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Dr. Eli Estey contributed extensively to this manuscript and passed away prior to submission.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

PATIENT CONSENT STATEMENT

All participants gave informed consent on Fred Hutch IRB-approved protocols for the use of their medical data and protected health identifiers in research.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author, subject to a data sharing agreement and documentation of appropriate IRB approval.

Elizabeth F. Krakow[1,2], Roland B. Walter[1,3], Julia M. Nathe[1], Tess Perez[1], Ali Ahmed[1], Nayak Polissar[4], Ljubomir Miljacic[4], Anna B. Halpern[1,3], Mary E. D. Flowers[1,2], Eli Estey[1,3]

[1]*Clinical Research Division of the Fred Hutchinson Cancer Research Center, Seattle, WA, USA*

[2]*Division of Medical Oncology, University of Washington, Seattle, WA, USA*

[3]*Division of Hematology, University of Washington, Seattle, WA, USA*

[4]*The Mountain-Whisper-Light: Statistics & Data Science, Seattle, WA, USA*

Correspondence

Elizabeth F. Krakow, Clinical Research Division of the Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Mailstop D5-297, Seattle, WA 98109, USA.

Email: efkrakow@fredhutch.org

ORCID

Elizabeth F. Krakow https://orcid.org/0000-0002-2690-3799

Roland B. Walter https://orcid.org/0000-0002-9268-3341

Anna B. Halpern https://orcid.org/0000-0002-4326-0748

Mary E. D. Flowers https://orcid.org/0000-0003-1631-0911

Eli Estey https://orcid.org/0000-0002-0987-8077

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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COVID-19 mRNA-1273 vaccine induces production of vaccine-induced immune thrombotic thrombocytopenia antibodies

To the Editor:

Recently, we reported on a 65-year-old male who developed bilateral pulmonary emboli and lower extremity deep venous thrombosis associated with severe thrombocytopenia (14 000/μL) ten days after receiving the second dose of the coronavirus disease 2019 (COVID-19) mRNA–1273 vaccine.[1] The patient was treated with intravenous immunoglobulin G and steroids for presumed immune thrombocytopenia, followed by unfractionated heparin. HIT testing obtained at two time points, immediately upon admission (first sample: prior to heparin therapy) and nine days into admission (second sample: after initiation of heparin therapy), demonstrated high optical densities (OD) in PF4-polyanion ELISA testing of 2.855 and 2.669, respectively.[1] A conventional serotonin release assay (that utilizes low concentrations of heparin) performed on the second sample was positive (51%; no functional testing was performed on the initial sample).[1] Despite the cessation of heparin and initiation of bivalirudin treatment, the patient worsened due to the development of cerebral venous sinus thrombosis, shock, lactic acidemia, compartment syndrome, sepsis, and ultimately died on day 12 of admission.[1] In the report, we suggested the possibility of vaccine-induced immune thrombotic thrombocytopenia (VITT), the first case of this type after mRNA vaccination. However, more recently, due to the rarity of this event (0.00855 per million mRNA-based COVID-19 vaccines[2]), and current dogma that VITT is an adenoviral-vector associated syndrome, the Centers for Disease Control and Prevention (CDC) recently concluded that this likely represented "…a background rate of spontaneous HIT or TTS associated with a different risk factor than cases associated with Ad26.COV2.S vaccination."[2]

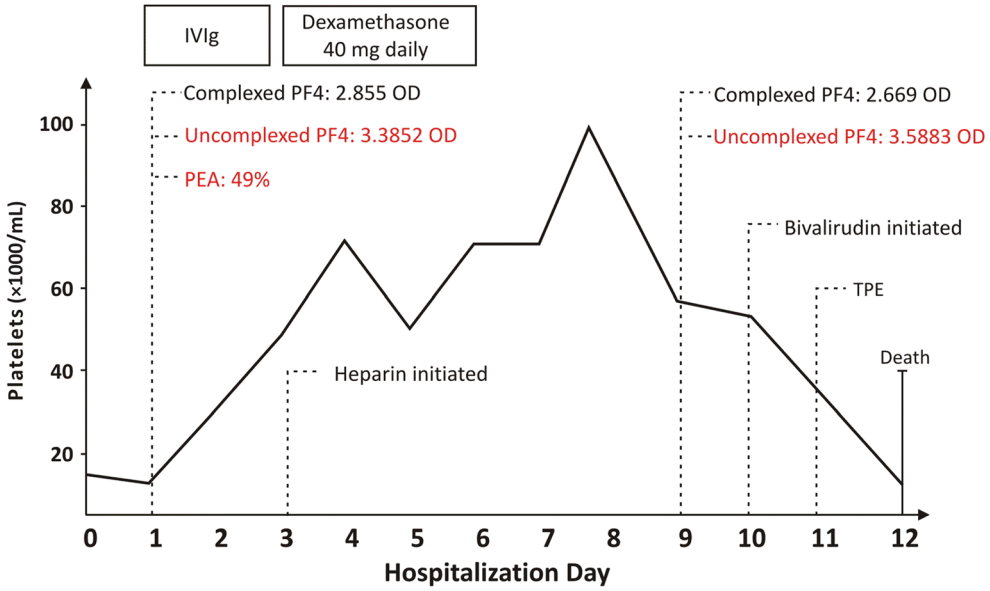

FIGURE 1 mRNA-1273 vaccine-associated antibodies recognize un-complexed PF4 targets. Key testing, intervention, and platelet count trending are provided. The abscissa denotes days of hospitalization, and the ordinate shows the platelet count. Complexed PF4:PF4-polyanion enzyme-linked immunosorbent assay (ELISA) (Lifecodes PF4 IgG); un-complexed PF4:antibody binding to un-complexed PF4 targets in an ELISA format; PEA, PF4-dependent P-selectin Expression Assay; TPE, therapeutic plasma exchange. Newly generated data since the prior report on this patient[1] are indicated in red. Some data are reproduced with permission from Sangli et al.[1] ©American College of Physicians

A challenge in distinguishing between VITT and the "background rate" of thrombotic thrombocytopenia due to anti-PF4 antibodies (i.e., spontaneous HIT) is a lack of tests capable of differentiating the two[3]; antibodies from both entities are detected in current ELISA and functional assays. In a just-published report in the *American Journal of Hematology*,[4] Kanack and colleagues make the novel finding that binding of antibodies to un-complexed PF4 can distinguish between these two syndromes. Thus, postmortem, the un-complexed PF4 ELISA was used to further characterize our patient's anti-PF4 antibodies. To avoid confounding antibodies that may have developed after heparin exposure, the preheparin (first) blood sample was initially tested. Figure 1 shows that this sample demonstrated a high OD of 3.3852, consistent with VITT antibodies, and was also found to be platelet-activating in the PF4-dependent P-selectin expression assay (PEA), an assay that uses PF4-treated platelets for the sensitive detection of VITT[4] and HIT antibodies.[5] The follow-up sample continued to be strongly positive in the uncomplexed PF4 ELISA (OD 3.5883), also consistent with VITT. These data are consistent with the possibility that nonadenoviral, mRNA-based vaccines can cause VITT in rare instances. To add to this case, the CDC reports two additional patients with a clinical/laboratory picture consistent with VITT after mRNA-1273 vaccination, including thrombosis (at unusual sites: cerebral venous sinus in one and mesenteric artery thrombosis in the other), thrombocytopenia, highly elevated d-dimers, and strong positive HIT ELISAs (OD > 1.0) in both patients.[2] To the best of our knowledge, samples from these patients have not been tested against un-complexed PF4 targets. In addition, a recent case of VITT has been reported after HPV vaccination (recombinant human papillomavirus quadrivalent vaccine), which uses nonadenoviral virus-like particle vaccine technology.[6] Thus, we believe the emerging data on VITT after nonadenoviral vector vaccines highlighted by our case suggests that VITT should remain on the differential diagnosis for thrombotic thrombocytopenic reactions seen after multiple different vaccine types so that an accurate diagnosis can be made and appropriate treatment interventions promptly instituted.

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CONFLICT OF INTEREST

AP reports pending/issued patents (Mayo Clinic, Retham Technologies and Versiti), equity ownership in Retham Technologies, and serving on the advisory board of Veralox Therapeutics. The remaining authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

Anand Padmanabhan and Swathi Sangli wrote the first draft and designed the figure. Adam J. Kanack performed un-complexed PF4 ELISA testing. Robert B. Kaplan and Adam J. Kanack provided helpful input. All authors edited the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Anand Padmanabhan[1], Adam J. Kanack[1], Robert B. Kaplan[2], Swathi Sangli[3]

[1]*Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA*

[2]*Department of Hematology and Cellular Therapy, Allegheny Health Network, Pittsburgh, Pennsylvania, USA*
[3]*Department of Pulmonary and Critical Care, Allegheny Health Network, Pittsburgh, Pennsylvania, USA*

Correspondence
Swathi Sangli, Division of Pulmonary and Critical Care, Allegheny Health Network, 4800 Friendship Avenue, Pittsburgh, PA 15224, USA.
Email: swathi.sangli@ahn.org

Anand Padmanabhan, Division of Hematopathology, Transfusion Medicine & Experimental Pathology, 200 1 Avenue, Rochester, MN 55905, USA.
Email: padmanabhan.anand@mayo.edu

ORCID

Anand Padmanabhan https://orcid.org/0000-0003-2519-4377

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