Description
Definite VITT	All five of the following criteria: Onset of symptoms 5–30 days after vaccination against SARS-CoV-2 (or ≤42 days in patients with isolated deep-vein thrombosis or pulmonary embolism) Presence of thrombosis Thrombocytopenia (platelet count <150,000 per cubic millimeter) D-dimer level >4000 FEU Positive anti-PF4 antibodies on ELISA
Probable VITT	D-dimer level >4000 FEU but one criterion not met (timing, thrombosis, thrombocytopenia, or anti-PF4 antibodies) or D-dimer level unknown or 2000–4000 FEU and all other criteria met
Possible VITT	D-dimer level unknown or 2000–4000 FEU with one other criterion not met, or two other criteria not met (timing, thrombosis, thrombocytopenia, or anti-PF4 antibodies)
Unlikely VITT	Platelet count <150,000 per cubic millimeter without thrombosis with D-dimer level <2000 FEU, or thrombosis with platelet count >150,000 per cubic millimeter and D-dimer level <2000 FEU, regardless of anti-PF4 antibody result, and alternative diagnosis more likely

* ELISA denotes enzyme-linked immunosorbent assay, FEU fibrinogen-equivalent unit, PF4 platelet factor 4, and SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.

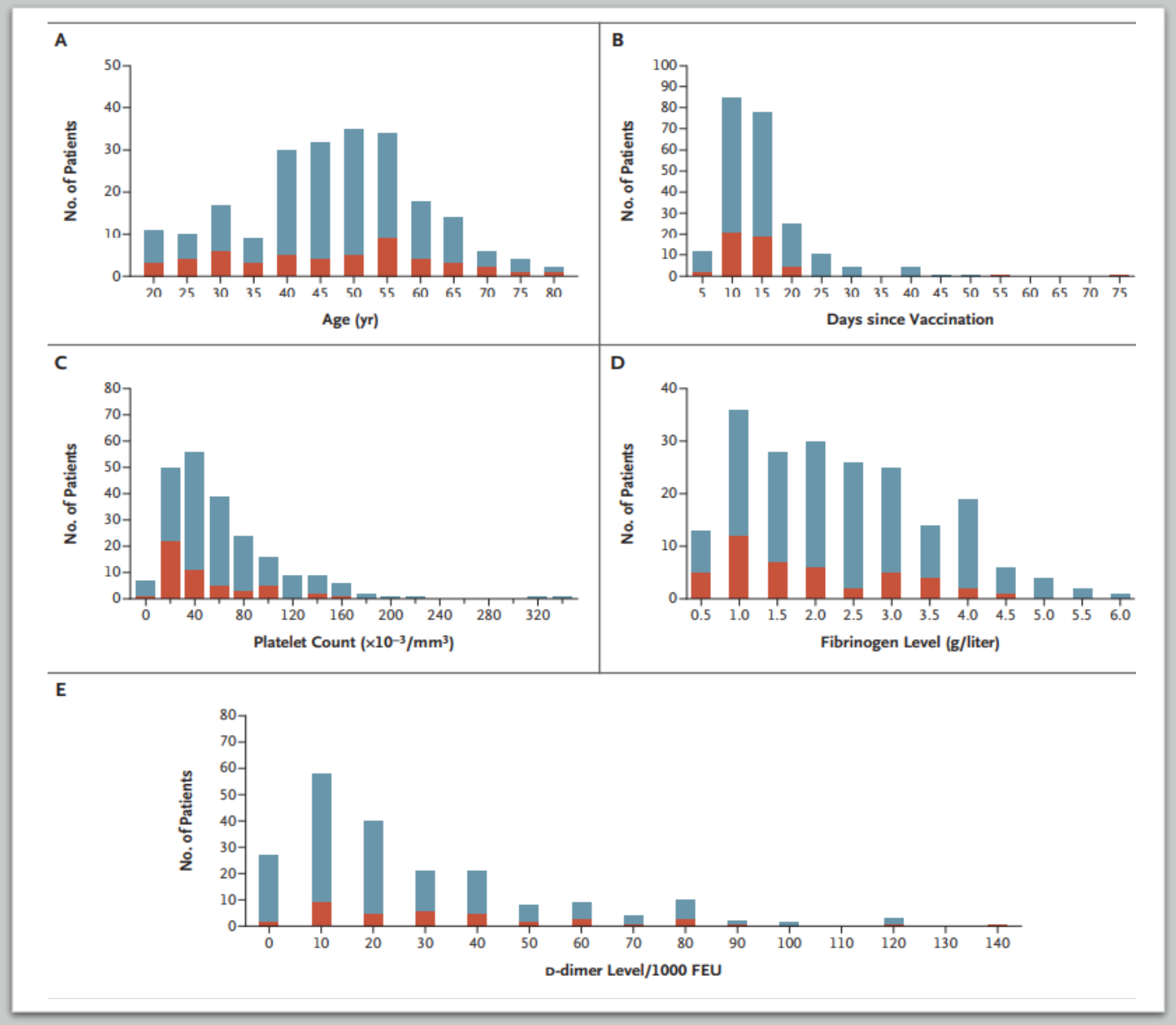
NEJM DOI: 10.1056/NEJMoa2109908

Baseline Characteristics of VITT Patients

22% of the patients died

Increased odds of death

- CVST
- Decrease in platelet count
- Increase in D-dimer
- Decrease in fibrinogen



NEJM DOI: 10.1056/NEJMoa2109908

TTS Following Ad26.COV2.S Vaccination

12 female patients 18-60 years old with CVST, 7 with concurrent intracranial hemorrhage

Symptom onset: 6-15 days following vaccination

Platelet count ranged from 9 – 127,000 / mcL

11 patients with anti-PF4 ELISA testing → all positive

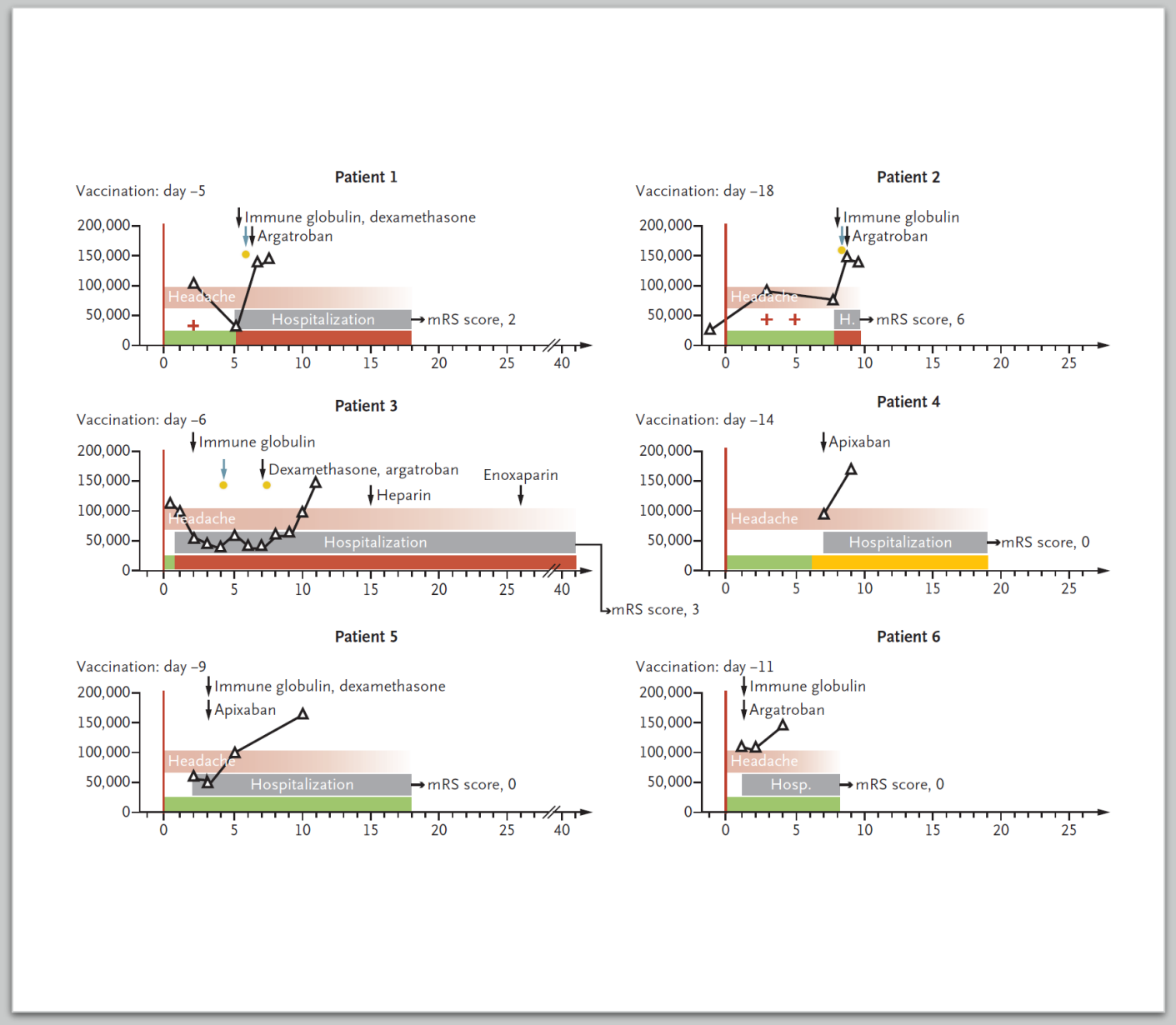
Outcomes at publication

- 3 death
- 3 intensive care unit
- 4 non-ICU hospitalization
- 4 discharged to home

JAMA. 2021;325(24):2448-2456. doi:10.1001/jama.2021.7517

Thrombocytopenia with Severe Headache

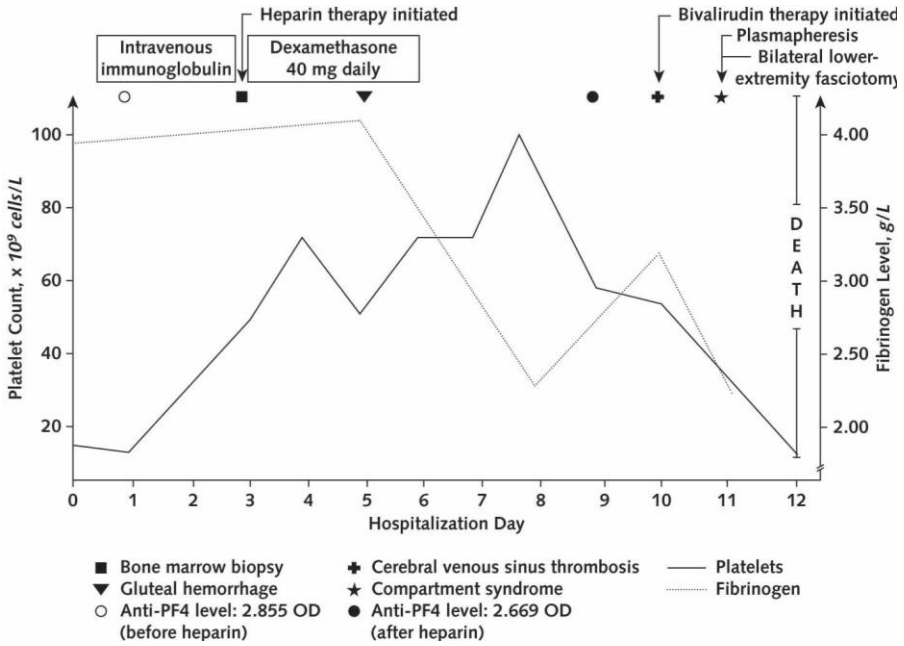
- 11 patients: thrombocytopenia without CVST 5-18 days after vaccination
  - Severe headache
  - Elevated d-dimer
  - Positive anti-PF4/heparin IgG ELISA
- Thrombotic complications did not occur in 7 patients, 6 of whom received early treatment (high dose IVIG, steroids, therapeutic dose anticoagulation)



NEJM doi: 10.1056/NEJMc2112974.

First Case Report of TTS After mRNA Vaccine

- 65-year-old man with chronic hypertension and hyperlipidemia 10 days after he received a second dose of the mRNA vaccine (Moderna)
- No known prior heparin exposure.
- Low platelets + bilateral acute pulmonary embolism, bilateral DVT



Editorial response:

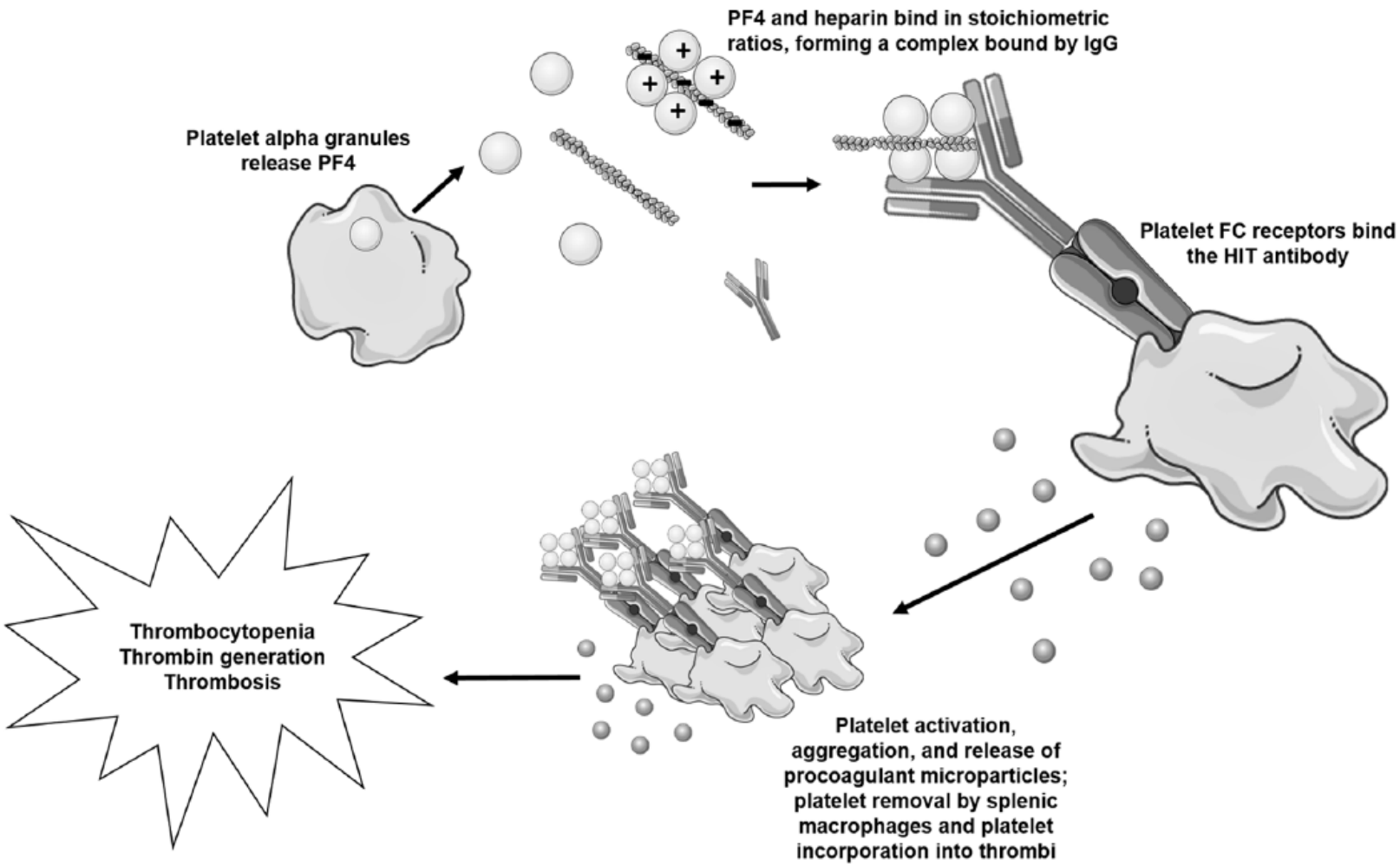
- Clinical case meets Brighton Collaboration and ISTH diagnostic criteria
  - Treatment of patient per TTS/VITT recommendations is warranted
- Critique of diagnosis
  - Weak (+) ELISA should not be the sole diagnostic criteria (7% + s/p vaccine)
  - Autoimmune / autoimmune HIT is a consideration
  - Causal / definitive link between mRNA vaccine and TTS/VITT has not been proposed

10/29/2021: Two confirmed cases of TTS following mRNA COVID-19 vaccination (Moderna) have been reported to VAERS after more than 394 million doses of mRNA COVID-19 vaccines administered in the United States.

Annals of Internal Medicine. DOI:10.7326/L21-0244
Annals of Internal Medicine. DOI:10.7326/M21-2680
https://www.cdc.gov/coronavirus/2019-ncov/vaccines/safety/adverse-events.html

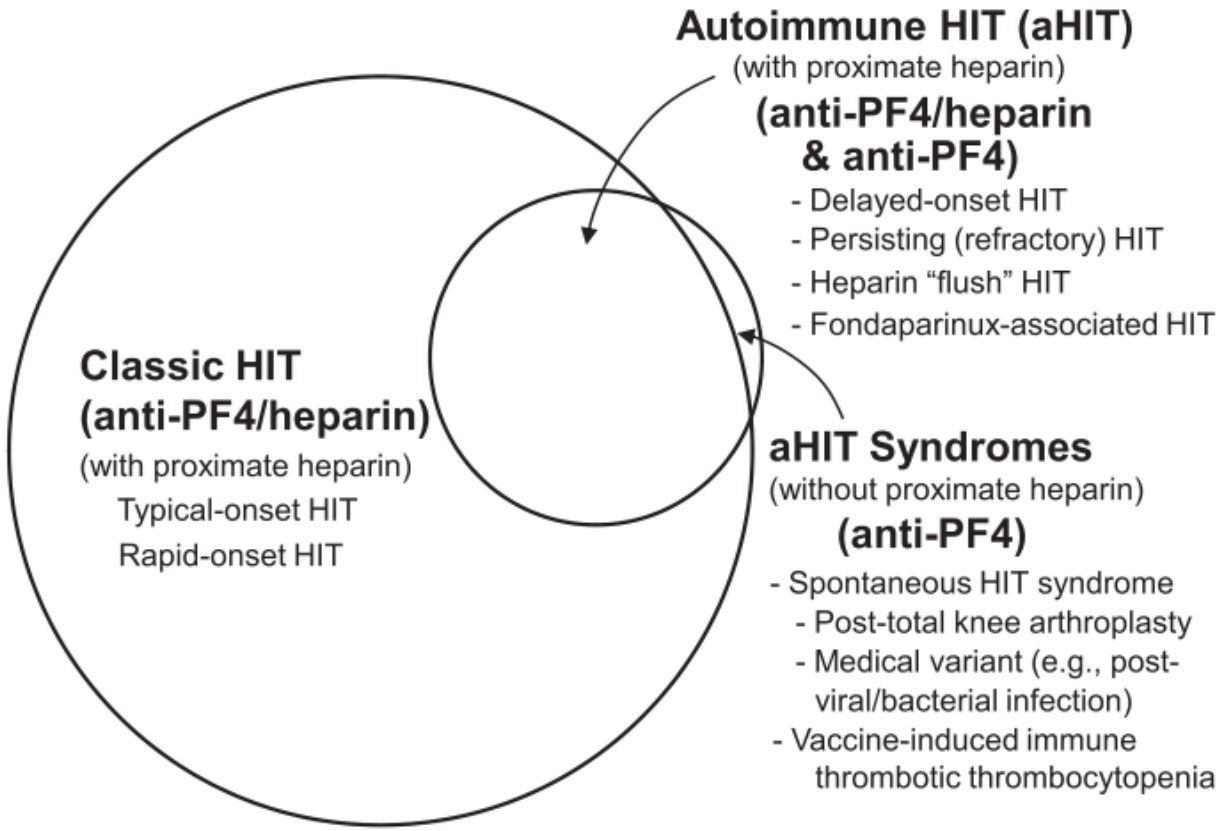
Pathophysiology of TTS

Heparin Induced Thrombocytopenia



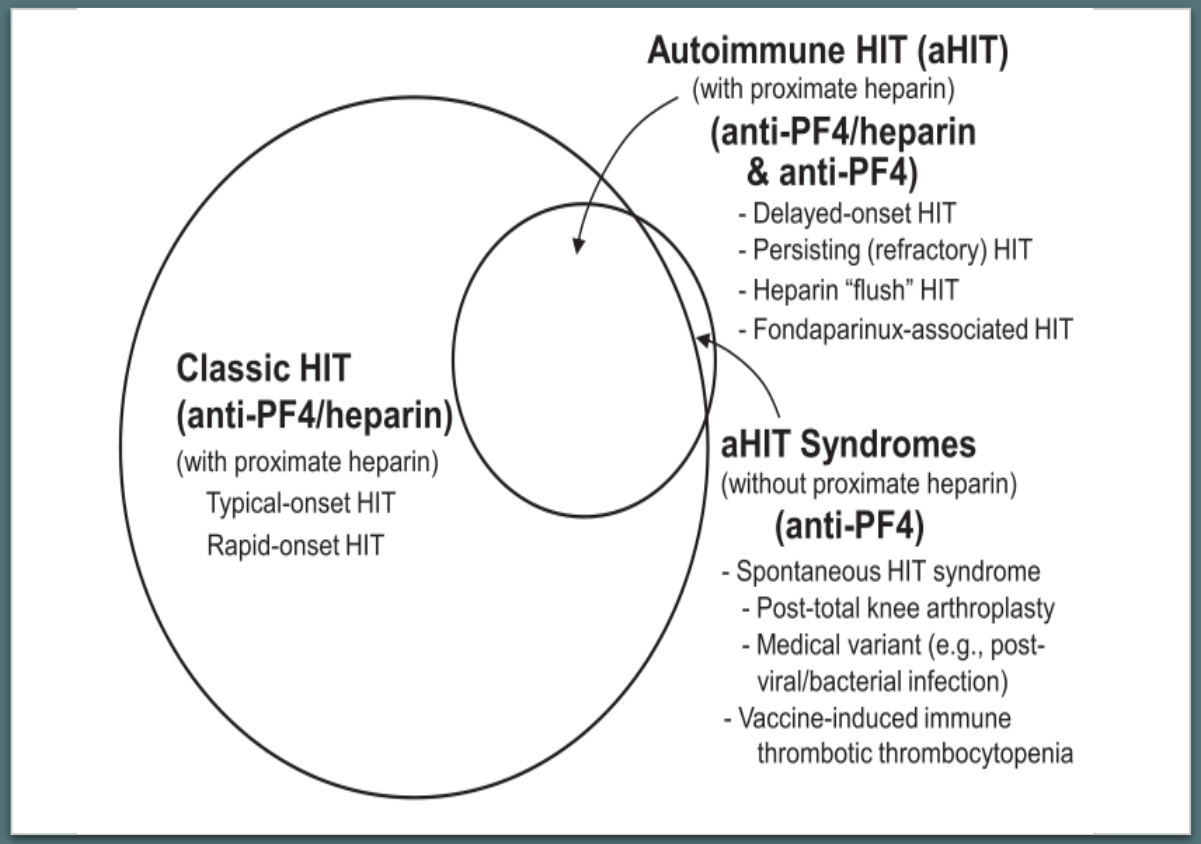
Vascular Medicine 2020, Vol. 25(2) 160–173

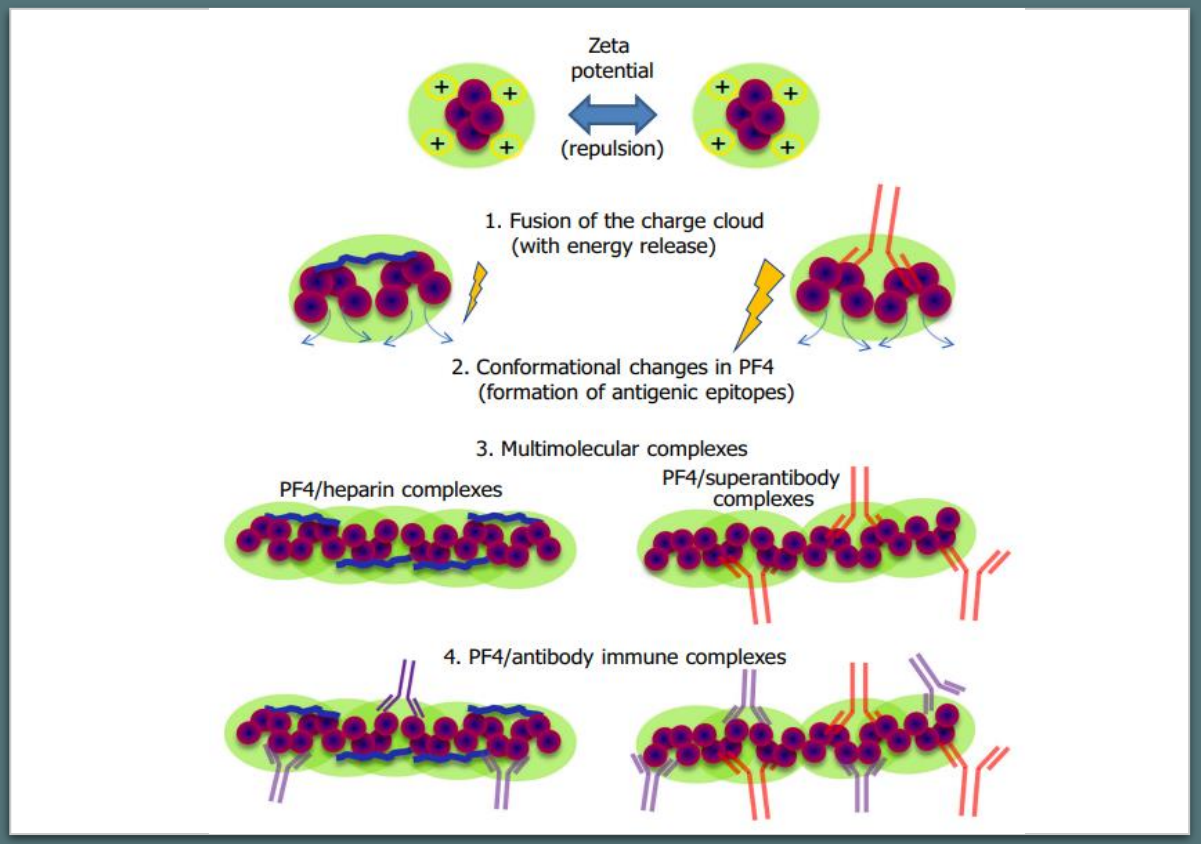
Interrelationship of HIT Variants



Thrombosis Research 2021:204;40-51.

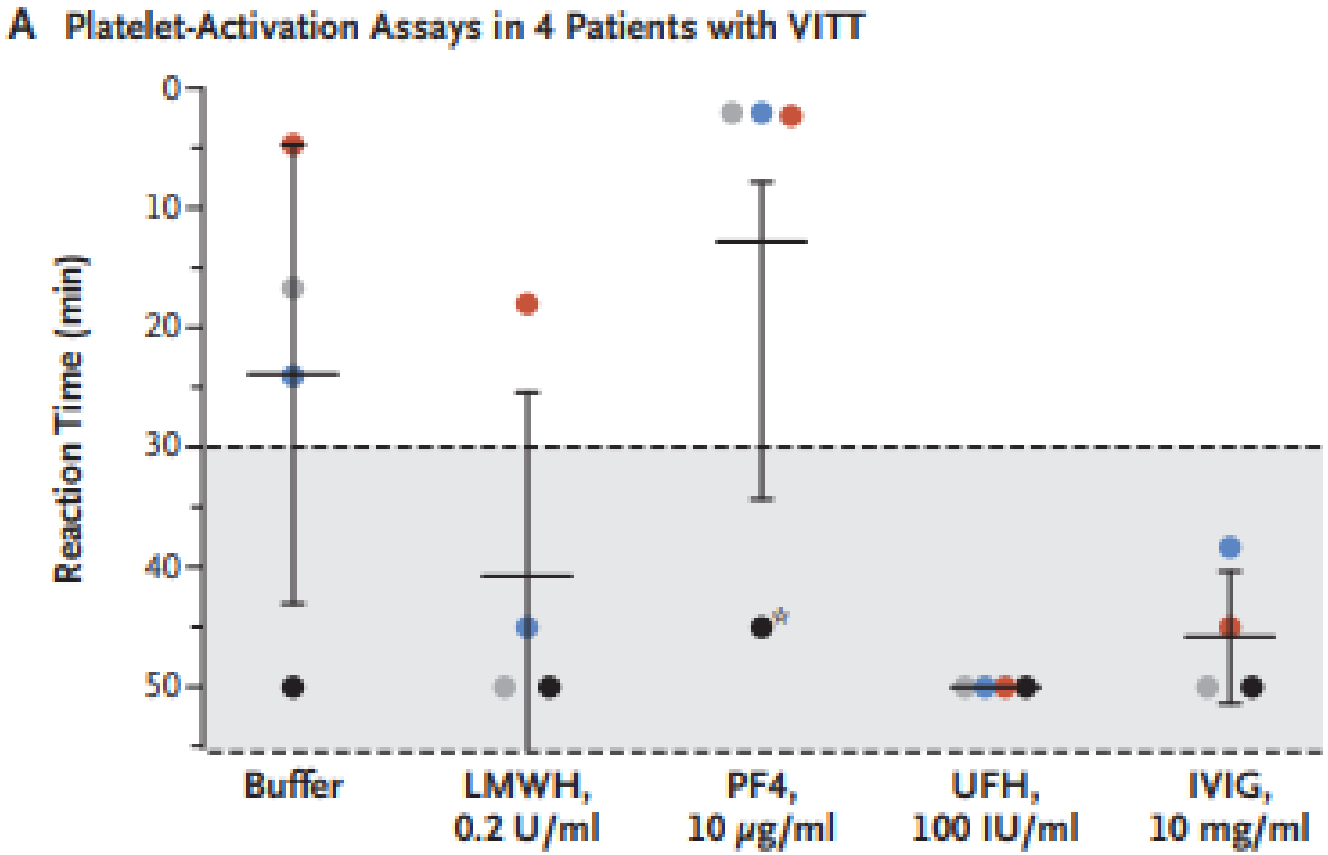
HIT Variants





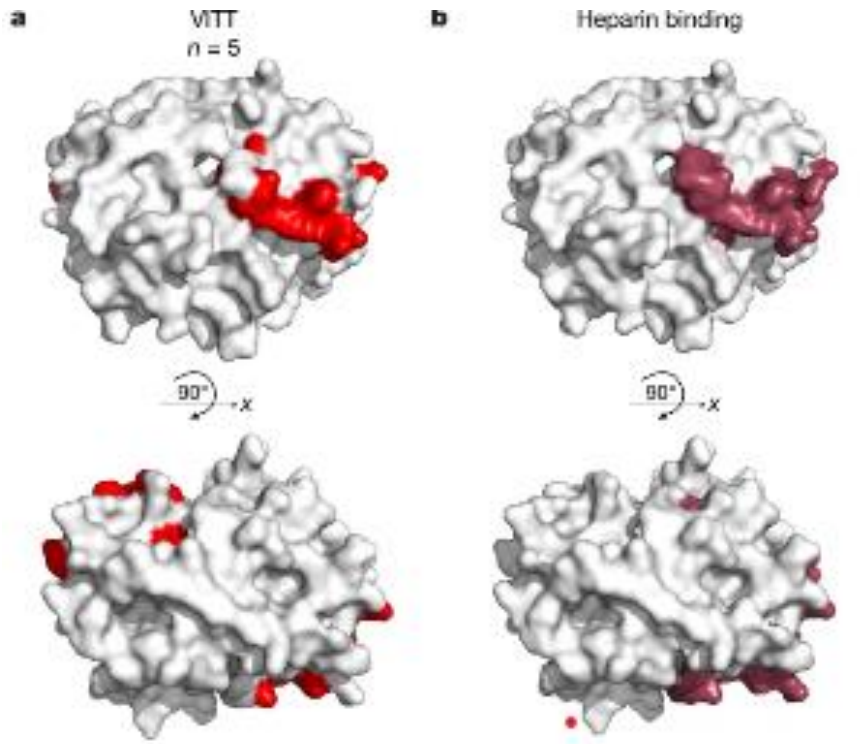
Thrombosis Research 2021:204;40-51.

Demonstration of Platelet Activation in VITT

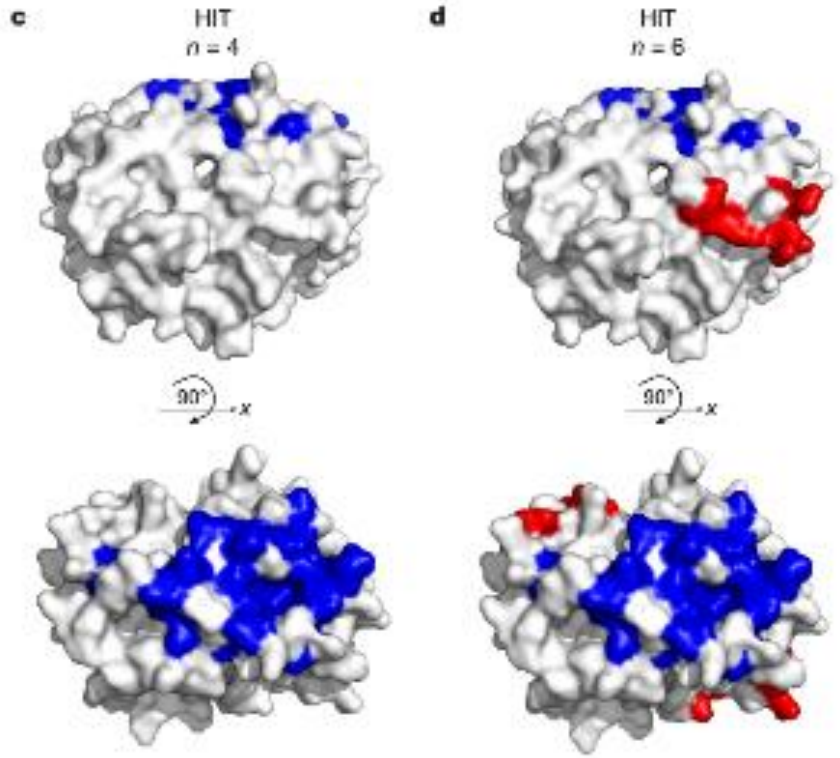


NEJM DOI: 10.1056/NEJMoa2104840

Characterization of VITT Antibodies



VITT antibodies bind PF4 at heparin binding site



HIT antibodies bind PF4 at heparin binding site & other locations

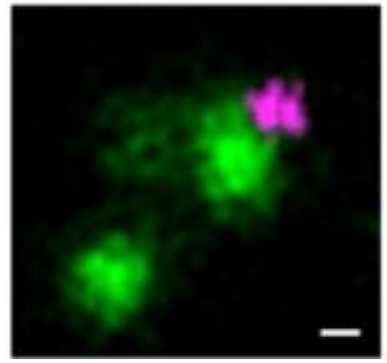
Nature. 2021 Aug;596(7873):565-569. doi: 10.1038/s41586-021-03744-4.

Mechanism of Platelet Activation

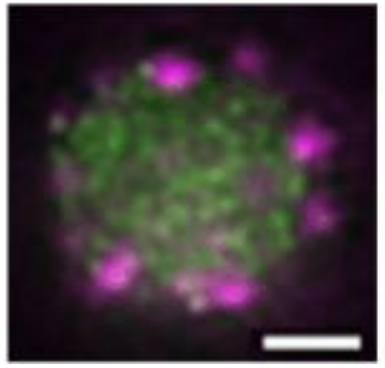
- VITT antibodies complex with PF4, platelets, and ChAdOx1 vaccine

- Which part of the vaccine?
  - Proteins from cell line
  - EDTA (0.1 nM in vaccine)
  - S2 subunit of spike protein
    - Although potential similar motifs, purified VITT antibodies do NOT bind spike protein

- Platelet activation increased by PF4 and DNA
  - NETs produced



PF4- Green
ChADOx virus-Purple



Platelets- Green
ChADOx virus-Purple

DOI: 10.21203/rs.3.rs-440461/v1 **DOI:** 10.21203/rs.3.rs-404769/v1

Proposed Sequence Causing VITT

Neoantigen generated

- Vaccine components (Adenovirus, cell culture proteins, EDTA) contact platelets→ platelet activation

Inflammatory co-signal stimulates immune response

- EDTA creates capillary leakage→ systemic dissemination

Prothrombotic reaction

- Anti-PF4 antibodies bind PF4 and platelets→ platelet activation
- Activated Platelets interact with neutrophils→ NETosis
- Extracellular DNA binds PF4 and anti-PF4 antibodies→ further platelet, neutrophil, ? monocyte & endothelial cell activation

DOI: 10.21203/rs.3.rs-440461/v1

Diagnostic Testing for TTS

Diagnostic Tests

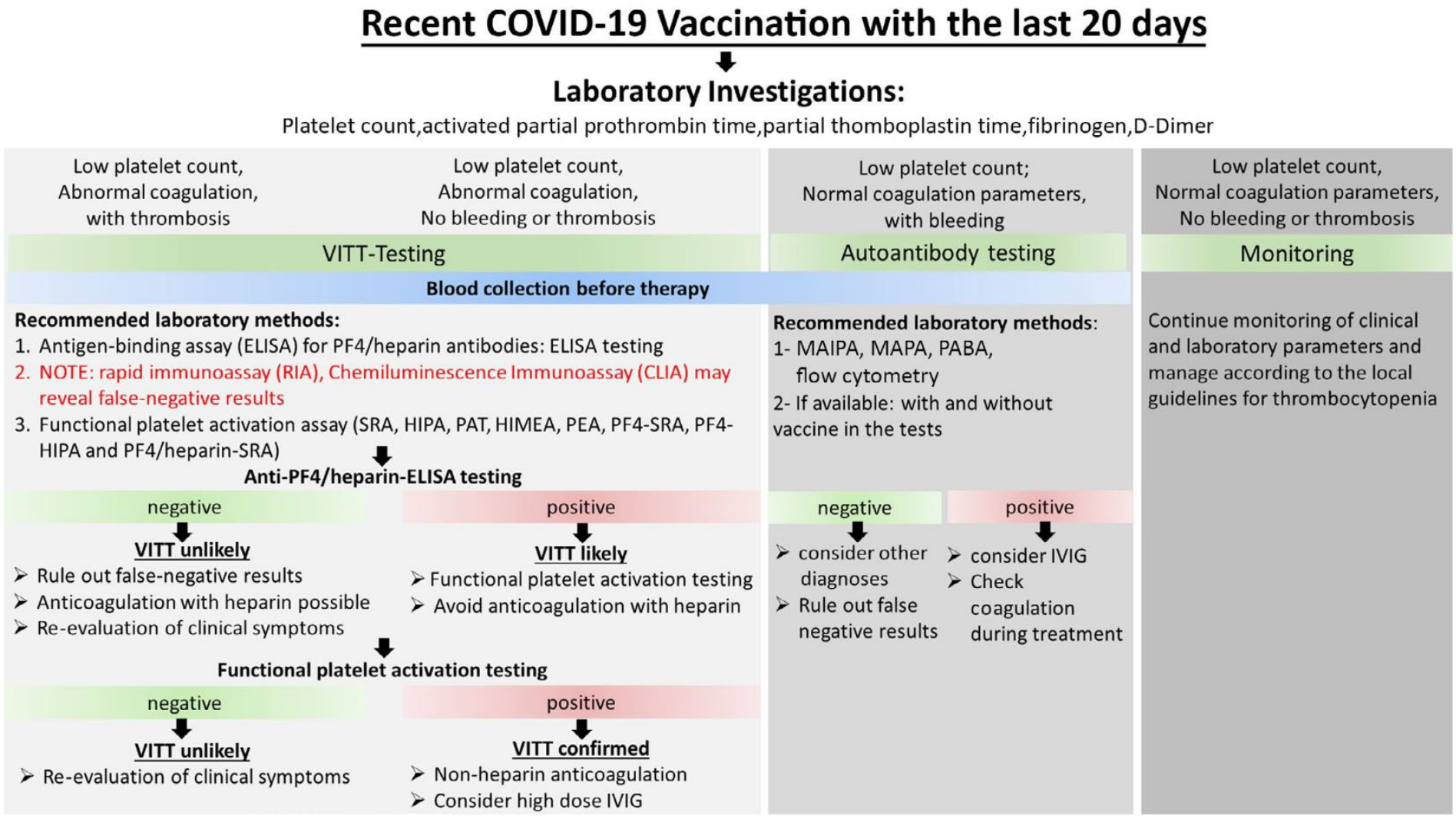
- CBC with platelet count
- Symptom directed imaging
  - CT with IV contrast for head and abdominal imaging
  - Ultrasound for DVT
- Fibrinogen & D-dimer
- Testing for TTS / VITT antibodies (HIT testing)

HIT Testing

Test	Purpose	Method
Heparin-platelet factor 4 (PF4) enzyme-linked immunosorbent assay (ELISA)	Immunologic HIT assay that measures the amount of HIT antibodies directed against PF4 (detects functional and nonfunctional antibodies)	Patient serum is added to a microtiter plate coated with PF4 (or heparin-PF4 complex) Alkaline-phosphatase linked secondary anti-human IgG antibody is added and incubated with the test samples After washing, a colorimetric substrate is added and the optical density of the colored product is measured after a 30-min incubation
Serotonin release assay (SRA)	Functional HIT assay that measures serotonin release from dense granules in platelets as a marker for platelet activation in the presence of high and low doses of heparin	Patient serum is added to donor platelets labeled with ^{14}C-serotonin in the presence of low (0.1 U/mL) and high (100 U/mL) concentrations of heparin (and in some specialized reference laboratories, buffer control) Platelet activation is determined by the amount of ^{14}C-serotonin released into the reaction supernatant
Latex immunoturbidimetric assay (LIA)	Functional immunologic HIT assay that detects the presence of PF4 HIT antibodies based on their ability to competitively inhibit agglutination of HIT-like monoclonal antibodies bound to latex particles	Patient plasma is added to latex particles coated with a HIT-like monoclonal antibody and PF4 molecules conjugated with fluorescent marker With negative plasma (those without HIT antibodies), the latex/monoclonal antibody particles and PF4 molecules agglutinate, which will increase detectable fluorescence Positive plasma will compete with the latex/monoclonal antibody particles leading to less agglutination and lower fluorescence
p-Selectin expression assay (PEA)	Functional HIT assay that measures platelet surface p-selectin expression as a marker of platelet activation in the presence and absence of high-dose heparin	Patient serum is incubated with platelets pretreated with PF4 purified from normal donor platelets ± high (100 U/mL) concentration of heparin Platelet activation is determined by measuring the presence of p-selectin (CD62p) by flow cytometry

JAMA. 2021;325(24):2448-2456. doi:10.1001/jama.2021.7517

ISTH Recommendations



J Thromb Haemost. 2021;19:1585-1588. DOI: 10.1111/jth15341

Evaluation of Anti-Platelet Factor 4 Antibodies

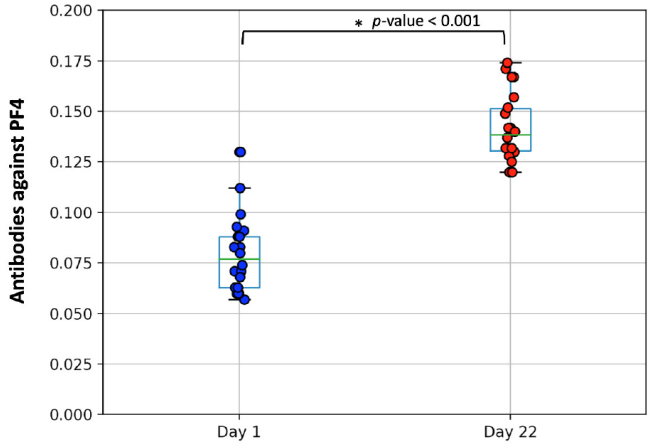
- 50 samples from 43 patients with suspected VITT s/p ChAdOx1 nCOV-19 vaccination
  - Cases per UK Expert Haematology panel
- Laboratory testing
  - IgG & polyspecific ELISA
  - Rapid assays (gel agglutination & chemiluminescent assays)
- Sensitivity for detection of VITT is quite different than HIT

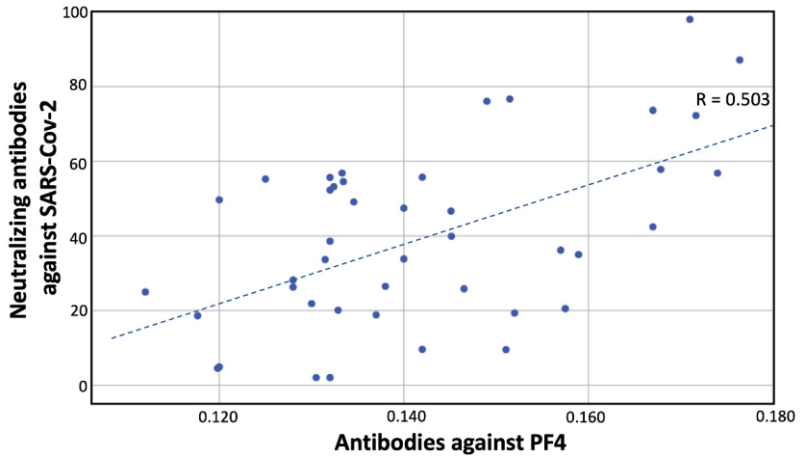
Assay	Sensitivity for VITT % (95% CI)	Specificity for VITT % (95% CI)
IgG-specific ELISAs		
AEKSULISA HiT II	70.6 (53.8–83.2)	88.9 (56.5–99.4)
Asserachrom HPIA IgG	91.1 (77.0–97.0)	100.0 (70.1–100.0)
Lifecodes PF4 IgG	94.1 (80.9–99.0)	77.8 (45.3–96.1)
Zymutest HIA IgG	94.1 (80.9–99.0)	77.8 (45.3–96.1)
Polyspecific ELISAs		
Asserachrom HPIA	94.1 (80.9–99.0)	100.0 (70.1–100.0)
Lifecodes PF4 Enhanced	100.0 (89.9–100.0)	55.6 (26.7–81.1)
Rapid tests		
Diamed PaGIA gel	45.5 (29.8–62.0)	66.7 (35.4–87.9)
HemosIL AcuStar HIT-$IgG_{(PF4\text{-}H)}$	5.9 (1.0–19.1)	100.0 (70.1–100.0)
HemosIL HIT-$Ab_{(PF4\text{-}H)}$	0.0 (0.0–17.6)	100.0 (67.6–100.0)
STic expert	4.2 (0.2–20.2)	100.0 (17.8–100.0)

J Thromb Haemost. 2021;19:2007-2013. DOI: 10.1111/jth15362.

ELISA Testing Can be Positive without Symptoms

- Low titer PF4/polyanion antibodies occur after vaccination with both adenoviral and mRNA vaccines
- Thiele et al
  - BNT162b2 vaccine: 8/143 (5.6%)
  - ChAdOx1 nCOV-19 vaccine: 11/138 (13.7%)
  - All except 1 OD values 0.5 – 1.0 units (weak)
- Terpos et al
  - ChAdOx1 nCOV-19 vaccine: 29/43 (67%)
    - non-platelet activating anti-PF4 antibodies
  - IgG antibodies against PF4 correlated linearly with the titer of neutralizing antibodies against SARS-CoV-2, but not with clinical characteristics of patients



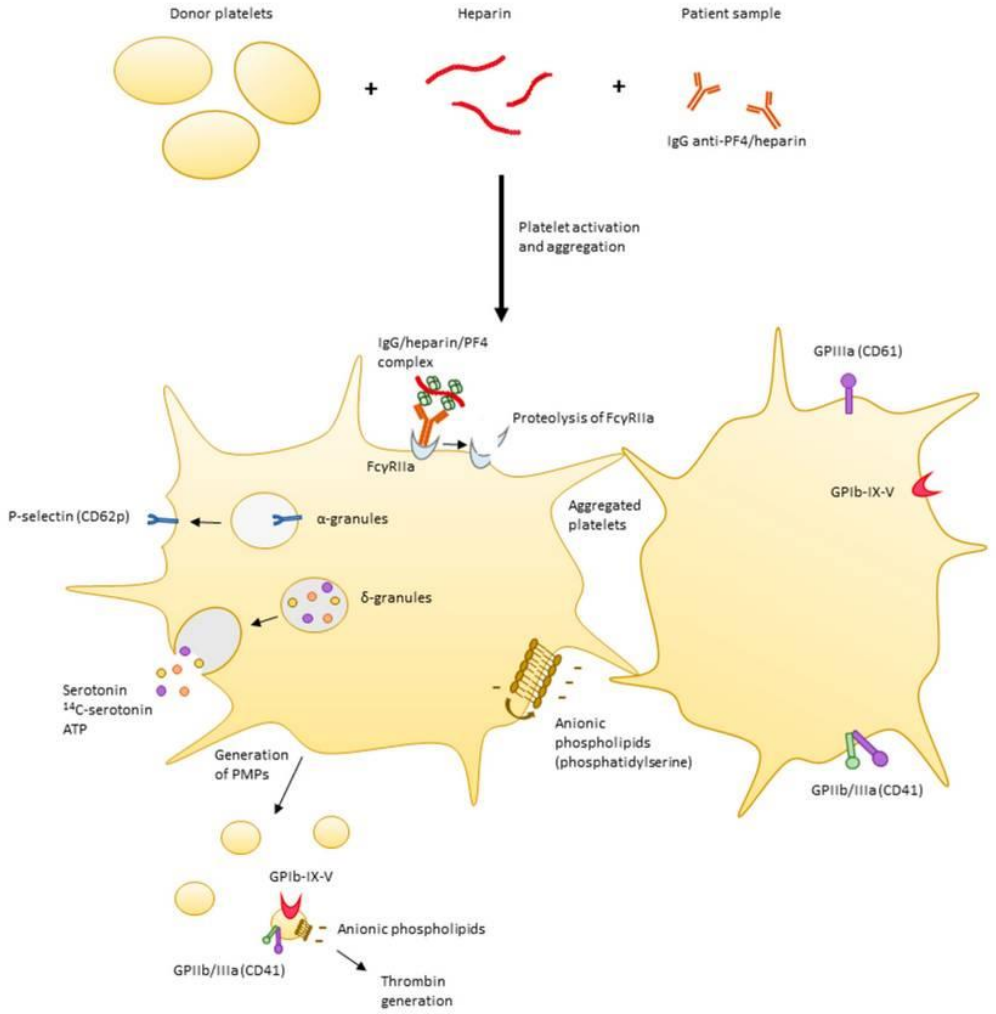


Vaccines 2201. DOI:10.3390/vaccines9070712
Blood 2021.138(4):299-303.

Functional Platelet Testing

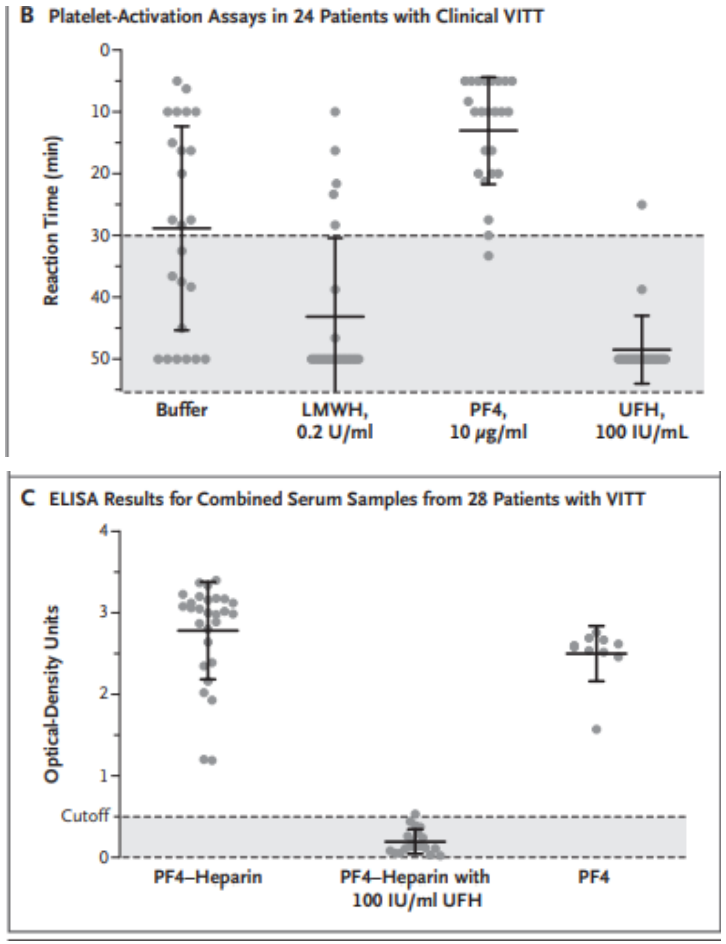
- Functional platelet testing is difficult and subject to considerable inter and intra-laboratory variability

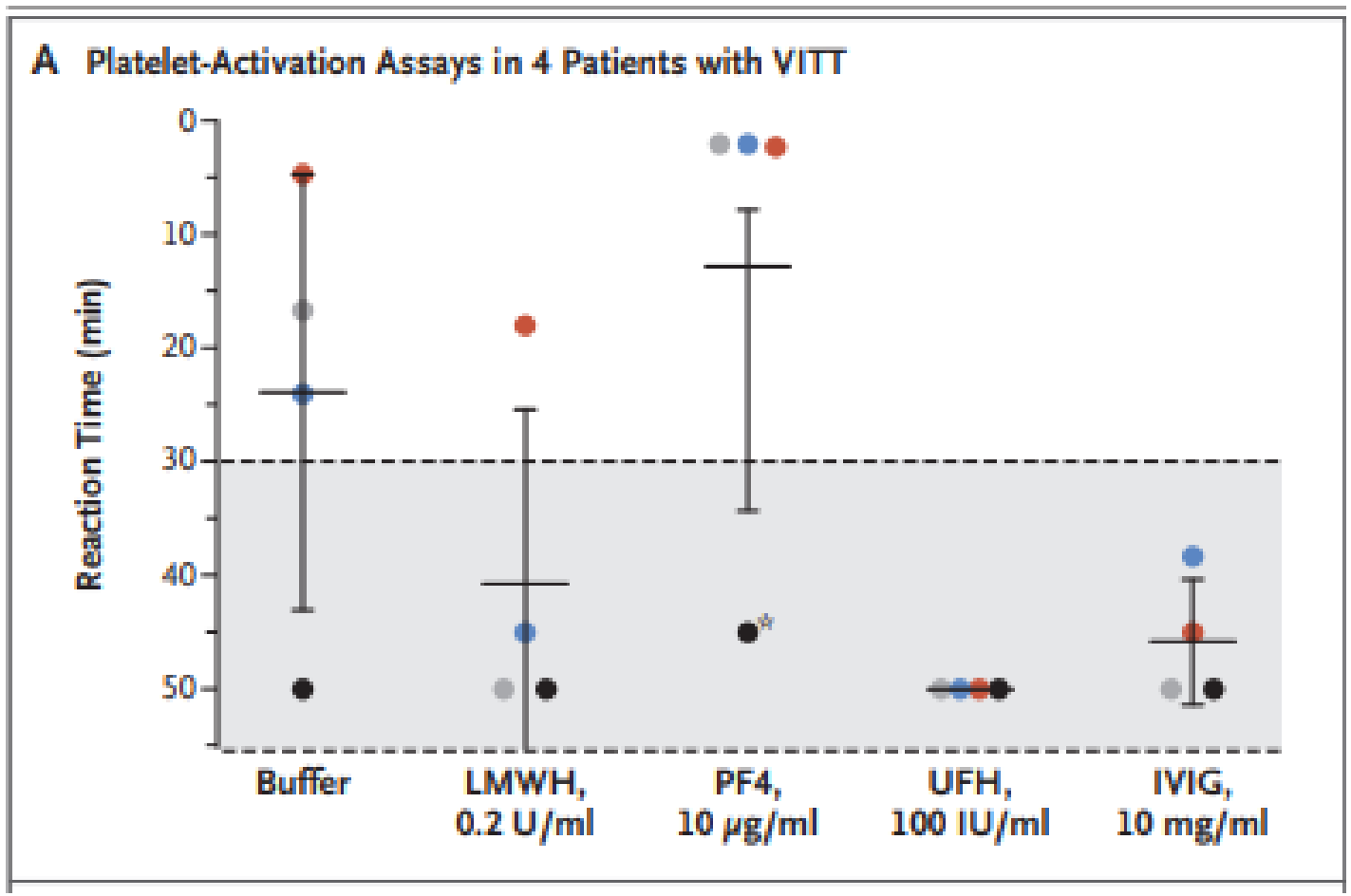
- Captures platelet changes with activation
  - Aggregation of platelets
  - Release of serotonin from dense granules
  - Translocation of p-selectin (CD62p) from alpha granules to platelet surface
  - Proteolysis of the FcR
  - Generation of and procoagulant activity of PMPs with thrombin generation
  - Translocation of anionic phospholipids to outer surface of platelets



Molecules 2017.22:617. DOI:10.3390/molecules22040617

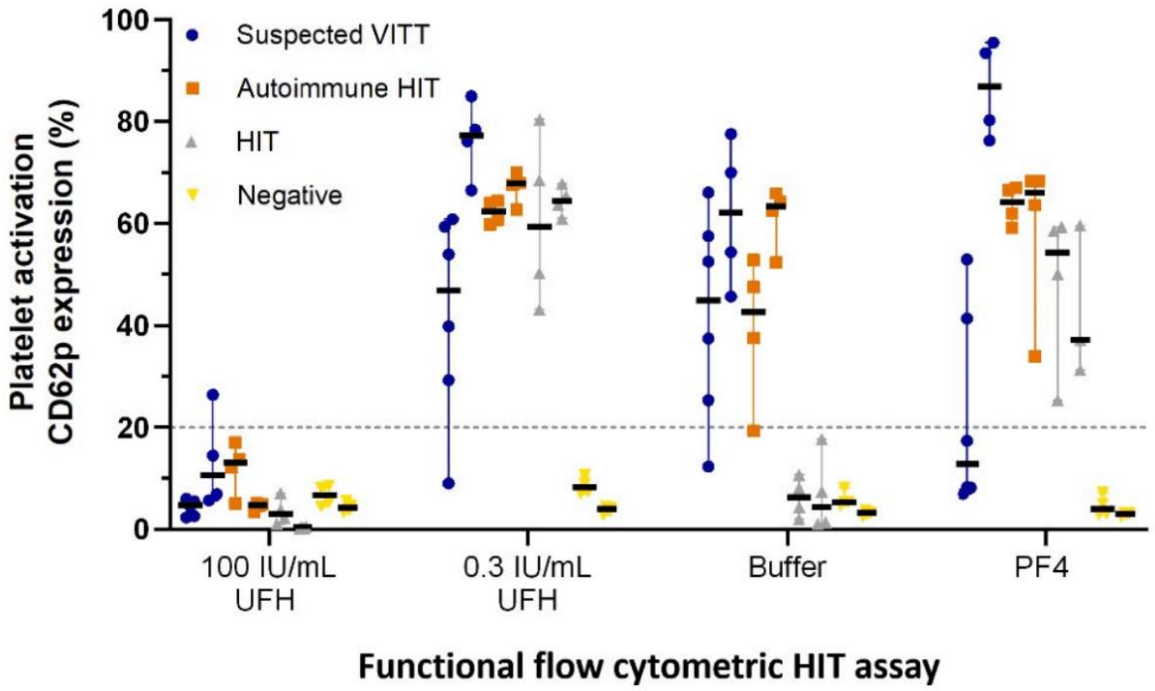
Platelet Activation in VITT

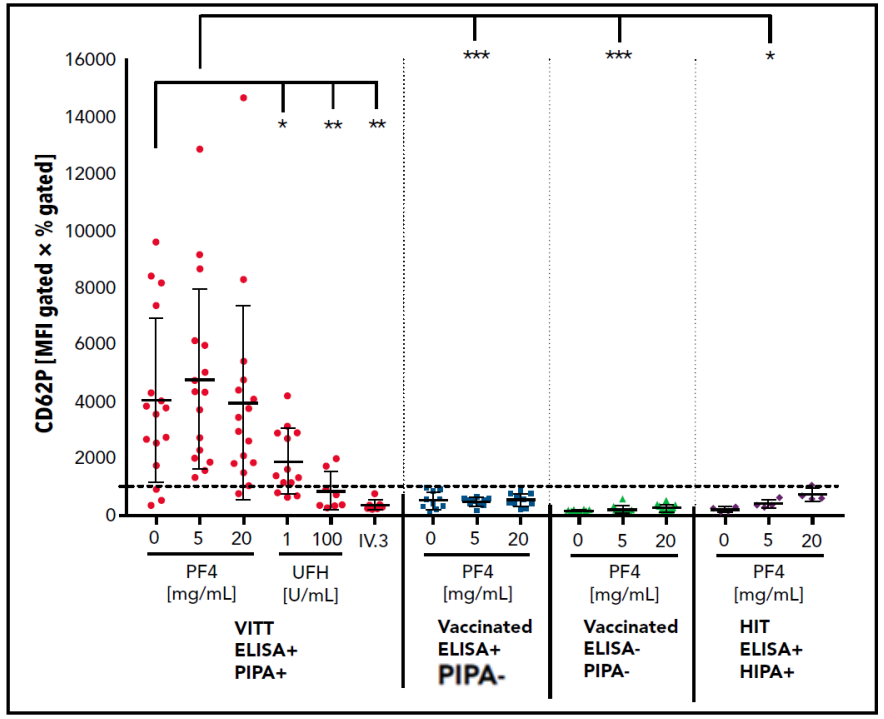




NEJM DOI: 10.1056/NEJMoa2104840

P-Selectin Assays





- Flow cytometry assay for P-selectin
  - Minimal activation in the presence of supra-therapeutic heparin
  - Activation in the presence of therapeutic heparin
  - VITT and autoimmune HIT identified in the absence of
  - PF4 enhancement resulted in variable expression

- PF4-induced flow cytometry platelet activation (PIFPA) test
  - Specificity for VITT patients
  - Excludes asymptomatic / non-functional antibodies
  - Excludes HIT antibodies

Blood 2021. DOI:10.1182/blood.2021012064.
J Thromb Haemost. 2021. DOI:10.1111/jth.15457

Serotonin Release Assay with Less Reliability

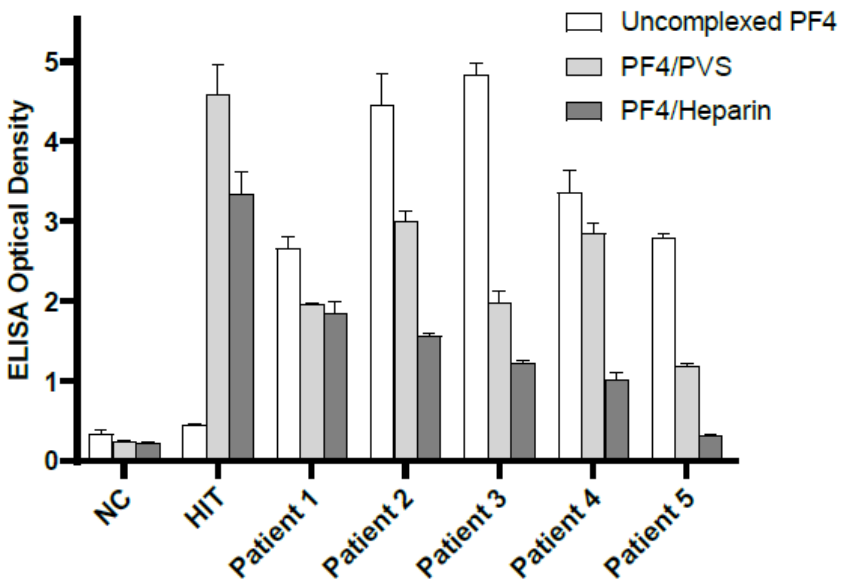
Table 3. Results From Hematology and SARS-CoV-2 Testing for Initial 12 Patients With Cerebral Venous Sinus Thrombosis and Thrombocytopenia Following Emergency Authorization Receipt of Ad26.COV2.S Vaccine—US, 2021[a]

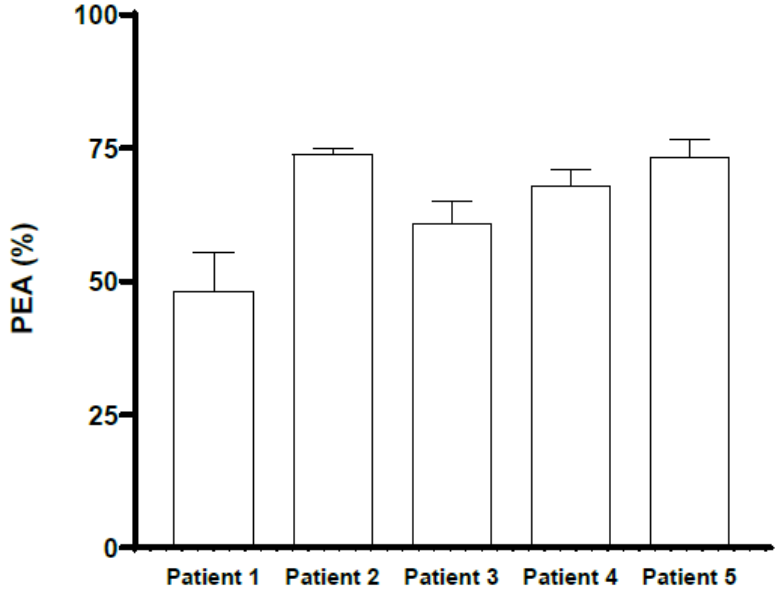
Patient No.	Platelet nadir, $\times 10^3/\mu L$[b]	D-dimer peak, µg/mL[b]	Fibrinogen nadir, mg/dL[b]	Initial		SARS-CoV-2		Heparin-PF4 ELISA test (optical density)[b,c]	Functional platelet assay test results[c]
				INR	aPTT, s	Serology	Viral assay		
1	12	>20.0	93	1.4	31	Not done	Negative (PCR)	Not done	Not done
2	69	1.1	166	1.2	22.3	Nucleocapsid antibody negative	Negative (PCR)	Positive (1.2)	Negative SRA
3	18	8.46	82	1.5	31.1	Not done	Negative (PCR)	Positive (2.7)	Negative SRA
4	127	5.45	240	1.1	31.2	Not done	Negative (PCR)	Positive (3.0)	Negative SRA
5	10	7.05	141	1.1	18.1	Antibody negative[d]	Negative (PCR)	Positive (1.6)	Negative SRA
6	13	112.07	59	1.3	34.5	Spike antibody negative	Negative (PCR)	Positive (3.2)	Negative SRA, negative LIA
7	64	7.84	77	1.2	-[e]	Not done	Not done	Positive (1.4)	Not done
8	90	6.7	239	0.9	28	Not done	Negative (antigen)	Positive (2.3)	Negative SRA
9	15	>4	332	1.1	26.9	Nucleocapsid antibody negative	Negative (PCR)	Positive (2.5)	Negative SRA
10	9	13.47	128	1.2	24.1	Not done	Negative (PCR)	Positive (2.2)	Not done
11	102	41.71	206	1.2	30.2	Not done	Negative (PCR)	Positive (2.6)	Negative SRA, negative LIA
12	20	45.57	149	-[f]	26.4	Not done	Negative (PCR)	Positive (2.1)	Positive SRA and PEA, negative LIA

JAMA. 2021;325(24):2448-2456. doi:10.1001/jama.2021.7517

Research Considerations in Testing

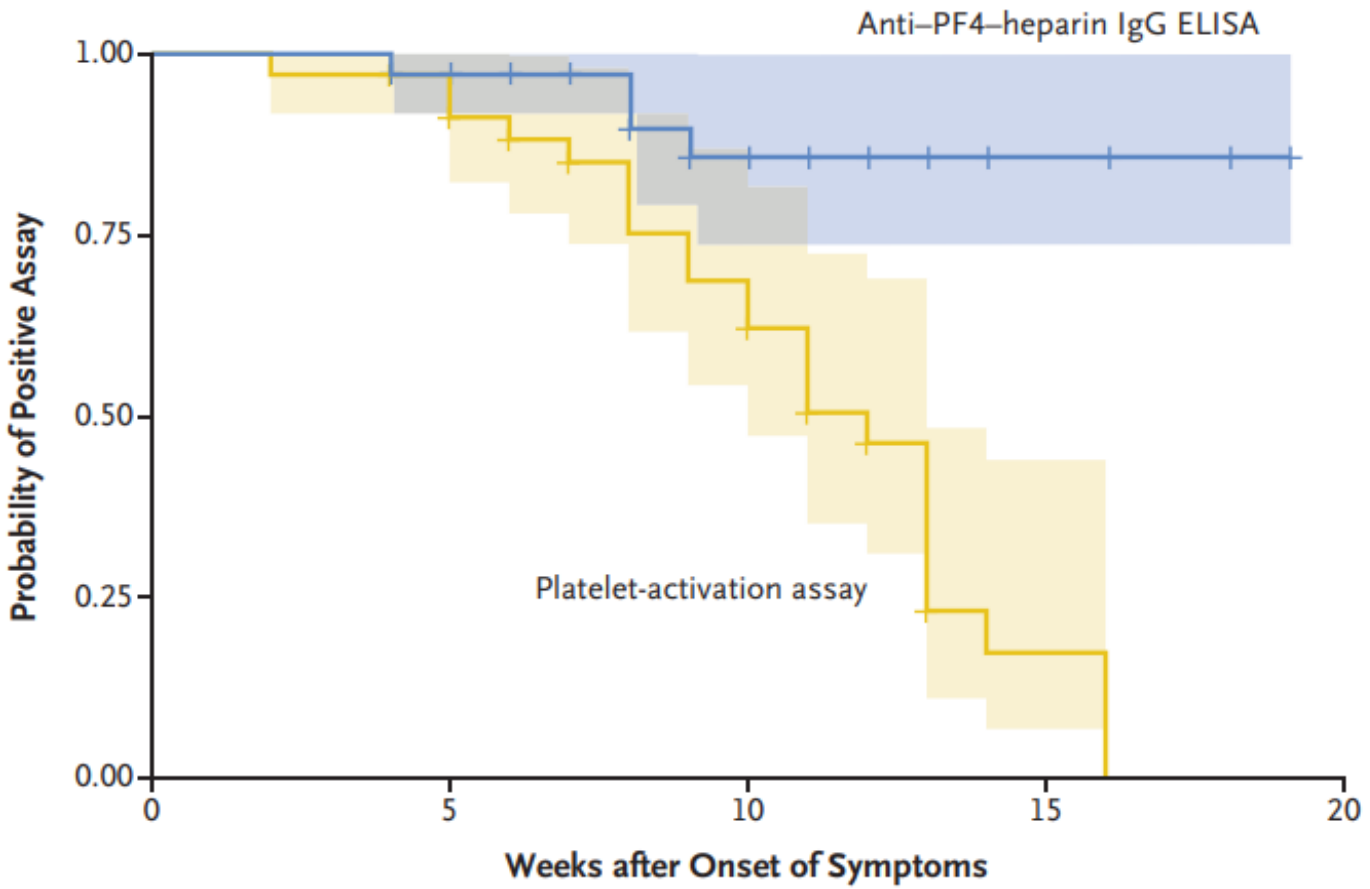
- Can ELISA testing be modified to improve sensitivity / specificity?
  - Modified ELISA plates by incubation with recombinant PF4, PF4 + polyvinyl sulfonate, PF4 + unfractionated heparin





medRxiv preprint doi: https://doi.org/10.1101/2021.09.23.21263047

Duration of VITT Antibodies



NEJM. DOI: 10.1056/NEJMc2112760

Management of VITT

Similar to autoimmune HIT

Avoid heparin & use non-heparin anticoagulant
IV Immunoglobulin (IVIG)
Avoid platelet transfusion*
Consider referral to tertiary care center for expertise in hemostasis

Anticoagulation

Non-Heparin anticoagulant

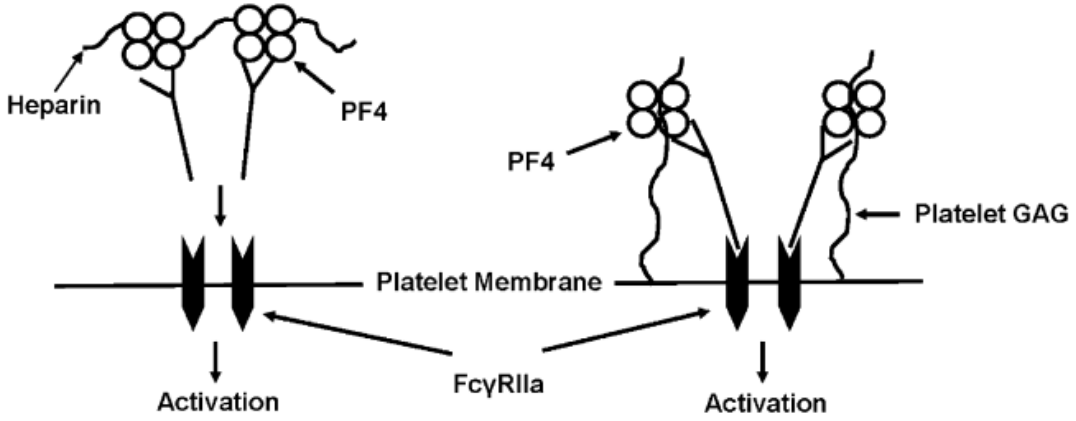
- IV direct thrombin inhibitor (bivalirudin, argatroban)
- Fondaparinux
- Apixaban or rivaroxaban

Treat for 3 months for provoked thrombosis

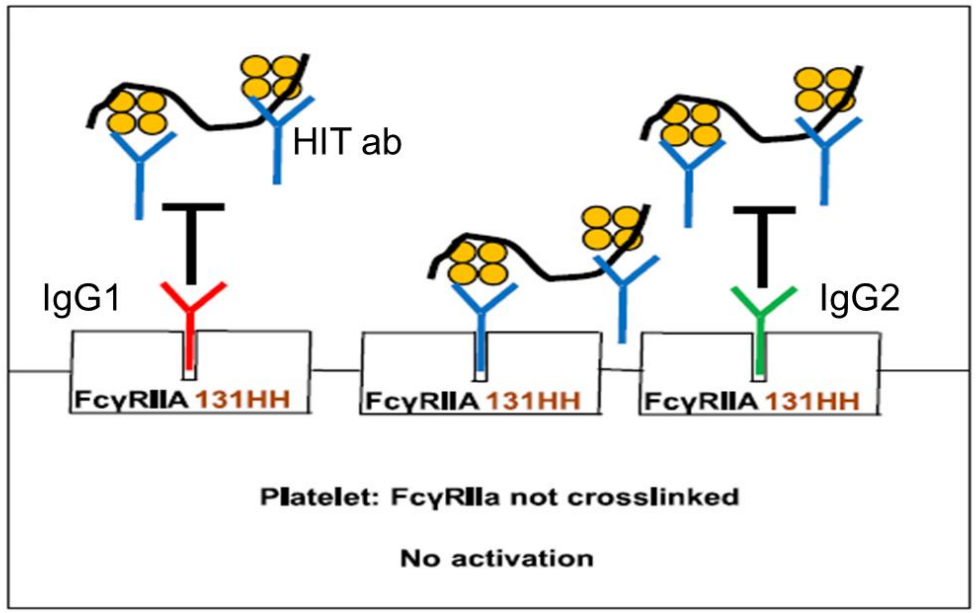
IVIG

- Decrease platelet activation
- 1-2 grams/kg IV in divided doses
- Give early if recognized
- Used in ITP also
  - Consider while awaiting PF4 ELISA

HIT

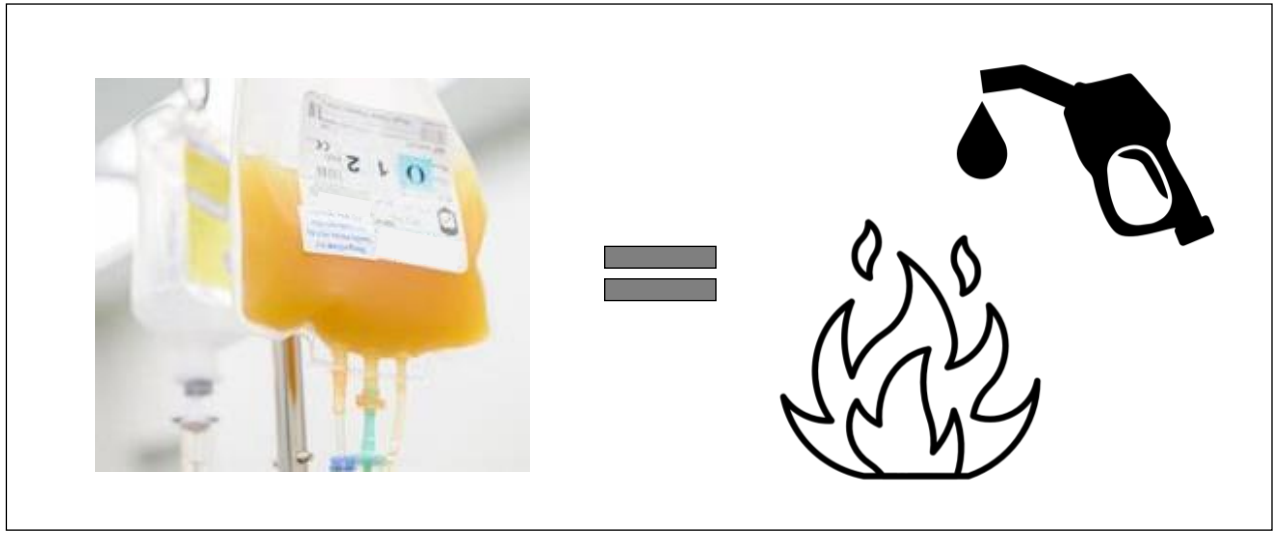


IVIG



https://b-s-h.org.uk/about-us/news/guidance-produced-by-the-expert-haematology-panel-ehp-focussed-on-vaccine-induced-thrombosis-and-thrombocytopenia-vitt/
Hamostaseologie 2021 Apr 1.doi: 10.1055/a-1469-7481. Padmanabhan et al, Blood 2015.

Platelet Transfusions



- Worse mortality in HIT with platelet transfusions→ Avoid platelet transfusions
- Cerebral vein thrombosis can have intracranial hemorrhage
  - Not a contraindication to anticoagulation
  - Present in 4 of 6 patients reported after J&J/Janssen vaccination
  - Occurred in 3 of 13 patients with CVT after AZ vaccination
    - Additional thrombotic events after receiving platelet transfusion or heparin
- Determine risk benefit ratio after IVIG if severe hemorrhage or emergent surgery

Blood. 2015 Feb 26; 125(9): 1470–1476. https://bit.ly/3sigzZd
NEJM DOI: 10.1056/NEJMoa2105385

Overlap with Disseminated Intravascular Coagulation?

- High D-dimer levels and low fibrinogen reported in cases of VITT

N	Vaccine	Low Fibrinogen	Elevated D-dimer	Reference
5	AZ	3/5 (60%)	5/5 (100%)	Schultz (DOI: 10.1056/NEJMoa2104882)
11	AZ	3/6 (50%)	7/7 (100%)	Greinacher (DOI: 10.1056/NEJMoa2104840)
1	J&J/Janssen	1 (100%)	1 (100%)	Muir (DOI: 10.1056/NEJMc2105869)
23	AZ	13/23 (57%)	21/21 (100%)	Scully (DOI: 10.1056/NEJMoa2105385)

- Consider correction of fibrinogen to >150 mg/dl
- Incidence may change as recognized earlier in disease course

https://b-s-h.org.uk/about-us/news/guidance-produced-by-the-expert-haematology-panel-ehp-focussed-on-vaccine-induced-thrombosis-and-thrombocytopenia-vitt/
Hamostaseologie 2021 Apr 1.doi: 10.1055/a-1469-7481.

What if….?

- Situations will arise as more people tested & early recognition of VITT
- Other reasons for thrombocytopenia & Thrombosis (e.g., Cancer Associated thrombosis)→ PF4 ELISA
- DVT or PE after vaccination without thrombocytopenia
  - Avoid heparin (consider DOAC)
  - Await PF4 ELISA results
  - Follow platelet count
- Thrombocytopenia & positive PF4 ELISA without thrombosis
  - Consider IVIG
  - Consider non-heparin anticoagulant

Should aspirin be given to patients after J&J vaccination?

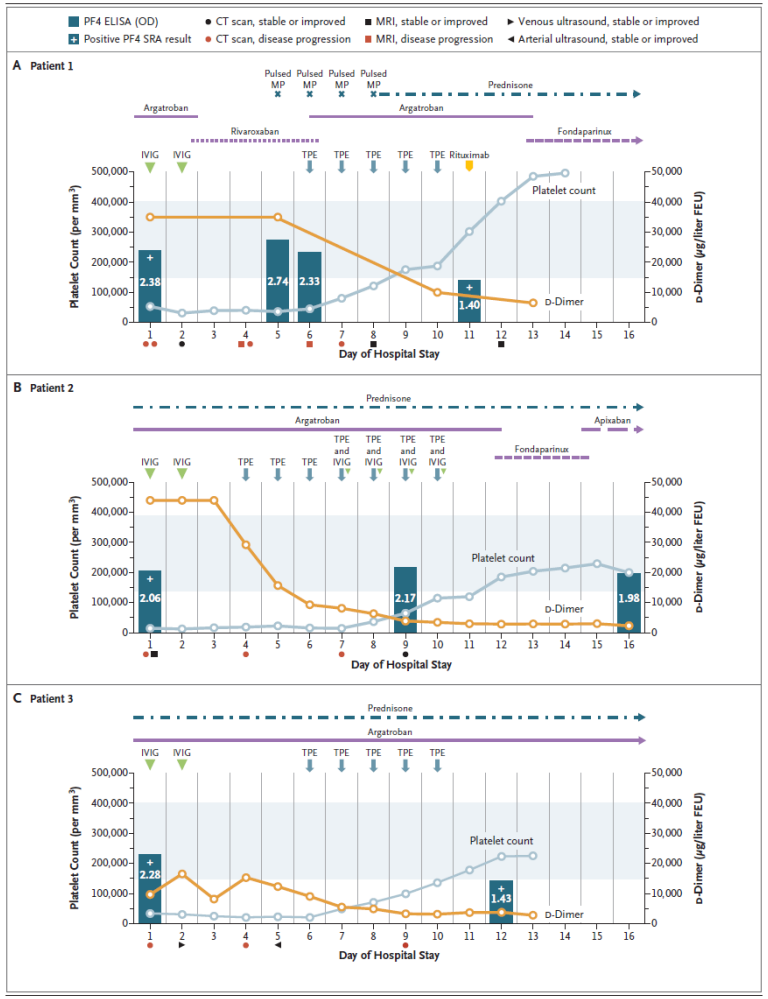
NO

- Blocking thromboxane does not block platelet activation in HIT
- Aspirin is associated with risk of bleeding (RR 1.3)
- Incidence of VITT is RARE

Polgar, et al. Blood 1998; 91 (2): 549-54.
NEJM 1989 Jul 20;321(3):129-35.

Therapeutic Plasma Exchange

- VITT unresponsive to initial therapy
- IgG-mediated, thus TEP is a reasonable consideration to facilitate antibody removal or neutralization
  - Plasma replacement fluid
- Adverse events / complications
  - Central line placement with low platelets
  - Hypotension
  - Hypocalcemia



NEJM doi: 10.1056/NEJMc2109465.
Transfusion and Apheresis Science 60 (2021) 103174.

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Thank you & Questions