Transient prothrombotic disorders caused by platelet-activating antibodies against platelet factor 4 (PF4) include heparin-induced thrombocytopenia (HIT), spontaneous HIT syndrome,1 and, most recently, vaccine-induced immune thrombocytopenia (VITT).2 Here, we identified prothrombotic, platelet-activating anti-PF4 antibodies, not associated with heparin treatment, in a patient with monoclonal gammopathy that resulted in a chronic hypercoagulability state.

Mid-2019, a 79-year-old caucasian female with a history of unprovoked right-lower-limb deep-vein thrombosis (DVT) had experienced 1 year earlier thrombocytopenia and recurrent DVT with subsequent pulmonary embolism and stroke despite therapeutic anticoagulation (apixaban, 5 mg twice daily) (Figure 1A). Anticoagulation was switched to a vitamin K antagonist. Her platelet count was low (<115×10⁹/L) but had been within the normal range (250–330×10⁹/L) in previous years. Prothrombin-international normalized ratio (PT-INR) values where within the therapeutic range and stable; however, platelet count remained persistently low. In July 2020, she was re-admitted with pulmonary embolism and stroke despite therapeutic anticoagulation (apixaban, 5 mg twice daily) and low-dose acetylsalicylic acid (100 mg daily), without new thromboembolic events as of September 2021. In 2020, repeated SARS-CoV-2 PCR analyses of nasopharyngeal swabs and antibodies against SARS-CoV-2 nucleocapsid, spike protein (receptor-binding domain), and trimeric spike protein were negative. The patient has not received a COVID-19 vaccine at time of reporting.

In August 2020, IgG-specific PF4/heparin (HIT)-enzyme-linked immunosorbent assay (ELISA) was positive (optical density >2.0 [reference range, <0.5]), while functional testing excluded presence of heparin-dependent, platelet-activating antibodies. She also tested negative for antiphospholipid syndrome, JAK2 V617F mutation, and paroxysmal nocturnal hemoglobinuria. There was no evidence for underlying malignancy (negative gastro-/colonoscopy and computerized tomography [CT] imaging of abdomen/pelvis) or rheumatologic diseases (antinuclear/ ds-DNA antibodies negative, no complement consumption) and bone marrow aspirate was without pathological findings. In June 2021, serum immunofluorescence electrophoresis revealed a monoclonal paraprotein of IgG-k type (M-gradient was 9.6%), with IgG-specific PF4/heparin-ELISA remaining strongly positive (Figure 1A). We re-analyzed the patient serum sample of August 2020 in a washed platelet aggregation assay. As typically seen in VITT, patient serum induced platelet activation that was amplified by addition of PF4. In contrast, addition of heparin did not enhance patient serum-triggered platelet aggregation (Figure 1B). Together the data indicate VITT-like anti-PF4 antibodies. In order to confirm the existence of VITT-like anti-PF4 antibodies in this unvaccinated monoclonal gammopathy patient, we used the deglycosylated monoclonal anti-PF4 antibody (DG-1E12)—which binds the identical epitope on PF4 as VITT antibodies, without activating platelets.3 DG-1E12 interfered with VITT patient serum-driven platelet aggregation in the presence of PF4 (see control in Figure 1B) and also markedly inhibited PF4-dependent platelet aggregation induced by the gammopathy patient serum. We affinity purified anti-PF4 antibodies from the gammopathy patient serum.4 The k-light chain IgG monoclonal band (equal to paraprotein) strongly cross-reacted with immobilized PF4/heparin complexes in an ELISA (optical density >2.0). Similar to the monoclonal gammopathy patient serum, immunopurified antibodies also initiated platelet aggregation strictly in a PF4-dependent manner (Figure 1B). Thus, our patient’s IgG-k paraprotein shares similarities with pathologic VITT antibodies, by (i) binding to PF4 and (ii) activating platelets in a Fc receptor-dependent manner. 

Our gammopathy patient, with a persisting PF4-reactive monoclonal IgG paraprotein that directly activates platelets leading to persistent thrombocytopenia and recurrent thrombosis, has a chronic hypercoagulability state that strongly correlates both with the degree of thrombocytopenia and D-dimer elevation (Figure 1C). A previous case of spontaneous HIT syndrome associated

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Figure 1. Continued on following page.
with IgG-κ paraprotein has been reported (although in that patient PF4-dependent reactivity profile was not reported).5

In conclusion, PF4-dependent platelet-activating antibodies causing chronic thrombocytopenia and persisting hypercoagulability may underly chronic prothrombotic disorders such as monoclonal gammopathy. The spectrum of anti-PF4 antibody mediated hypercoagulability states should be extended beyond heparin (HIT) and vaccine-induced thrombotic thrombocytopenia (VITT) to paraproteins in neoplastic disease.

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Figure 1: History of platelet counts, thromboembolic events, and PF4-based diagnostic tests in a patient with monoclonal gammopathy. (A) The shaded area indicates the normal reference range of peripheral platelet counts (150–400×10^9/L). (B) Patient serum or affinity purified anti-PF4 antibodies were tested with washed platelet from 3 healthy donors in the presence of buffer, low-molecular-weight heparin (reviparin, 0.2 aFXaU/mL), PF4 10 µg/mL, or deglycosylated anti-PF4 antibody DG-1E12 (100 µg/mL) in the functional heparin-induced platelet activation test (HIPA). The lag time until platelet aggregation occurred is indicated in minutes (min). Shorter lag time indicates stronger platelet activation. As reactivity between different platelet donors can vary, reactivity of each platelet preparation is given as a data point. Serum samples from August 2020 and November 2020 as well as affinity purified anti-PF4 antibodies induced platelet activation in the presence of PF4, but were negative following addition of buffer or heparin both at low (LMWH 0.2 aFXaU/mL, or high heparin (100 IU/mL) not shown). Also the monoclonal antibody IV.3 inhibited platelet activation in the presence of patient serum or the affinity purified anti-PF4 antibody fraction (data not shown). The serum of a vaccine-induced immune thrombotic thrombocytopenia (VITT) patient was used as positive control. (C) Correlation between peripheral platelet counts and plasma D-dimers during the course of treatment indicates that D-dimer levels increased when platelet counts decreased, a finding consistent with platelet count reduction due to procoagulant activation and consumption. DVT: deep vein thrombosis; PE: pulmonary embolism; VKA: vitamin K antagonist; LMWH: low-molecular-weight heparin; FPX: fondaparinux; ASA: acetylsalicylic acid; PF4: platelet factor 4; ELISA: enzyme-linked immunosorbent assay; OD: optical density; FEU: fibrinogen equivalent units; aFXaU/mL: anti-factor Xa activity in units/mL; DG-E12: deglycosylated monoclonal antibody E12; Ig: immunoglobulin.
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References
1. Warkentin TE, Greinacher A. Spontaneous HIT syndrome: knee replacement, infection, and parallels with vaccine-induced immune thrombotic thrombocytopenia. Thromb Res. 2021;204:40-51.
2. 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.
3. Vayne C, Nguyen TH, Rollin J, et al. Characterization of new monoclonal PF4-specific antibodies as useful tools for studies on typical and autoimmune heparin-induced thrombocytopenia. Thromb Haemost. 2021;121(3):322-331.
4. Greinacher A, Selleng K, Mayerle J, et al. Anti-platelet factor 4 antibodies causing VITT do not cross-react with SARS-CoV-2 spike protein. Blood. 2021;138(14):1269-1277.
5. Faille D, Hurtado-Nedelec M, Ouedrani A, et al. Isolation of a monoclonal IgG kappa with functional autoantibody activity against platelet factor 4/heparin from a patient with a monoclonal gammopathy of undetermined significance and clinically overt heparin thrombocytopenia. Res Pract Thromb Haemost. 2017;1(Suppl 1):S185.