



Procoagulant microparticles: a possible link between vaccine-induced immune thrombocytopenia (VITT) and cerebral sinus venous thrombosis

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Abbreviations

CDC	Centers for Disease Control and Prevention
CSVT	Cerebral Sinus Venous Thrombosis
FDA	Food and Drug Administration
HIT	Heparin-induced thrombocytopenia
MPs	Microparticles
PF4	Platelet factor 4
PS	Phosphatidylserine
TF	Tissue factor
VITT	Vaccine-induced immune thrombotic thrombocytopenia
VWF	Von Willebrand factor

To the Editor,

Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT) (also termed vaccine-induced thrombotic thrombocytopenia or vaccine-induced immune thrombocytopenia or thrombosis with thrombocytopenia syndrome (TTS) by the CDC and FDA) is characterized by (i) venous or arterial thrombosis; (ii) mild to severe thrombocytopenia; (iii) positive antiplatelet factor 4 (PF4)–polyanion antibodies or anti-PF4-heparin antibodies detected by the HIT (heparin-induced thrombocytopenia) ELISA assay (iv) occurring five to 24 days after ChAdOx1 nCoV-19 or Ad26.COV2.S vaccination [1]. In initial reports, patients were likely young and under 50 years, female (more than two thirds), median

platelet counts at diagnosis about 20 to $30 \times 10^9 \text{ L}^{-1}$, no risk factors for thrombosis and faced unusual sites for thrombosis including cerebral sinus venous thrombosis (more than two thirds) or portal vein with high fatality rates. VITT is associated with the detection of anti-PF4 antibodies, unrelated to previous use of heparin therapy. Heparin-independent platelet activation, known as autoimmune heparin-induced thrombocytopenia (aHIT) was previously described in patients with positive antiPF4–polyanion antibodies [2]. Similarly to aHIT, PF4 antibodies are sought to activate platelets via the platelet FcγRIIA receptors, but there is to date no clear data supporting that PF4 is either a bystander component within an immune complex that activates platelets, or directly contribute directly to platelet aggregation [3]. Based on current evidence, the precise mechanisms and molecular pathways triggering the production of anti-PF4 antibodies after adenovirus vectored vaccines remain to be determined. The Ad26.COV2.S and ChAdOx1 nCoV-19 vaccines have different vaccine phenotypes with different host cell receptors and biological effects making unclear which component of the vaccine (adenoviral sequence, spike protein, other component) may be held responsible for the production of anti-PF4 antibodies.

Potential risk factors for VITT may include young age and female sex while estrogen-replacement therapy or oral contraceptives were inconstantly reported among women. Moreover, no evidence to date is sufficient to establish a causal relationship between these events and hormonal therapy. The unusual topography and rare incidence for acute cerebral sinus venous thrombosis (CSVT), have led us to the hypothesis that procoagulant microparticles (MPs) enriched in phosphatidylserine (PS) and tissue factor (TF) may be important cofactors in the pathogenesis of VITT.

Microparticles (MPs) refer to small vesicles, ranging from 0.1 to $2 \mu\text{m}$, originating from the plasma membrane of stimulated or apoptotic cells including, platelets,

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leukocytes and endothelial cells. MPs are constituted of material from their cells of origin and carry or express pro-inflammatory lipids, specific membrane glycoproteins (selectins, adhesion molecules, CDs...), antigens, mRNA etc. Procoagulant MPs (platelet and monocyte-derived) are circulating MPs that express phosphatidylserine (PS) and tissue factor (TF) and contribute to pro-coagulant responses. So what links circulating procoagulant microparticles, VITT and CSVT?

First, acute heparin-induced thrombocytopenia (HIT) is characterized by an increased level of procoagulant circulating MPs and a distinct phenotype of circulating PF4-bearing MPs associated with an increased risk of thrombosis [4]. Nevzorova et al. confirmed that PF4-containing pathogenic immune complexes lead to platelet activation, phosphatidylserine and P-selectin exposure on the outer leaflet of the platelet plasma membrane together with the shedding of procoagulant MPs that express PS [5]. In addition, HIT Ab complexes induced tissue factor expression by monocytes and the release of TF-bearing MPs. Altogether these data advocated the importance of a TF dependent and driven

prothrombotic state supported by platelet and leukocyte-derived MPs in HIT [5, 6].

Second, the atypical cerebral venous distribution of thrombi in VITT may be explained by the fact that (i) circulating TF is more likely to play a role in venous thrombosis that is not associated with vessel damage [7] and (ii) TF involvement is crucial in cerebral microvascular thrombogenesis, with endothelial cell-associated TF mediating this response in venules, but not arterioles [8]. This gives echo to the platelet–neutrophil interaction triggered by HIT antibodies known to activate vascular endothelium [9].

Therefore, we formulate the hypothesis that the pathogenesis of VITT involves (i) a FcγRIIA receptors pathway with circulating PF4 antibodies complexes that bind platelets and monocytic FcγRIIA receptors, causing cell monocytic activation and release of procoagulant MPs (ii) a direct activation of the endothelium by HIT Ab complexes leading to enhanced thrombogenicity through the release of P-selectins, E-selectins, von Willebrand factor and IL-6 [10]. Altogether, this results in enhanced PS and TF expression and subsequent thrombin generation (Fig. 1) more likely to occur in

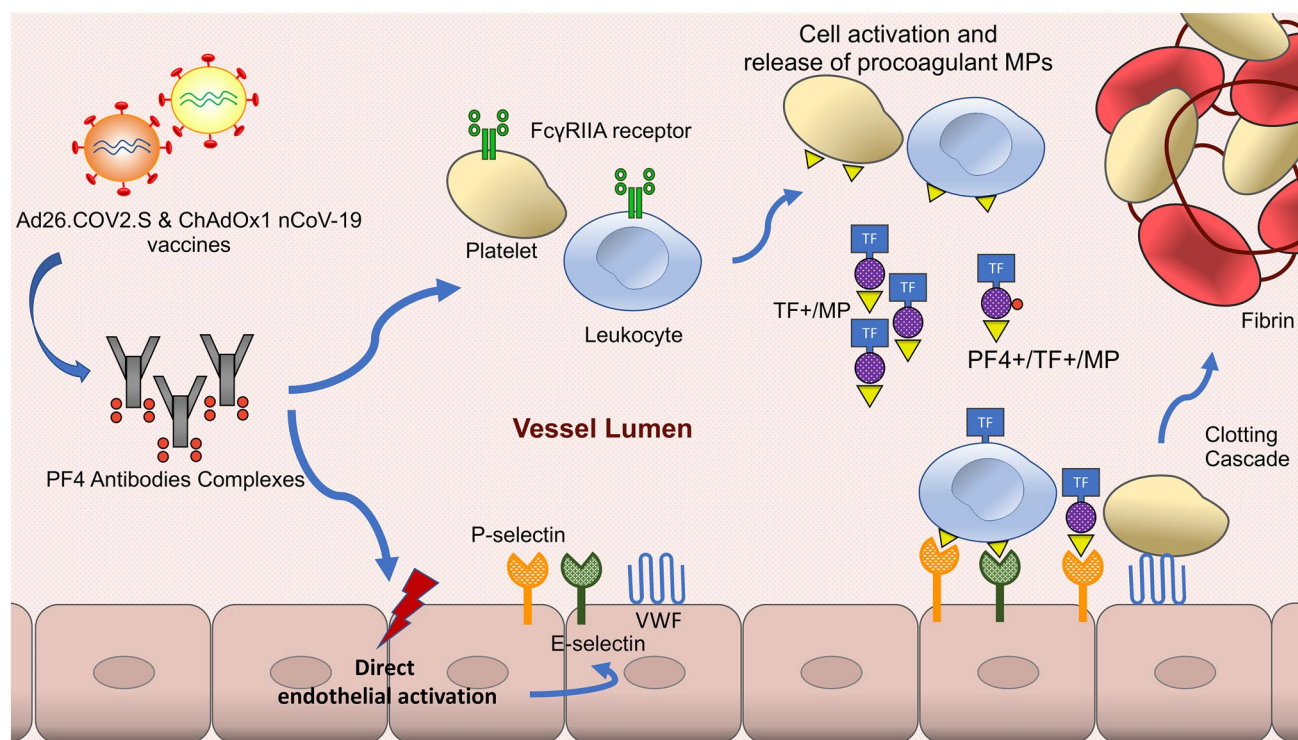


Fig. 1 Proposed mechanisms for cerebral sinus venous thrombosis in vaccine-induced immune thrombocytopenia (VITT). We proposed that venous thrombosis can be divided into two complementary and synergistic pathways in VITT. First, circulating PF4 antibodies complexes bind platelets and monocytic FcγRIIA receptors, causing cell activation and release of procoagulant microparticles (MPs). Second, the endothelium is directly activated by PF4 antibodies complexes and expresses the adhesion proteins E-selectin P-selectin and von

Willebrand factor (VWF). Circulating TF+MPs, PF4+TF+MPs, platelets and leukocytes bind to the activated endothelium and lead to the activation of the coagulation cascade. Cerebral sinus venous thrombosis in VITT is likely the consequence of (i) the key role played by circulating TF in venous thrombosis unrelated to vessel damage and (ii) the key role of endothelial cell-associated TF pathway in cerebral microvascular thrombogenesis. *MPs* microparticles, *PF4* platelet factor 4, *TF* tissue factor, *VWF* Von Willebrand factor

the cerebral venous system due to its specific thrombogenesis process.

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Declarations

Conflict of interest All authors have nothing related to this paper to disclose. OM received Institutional Research Grants from Fédération Française de Cardiologie.

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