Case report

A case of vaccine-induced immune thrombotic thrombocytopenia with massive artero-venous thrombosis

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Heparin-induced thrombocytopenia (HIT) is a drug-induced autoimmune disorder, characterised by thrombocytopenia, an increased incidence of venous, arterial and/or microvascular thrombosis, and auto-antibodies that recognise multimolecular complexes of platelet factor 4 (PF4) bound to heparin or other polyanions[1].

Administration of the ChAdOx1 nCoV-19 Astra Zeneca vaccine is frequently followed by flu-like symptoms, such as fatigue, fever, headache, muscle and joint pain, which subside after 1 or 2 days, but in some cases, symptoms can persist beyond the third day. Recently, sinus/cerebral vein thrombosis, pulmonary embolism and splanchnic vein thrombosis, associated with low platelet counts and elevated D-dimer levels, have been reported 5 to 20 days after the first dose of the ChAdOx1 nCoV-19 Astra Zeneca vaccine. This constellation of events has recently been assigned the operative acronym of VITT (vaccine-induced immune thrombotic thrombocytopenia).

CASE REPORT

A previously healthy 57-year-old female received her first dose of the ChAdOx1 nCoV-19 Astra Zeneca vaccine on March 11th, 2021. The following day she developed fever, arthromyalgia and headache. Because of the persistence of these symptoms, on the sixth day she was referred to the Emergency Unit in Pescara. Blood tests showed mild platelets decrease (120×10^9/L, normal values [nv] 130-400×10^9/L) and an increase of D-dimer (1.3 mg/L, nv 0.00-0.50 mg/L). A cerebral computed tomography (CT) scan revealed no vascular or parenchymal alteration. The woman was discharged with symptomatic therapy.

On the 11th day she returned to the Emergency Unit because of persisting flu-like symptoms and the onset of purpuric lesions on both legs. Her platelet count was 10×10^9/L, while her D-dimer had risen to 5.09 mg/L. Contrast-enhanced cerebral, chest and abdomen CT scans were performed, documenting a large thrombosis (9 mm thick) in the abdominal aorta. During hospitalisation, the D-dimer concentration rose to a maximum of 37.8 mg/L while the platelet count increased to 70×10^9/L after administration of intravenous immunoglobulins (IVIG) 1 g/kg, on the first day only and methylprednisolone 1 mg/kg iv for the next 2 days. The patient was then discharged, with a prescription of prednisone 62.5 mg/day, gabapentin 100 mg bid, and morphine as needed for persisting generalised pain. Five days later she was readmitted, because of severe chest and abdominal pain. Blood tests documented a platelet count of 55×10^9/L and D-dimer level of 7.1 mg/L; liver transaminases were normal. Contrast CT of chest and abdomen revealed pulmonary embolism, thrombosis of the portal vein and splenic artery, with splenic infarction, parietal

thrombus in the thoracic aorta and increased size of the thrombus in the abdominal aorta. On day 10 venous blood from the patient was sent for diagnostic tests to Florence, and the results received on the following day showed a negative immunoassay for IgG anti-PF4-heparin, but a positive heparin-induced platelet activation (HIPA) test at low-dose heparin and also in its absence. In the Intensive Care Unit the patient was treated with fondaparinux 7.5 mg/qd, IVIG 1 g/kg/qd, and dexamethasone 40 mg. In the following days her condition worsened due to liver failure, although platelet counts progressively increased up to 145×10^9/L (**Figure 1**) and liver failure resolved after 3 days. IVIG were then discontinued, and fondaparinux therapy was replaced by dabigatran and acetylsalicylic acid. To exclude other possible diagnoses, we also measured ADAMTS-13 activity, which was normal (80.7%). Homocysteine, protein C, protein S, lupus anticoagulant, antibodies to beta-2 glycoprotein and cardiolipin, and antithrombin III values were normal or negative. Genetic tests for factor V Leiden, prothrombin and *JAK2* mutations were negative, and the paroxysmal nocturnal haemoglobinuria phenotype was not present. The main laboratory findings during the patient's hospital admission are shown in **Table I**.

Laboratory assay for vaccine-induced immune thrombotic thrombocytopenia

On day 10, whole venous blood was collected in test-tubes with sodium citrate 3.2% and in tubes without anticoagulant. The test-tubes were centrifuged at room temperature at 1,500*g* for 15 min, and the supernatants were aliquoted by the Laboratory in Pescara and shipped on dry ice to the Thrombosis Centre Laboratory at Careggi Hospital.

On the citrated sample we performed an immunoassay to detect the presence and quantify the titre of PF4-heparin antibodies by an IgG specific chemiluminescence test for heparin-PF4 antibodies (Hemosil AcuStar HIT-IgGPF4-H; IL, Bedford, MA, USA). The functional HIPA test was also performed on a serum sample to assess the ability of PF4-heparin antibodies to bind and activate platelets.

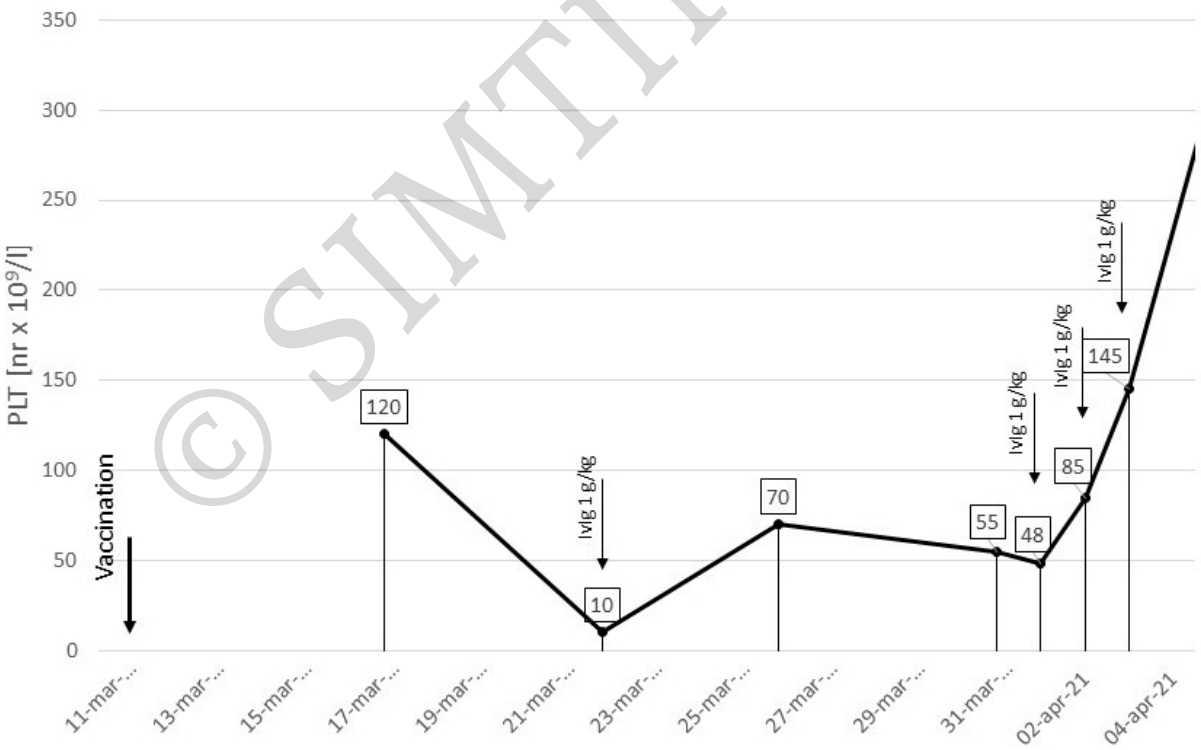

Figure 1 - Platelet counts and administration of intravenous immunoglobulins during observation of the case
PLT: platelets; IvIg: intravenous immunoglobulins.

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Table I - *Laboratory findings*

	Day 6	Day 11	Day 16	Day 20	Day 21	Day 23	Day 28
Haemoglobin g/dL (nv 12-16)	14.9	13.4	11.6	13.3	12.0	9.1	9.8
Platelet count *10³/µL (nv 130-400)	120	10	79	55	48	145	364
Leukocytes *10³/µL (nv 4.8-10.8)	7.3	11.7	12.3	21.2	22.5	29.9	23.4
Fibrinogen (mg/dL) nv 180-400	458	345	265	86	240	202	190
D-dimer (mg/L) nv 0.0-0.5	1.31	5.09	37.8	7.10	4.82	4.55	3.82
Aspartate-aminotransferase (U/L) nv 5-34	30	66	221	66	1782	330	45
Alanine-aminotransferase (U/L) nv 0-55	25	43	103	68	636	394	68
Lactate dehydrogenase (U/L) nv 125-220	227	264	284	635	2126	677	368
C-reactive protein (mg/L) nv 0.0-5.0	41.99	70.58	14.23	16.36	30.26	10.44	63.69

Results of blood examinations performed on the days indicated. nv: normal value.

This assay tests the ability of patient's serum to aggregate control platelets in the presence of low (0.2 IU/mL) and high (100 IU/mL) heparin concentrations and in the absence of heparin (buffer control)[2].

The immunological assay was negative with an optical density of 0.04 (cut-off: 1.0). The HIPA test was positive demonstrating platelet activation in the absence of heparin (buffer control) and in the presence of a low concentration of heparin.

DISCUSSION

VITT is a rare adverse event recently reported after administration of the ChAdOx1 nCoV-19 Astra Zeneca vaccine[3,4]. Recognition of early signs of the pathogenic cascade leading to VITT may significantly reduce its mortality, as early identification may prompt effective treatment.

The ChAdOx1 nCoV-19 Astra Zeneca vaccine can induce a HIT-like syndrome, also in the absence of exposure to heparin, but the mechanisms by which this vaccine may stimulate the production of anti-PF4 antibodies are still unknown, despite the demonstration that adenoviruses can bind to platelets and cause platelet activation[5]. One possible trigger of the PF4-reactive antibodies could be free DNA in the vaccine[6].

In healthy individuals, extracellular nucleic acid concentrations in plasma range from 0 to >1,000 ng/mL[7] and include chromosomal and extrachromosomal DNA, mitochondrial double-stranded DNA[8], as well as several types of RNA. From the killing of bacteria or viruses, cell apoptosis or tissue damage, the amount of extracellular nucleic acids can rise up to 2,000 µg/mL. Nucleic acids induce structural changes in PF4 (like heparin), and PF4/nucleic acid complexes can be immunogenic through the exposure of neo-epitopes recognised by anti-PF4/heparin antibodies[9].

Two types of laboratory tests were performed: (i) an immunoassay (chemiluminescence) to detect the presence and quantify the titre of PF4-heparin antibodies, which was negative; (ii) a functional assay which demonstrated the presence of antibodies able to activate platelets *in vitro* independently of heparin.

The apparent discrepancy between the two methods may be ascribed to the high specificity of the chemiluminescence test in detecting only anti-heparin/PF4 antibodies. In this case, the high specificity for heparin failed to detect the polyanion linked to PF4, but the functional assay confirmed the ability of the patient's serum to activate platelets from human donors *in vitro*.

After vaccination, when flu-like symptoms persist, or dyspnoea, chest/abdominal pain, or focal neurological symptoms develop, haematological evaluations and instrumental examinations are recommended, to determine whether VITT is present.

VITT is a novel syndrome, whose management is derived from the therapy of HIT[10]. The administration of IVIG is reported to be beneficial in this condition[11], but its timing and amount remain to be determined. Anticoagulation with heparin should be avoided and, alternatively, HIT-compatible anticoagulants should be used, i.e, fondaparinux or direct oral anticoagulants[12].

In our case IVIG were administered immediately after the detection of thrombocytopenia; despite the early start of therapy, our patient developed multiple district

thrombosis. This would suggest that IVIG administration did not arrest the early coagulation process. During the second hospital admission, IVIG were given for 3 days, which is longer than usual, and associated with steroids and direct oral anticoagulants. Since the patient's condition improved quickly and progressively, this type of combined treatment should be preferred.

AUTHORSHIP CONTRIBUTION

RP and RM contributed equally as senior Authors.
MCT: conception of the work; FS: data collection and analysis; AMG, AAR, BG and FC: performed lab tests; GP: collaboration in the preparation of the submitted manuscript; AA and GDG: followed the patient in the Emergency Unit; LA: followed the patient in the Intensive Care Unit; PR: followed the patient in the centre for haemorragic, thrombotic and rare haematological diseases; SP: followed the patient in the Haematology unit; RP: critical revision of the article; RM supervised lab tests. All Authors approved the final version of the manuscript.

Keywords: *thrombocytopenia, vaccine, thrombosis, heparin, COVID19.*

The Authors declare no conflicts of interest.

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