

Thrombosis with thrombocytopenia after the second ChAdOx1 nCoV-19 vaccination: A possible immunological mechanism independent of anti-platelet factor 4 antibody

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Abstract

Background: Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a distinctive syndrome characterized by unusual site thrombosis accompanied by thrombocytopenia following adenoviral vector vaccines against severe acute respiratory syndrome coronavirus 2. Platelet-activating anti-platelet factor 4-dependent antibodies (anti-PF4 Abs) have been identified as pathogenic antibodies in almost all patients.

Objective: We proposed an immunological mechanism of VITT independent of anti-PF4 Abs.

Method: Case report.

Results: A 68-year-old Thai woman developed pulmonary embolism and deep vein thrombosis with thrombocytopenia one week after the second ChAdOx1 nCoV-19 vaccination with undetectable anti-PF4 Abs. The platelet count responded rapidly to intravenous immunoglobulin and steroids. Therefore, the high clinical suspicion is essential for early recognition and prompt management irrespective of anti-PF4 Ab results.

Conclusion: We hypothesize that platelet and endothelial activation following ChAdOx1 nCoV-19 vaccination may lead to generation of pathogenic antibodies which account for VITT independent of anti-PF4 Abs.

Key words: thromboembolism, platelet factor 4, vaccine, adenoviral vector, antibody

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Introduction

Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a clinically distinctive syndrome characterized by unusual site thrombosis accompanied by thrombocytopenia and markedly elevated D-dimer levels 5-30 days following ChAdOx1 nCoV-19 and Ad26.COV2.S adenoviral vector vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).¹⁻⁵ Although the pathogenesis of VITT remains largely unknown, platelet-activating anti-platelet factor 4-dependent antibodies (anti-PF4 Abs) have been identified as pathogenic antibodies using enzyme-linked immunosorbent

assay (ELISA) detecting anti-PF4/polyanion IgG antibodies in almost all patients.¹⁻⁵ Therefore, VITT may represent a vaccine-related variant of autoimmune heparin-induced thrombocytopenia (HIT), which were previously reported following orthopedic surgery and both bacterial and viral infections.^{1,2} In the HIT model, an immune complex of anti-PF4/heparin Abs binds to FcγRIIa on platelets leading to platelet activation and thrombocytopenia, while binding to FcγRIIa on neutrophils leading to formation of neutrophil extracellular trap (NETosis) and thrombus formation.^{6,7}

VITT typically occurs after the first ChAdOx1 nCoV-19 dose, while it is very rare after the second dose. We herein reported the case of thrombosis with thrombocytopenia after the second ChAdOx1 nCoV-19 vaccination with undetectable anti-PF4 Abs. Our case raises the concern of an unknown immunological mechanism underlying VITT independent of anti-PF4 Abs.

Case report

A 68-year-old female with early Alzheimer’s disease was taking memantine of 10 mg per day. She was able to independently perform all daily activities. She developed right leg edema and acute dyspnea on exertion one week after the second ChAdOx1 nCoV-19 vaccination on July 26, 2021. She became dyspneic at rest and visited the emergency department on Aug 8, 2021. Physical exam showed tachypnea with a pulse oximeter saturation of 90% and pitting edema on the right leg. An initial complete blood count showed a platelet count of $139 \times 10^9/l$. Her D-dimer (Vidas, Biomerieux, Marcy-l’Étoile, France) and fibrinogen levels were 4,280 ng/ml and 5.0 g/l (1.7-4.0 g/l), respectively. A SARS-CoV-2 nasopharyngeal swab test using reverse transcription-polymerase chain reaction was negative. Severely dilated right ventricle and acute right ventricular dysfunction with elevated pulmonary arterial pressure was documented by echocardiography. Doppler ultrasonography showed non-compressible right popliteal vein. Computed tomography pulmonary angiogram revealed acute pulmonary embolism of bilateral main pulmonary arteries extending to lobar and subsegmental branches of all lobes of both lungs. As VITT was suspected, intravenous immunoglobulin (IVIG 1 g/kg/day for 2 days) and methylprednisolone (1 mg/kg/day for 3 days) were promptly administered.

Therapeutic enoxaparin (1 mg/kg twice daily) was initially prescribed on the first day and subsequently changed to apixaban (10 mg twice daily). Her platelet counts were rapidly increased to 233, 278 and $338 \times 10^9/l$ on day 3, 5 and 7 after IVIG suggestive of antibody-mediated thrombocytopenia (Figure 1). On day 11, her symptoms and hypoxia were resolved. Her platelet count was $298 \times 10^9/l$. Echocardiography revealed normal right ventricular structures and function. Thus, she was discharged with therapeutic apixaban (5 mg twice daily). However, anti-PF4 Abs by an ELISA (Asserachrom HPIA IgG, Stago, Asnières-sur-Seine, France; the cutoff optical density value of 0.3) were undetectable. Heparin-induced platelet activation test showed no platelet activation at different concentrations of heparin. This case was defined as probable VITT following the case definition criteria for VITT according to the British expert hematology panel (onset of symptoms 5–30 days after vaccination against SARS-CoV-2, presence of thrombosis, platelet count $< 150 \times 10^9/l$ and D-dimer > 4000 ng/ml).⁴ To the best of our knowledge, this is the first case of VITT after the second ChAdOx1 nCoV-19 dose documented in Thailand.

Discussion

Demonstration of circulating anti-PF4 Abs in conjunction with thrombocytopenia and thrombosis in almost all VITT cases suggests that VITT is a distinct clinical syndrome which is related to anti-PF4 Abs. However, undetectable anti-PF4 Abs were reported in a small proportion of VITT cases. The largest VITT cohort from the United Kingdom documented 170 definite and 50 probable VITT cases. All the patients presented after the first ChAdOx1 nCoV-19 vaccination. Of 198 definite or probable cases with available anti-PF4 Ab results,

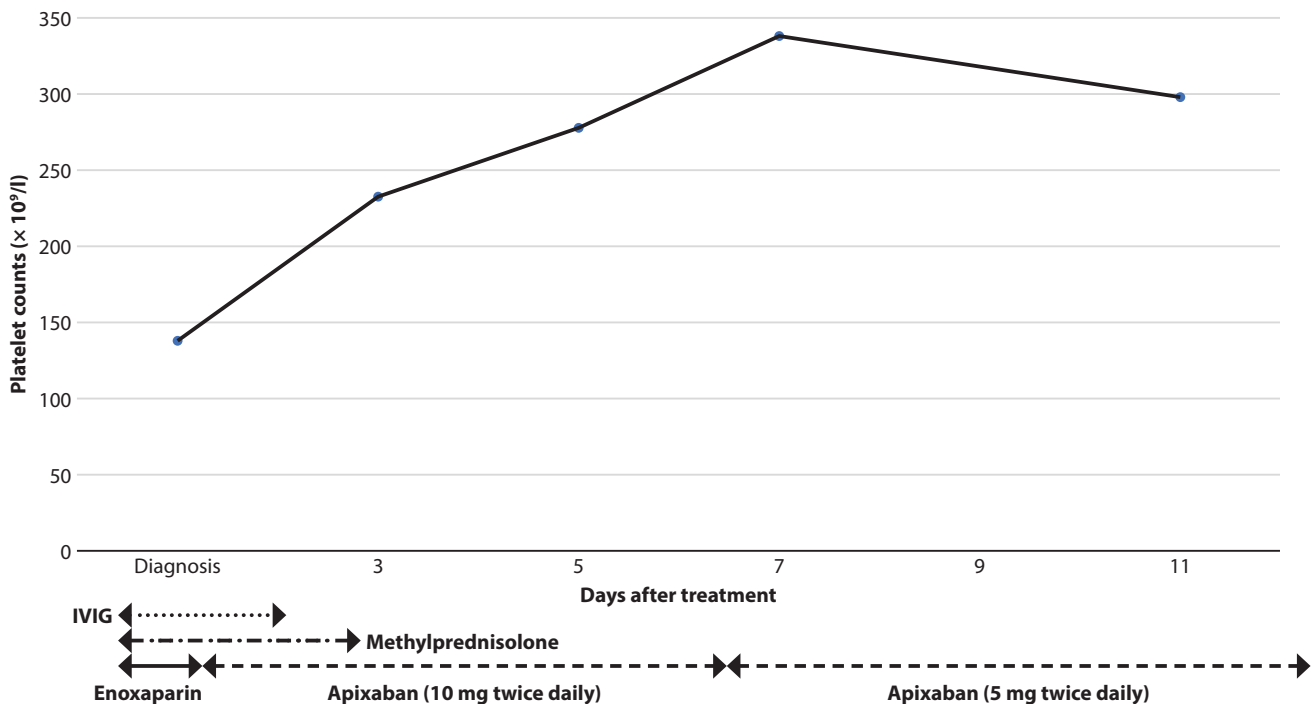


Figure 1. Platelet counts over time after treatment of vaccine-induced immune thrombotic thrombocytopenia. IVIG, intravenous immunoglobulin.

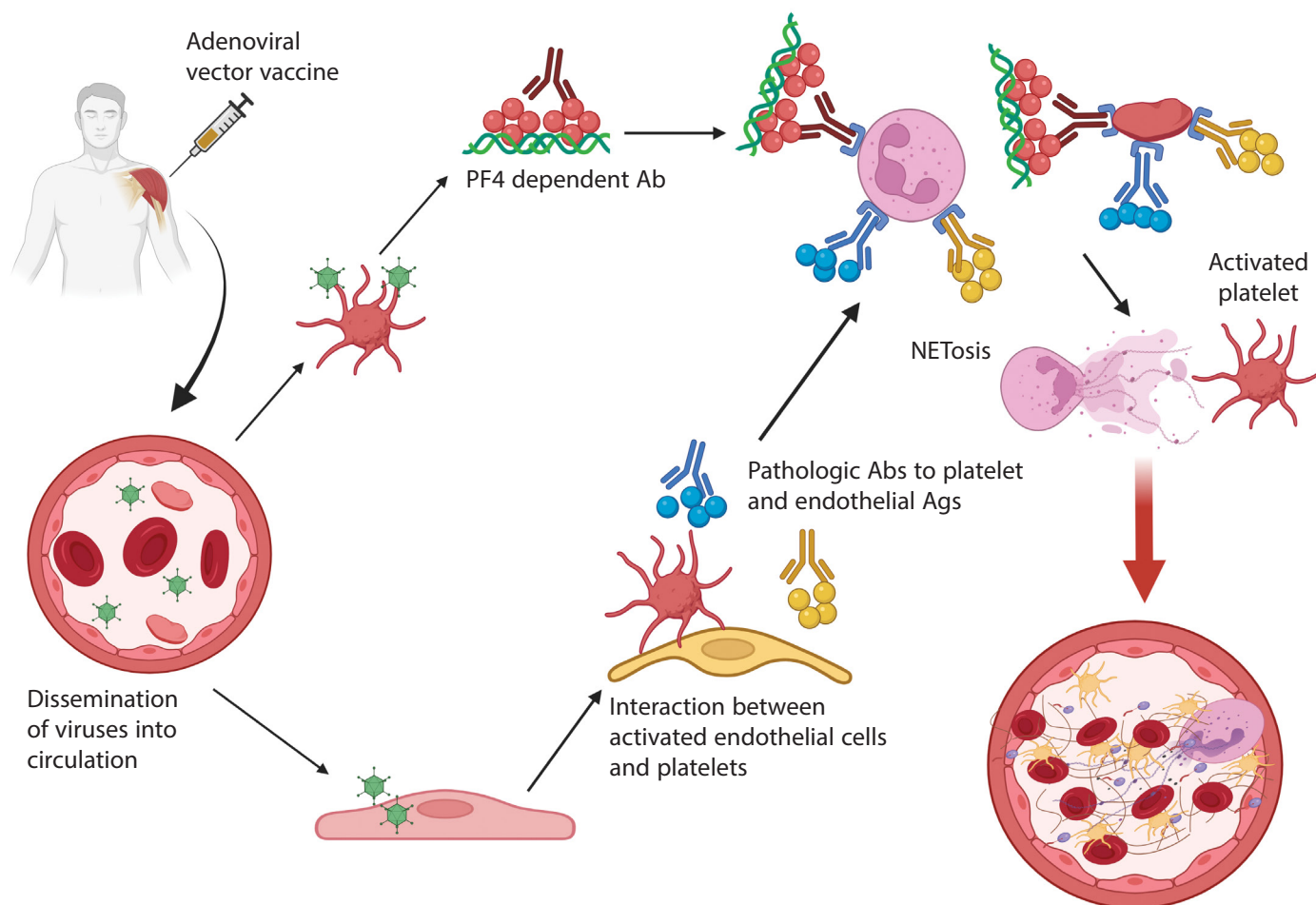


Figure 2. Local immune reaction leads to an increase in vascular permeability and dissemination of adenovirus into systemic circulation. Direct and indirect interactions among viruses, vaccine components, platelets and endothelial cells leads to platelet and endothelial activation. Canonically, generation of anti-platelet factor 4 antibodies can activate neutrophils leading to formation of neutrophil extracellular trap or NETosis. The immune complex of anti-PF4 dependent antibodies is a pathogenic antibody found in typical vaccine-induced immune thrombotic thrombocytopenia (VITT). In contrast, antibodies to unknown platelet or endothelial antigens may alternative pathologic antibodies which are able to trigger NETosis and platelet activation leading to thrombosis with thrombocytopenia in VITT with undetectable anti-PF4 antibodies. This figure is created with BioRender.com.

Ab, antibody; Ag, antigen; PF4, platelet factor 4

there were 6 (3.0%) patients with undetectable anti-PF4 Abs.⁴ Additionally, a recent review by Gresele and colleague identified 92 VITT cases reported in the literature up to July 10th, 2021 after the first vaccination (78 with the ChAdOx1 nCoV-19 vaccine and 14 with the Ad26.COV2.S vaccine). There were 5 (5.4%) cases yielding negative anti-PF4 Ab results.⁸ The global AstraZeneca safety database revealed the estimated rates of thrombosis with thrombocytopenia within 14 days after the first and the second dose of the ChAdOx1 nCoV-19 vaccine of 8.1 and 2.3 per million persons, respectively. There were 13 cases identified within 14 days after the second ChAdOx1 nCoV-19 dose. Only 3 (2 pulmonary emboli and 1 deep vein thrombosis with cerebral hemorrhage) of these cases had available anti-PF4 Abs results, all of which yielded negative.⁹ Therefore, undetectable anti-PF4 Abs could not exclude VITT in patients developing thrombosis with thrombocytopenia after ChAdOx1 nCoV-19 vaccination. These data altogether suggested that VITT may occurred independent of anti-PF4 Abs.

We recently reported the anti-PF4 Ab prevalence of 3.1% in 521 Thais after the first ChAdOx1 nCoV-19 vaccination. All detectable anti-PF4 Abs in our study were non-pathogenic.¹⁰ In addition, a second dose of the ChAdOx1 nCoV-19 vaccine does not alters anti-PF4 Ab functionality in people with pre-existing non-pathogenic anti-PF4 Abs.¹¹ In an animal model, adenovirus may induce platelet activation and thrombocytopenia from complex platelet-endothelial-leukocyte interaction triggering development of microthrombi in liver sinusoids and disseminated intravascular coagulation.^{8,12} A typical onset of 1-2 weeks after adenoviral vector vaccination suggests an immune pathogenesis and a dramatic platelet response to IVIG implies an antibody-mediated mechanism. Therefore, we hypothesize that adenoviral vector vaccines may induce platelet and endothelial activation that elicits generation of antibodies to platelet or endothelial antigens. This pathologic immune complex is able to activate neutrophils and platelets leading to thrombosis with thrombocytopenia syndrome (**Figure 2**).

VITT after the second dose of the ChAdOx1 nCoV-19 vaccine is extremely rare. In the UK experience of more than 300 suspected VITT cases, there were only one definite and one possible case of VITT following the second ChAdOx1 nCoV-19 vaccination.¹³ Therefore, baseline characteristics, clinical course and pathogenesis of VITT after the second dose of the ChAdOx1 nCoV-19 vaccine remain largely unknown. Herein, we reported clinical and laboratory characteristics and treatment outcomes of the probable VITT independent of anti-PF4 Abs following the second ChAdOx1 nCoV-19 vaccination.

There are some limitations of the study. Biomarkers of platelet and endothelial activation, such as soluble P-selectin, soluble thrombomodulin and endothelin-1, to support our hypothesis were not tested as they are not routinely performed in clinical practice. In addition, thrombophilia profiles were not determined as the testing is not indicated in an elderly patient. Venous thromboembolism is common in these patients, and low natural anticoagulant levels are likely from a coexisting condition rather than a pathogenic factor in such cases.

Although VITT is very rare, especially after the second ChAdOx1 nCoV-19 vaccination, it is associated with a high mortality.¹⁻⁴ High clinical suspicion is essential for early recognition and prompt management in patients with thrombosis and thrombocytopenia, especially when accompanied by very high D-dimer levels, irrespective of anti-PF4 Ab results. Additionally, our VITT case highlights a possibility of an unknown mechanism of VITT independent of anti-PF4 Abs. Further studies to elucidate the alternative pathway inducing thrombosis with thrombocytopenia after ChAdOx1 nCoV-19 vaccination are warrant.

Conflict of interest

The authors report no conflicts of interest for the submitted manuscript.

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Author contributions

Dr. Noppacharn Uaprasert was involved in patient care, data collection, and writing the final draft of manuscript. Dr. Ponlapat Rojnuckarin was involved in appraisal and editing the manuscript.

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