

# Is Molecular Mimicry between hPF4 and SARS-CoV-2 Spike Protein a Potential Basis for Autoimmune Responses in Vaccinated and Naturally Infected Patients?

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Vaccination against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) will hopefully terminate the pandemic of coronavirus disease 2019 (COVID-19) and several vaccines are now available. The European Medical Agency has approved four vaccines<sup>1</sup> for the prevention of symptomatic COVID-19: two messenger RNA (mRNA)-based vaccines (mRNA BNT162b2-BioNTech/Pfizer and mRNA-1273-Moderna) encoding the spike protein antigen of SARS-CoV-2 and two recombinant adenoviral vector-based vaccines (ChAdOx1-AstraZeneca and Ad26.COV2.S -Janssen) encoding the spike glycoprotein of SARS-CoV-2.

In Europe since March 2021, ~100 patients<sup>2</sup> with venous thromboses at unusual sites (cerebral venous sinus thrombosis and splanchnic vein thrombosis) in combination with moderate-to-severe thrombocytopenia were observed in individuals within 1 month from the vaccination with the ChAdOx1 nCoV-19 COVID-19 vaccine.<sup>3–5</sup> Similar complications have also been reported after vaccination with the Ad26.COV2.S Janssen COVID-19 vaccine.<sup>6,7</sup> Known as vaccine-induced immune thrombotic thrombocytopenia (VITT) or thrombosis with thrombocytopenia syndrome,<sup>8</sup> the condition presents with thromboses, often at unusual sites, low platelet count, high D-dimer, and high levels of anti-platelet factor 4 (anti-PF4) antibodies,<sup>4,9,10</sup> which are otherwise usually seen in patients with heparin induced thrombocytopenia or autoimmune HIT.<sup>11</sup> It is currently unknown what triggers the formation of these antibodies after vaccination against COVID-19.

Recent findings, especially considering the clinical profile, suggest the production of two types of antibodies, as described elsewhere.<sup>11</sup> These different types of antibodies, analogous to the reference monoclonal IgG<sub>2bK</sub> (designated KKO) and anti-PF4 (designated RTO) antibodies monoclonal antibodies,<sup>11</sup> are directed against overlapping, yet different epitopes of human PF4. KKO-like antibodies were found to induce an autoimmune response in heparin-treated patients that results in thrombocytopenia and a prothrombotic state, while RTO-like antibodies do not promote platelet activation and thrombosis.<sup>11</sup> This response was observed in ChAdOx1 nCoV-19-vaccinated people and might be occurring in COVID-19 patients as well, since a prothrombotic state with thrombocytopenia was observed in patients with thrombotic complications of the pulmonary and gastroenteric districts.<sup>12</sup>

Considering this background, human PF4 (hPF4; Reference Sequence: NG032897) and surface spike glycoprotein (Reference Sequence: YP\_009724390.1) have been downloaded from NCBI genome database and manually edited using BioEdit.<sup>13</sup> Subsequently Swiss-Model<sup>14</sup> and HHpred<sup>15</sup> online web-tools have been used to identify eventual amino-acidic domains showing homology or similarity between the two protein structures. For this purpose, the BepiPred 2.0 online tool has been used to survey eventual common B cells epitopes.<sup>16</sup> The performed analyses did not show any statistically significant homologies or similarities between the

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hPF4 and the spike glycoprotein structures and no potentially overlapping B cell epitope was found. These results suggest that probably the molecular mimicry between the hPF4 and the SARS-CoV-2 spike protein is not responsible for the prothrombotic events associated with VITT. However, binding experiments with KKO monoclonal antibody against the spike by using both surface plasmon resonance and immunoprecipitation should be performed to confirm this evidence.

Previous articles have highlighted how SARS-CoV-2 can directly activate platelets and trigger procoagulant events. Moreover, they suggested the possibility of cross-reactivity between antivector antibodies and PF4.<sup>17</sup> Probably this rare emergent autoimmune response can be induced by the cross-reaction with the adenovirus that brings viral DNA and PF4 together in the extracellular space inducing a possible reaction with antigen presenting cells.<sup>17</sup> This potentially explains why thrombotic complications in patients vaccinated with mRNA-lipid nanoparticle are less frequent compared with adenovirus as vaccine vector. Further investigations are needed to clarify the safety of adenoviral vector vaccines in patients with risk factors for thrombosis or chronic thrombocyte disorders.<sup>17</sup>

#### Conflict of Interest

None declared.

#### References

- 1 Cavaleri M, Enzmann H, Straus S, Cooke E. The European Medicines Agency's EU conditional marketing authorisations for COVID-19 vaccines. *Lancet* 2021;397(10272):355–357
- 2 Paul-Ehrlich-Institut (Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel) Service-Presse-Sehr seltene Fälle des "Thrombose-mit-Thrombozytopenie Syndroms (TTS) nach Impfung. 2021. Accessed December 13, 2021 at: <https://www.pei.de/DE/service/presse/aktuelles/aktuellesinhalt.html%20approached%20May%2031>
- 3 Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *N Engl J Med* 2021;384(22):2092–2101
- 4 Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *N Engl J Med* 2021;384(22):2124–2130
- 5 Scully M, Singh D, Lown R, et al. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. *N Engl J Med* 2021;384(23):2202–2211
- 6 Muir K-L, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. *N Engl J Med* 2021;384(20):1964–1965
- 7 Sadoff J, Davis K, Douoguih M. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination—response from the manufacturer. *N Engl J Med* 2021;384(20):1965–1966
- 8 Arepally GM, Ortel TL. Vaccine-induced immune thrombotic thrombocytopenia: what we know and do not know. *Blood* 2021;138(04):293–298
- 9 Rzymiski P, Perek B, Flisiak R. Thrombotic thrombocytopenia after COVID-19 vaccination: in search of the underlying mechanism. *Vaccines (Basel)* 2021;9(06):559
- 10 McGonagle D, De Marco G, Bridgewood C. Mechanisms of immunothrombosis in vaccine-induced thrombotic thrombocytopenia (VITT) compared to natural SARS-CoV-2 infection. *J Autoimmun* 2021;121(121):102662
- 11 Cai Z, Greene MI, Zhu Z, Zhang H. Structural features and PF4 functions that occur in heparin-induced thrombocytopenia (HIT) complicated by COVID-19. *Antibodies (Basel)* 2020;9(04):52
- 12 Carnevale S, Beretta P, Morbini P. Direct endothelial damage and vasculitis due to SARS-CoV-2 in small bowel submucosa of COVID-19 patient with diarrhea. *J Med Virol* 2021;93(01):61–63
- 13 Hall TA. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 1999;(41):95–98
- 14 Waterhouse A, Bertoni M, Bienert S, et al. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* 2018;46(W1):W296–W303
- 15 Zimmermann L, Stephens A, Nam SZ, et al. A completely reimplemented MPI bioinformatics toolkit with a new HHpred server at its core. *J Mol Biol* 2018;430(15):2237–2243
- 16 Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic Acids Res* 2017;45(W1):W24–W29
- 17 Tsilingiris D, Vallianou NG, Karampela I, Dalamaga M. Vaccine induced thrombotic thrombocytopenia: the shady chapter of a success story. *Metabol Open* 2021;11(11):100101