

COMMENTARY

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Explaining COVID-19 postvaccination-related immune thrombotic thrombocytopenia: a hypothesis-generating *in-silico* approach

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ABSTRACT

Cases that experienced COVID-19 postvaccination-related thrombosis have been reported after the first dose of ChAdOx1 nCov-19 (Vaxzevria, AstraZeneca) or Ad26.COV2.S (Johnson & Johnson/Janssen) vaccine. These rare thrombotic events were observed within the expected vaccine-induced seroconversion period and could be attributed to platelet-activating (auto)antibodies against platelet factor 4 (PF4). Newly, vaccine-induced, cross-reactive anti-PF4 antibodies could explain this observation. An *in-silico* analysis using the Basic Local Alignment Search Tool was used to identify sequence similarity between PF4 and antigens contained in or encoded by ChAdOx1 nCov-19 or Ad26.COV2.S vaccines. Only one sequence within the signaling peptide of the SARS-CoV-2 spike protein exhibited a high percent identity (85.71%) with PF4. This sequence overlaps with a proven immunogenic peptide recognized from convalescent COVID-19 sera and could be responsible for the formation of platelet-activating immunocomplexes in susceptible patients. Manipulation of the immunogenicity of this particular sequence within the encoded SARS-CoV-2 spike protein signaling peptide may eliminate this iatrogenic severe adverse effect.

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease 2019 (COVID-19), an entity that is characterized by symptoms ranging from a mild cough to severe respiratory distress and death. The core pathophysiology of COVID-19 arises from hyperactivation or dysfunction of the endothelium of the capillary related to the coagulation cascade.¹

Until now, Hippocrates' quote "Prevention is better than Cure" has been the best approach that could be offered under these extraordinary emergency conditions of SARS-CoV-2 pandemic. Highly effective vaccines have been developed with unprecedented speed. As expected with all medications, side effects do occur. However, considering the number of vaccinated people, this risk has been remarkably low. Apart from very rare severe allergic reactions, immunological-based adverse events have been described, among which immune thrombotic thrombocytopenia is one of the most severe or even fatal, although rare.²

Cases that experienced COVID-19 postvaccination-related thrombosis have been reported after the first dose of the ChAdOx1 nCov-19 (Vaxzevria, AstraZeneca) vaccine^{3–5} or in recipients of the first dose of the Ad26.COV2.S vaccine (Johnson & Johnson/Janssen).^{6–8} This rare entity has been named Vaccine-Induced Immune Thrombotic Thrombocytopenia (VITT). VITT resembles heparin-induced thrombocytopenia, a prothrombotic disorder attributed to platelet-activating antibodies that recognize complexes between anionic heparin and cationic anti-platelet factor 4 (PF4).^{9,10}

PF4 or chemokine (C-X-C motif) ligand 4 (CXCL4) is stored in platelet α -granules. Its physiological role is to promote blood coagulation, e.g., in trauma repair, although it can

be released upon any type of platelet activation.¹¹ The SARS-CoV-2 can activate platelets either indirectly through the epithelial damage¹¹ or directly by infecting them via angiotensin converting enzyme-dependent¹² or independent^{13,14} mechanisms.

No risk factors for VITT have been identified until now,² including known history of thrombosis or prothrombotic conditions, inherited and acquired thrombophilia including protein C-, protein S- and antithrombin-deficiency, factor V Leiden mutation, prothrombin mutation or antiphospholipid-antibodies. Interestingly, the majority of the reported patients did not recall heparin exposure in the past.^{2–5,15–18} All reported VITT cases occurred around a mean time of 14–21 days, (range 5–28 days) postvaccination, namely within the expected vaccine-induced seroconversion period.

PF4 induced antibodies were found to be essential for the observed platelet activation that seemed to occur through platelet Fc γ receptors signaling. Affinity-purified sera from VITT patients with either immobilized PF4 or immobilized PF4-heparin showed the same platelet-activation pattern as the original sera, suggesting that free circulating anti-PF4 or anti-PF4-heparin antibodies did not contribute to this activation. A prerequisite for this activation was the presence of PF4. Considering the affinity-purified process, only cross-reactive anti-PF4 antibodies that escaped purification due to lower affinity/avidity could explain this observation.⁵ Antigens with immunogenic amino acid sequences homologous to PF4 contained in or encoded by the administered vaccine could induce these antibodies.

On this basis, Basic Local Alignment Search Tool (BLAST) was used to identify sequence similarity between PF4 and antigens contained in or encoded by Vaxzevria or Janssen COVID-19 vaccine. Sequences with a homology of at least 50% were considered significant. A pairwise sequence similarity searching was performed to compare PF4 (UniProt accession number: P02776) with all known proteins with unique accession numbers in the UniProt database (December 22nd, 2021) that were contained in or encoded by the vaccine. These included: i) all known proteins from chimpanzee adenovirus Y25 (UniProt accession numbers: G9G840–9, G9G850–9, G9G860–9, and G8G870), ii) all known proteins from human adenovirus 26 (UniProt accession numbers: A4ZKK7–9, A4ZKL0–9, A4ZKM0–1, A5×2P6, B2ZWZ4, B5MEQ4, C8Z266, E2CUU4, H9XUH0–2, H9XUI6, H9XUJ6, Q2Z0K1, Q3S8B3, Q49QU7, Q4EW99, Q5ICR7, Q64LE9, Q67759, Q76I59, and Q9QB27) and iii) the spike protein antigen of the SARS-CoV-2 (UniProt accession number: P0DTC2).¹⁹

Only one sequence, the peptide MFVFLVLLPLVS between one to eleven (1–11) positions within the signaling peptide region of the SARS-CoV-2 spike protein, exhibited a high percent identity of 85.71%, with PF4's peptide LFLGLLPLVVA located between 15 to 25 positions (Figure 1). The peptide MFVFLVLLPLVS between one to eleven (1–11) positions within the signaling peptide region of the SARS-CoV-2 spike protein SARS-CoV-2 is conservative among variants Alpha/B.1.1.7, Delta/B.1.617.2 and Omicron/B.1.1.529.

In a recent study, convalescent COVID-19 sera were found to contain antibodies against the signaling peptide of the spike protein. The peptide identified above overlaps (7–11 positions) with a sequence between 7 to 18 (7–18) positions, spanning the second half of the signaling peptide and the beginning of the N-terminal domain of the SARS-CoV-2 spike protein. This

sequence has been shown to be immunogenic.²⁰ The sera response frequency of peptide MFVFLVLLPLVS is not available, but it is expected to be low, offering a possible explanation why VITT is uncommon.

It has been suggested that COVID-19 vaccines using adenoviral vector platform may be related to VITT.⁷ However, the vectors used are from different species (D and E, respectively) and they use different host cellular receptors, namely, they are expected to have different biologic effects.⁶ Nevertheless, their known proteins do not exhibit homology with PF4.

A few cases of thrombocytopenia have been reported to occur with the two messenger RNA-based vaccines - BNT162b2 (Pfizer–BioNTech) and mRNA-1273 (Moderna).²¹ Additionally, COVID-19 has been associated with a higher risk of thrombosis than vaccinations.²² Based on the proposed cross-reactivity, it can be hypothesized that individuals susceptible to autoimmunity may develop thrombosis mediated by anti-signaling-peptide antibodies that cross-react with PF4. These patients would probably have experienced similar consequences if they were infected by the SARS-CoV-2 before vaccination.

There are no differences between the signaling peptide regions among SARS-CoV-2 variants Alpha/B.1.1.7, Delta/B.1.617.2,²³ and Omicron/B.1.1.529. Therefore, if the cross-reactivity between the proposed peptide's region with PF4 could explain the auto-immunological phenomena triggered to cause VITT, then the incidence of VITT is not expected to be different in the case of COVID-19 infection with any of the above variants in susceptible individuals.

In VITT, PF4/anti-PF4 autoantibodies immune complexes (ICs) may activate platelets.⁵ ICs composed of antibodies against the PF4 and the homologous identified peptide MFVFLVLLPLVS may also contribute to this activation. The

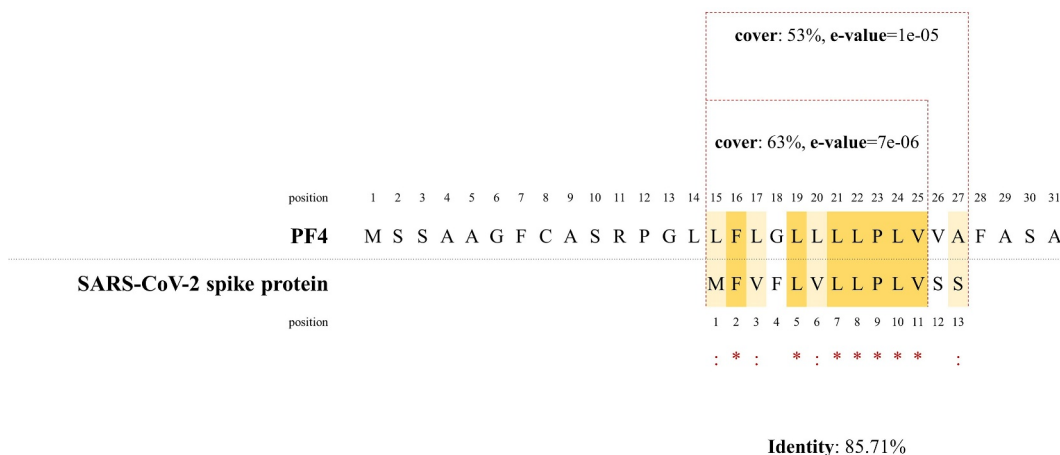


Figure 1. Sequence alignment of the signaling peptides of Platelet Factor 4 (PF4) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spike protein antigen. MFVFLVLLPLVS was the only significant sequence identified after pairwise sequence similarity searching between the PF4 (UniProt accession number: P02776) and all known proteins contained in or encoded by Vaxzevria (AstraZeneca) vaccine represented by unique accession numbers in the UniProt database on December 22nd, 2021 [proteins from chimpanzee adenovirus Y25 (UniProt accession numbers: G9G840–9, G9G850–9, G9G860–9, and G8G870), proteins from human adenovirus 26 (UniProt accession numbers: A4ZKK7–9, A4ZKL0–9, A4ZKM0–1, A5×2P6, B2ZWZ4, B5MEQ4, C8Z266, E2CUU4, H9XUH0–2, H9XUI6, H9XUJ6, Q2Z0K1, Q3S8B3, Q49QU7, Q4EW99, Q5ICR7, Q64LE9, Q67759, Q76I59, and Q9QB27) and the spike protein antigen of the SARS-CoV-2 (UniProt accession number: P0DTC2)]. An * (asterisk) indicates positions that have a single, fully conserved residue. A : (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. Cover: the percent of the query length that is included in the aligned segments. Identity: the highest percent identity for a set of aligned segments to the same subject sequence.

therapeutic role heparin has on VITT enhances this possibility since heparin binds the SARS-CoV-2 receptor-binding domain to cause conformational changes that alter binding specificity, which can inhibit platelet activation.¹²

If the cross-reactivity of SARS-CoV-2 spike protein with PF4 is responsible for the formation of platelet-activating ICs, then manipulation of the immunogenicity of this particular sequence within the encoded SARS-CoV-2 spike protein signaling peptide by specific amino acid substitutions may eliminate the iatrogenic adverse effect of VITT.

Cross-reactivity between SARS-CoV-2 signaling-peptide and PF4 may trigger autoimmune responses that could explain VITT. The proposed mechanism seems to be independent of the vector used. The adenoviruses could explain, though, direct or indirect platelet and endothelial activation, which subsequently induces PF4 release.²⁴ The newly vaccine-induced antibodies against peptide MFVFLVLLPLVS within the spike protein may accidentally play the role of anti-PF4 autoantibodies due to cross-reactivity, explaining the pathophysiological cascade causing thrombotic thrombocytopenia.

Additional experimental and clinical work is needed to confirm this *in-silico* based hypothesis by examining sera response frequencies of the peptide MFVFLVLLPLVS among patients experiencing VITT or the antibody responses to vaccines that lack this sequence.

Abbreviations

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus disease 2019
VITT	Vaccine-Induced Immune Thrombotic Thrombocytopenia
PF4	Anti-platelet factor 4
IC	Immune complexes

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