Autoimmune Thrombotic Thrombocytopeny associated with COVID-19 Infection or Vaccination: Learning from Heparin-induced Thrombocytopenia

Michel Goldman, MD, PhD
Institute for Interdisciplinary Innovation in healthcare (I3h)
Université libre de Bruxelles, Belgium
mgoldman@i3health.eu

Abstract: Prothrombotic thrombocytopeny mimicking heparin-induced thrombocytopenia has been observed in patients with severe COVID-19 and after immunisation with the Vaxzevria vaccine. Herein, we discuss the possible pathogenesis of this disorder focusing on the possible involvement of anti-platelet factor 4 (PFA) autoantibodies.

Keywords: COVID-19; vaccine; heparin; thrombocytopenia; thrombosis; platelet; endothelial cell; autoimmunity; proteoglycan; B cell; coagulation

Introduction
There is convincing evidence that autoimmunity is involved in the pathogenesis of COVID-191,2. Regarding the severe forms of the disease in which thromboinflammation is prominent, both endothelial cells and platelets might be affected by autoimmune reactions in addition to direct viral infection and cytokine-mediated activation3,4. Indeed, multiple anti-phospholipid antibodies have been detected in the blood of hospitalized patients in relation with the severity of the disease and the formation of neutrophil extracellular traps known to contribute to thrombotic events5. A recent study further established that among anti-phospholipid autoantibodies detected in COVID-19 patients, IgG to cardiolipin and phosphatidylserine/prothrombin might be the ones driving endothelial cell activation6. In addition, anti-annexin A2 autoantibodies found in critically ill patients were suggested to contribute to small vessel damages in the lungs7.

Besides endothelial cell damages, activation of platelets is the other cornerstone of the prothrombotic state characteristic of COVID-194. Several factors are involved including mitochondrial disturbances caused by hypoxia, mediators of inflammation and other stressors, leading to platelet hyperactivation and apoptosis4,8. Furthermore, infection of platelets by the SARS-CoV-2 virus might also contribute to their activation via ACE2-dependent9 as well as non-ACE2 mechanisms involving heparan sulfate10 or CD14711. Following viral entry, SARS-CoV-2 ssRNA might trigger intracellular Toll-like receptor 7-dependent activation pathways as in the case of influenza infection12. Antibody-mediated mechanisms involving engagement of Fc receptor RIIA on platelets were also shown to contribute to procoagulant activity in severe COVID-1913,14. Although the antigenic specificity of these antibodies could not always be defined, antibodies to platelet factor 4 were shown to be involved in several cases15–22.
Platelet factor 4, also called CXCL4, is a tetrameric chemokine stored in platelet alpha-granules. Upon platelet activation, PF4 is released and binds polyanions with high affinity. Indeed, PF4 was shown to play a critical role in heparin-induced thrombocytopenia (HIT). Below, we summarize the key features of HIT before proposing that COVID-19 causes an autoimmune thrombotic thrombocytopenia mimicking HIT.

**PF4 autoimmunity in heparin-induced thrombocytopenia (HIT)**

Heparin-induced thrombocytopenia (HIT) is a severe prothrombotic condition that occurs in less than 5% of patients receiving intravenous unfractionated heparin, usually between 4 and 10 days after initiation of the treatment. Thrombotic complications can develop in unusual locations such as cerebral venous sinuses. Thrombocytopenia is a hallmark of HIT, blood platelet counts decreasing by more than 50% in most patients. After exclusion of other causes of thrombocytopenia, the clinical diagnosis of HIT is established by immune-enzymatic detection of circulating antibodies to PF4/heparin complexes, followed by a functional assay demonstrating platelet activation by patient’s serum in presence of heparin.

The fine specificity of PF4 autoantibodies causing HIT has been identified through a series of elegant studies which established that the epitope recognized on PF4 tetramers is exposed upon conformational changes induced by their interaction with heparin or other long polyanions. Indeed, injection of heparin has been shown to induce the release of PF4, resulting in the assembly of PF4/heparin complexes which activate complement and bind circulating B lymphocytes in a complement-dependent manner. B cells responsible for the synthesis of PF4 autoantibodies display unique characteristics which enable them to rapidly mount an IgG response following a first exposure to heparin. Indeed, B cells able to produce anti-PF4 antibodies are present in healthy individuals in an anergic state that normally prevents the development of PF4 immune response. This B cell tolerance might be broken upon heparin exposure and under some inflammatory conditions. In these situations, anti-PF4 IgG antibodies elicit thrombus formation and thrombocytopenia via multiple mechanisms. Immune complexes assembled with PF4 bound to heparin induce platelet activation and aggregation by cross-linking FcyRIIa receptors. Anti-PF4 antibodies also activate the procoagulant activity of monocytes by cross-linking their FcyRI receptors, and of endothelial cells via the recognition of PF4 firmly attached to surface proteoglycans. Thrombocytopenia results from enhanced apoptosis and clearance of antibody-decorated platelets.

A prothrombotic syndrome with all the features of HIT has been reported in absence of heparin exposure. These observations led to define a so-called “spontaneous HIT” caused by anti-PF4 autoantibodies elicited by polyanions reproducing the conformational changes induced in PF4 tetramers by heparin. Potential polyanions triggering “spontaneous HIT” include bacterial wall components, nucleic acid materials or endogenous proteoglycans released by damaged cells.
Antibody-mediated thrombotic thrombocytopathy during COVID-19: an autoimmune reaction induced by SARS-CoV-2?

The high incidence of thrombotic and thromboembolic events during severe COVID-19 results in the frequent administration of heparin in affected patients. When thrombocytopenia develops in this setting, HIT must be considered as possible cause. Indeed, several studies report the presence of anti-PF4/heparin antibodies in COVID-19 patients. However, these antibodies sometimes occur in absence of heparin administration. Furthermore, they do not always activate platelets in presence of heparin/PF4 complexes although they do so in presence of PF4 alone, suggesting that they were induced by another mechanism than classical HIT.

Indeed, IgG antibodies present in the serum of severe COVID-19 patients were found to induce platelet apoptosis and procoagulant activity via FcγRIIA receptor-dependent mechanisms. The antigenic specificity of these antibodies was not defined but one can speculate that at least some of them are directed against PF4.

The model that we are proposing is first based on the hyperactivation of platelets during COVID-19, resulting in the release of PF4 in the circulation. Circulating PF4 could form complexes with endogenous polyanionic proteoglycans (PG) released by damaged endothelial cells. Syndecan-1 and endocan are potential PG candidates as since their serum levels are increased in severely ill COVID-19 patients in association with other markers of endothelial injury. Complexes formed between PF4 and endothelial cell-derived polyanionic PG would then stimulate extrafollicular B cells producing anti-PF4 antibodies. Indeed, autoimmune responses elicited by extrafollicular B cells were previously suggested to be involved in the pathophysiology of severe COVID-19. Recapitulate the sequence of events responsible for HIT (figure 1). Besides anti-PF4 autoantibodies, anti-phospholipid antibodies could also contribute to platelet activation as well as anti-SARS-CoV-2 antibodies as observed in other viral diseases.
Figure 1. Induction of thrombotic thrombocytopenia during COVID-19

A Sars-CoV-2 induces the release of platelet factor 4 (PF4) by activated platelets and of polyanionic proteoglycans (PG) by endothelial cells (e.g. syndecan, endocan)

B Complexes of PF4 and PG expose PF4 immunogenic epitopes which activate extrafollicular B lymphocytes secreting PF4 autoantibodies

C PF4 autoantibodies bind complexes of PF4 and PG on platelets and endothelial cells and stimulate their procoagulant activities. Cross-linking of Fcγ receptor IIA also promote apoptosis and clearance of antibody-decorated platelets.

Possible relevance to prothrombotic thrombocytopenia following COVID-19 vaccination

Several observations of prothrombotic thrombocytopenic events following vaccination with the Oxford–AstraZeneca chimpanzee adenovirus vectored vaccine ChAdOx1 nCoV-19 (Vaxzevria) were recently reported. The incidence of these events is very low (around 1 in 100,000 recipients) but appears superior to what would have been occurred by pure coincidence. As the clinical presentation is often reminiscent of HIT, the hypothesis of a vaccine-induced autoimmune response to PF4 seems plausible. Indeed, Greinacher et al. recently provided strong biological evidence to support this hypothesis by identifying platelet-activating anti-PF4 antibodies in sera of patients suffering from unusual thrombotic events associated with thrombocytopenia within 4 to 16 days after injection of the Vaxzevria vaccine. The induction of this possible vaccine-induced anti-PF4 autoimmune response might depend on mechanisms similar to those proposed above for the prothrombotic thrombocytopenia induced by the SARS-CoV-2 virus itself. Indeed, the SARS-CoV-2 spike protein encoded by the Vaxzevria vaccine was found to induce endothelial
damage through CD147-mediated cell signaling\textsuperscript{46} as well as ACE 2 receptor-dependent which could also contribute to platelet activation\textsuperscript{47}.

**Concluding remarks**

Autoantibodies appear to play a critical role in the prothrombotic thrombocytopenic disorders that may occur in the course of COVID-19 or occasionally after vaccination. It is of utmost importance to understand the pathogenetic mechanisms involved in order to design the most appropriate preventive and therapeutic strategies. Regarding vaccine-induced events, the respective roles of the Vaxzevria adenoviral vector and the encoded spike protein should be deciphered as well as the factors that may influence migration of vaccine components from the injection site.

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