

SARS-CoV-2 spike-dependent platelet activation in COVID-19 vaccine-induced thrombocytopenia

Tracking no: ADV-2021-005050R2

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Abstract:

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: J Appelbaum provided clinical care of the patient, analyzed and interpreted data and wrote the manuscript. D.M. Arnold designed the research and wrote the manuscript. J.G. Kelton designed the research and wrote the manuscript. T. Gernsheimer provided clinical care of the patient, analyzed and interpreted data and wrote the manuscript. S.D. Jevtic analyzed and interpreted data and wrote the manuscript. N. Ivetic carried out additional experiments and analyses. J.W. Smith carried out the described studies, analyzed data, and wrote the manuscript. I. Nazy designed the research, analyzed and interpreted data, and wrote the manuscript.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Emails to the corresponding author

Clinical trial registration information (if any):

Title: SARS-CoV-2 spike-dependent platelet activation in COVID-19 vaccine-induced thrombocytopenia

Short: SARS-CoV-2 spike vaccine-induced thrombocytopenia

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Word count: 1132

Table and Figures: 2 Figure

References: 12

Category: Platelets and Thrombopoiesis

Introduction

Coronavirus disease 2019 (COVID-19) is a severe viral illness that has resulted in significant morbidity and mortality worldwide. Several vaccines have been created that can prevent disease transmission, as well as severity and mortality. All COVID-19 vaccines use the SARS-CoV-2 Spike protein as the antigenic substrate. However, serious adverse reactions have been reported, including more than 150 cases of thrombocytopenia following vaccination¹. A precise mechanism linking COVID-19 vaccination and severe thrombocytopenia has yet to be confirmed. Identifying this mechanism could facilitate diagnostic test development.

The serotonin release assay (SRA) is the gold-standard diagnostic test for heparin-induced thrombocytopenia², which is characterized by severe thrombocytopenia and a high risk of thrombosis. Using the SRA, we recently showed that a subset of critically ill COVID-19 patients test positive for platelet-activating immune complexes³. Similarly, Althaus et al. showed IgG antibodies from critically ill COVID-19 patients can also activate platelets and lead to thrombotic events⁴. Here, we employ a modified SRA to demonstrate Spike-dependent, platelet-activating immune complexes in a patient with vaccine-induced thrombocytopenia (VIT) after receiving the Moderna vaccine.

Blood samples were referred to the McMaster Platelet Immunology Laboratory (MPIL) for testing. Clinical data was obtained with patient consent and additional testing was completed in keeping with ethics approval by the Hamilton Integrated Research Ethics Board.

Anti-PF4/heparin antibody testing was done using an anti-PF4/heparin enzymatic immunoassay (EIA, LIFECODES PF4 enhanced assay, Immucor GTI Diagnostics, Waukesha, Wisconsin) for IgG, IgM, and IgA PF4/heparin antibodies. Standard SRA testing was conducted in the absence and presence of heparin (0.1, 0.3, and 100 U/mL) or with exogenous PF4 added as previously

described^{2,5,6}. A modified SRA (Spike-SRA) was performed through exogenous administration of SARS-CoV-2 Spike protein at varying concentrations (0, 0.5, 5, 50, 100 µg/mL). Testing was also completed with varying amounts of Moderna vaccine or polyethylene glycol (PEG2000). Anti-human CD32 antibody (IV.3) or intravenous immunoglobulin (IVIg) was added to the SRA to confirm FcγRIIA signalling.

Platelet autoantibody binding was completed using washed donor platelets and tested using flow cytometry, platelet glycoprotein-specific enzyme immunoassay (PakPlus, Immucor), and radioimmunoprecipitation assay, as previously described^{7,8}.

Our patient was a 25-year-old woman who presented to hospital ten days after receiving the Moderna mRNA COVID-19 vaccine with fatigue, petechiae and wet purpura. The initial platelet count was 1,000 per cubic millimeter without evidence of schistocytes on blood smear. Coagulation studies were within the normal range including PT (13.6 s, normal 10.7 – 15.6 s), INR (1.1, normal 0.8 – 1.3), and PTT (30 s, normal 22 – 35 s). This also likely excludes the presence of a lupus anticoagulant, given the use of a lupus-sensitive reagent for PTT testing. Anti-platelet factor 4 (PF4)/heparin antibodies were not detected (OD = 0.221) and the classic SRA test, with or without heparin or exogenous PF4, was negative. Assays for drug-induced immune thrombocytopenia with washed donor platelets were also negative for platelet binding with vaccine, PEG2000, or SARS-CoV-2 Spike protein; assays included flow cytometry, platelet glycoprotein-specific enzyme immunoassay, and radioimmunoprecipitation.³ These results support an alternative diagnosis to drug-induced immune thrombocytopenia, although it cannot be fully excluded. The patient was treated with dexamethasone and intravenous immune globulin (IVIg) for a presumed immune thrombocytopenic purpura. The platelet count normalized by day seven of treatment (Figure 1A).

Additional serum testing identified SARS-CoV-2 Spike protein antibodies of the IgG (optical density [OD] = 2.847), IgA (OD = 3.130) and IgM (OD = 1.168) classes⁹. Antibodies against SARS-CoV-2 nucleocapsid protein were absent, confirming vaccine-induced antibodies without prior infection. To further investigate the mechanism of thrombocytopenia, we tested the patient's serum using a modified SRA with addition of recombinant SARS-CoV-2 Spike protein (Spike-SRA). We observed dose-dependent platelet activation with increasing SARS-CoV-2 Spike protein (0, 0.5, 5, 50, 100 $\mu\text{g}/\text{mL}$; Figure 1B). The reaction was inhibited by an Fc γ RIIa blocker (IV.3; 5 $\mu\text{g}/\text{mL}$) and IVIg (400 $\mu\text{g}/\text{mL}$), confirming Fc γ RIIa-dependent platelet activation. Platelet activation was also demonstrated to a lesser degree with increasing amounts of Moderna vaccine (Figure 1C) and the excipient PEG2000. Spike-SRA platelet activation was not observed in patients with high titre anti-Spike antibodies who had recovered from severe (n = 5) or mild (n = 3) COVID-19. Furthermore, platelet activation was not detected in a control sample from a patient who had received the Moderna vaccine and had not developed thrombocytopenia; this was measured by P-selectin expression using flow cytometry (data not shown). Circulating Spike protein was detected in our patient's serum using enzyme immunoassay testing (OD = 10.4; positive control OD = 17.8; negative control OD = 0.4). Together, these results suggest that the thrombocytopenia in this patient was secondary to Fc γ RIIa-mediated platelet activation by SARS-CoV-2 Spike immune complexes.

Our serological investigations highlight a potential mechanism for COVID-19 VIT involving SARS-CoV-2 Spike-dependent, Fc γ RIIa-mediated platelet activation. Similar immune complex mediated platelet activation has also been observed with severe COVID-19 infection.^{2,4} The mechanism described here resembles platelet activation seen in HIT but does not involve anti-PF4/heparin antibodies. While HIT serves as a useful analogy, certain key differences are noted

in our case. Notably, the patient presented with bleeding symptoms as opposed to thrombosis; however, in parallel to HIT, not all patients with platelet-activating antibodies develop thrombosis¹⁰. Finally, it is unclear why only a minority of patients with anti-Spike antibodies feature such thrombocytopenia and platelet activation. One hypothesis is that platelet activation is dependent on unique Spike protein epitopes, which are only recognized by a minority of identified antibodies, as seen in HIT¹¹. Therefore, using our knowledge of platelet activation from studying HIT, we propose this mechanism for COVID-19 VIT involving SARS-CoV-2 anti-Spike antibodies.

It is important to recognize that the mechanism described here is unique from the recently reported HIT-like syndrome associated with AstraZeneca vaccination¹². In this syndrome, patients present with life-threatening thrombosis in the context of strongly positive, platelet activating anti-PF4/heparin antibodies. The mechanism of VIT proposed here is independent of anti-PF4/heparin antibodies and presents differently.

Our case also highlights the applicability of the SRA to detect platelet activation disorders aside from HIT. Although classically done in the presence of heparin, it can be modified to include various antigens to elicit immune complex formation and identify platelet activation. In our case, addition of Spike protein led to significant platelet activation that was inhibited by IV.3 and IVIg, suggesting immune complex signalling. The SRA may thus prove useful in a variety of other clinical scenarios involving platelet activation, as has been shown here.

Ultimately, the role of SARS-CoV-2 Spike protein requires further clarification in regards to platelet activation, as well as the role of vaccine- and PEG-dependent platelet activation. We postulate that a small subset of antibodies against the Spike protein, formed after vaccination, can activate platelets and cause thrombocytopenia. The prevalence of this phenomenon remains

to be clinically determined. Regardless, the modified SRA presented here may be a useful diagnostic test as more cases of vaccine-induced thrombocytopenia are recognized.

Data sharing statement

For data sharing, contact the corresponding author: nazyi@mcmaster.ca.

Acknowledgements

The authors thank the laboratory of Dr. Matthew Miller at McMaster University for providing the Spike protein. Funding support for this work was provided by COVID-19 Rapid Research Fund, Grant/Award Number: #C-191-2426729-NAZY; Canadian Institute of Health Research (CIHR)-COVID-19 Immunity Task Force (CITF), Grant/Award Number: #VR2-173204; Ontario Research Fund, Grant/Award Number: 2426729 awarded to Dr. Ishac Nazy, and Academic Health Sciences Organization (HAHSO) grant awarded to Dr. Donald M Arnold (#HAH-21-02).

Authorship Contributions

J Appelbaum provided clinical care of the patient, analyzed and interpreted data and wrote the manuscript. D.M. Arnold designed the research and wrote the manuscript. J.G. Kelton designed the research and wrote the manuscript. T. Gernsheimer provided clinical care of the patient, analyzed and interpreted data and wrote the manuscript. S.D. Jevtic analyzed and interpreted data and wrote the manuscript. N. Ivetic carried out additional experiments and analyses. J.W. Smith carried out the described studies, analyzed data, and wrote the manuscript. I. Nazy designed the research, analyzed and interpreted data, and wrote the manuscript.

All authors reviewed and approved the final version of the manuscript.

Disclosure of Conflicts of Interest

The authors declare no competing financial interests.

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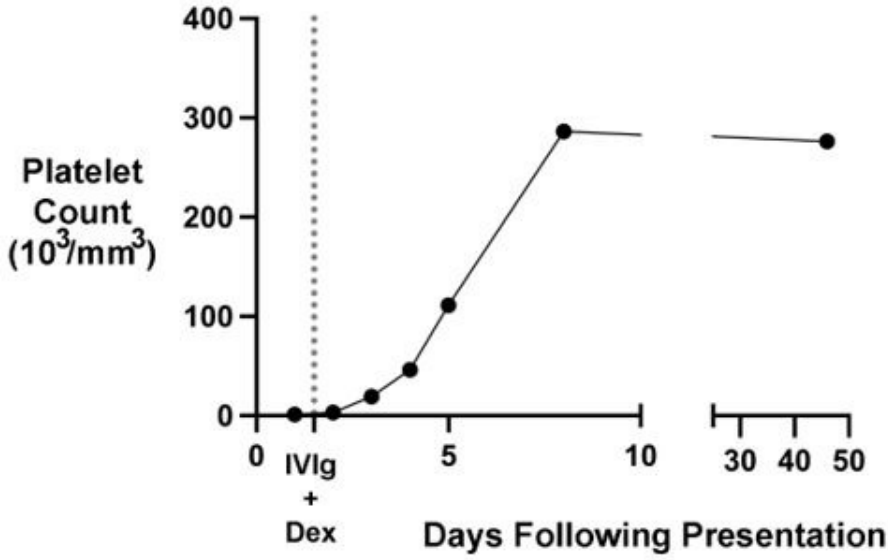
Figure Legend:

Figure 1. Patient Platelet Count and Functional Activation in Vaccine-Induced Thrombocytopenia. Patient platelet count (Panel A) and investigation of platelet activation using a modified Serotonin Release Assay (SRA) with exogenous addition of Spike protein (Panel B) or vaccine (Panel C). The platelet count fully recovered by day 7 of treatment with dexamethasone and intravenous immunoglobulin (IVIg). Serum from the patient (black squares) caused dose-dependent platelet activation and serotonin release with spike protein (93%, 100 $\mu\text{g}/\text{mL}$) and with vaccine (53%, 50 $\mu\text{L}/\text{mL}$). This effect was not observed with plasma from recovered COVID-19 subjects with severe (n=5, black circles) or mild infection (n=3, white circles). The activation was inhibited with Fc γ RIIa blockade using the monoclonal antibody IV.3 (5 $\mu\text{g}/\text{mL}$) or IVIg (400 $\mu\text{g}/\text{mL}$).

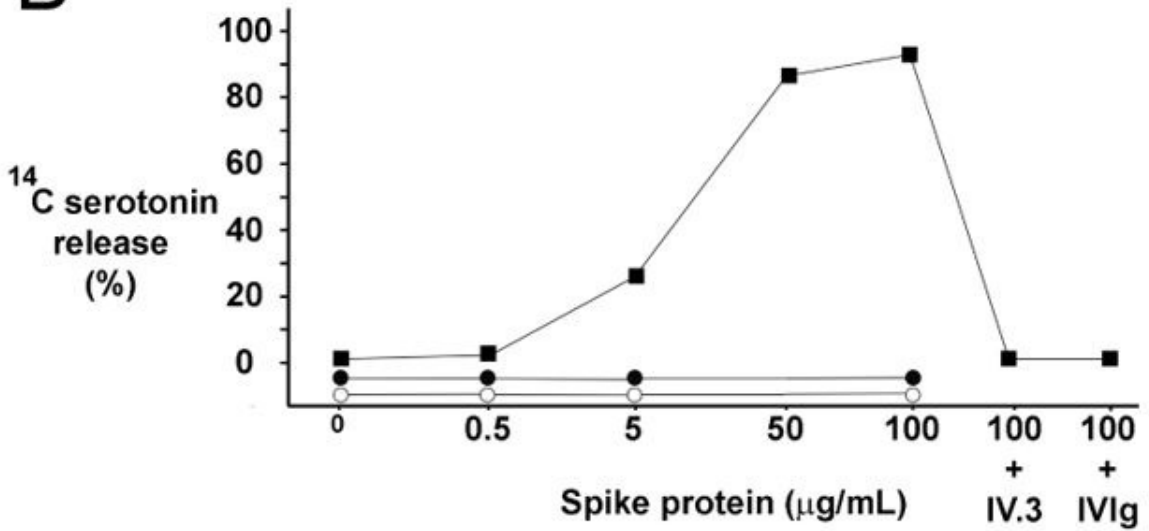
Figure 2. Flow Cytometry of IgG Binding to the Platelet Surface. Platelets incubated with plasma sample from our patient (Green) failed to show IgG binding with exogenous Spike protein administration (Red). Negative buffer control (Black) and positive Glanzmann thrombasthenia (Blue) are shown for comparison.

Figure 1

A



B



C

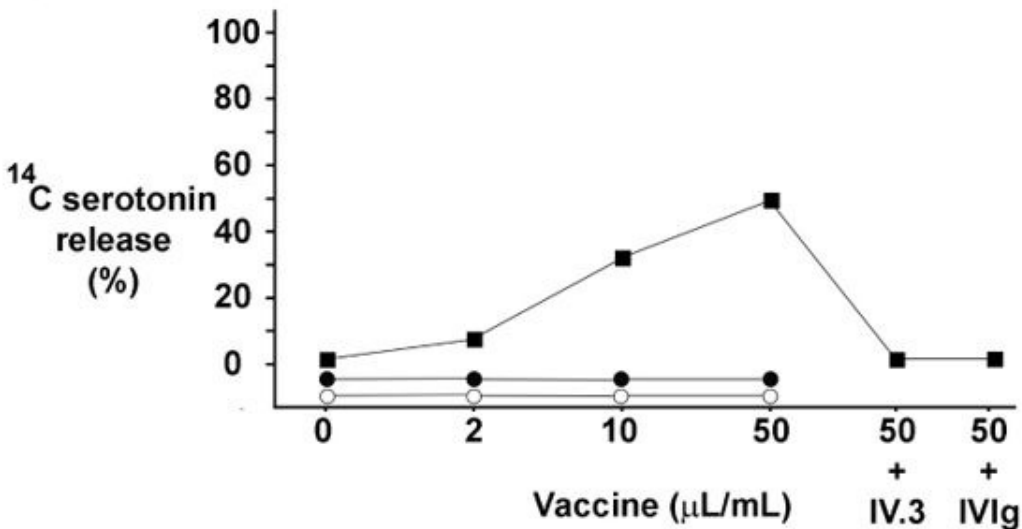
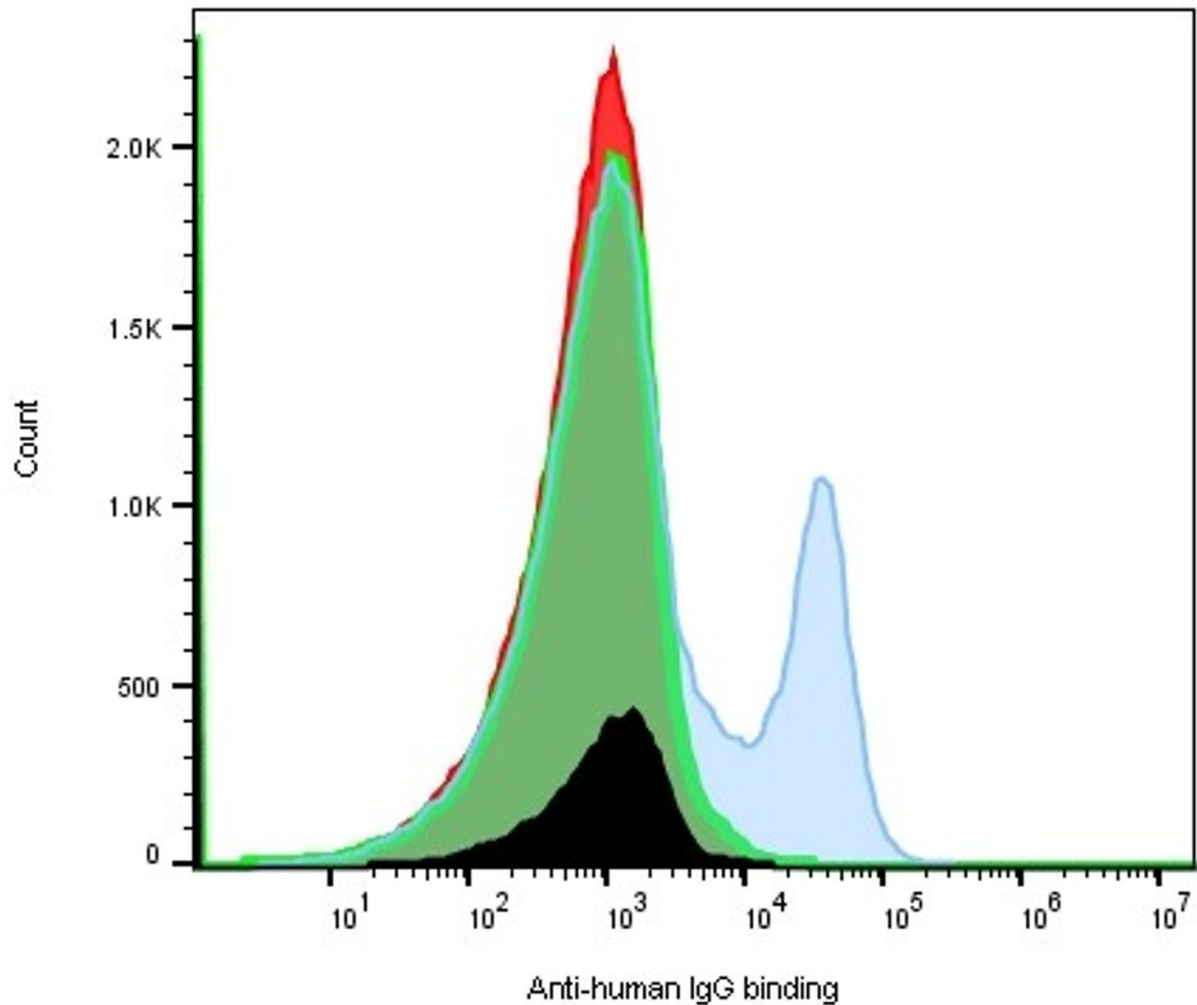


Figure 2



- Buffer
- Glanzmann plasma
- Patient plasma
- Patient plasma + Spike protein