



Journal of The Ferrata Storti Foundation

Massive cerebral venous thrombosis due to vaccine-induced immune thrombotic thrombocytopenia

by Sara Bonato, Andrea Artoni, Anna Lecchi, Ghil Schwarz, Silvia La Marca, Lidia Padovan, Marigrazia Clerici, Chiara Gaudino, Giacomo Pietro Comi, Armando Tripodi, and Flora Peyvandi

Haematologica 2021 [Epub ahead of print]

*Citation: Sara Bonato, Andrea Artoni, Anna Lecchi, Ghil Schwarz, Silvia La Marca, Lidia Padovan, Marigrazia Clerici, Chiara Gaudino, Giacomo Pietro Comi, Armando Tripodi, and Flora Peyvandi. Massive cerebral venous thrombosis due to vaccine-induced immune thrombotic thrombocytopenia. Haematologica. 2021; 106:xxx
doi:10.3324/haematol.2021.279246*

Publisher's Disclaimer.

E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.

Massive cerebral venous thrombosis due to vaccine-induced immune thrombotic thrombocytopenia

Sara Bonato¹, Andrea Artoni², Anna Lecchi², Ghil Schwarz¹, Silvia La Marca², Lidia Padovan², Marigrazia Clerici², Chiara Guadino³, Giacomo Pietro Comi^{4,5}, Armando Tripodi², Flora Peyvandi^{2,6}

¹*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Stroke Unit, Milan, Italy.*

²*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, and Fondazione Luigi Villa, Milan, Italy.*

³*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuroradiology Unit, Milan, Italy*

⁴*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy.*

⁵*University of Milan, Dino Ferrari Center, Department of Pathophysiology and Transplantation, Milan, Italy*

⁶*University of Milan, Department of Pathophysiology and Transplantation, Milan, Italy*

Running head: A case report of VITT in a young woman

Corresponding author:

Flora Peyvandi

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center,

via Pace 9, 20122 Milan, Italy.

Tel: +39 02 55035414

Fax: +39 0254100125

Email: flora.peyvandi@unimi.it

WORD COUNT: 1461

ACNOWLEDGMENTS

The authors thank Luigi Flaminio Ghilardini (Università degli Studi di Milano, Milan) for

his help with figures.

This work was partially supported by the Italian Ministry of Health-Bando Ricerca Corrente 2020.

Authors' contribution

S. Bonato managed the clinical case, collected clinical data and wrote the clinical part of the manuscript. A. Artoni, G. Schwarz, C. Gaudino and G.P. Comi managed the clinical case, collected clinical data and critically reviewed the manuscript. A. Lecchi performed the laboratory assays, interpreted the results and contributed to write the manuscript. S. La Marca, L. Padovan and M. Clerici performed the laboratory assays. A. Tripodi organized the hemostatic assays, interpreted the results and contributed to write the manuscript. F. Peyvandi designed the study and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest disclosure

All authors do not have any conflict of interest to disclose related to this manuscript.

Since the breakthrough of coronavirus disease (COVID-19) more than 3 million people died worldwide¹ and different vaccines were developed, tested in phase 3 clinical trials and used in the general population. Few reports of moderate-to-severe thrombocytopenia and thromboses (especially cerebral-venous and splanchnic-vein thromboses) developing approximately 4-14 days after vaccination were reported. These events were related to the adenovirus vector-based DNA vaccines ChAdOx1 nCoV-19 [Oxford–AstraZeneca]^{2,3,4} or Ad26.COV2.S [Johnson&Johnson/Janssen].⁵ Recently, this new rare autoimmune syndrome that mimics heparin-induced thrombocytopenia (HIT)³ was defined as vaccine-induced immune thrombotic thrombocytopenia (VITT).⁴ Even though details on pathophysiology are still scanty, diagnostic and therapeutic recommendations were proposed by international scientific organizations.⁶⁻⁸ No definite data on risk factors are reported and it is unknown whether or not the therapeutic options currently adopted for HIT are also valid for VITT.

With this background, we describe an Italian case of severe VITT-related cerebral venous thrombosis (CVT) and bi-hemispheric hemorrhage, who was successfully treated with argatroban, intravenous Ig (IVIg) and corticosteroids. The case report is described according to CARE (CAse REport)-Statement and Checklist.⁹

A previously healthy 26-year-old female presented to the emergency department 14 days after the first injection of ChAdOx1 nCoV-19 vaccine with a headache non-responsive to anti-inflammatory drugs. On admission, she had right-sided weakness and visual disturbances. She has been on combined (estrogen-progestogen) contraceptives for more than 10 years but her past medical history was otherwise unremarkable and there was no prior exposure to heparin.

While general examination and vital signs were normal, neurological examination found a severe right-sided weakness but no visual field defects. CT scan at admission showed hyperdense rectus sinus and vein of Galen (Figure 1, panel A). MRI venography showed multifocal venous thrombosis with bilateral occlusion of parietal cortical veins, straight sinus, vein of Galen, internal cerebral veins and inferior sagittal sinus. Transverse sinuses were also partially involved but still patent (Figure 1, panel B). At the right parietal and left frontoparietal lobes an extensive venous infarction with hemorrhagic transformation was present (Figure 1, panel C). D-dimer was dramatically raised to 12,204 µg/L (reference value <500) and the platelet count was 134x10⁹/L. Given her recent exposure to ChAdOx1 nCoV-19 and clinical presentation, she was first treated with fondaparinux (5mg subcutaneously) and admitted to

ICU. Her clinical condition rapidly deteriorated with decreased consciousness, right-sided hemiplegia and complete Balint syndrome.

To perform an extensive hemostasis laboratory work-up before and after therapies, blood was collected at different time-points (T0=April 13th; T1=April 15th, and T2=April 20th, 2021) into vacuum-tubes containing 1/10 volumes of trisodium-citrate 0.109M, K-EDTA or plain tubes. Activated partial thromboplastin time (aPTT), prothrombin time (PT), D-dimer, fibrinogen and factor (F)VIII were obtained. Platelet-factor 4 (PF4)–heparin IgG antibodies (aPF4) were evaluated by a commercially-available ELISA (Immucor, Waukesha, WI, USA). Platelet-activating antibodies were evaluated by a platelet-activation test (PAT).^{2,10} Platelet function was also evaluated by using the Total Thrombus-Formation Analysis device (T-TAS®, Zacros, Fujimori-Kogyo, Tokyo, Japan),¹¹⁻¹² a flow-chamber device that assesses platelet-mediated thrombus formation in capillary channels by means of the following parameters: area under the flow-pressure curve (AUC), occlusion start-time (OST) and occlusion time (OT). Thrombin generation (TG) was measured in platelet-poor plasma.¹³ Controls were plasma samples from subjects negative for aPF4 and normal TG.

Coagulation. PT, aPTT and fibrinogen were within normal range; FV-Leiden and G20210A-prothrombin mutations were absent; antithrombin and protein C/S were normal; lupus anticoagulant and antiphospholipid antibodies were negative.

aPF4. Patient serum (T1) was positive to aPF4-Heparin ELISA (OD=1.918, reference value <0.4) and was inhibited (OD<0.5) by 100U/mL heparin.

PAT. Patient serum (T1) showed strong platelet activation on platelet poor plasma (PRP) from two controls in the presence and absence of low-dose heparin, whereas control serum showed no platelet activation. Five-days afterwards (T2), the patient serum showed significant reduction of aPF4 reactivity (OD=0.6) and no longer did activate platelets (Figure 2, panels A-C).

T-TAS. At T0, platelet thrombus formation was impaired, AUC was smaller and OT longer than the reference range. In contrast, at T1 and T2 thrombus stability improved and T-TAS parameters as well as platelet count also improved (Figure 2, panels D-F).

TG. Results at the time of hospital admission (T1) showed a marked state of hypercoagulability when compared to control, as indicated by short lag-time (8.5-vs-21.3min), increased thrombin-peak (289-vs-115nM), short time-to-peak (11.8-vs-26.2min), increased ETP (2,158-vs-1,684nMxmin) and ETP-TM ratio (0.99-vs-0.79) (Figure 3). FVIII, one of the

most potent procoagulants, was higher (200U/dL) than the upper limit of the reference range (<150U/dL). In contrast PC, the physiological inhibitor to activated FVIII was normal (88U/dL). The imbalance between FVIII and PC corresponded to an increased FVIII/PC ratio (2.3), considerably greater than the expected unity and consistently with the hypercoagulability shown by TG. There are potential limitations of the TG assessment. First, measurements (owing to assay complexity and limited blood volumes) were performed only in PRP. Therefore, the potential role of procoagulant platelets in supporting TG could not be assessed. Second, TG could not be assessed on samples obtained during the time course of the disease because soon after onset of the symptoms and preliminary diagnosis the patient was treated with anticoagulation, so that TG results would have been unreliable.

In consideration of the clinical conditions and laboratory results, IVIG (1g/kg o.d. for 2 days) and dexamethasone (40mg/day, for 4 days) were started.⁶⁻⁸ Owing to the possible need for a sudden decompressive neurosurgical intervention, anticoagulation with fondaparinux was replaced by the short-acting drug argatroban [starting dosage 1µg/kg x min with an aPTT-ratio (patient/normal) target of 1.5-2.0]. Argatroban was subsequently increased to 3 µg/kg x min. Patient's neurological conditions improved in the next few days. She was awake and fully responsive to stimuli with a progressive recovery of right upper-limb strength, partial optic ataxia and regression of apraxia. On a follow-up CT scan, the rectus sinus and the vein of Galen showed normal density with oedema in the brain tissue on both hemispheres (Figure 1, panel D). Follow-up MRI venography showed restored venous flow in the rectus sinus and the vein of Galen; right internal cerebral vein and bilateral frontoparietal cortical veins were still occluded (Figure 1, panel E), and the large intraparenchymal venous infarction was unchanged (Figure 1, panel F).

In the next few days, platelet count progressively increased to $339 \times 10^9/L$ and D-dimer decreased to normal levels, in parallel with a significant reduction of aPF4 reactivity after 3 days (OD=0.9), and after one week (OD=0.6) patient serum was no longer able to activate platelets (Figure 2). Currently (nearly two months after symptoms onset), patient has moderate disability: she has no neuropsychological deficits, can walk unassisted for short distances (sustained clonus and spasticity coexist in her right leg) and her right arm almost fully recovered. Fondaparinux was replaced with oral vitamin K antagonist.

In summary, we report a case of 26-year-old female who developed VITT following the first dose of ChAdOx1 nCoV-19. The patient had high-titer aPF4 and signs of platelet activation.

Various treatment regimens have been suggested for this rare syndrome, being all based on HIT-derived approaches. Our treatment protocol, based upon IVIG, dexamethasone and argatroban was successful with almost complete clinical, laboratory and radiological response.

VITT should be considered in post-vaccination cases of thrombosis at unusual sites, even when apparent prothrombotic risk factors are identified (oral contraceptives in our case) and irrespective of the baseline platelet count. Indeed, this patient had mild thrombocytopenia on admission, but historical testing carried out before VITT recorded a platelet count of $275 \times 10^9/L$. Thus, the platelet count had decreased by approximately 50%, in agreement with HIT and VITT diagnostic criteria. Aware of the possible diagnosis of VITT, we initially avoided a potentially detrimental heparin treatment, and this decision is likely to have played a major role in determining the positive outcome.^{2,3} Another important decision was to promptly start immune modulating therapy which caused the reduction of aPF4 titer and D-dimer. The causative prothrombotic mechanism in the reported patient is likely to be due to the antibodies to PF4 that induced a strong platelet activation even in absence of heparin exposure. The fact that the patient started to improve soon after the antibody titer decreased strongly supports this mechanism. One of the potential mechanisms that can explain the loss of serum activity in the functional assays (PAT) could be the IVIG blockade of FC α platelet receptors and/or the antibody suppression. Interestingly, platelets' ability to promote thrombus formation in vitro was greatly reduced at admission, probably as a consequence of in vivo platelet activation and the formation of exhausted platelets as observed in other pathological conditions such as disseminated intravascular coagulation or sepsis. Laboratory tests correlated well with the clinical and radiological course. All in all, our experience supports the application of an early and multidisciplinary therapeutic approach in cases of VITT, with the possibility to avoid fatalities and obtain a resolution of the syndrome as in this case.

REFERENCES

1. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *JAMA*. 2020;324(08):782-793.
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. Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med*. 2021;84(22):2124-2130.
4. Scully M, Singh D, Lown R, et al. Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med*. 2021;384(23):2202-2211.
5. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic Thrombocytopenia after Ad26.COV2.S Vaccination. *N Engl J Med*. 2021;384(20):1964-1965.
6. Oldenburg J, Klamroth R, Langer F, et al. Diagnosis and Management of Vaccine-Related Thrombosis following AstraZeneca COVID-19 Vaccination: Guidance Statement from the GTH. *Hamostaseologie*. 2021 April 1. [Epub ahead of print]
7. Nazy I, Sachs UJ, Arnold DM, et al. Recommendations for the clinical and laboratory diagnosis of vaccine-induced immune thrombotic thrombocytopenia (VITT) for SARS-CoV-2 infections: Communication from the ISTH SSC Subcommittee on Platelet Immunology. *J Thromb Haemost*. 2021;19(6):1585-1588.
8. Gresele P, Marietta M, Ageno W, et al. Management of cerebral and splanchnic vein thrombosis associated with thrombocytopenia in subjects previously vaccinated with Vaxzevria (AstraZeneca): a position statement from the Italian Society for the Study of Haemostasis and Thrombosis (SISST). *Blood Transfus*. 2021 April 15. [Epub ahead of print]
9. Riley DS, Barber MS, Kienle GS, et al. CARE guidelines for case reports: explanation and elaboration document. *J Clin Epidemiol*. 2017;89:218-235.
10. Brodard J, Alberio L, Angelillo-Scherrer A, Nagler M. Accuracy of heparin-induced platelet aggregation test for the diagnosis of heparin-induced thrombocytopenia. *Thromb Res*. 2020;185:27-30.

11. Hosokawa K, Ohnishi T, Kondo T, et al. A novel automated microchip flow-chamber system to quantitatively evaluate thrombus formation and antithrombotic agents under blood flow conditions. *J Thromb Haemost.* 2011;9(10):2029-2037.
12. Ghirardello S, Lecchi A, Artoni A, et al. Assessment of Platelet Thrombus Formation under Flow Conditions in Adult Patients with COVID-19: An Observational Study. *Thromb Haemost.* 2021 Feb 5. [Epub ahead of print]
13. Tripodi A. Detection of procoagulant imbalance. Modified endogenous thrombin potential with results expressed as ratio of values with-to-without thrombomodulin. *Thromb Haemost.* 2017(5);117:830-36.
14. Cattaneo M, Cerletti C, Harrison P, et al. Recommendations for the standardization of light transmission aggregometry: A consensus of the working party from the platelet physiology subcommittee of ssc/isth. *J Thromb Haemost.* 2013;11(6):1183-1189.

FIGURE LEGENDS

Figure 1. Neuroradiological findings at baseline and follow-up

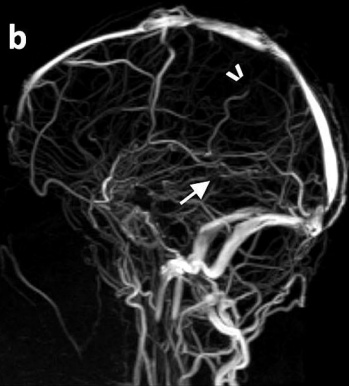
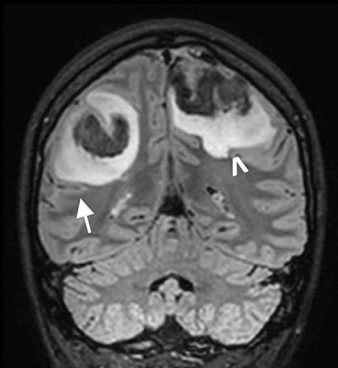
PANEL A: baseline CT (at admission) shows hyperdense rectus sinus and vein of Galen as signs of thrombosis (arrow). PANEL B: MRI examination (day 1) confirms complete occlusion of the rectus sinus, vein of Galen, right internal cerebral vein (arrow) and frontoparietal cortical veins on both sides (arrow head) on venous angiography. PANEL C: On coronal T2-FLAIR images extensive venous infarctions with hemorrhagic transformation can be seen in right parietal (arrow) and left frontoparietal (arrow head) regions. PANEL D: On follow-up CT (day 6) rectus sinus and vein of Galen show normal density (arrow) with oedema in brain tissue on both hemispheres (arrow head). Follow-up MRI (day 7) shows (PANEL E) restored venous flow in the rectus sinus and the vein of Galen (arrow); right internal cerebral vein and bilateral frontoparietal cortical veins are still occluded. PANEL F: On T2 weighted images the large bilateral venous infarctions are still visible with hemorrhagic transformation in pre- and postcentral gyrus in the left side (arrow).

Figure 2. Platelet activation test and thrombus formation analysis. Panels A-C show results of the platelet activation test (PAT) assessed under various conditions. Citrated blood from two healthy donors was collected and platelet-rich and platelet-poor plasma (PRP, PPP) were prepared by centrifugation (15min) at 200g or 1400g, respectively and kept at 37°C.¹⁴ Platelet counts for PRPs were $5,6 \times 10^9/L$. Heat-inactivated (56°C, 30min) serum from patient or controls (negative for aPF4) was incubated with PRP with or without LMWH 0.2U/mL or unfractionated-heparin (UFH) 100U/mL. Aggregation was assessed by means of a light-transmission aggregometer (Chrono-log, Mascia-Brunelli, Milano, Italy). Results were expressed as increase of light-transmission (%LT). PAT was considered positive if aggregation occurred in at least one of the two donors in the absence and presence of low LMWH and was inhibited by UFH. Panel A shows PAT results at T1 with patient serum that caused platelet activation in presence of LMWH (2), but also in absence of heparin (1) (85%, 77% LT, respectively), in contrast, high levels of UFH inhibited the reaction (3) (0% LT). The negative control serum did not cause aggregation (4) (0%LT). Similar results were obtained with the PRP of the other healthy donor. Panel B shows PAT results at T2 with patient serum that behaved like the negative control serum (i.e., it did not activate platelets under any of the

conditions) (1-2-3-4) (0%LT). Panel C shows PAT results with serum of an asymptomatic subject positive to aPF4 ELISA, but negative to PAT (1-2-3-4) (0%LT).

Panels D-F show results of Total Thrombus formation Analysis (T-TAS). To analyze thrombus formation under flowing conditions, whole blood was applied to type 1 collagen-coated chips at a flow rate of 24 $\mu\text{L}/\text{min}$ which corresponds to a wall shear stress of $2,000\text{s}^{-1}$. Thrombus-formation was analyzed through the following parameters: AUC, defined as the area under the flow pressure curve (Panel D), OST (min), defined as occlusion start time (i.e, the time needed to reach the baseline pressure indicating the onset of the platelet thrombus formation) (Panel E), and OT, defined as occlusion time (i.e., the time at which the occlusion pressure is reached (Panel F). The dotted horizontal lines represent reference ranges (2.5th-97.5th centiles).

Figure 3. Thrombin generation (TG). TG was assessed as previously described¹³ following in vitro coagulation activation of platelet poor plasma by calcium chloride with no addition of exogenous triggers. The TG reaction was monitored by means of fluorogenic substrate for 20 min in a dedicated fluorimeter (Ascent, Fluorocan, Thermolab System, Helsinki, Finland). The procedure was carried out in the absence (panel A) or presence (panel B) of soluble rabbit thrombomodulin (TM) (2nM), that which acts as the main physiological protein C (PC) activator and is located on endothelial cells. TM was titrated to reduce the ETP of the normal plasma by 50%.¹³ The assessed parameters were the lag-time, defined as the time (min) needed to start TG, thrombin peak height (nM), the time to reach the peak (min) and the area under the curve, defined as endogenous thrombin potential (ETP) (nMxmin). ETP represents the net amounts of thrombin that plasma can generate under the opposing driving forces of the pro- and anticoagulants operating in plasma. ETP results were also expressed as ETP-TM ratio by dividing the ETP in the absence to the ETP in the presence of TM. ETP-TM ratio represents the resistance to the anticoagulant activity of TM and is sensitive to the procoagulant imbalance between factor (F)VIII and PC (the higher the ETP-TM ratio, the greater the FVIII/PC ratio and hence hypercoagulability).¹³ Solid and broken lines represent patient and control plasma.

Baseline**a****b****c****Follow-up****d****e****f**

