Long-term persistence of high anti-PF4 antibodies titer in a challenging case of AZD1222 vaccine-induced thrombotic thrombocytopenia

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ABSTRACT

A syndrome occurring after adenoviral vector anti-SARS-CoV-2 vaccination, characterized by thrombocytopenia, venous thrombosis, and circulating anti-PF4 antibodies, known as vaccine-induced immune thrombotic thrombocytopenia (VITT), is well described. Data on the long-term course of this syndrome are lacking. Our aim is to report the clinical and laboratory features of a patient with VITT from diagnosis and during 21 months of follow-up. Cerebral venous thrombosis associated with elevated D-dimer, low fibrinogen, thrombocytopenia, and anti-PF4 antibodies positivity occurred in this patient after ChAdOx1 nCoV-19 vaccination. Cerebral thrombosis required a revascularization procedure and decompressive craniectomy. Upon dexamethasone and anticoagulant treatment initiation, the platelet count recovered. However, a persistently high anti-PF4 antibody titer, without thrombosis recurrence, was observed. Little is known about the long-term persistence of anti-PF4 antibodies, their clinical significance, and their possible role in guiding therapeutic decisions. In our patient, we decided to continue anticoagulant treatment beyond 21 months with parallel anti-PF4 antibody monitoring.

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Citation: Barcella L, Ambaglio C, Gritti P, et al. Long-term persistence of high anti-PF4 antibodies titer in a challenging case of AZD1222 vaccine-induced thrombotic thrombocytopenia. Bleeding, Thrombosis, and Vascular Biology 2023;2:72.

Key words: anti-SARS-CoV2 vaccine, thrombosis, thrombocytopenia, anti-PF4 antibody, immune.

Contributions: LB, CA, PG, FS, VB, MM collected the data, contributed to the interpretation of the results and wrote the manuscript. MM and ES performed laboratory testing. AF wrote and critically revised the manuscript.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: the study was partially supported by the Fondazione ARTET Onlus-Bergamo, Italy.

Availability of data and materials: data and materials are available by the authors.

Informed consent: written informed consent for publication was obtained from the patient.

Acknowledgments: the authors thank the Fondazione ARTET Onlus-Bergamo for its support.

Received: 18 March 2023.
Accepted: 1 June 2023.

Bleeding, Thrombosis and Vascular Biology 2023; 2:72
doi:10.4081/btvb.2023.72

Introduction

Several reports are currently available on ChAdOx1 nCoV-19 (AZD1222, AstraZeneca) vaccinated patients, who developed thrombocytopenia, venous thrombosis at unusual sites, and elevated anti-platelet factor 4 (PF4) antibody levels with platelet-activating capacity, which characterize a specific medical emergency called vaccine-induced thrombotic thrombocytopenia (VITT).[1-3]

While the first cases presented high mortality rates, awareness to encourage early presentation combined with a clearer pathway for diagnosis and immediate treatment had reduced mortality to less than 5%, as described in Australian patients (https://www.tga.gov.au/periodic/covid-19-vaccine-weekly-safety-report/current).

Although diagnosis and acute-phase treatment recommendations have been released,[4-7] data on the long-term course and management of this syndrome is lacking. The evolution of antibodies and their ability to activate platelets remains not well-defined.

Early experience suggests that anti-PF4 antibodies persist at least for several months;[8] the National Institute for Health and Care Excellence guidelines suggests anti-PF4 antibody measurements weekly for 4 weeks and then monthly for 6 months.[9]

A decline over time of pathogenic antibodies against PF4 has been recently described.[10-12] In a German case series, only five patients (7.5%) showed persistent platelet-activating antibodies and high IgG anti-PF4/heparin antibody levels for more than 11 weeks. In a UK cohort of patients, the median duration of positivity of the PF4 assay is 87 days with 72% of patients remaining

positive after a median follow-up of 105 days. The rate of relapse was 12.6% with all cases exhibiting persistently positive PF4 antibodies and falling platelet count, in one case with extension of thrombosis.[13]

Here we report on the clinical and laboratory features of a patient with VITT at diagnosis and after 21 months of follow-up, showing a long-term persistence of high titer of anti-PF4 antibodies.[14]

Case Report

A 61-year-old woman with a personal history of SARS-CoV2 infection in March 2020 received her first dose of the AZD1222 vaccine on May 5th, 2021. Known comorbidities were arterial hypertension, nephrolithiasis, and hyperuricemia, all treated by specific medications. Nonspecific antinuclear antibody positivity was also known since 2019. Twelve days after vaccination, she developed an acute frontal headache unresponsive to analgesics, and subsequent disorientation and drowsiness.

The Out-of-Hospital Emergency Service was alerted due to the severe clinical conditions of the patient, who was urgently transferred to the Emergency Department of our Hospital. Her complete blood cell counts were normal except for thrombocytopenia (44×10^9/L). Prothrombin time ratio and partial thromboplastin time ratio were normal, fibrinogen level was 70 mg/dL, and D-Dimer level >35,000 ng/mL (Table 1). The direct Coombs test was negative, and no schistocytes were observed in the peripheral blood smear. Molecular and antigenic nasal swab tests for SARS-CoV-2 were negative. A non-contrast brain computed tomography (CT) scan showed a temporal intracerebral hemorrhage and a left subdural hematoma, while a contrast brain CT scan showed thrombosis of the left transverse and sigmoid sinuses and jugular vein (Figure 1A-C), subsequently confirmed by digital angiography (Figure 1D).

In the suspicion of VITT, anticoagulation was started with low dose fondaparinux 2.5 mg daily, and the patient was transferred to the Intensive Care Unit. A blood sample for anti-PF4 IgG antibody testing was immediately sent to our specialist laboratory (Immunohematology and Transfusion Medicine). The enzyme linked immunosorbent assay, which detects IgG against PF4/polyanions complexes (ELISA, PF4 Enhanced, IMMUCOR), was strongly positive [2.810 Optical Density (OD); normal values <0.400 OD] (Table 1 and Figure 2). The multiple-electrode aggregometry (MEA), performed to verify whether these antibodies have platelet-activating properties, showed that the patient's plasma could induce the aggregation of platelets from normal control subjects, an effect that was potentiated in the presence of a low-dose of heparin (0.5 IU/mL) and inhibited at a high heparin dose (200 IU/mL). High titer (IgG 4+) of anti-SARS-CoV2 IgG was detected by the chromatographic immunoassay qSars-CoV-2 IgG Cassette Rapid Test (Cellex). This positivity was further

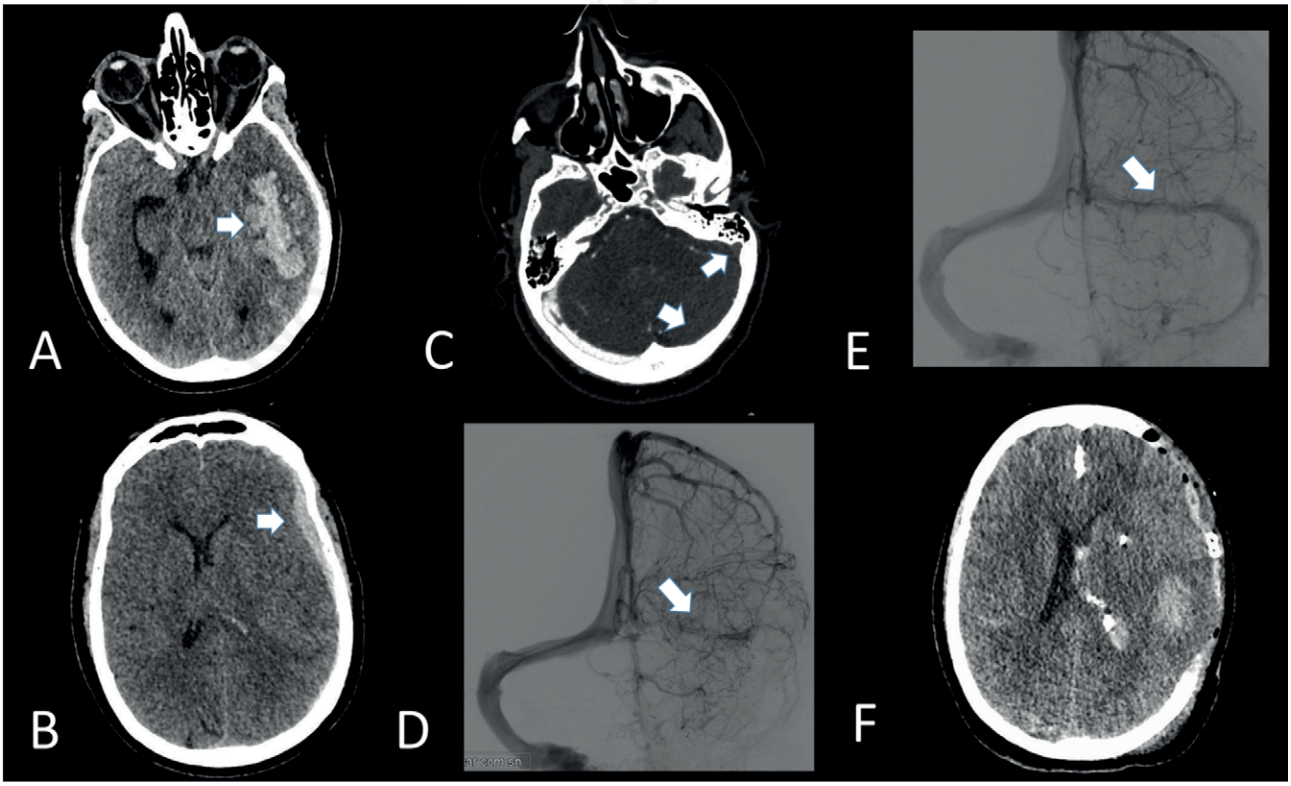

Figure 1. Non-contrast brain computed tomography (CT) scan showing left temporal intracerebral hemorrhage (A) and subdural hematoma (B). CT venogram revealed a filling defect in the left transverse sigmoid sinus (C), while digital angiography confirmed thrombosis of the left transverse and sigmoid sinuses and jugular vein (D) that was partially resolved at the end of endovascular treatment (E). Non-contrast brain CT scan showing left decompressive craniectomy of the patient (F).

confirmed by the measurement of specific anti-Spike IgG (anti-S SARS-Cov2 IgG, Abbott). The diagnosis of VITT was made and dexamethasone (40 mg per day for 4 days) was started.

Due to the severity of the intracerebral hemorrhage and the risk of intracranial hypertension, the patient underwent a cerebral thrombosis revascularization procedure (Figure 1D,E), followed by left decompressive craniectomy (Figure 1F). After these invasive procedures, anticoagulation was resumed by intravenous (i.v.) continuous infusion argatroban, targeting an aPTT ratio of 2.0, range 1.5-2.5, as per ISTH-SSC recommendations.[4] The platelet counts markedly improved in the subsequent days. After 27 days, she underwent autologous cranioplasty and a week later she stopped i.v. argatroban and switched to subcutaneous fondaparinux at the dose of 7.5 mg once daily. About 2 weeks later, on July 2nd, 2021, she was transferred to the rehabilitation Institute, where she switched to oral anticoagulant therapy with warfarin targeting a PT-INR of 2.5 (therapeutic range 2.0-3.0).

In September 2021, after 4 months, she was discharged from rehabilitation in good general conditions, with notes of fluent aphasia (Wernicke's aphasia) in marked improvement and a Barthel Index of 95/100.

At follow-up visits at the outpatient Hemostasis and Thrombosis Clinics in December 2021 and July 2022 (respectively +198 and +413 days from the onset of the VITT) the patient was in good health. As shown in Table 1, blood cell counts, including platelets, were normal, but anti-PF4 antibodies resulted persistently positive.

In October 2022, she underwent urgent cholecystectomy for acute emphysematous cholecystitis; anticoagulant therapy with warfarin was suspended and fondaparinux 2.5 mg/day was introduced postoperatively. After a week Fondaparinux, oral anticoagulant therapy was resumed with apixaban 2.5 mg every 12 hours. At follow-up visits at the outpatient Hemostasis and Thrombosis Clinics in January 2023 (+610 days from VITT onset), blood cell counts, including platelets, were normal, however anti-PF4 antibodies resulted still positive. We decided to continue apixaban 2.5 mg bid.

Written informed consent for publication was obtained from the patient.

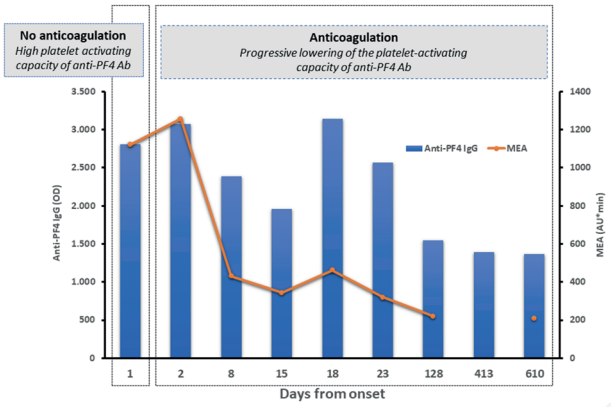

Figure 2. Modifications in anti-PF4 IgG antibodies and their platelet activating capacity over time.
At onset, the high titer of anti-PF4 antibodies (by ELISA) displaying a full platelet activating capacity by multiple-electrode aggregometry (MEA) contributed to the development of the thrombotic event. After diagnosis, the continuous administration of an anticoagulant therapy together with the progressive decrease in the platelet activating capacity of anti-PF4 antibodies, in the absence of the triggering factor (*i.e.*, the vaccine), was effective in the prevention of a thrombotic recurrence in this patient.

Discussion

ChAdOx1 nCov-19 vaccine against SARS-CoV2, currently named as AZD1222, employs an Adenovirus derived from the chimpanzee. The added sequence encodes for the full-length S protein with a tissue plasminogen activator signal sequence. Starting from late February 2021, some reports documented the appearance in healthy individuals of complications such as thrombocytopenia and thrombosis at atypical sites (in particular, cerebral and/or splanchnic) within 2 weeks from ChAdOx1 nCov-19 vaccination. This new syndrome, named VITT, has been associated with AZD1222 or Ad26.COV2.S vaccines.[15]

In the first reports, patients were primarily younger than 60

Table 1. Laboratory parameters of the patient at hospital admission (day 0) and during the 21 months of follow-up

Days	Hb, g/L (120-160)	WBC, 10^9/L (4.2-9.4)	Platelets, 10^9/L (150-400)	Fibrinogen,L mg/d (150-400)	D-dimer, ng/mL (< 500)	CRP, mg/dL (0.0-0.5)	Anti-PF4 IgG, OD (<0.400)	MEA, AUmin (<50)
0	133	7.46	44	70	> 35,000	1.5	-	-
+1	114	6.52	38	184	-	-	2.810	1,120
+2	87	4.79	72	132	17,882	3.3	3.070	1,256
+8	88	6.53	217	507	4,312	5.0	2.387	431
+15	100	4.41	210	547	-	4.5	1.960	343
+18	101	4.94	205	584	-	2.1	3.140	461
+23	101	6.35	262	501	-	11.2	2.565	320
+128	138	3.89	213	-	-	-	1.550	220
+413	149	5.43	221	-	-	0.1	1.388	-
+610	144	4.07	214	-	<190	-	1.370	210

Hb, hemoglobin; WBC, white blood cells; OD, Optical Density; CRP, C-reactive protein; MEA, multiple-electrode aggregometry; AU, arbitrary units.

years and were mostly females. None had received heparin earlier, and few had other known risk factors for thrombosis. Many of the patients were critically ill by the time thrombosis with thrombocytopenia was diagnosed, and mortality rate was high.[16] The pathogenic mechanisms of VITT have been linked to the production of IgG autoantibodies targeting PF4 which activate platelets via their FcgRIIa receptors, as occurring in autoimmune heparin-induced thrombocytopenia (HIT).[17] In HIT, PF4/heparin complexes progressively decrease in circulation with parallel reduction and negativization of the antibody titer in the following 7-12 weeks, upon interruption of the heparin treatment. Less is known in the VITT setting. One study, in 35 patients with VITT followed-up for a median time of 11 weeks (range, 4-19), showed that in more than 90% of patients, pathologic, platelet-activating anti-PF4 antibodies disappeared within 12 weeks, while the serological reversion (*i.e.,* negative ELISA result) occurred only in 3 patients.[12]

Our patient, after 21 months, still shows a persistently high titer of antibodies, but a normal platelet count. According to the above theory, the presence of anti-PF4 antibodies is the underlying condition causing VITT, however, our case of positive anti-PF4 titer in clinical remission phase suggests that other factors may intervene in the acute setting to determine the activation of coagulation, mainly in the cerebral or splanchnic venous districts. Alternatively, we can assume that anti-PF4 antibodies are less reactive to platelets over time, as demonstrated by the decrease in the platelet activating capacity by the functional assay, or that the patient is protected by the anticoagulant therapy.

We believe in the protective role of anticoagulation and indeed we kept our patient on long-term anticoagulation, without bleeding complications or thrombotic recurrences. Differently from our case, a complicated VITT case with extremely high anti-PF4-IgG titers over three months was characterized by recurrent thrombocytopenia despite treatment with i.v. IG and anticoagulation and ended with a massive hemorrhage in the left parietal and temporal lobe without signs of new thrombosis.[11] This supports the relevance of deciding the optimal timing and dosing of anticoagulant therapy in VITT patients with persisting high titer anti-PF4 antibodies.

Conclusions

Little is known about long-term follow up of patients who survived to VITT. The case here described shows how rapid recognition and treatment of VITT is crucial for the short and long-term clinical outcomes of this severe syndrome. Also, laboratory monitoring of anti-PF4 IgG antibody and their platelet activating capacity, together with platelet count, over time, can be of importance in these patients, as anticoagulant therapy might continue until the pathogenic mechanism is active. The disappearance of pathogenic antibodies is an important criterion also for deciding for the subsequent vaccination with a mRNA vaccine. However, when no platelet-activation assay is available, a substantial decline (more than 50%) in the optical density of anti–PF4–heparin IgG ELISA has been suggested as a decision criterion.[12]

Whether the presence or not of high titer anti-PF4 IgG antibodies may be a tool for deciding on duration of anticoagulation and/or the necessity of additional immunosuppression remains a challenge and is to be established by large studies.

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