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EXPERT OPINION

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The known knowns and known unknowns of vaccine-induced thrombotic thrombocytopaenia

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The COVID-19 pandemic has, to date, resulted in over 200 million infections and over 4.25 million deaths and continues to significantly disrupt health systems and societies worldwide. However, in a remarkable triumph of medical science, a number of highly effective vaccines have been developed in a previously inconceivable short time period and are now being delivered globally. A significant issue to emerge in the midst of such a large-scale vaccination effort has been the recognition of rare adverse events such as vaccine-induced thrombotic thrombocytopaenia (VITT), which has been predominantly associated with the adenovirus vector vaccines, ChAdOx1 nCoV19 (Oxford/Astra-Zeneca) and the Ad26.COV2.S COVID-19 (Janssen/Johnson & Johnson) vaccine.[1–3]

The known knowns

VITT is characterized by the development of potentially life-threatening thrombotic complications, which typically manifest as deep venous thrombosis, pulmonary embolism, cerebral venous sinus thrombosis, or splanchnic (portal, mesenteric) vein thrombosis (*Figure 1*). These thrombotic complications occur in concert with thrombocytopaenia, a markedly elevated D-dimer and the presence of anti-platelet factor 4 (PF4)/polyanion antibodies. The clinical features of VITT share similarities with heparin-induced thrombocytopaenia (HIT), a condition that is driven by pathological antibodies against complexes of the highly positively charged PF4 and negatively charged heparin. Similarly, VITT is characterized by pathological antibodies that react with PF4, potentially complexed with an unknown co-factor, to render this naturally occurring protein immunogenic. The Fc portion of VITT antibodies bind to platelet FcγIIa receptors, which in turn clusters FcγRIIa, ultimately resulting in strong platelet activation (*Figure 1*). Indeed, recent studies with plasma from patients with VITT have demonstrated that these IgG antibodies can induce activation and aggregation of platelets from healthy donors.[2] Importantly, a recent report provided direct proof that significant numbers of platelets are circulating in an activated state in the blood of a VITT patient.[4] The platelet-activating effects of VITT antibodies can be inhibited by an FcγRIIa-blocking antibody, with recent evidence supporting a central role of intravenous immunoglobulin in ameliorating the effects of this profound antibody-induced platelet activation.[4,5]

One of the early treatment recommendations for VITT has been the use of non-heparin anticoagulants given the presence of anti-PF4/polyanion antibodies in both VITT and HIT. However, in stark contrast to HIT, the pathological antibodies in VITT develop independent of heparin exposure and high-dose heparin can inhibit *in vitro* antibody binding to PF4. Utilizing alanine screening mutagenesis, Huynh *et al.*[6] identified that VITT antibody binding to PF4 is restricted to an eight amino acid sequence. This sequence overlaps with the heparin-binding site, thus explaining how high-dose heparin can inhibit this interaction *in vitro*. Importantly, given VITT antibodies bind to a similar region to PF4 as heparin, it is proposed that VITT antibodies can mimic the function of heparin, therefore facilitating the clustering of PF4 tetramers that form immune complexes which can efficiently cluster FcγIIa receptors and thereby activate platelets.[6]

Alternatively, it has also been speculated that platelet-activating antibodies present in VITT may be due to the development of antibodies directed against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein or some of its splice variants. This hypothesis emerged given the observation that anti-spike protein antibodies from COVID-19 patients can induce FcγR-dependent platelet activation. However, whilst the SARS-CoV-2 spike protein and PF4 share epitopes, importantly, antibodies from VITT patients did not cross-react with the SARS-CoV-2 spike protein, suggesting this mechanism does not play a significant role in the pathogenesis of VITT.[7]

The known unknowns

Although most of the reports on VITT have focused on the role of platelets, it is likely that VITT pathogenic antibodies bind and activate other cells that express FcγRIIa, notably leucocytes (*Figure 1*) and endothelial cells. Emerging data have indicated that patients with VITT display increased platelet-leucocyte aggregates and sera from VITT patients can induce neutrophil extracellular trap formation (*Figure 1*),[4,8] which may

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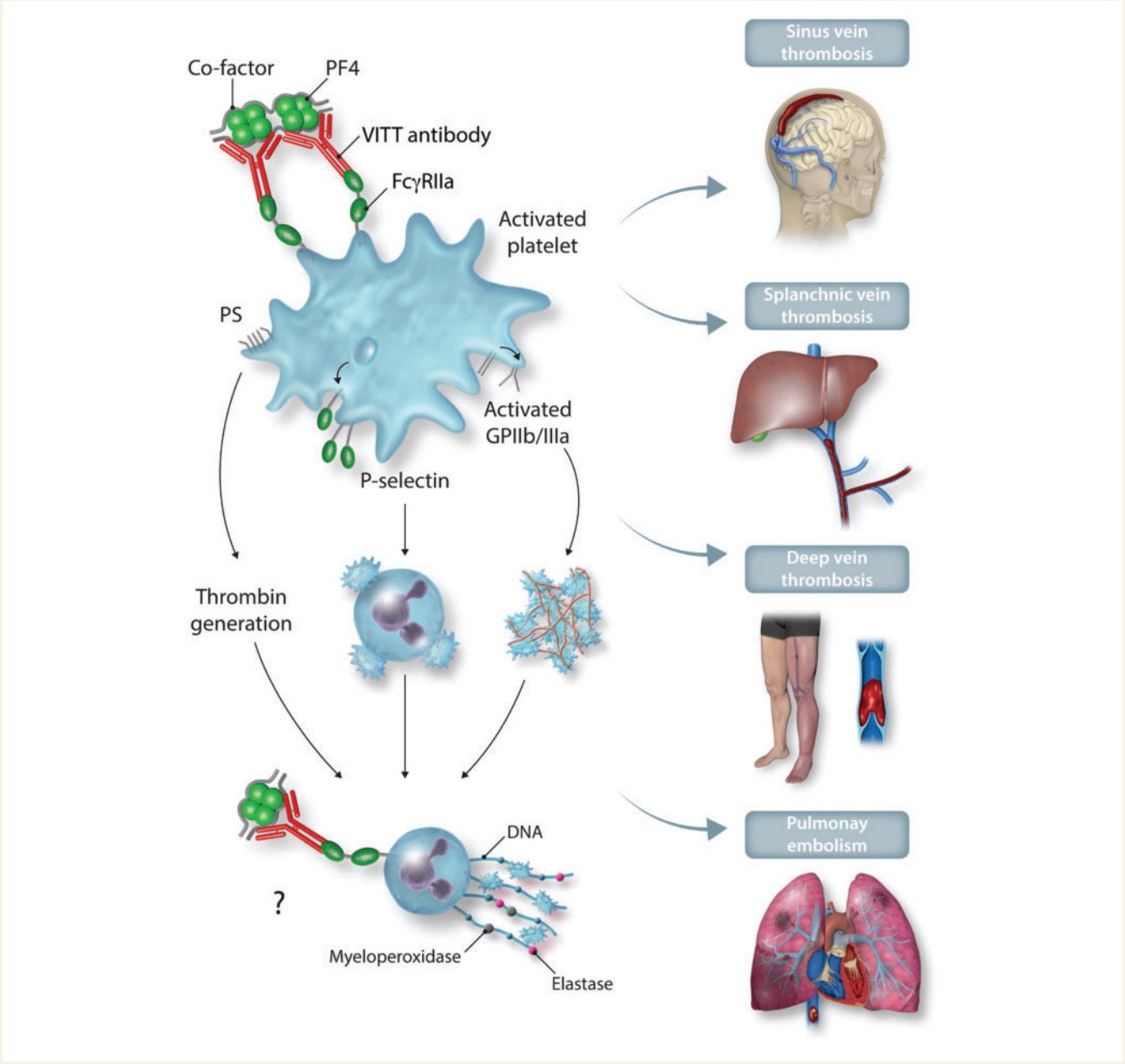

Figure 1 Pathological VITT antibodies bind PF4 and induce cross-linking of platelet FcγRIIa and subsequent platelet activation leading to activation of the GPIIb/IIIa receptor, degranulation and P-selectin expression, and the exposure of PS. This results in enhanced thrombin generation, platelet-leucocyte aggregate formation, and platelet aggregation and FcγRIIa is also expressed on leucocytes, and it is currently proposed that VITT antibodies induce neutrophil extracellular trap (consisting of extracellular DNA with trapped myeloperoxidase, elastase, adhering platelets, and bound coagulation factors) formation, which can further propagate thrombus formation. Notably, thrombus formation often involves multiple vascular beds including cerebral venous sinus, splanchnic veins, deep veins in the legs, and pulmonary embolism. PF4, platelet factor 4; PS, phosphatidylserine; VITT, vaccine-induced thrombotic thrombocytopaenia.

promote thrombus formation via the contact pathway of coagulation and by facilitating platelet adhesion. However, why there appears a predilection of cerebral venous sinus and splanchnic vein thrombosis remains unanswered. Likewise, whether Fc receptor polymorphisms, or Fc receptor expression levels modulate the thrombotic response has yet to be explored.

One of the inherent and unsolved paradoxes of VITT is the fact that most individuals have B cells which produce antibodies that bind to PF4/heparin complexes. This is thought to be due to the role of PF4 in the innate immune response where it can bind bacteria and facilitate phagocytosis, operating in a non-cognate fashion. However, VITT, like HIT is an extremely rare condition. Therefore, a critical outstanding question

relates to the 'co-factor' that may bind to PF4 to induce immunogenic neoepitopes, which may trigger the generation of pathological PF4 antibodies, and the potential role of co-factors such as extracellular DNA, danger signals, and T cells, which may augment the antibody response. In this regard, Greinacher *et al.* systematically investigated the components of the ChAdOx1 nCoV-19 vaccine and demonstrated that PF4 binds to constituents of this vaccine, including the adenovirus vector, which results in the formation of large multimolecular aggregates. This interaction of PF4 with vaccine constituents appears to be charge-dependent since high doses of heparin retain the ability to inhibit these interactions. This is further supported by recent work demonstrating that the ChAdOx1 viral capsid possesses a strong electronegative potential and thus can bind positively charged PF4.[9] Interestingly, the vaccine appears to contain a large number of human proteins, however, whether any of these proteins constitute a specific PF4 binding partner(s), or merely provides an inflammatory co-signal remains to be elucidated.

Whilst the association of thrombotic complications with the ChAdOx1 nCoV-19 and the Ad26.COV2.S COVID-19 vaccines appears a cruel irony given the often severe thrombotic complications associated with COVID19,[10] the rapid recognition of a rare vaccine complication, development of diagnostic tests, and therapeutic paradigms for VITT is a strong testament to the medical community's responsiveness and provides significant hope that the known unknowns of VITT can be elucidated in the near future. Indeed, understanding why only a tiny fraction of vaccine recipients develop VITT, and the so far unknown potential conspirators that turn a protective immune response to potentially fatal thrombotic complications are key outstanding questions. However, as we have learnt during the COVID-19 pandemic, there may be unknown unknowns that come to light as the scientific community seeks to conquer rare vaccine side effects such as VITT. The elucidation of these unknowns will hopefully provide new insights into VITT that will pave the way for engineering COVID-19 vaccines to SARS-CoV-2 variants that are not associated with thrombotic complications.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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Biography: Karlheinz Peter is Professor and Head of the Baker Department of Cardiometabolic Health at the University of Melbourne and Deputy Director and Head of the Atherothrombosis and Vascular Biology Program at the Baker Heart and Diabetes Institute. He also works as an interventional cardiologist at the Alfred Hospital in Melbourne. Karlheinz undertook medical training at the University of Freiburg and Heidelberg, Germany; Johns Hopkins Medical School; Scripps Research Institute; and the University of North Carolina at Chapel Hill, USA. Prior to moving to the Baker Heart and Diabetes Institute, Karlheinz was Director of the Cardiac Catheter Laboratory at the University of Freiburg. His research is focused on the role of platelets, coagulation, and inflammation in the development of thrombosis, atherosclerosis, and myocardial infarction. This work has led to the development and numerous patents of novel biomarker and molecular imaging strategies for thrombosis, inflammation, and unstable atherosclerotic plaques using magnetic resonance imaging, positron emission tomography, computed tomography, ultrasound, and fluorescence imaging as well as innovative biotechnological anti-thrombotic and anti-inflammatory approaches. He is also a Deputy Editor of *Cardiovascular Research*.