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Vaccine-induced severe thrombotic thrombocytopenia following COVID-19 vaccination: a report of an autoptic case and review of the literature

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Abstract. – OBJECTIVE: Vaccine-induced immune thrombocytopenia (VITT) is a new syndrome occurring primarily in healthy young adults, with a female predominance, after receiving the first dose of ChAdOx1 nCoV-19 vaccine. We describe VITT syndrome characterized by severe thrombosis and thrombocytopenia found in our patient, with fatal outcome.

CASE REPORT: A 58-year-old man, after 13 days from the first administration of ChAdOx1 nCoV-19 vaccine (AstraZeneca), presented with abdominal pain, diarrhea and vomitus. Laboratory tests revealed a severe thrombocytopenia, low fibrinogen serum levels and marked increase of D-dimer serum levels. The patient quickly developed a multiple organ failure, till death, three days after the hospital admission. RESULTS: At histology, in the lungs, interalveolar septa appeared thickened with microthrombi in the capillaries and veins. Interalveolar septa appeared thickened and showed vascular proliferation. Thrombi were detected in the capillaries of glomerular tufts. In the hearth, thrombi were observed in veins and capillaries. In the liver, voluminous fibrin thrombi were diffusely observed in the branches of the portal vein. Microthrombi were also found in the vasa vasorum of the wall of abdominal aorta. In the brain, microthrombi were observed in the capillaries of the choroid plexuses. Diffuse hemorrhagic necrosis was observed in the intestinal wall with marked congestion of the venous vessels.

CONCLUSIONS: In our patient, the majority of data necessary for a VITT final diagnosis were present: thrombocytopenia and thrombosis in pulmonary, portal, hepatic, renal and mesenteric veins, associated with a marked increase

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of D-dimer serum levels. The finding of cerebral thrombosis in choroid plexuses, is a new finding in VITT. These features are suggestive for a very aggressive form of VITT.

Key Words:
VITT, COVID-19, Thrombotic thrombocytopenia, COVID-19 vaccination.

Introduction

According with a report from the World Health Organization (WHO) of May 5th, 2021, more than 1,047 billion doses of vaccine against coronavirus disease 2019 (Covid-19) have been administered globally[1]. On March 2021, multiple European countries – including Denmark, Austria, Norway, Iceland, Estonia, Lithuania, Luxemburg, Latvia and Italy – decided to suspend use of the ChAdOx1 nCoV-19 AstraZeneca vaccine, a recombinant chimpanzee adenoviral vector encoding the spike protein of SARS-CoV-2. The decision was taken on the basis of multiple reports of blood clots after vaccination[2-4]. Some patients recovered, whereas in others outcome was fatal. According with the European Medicines Agency (EMA), on March 10th, 2021, 30 cases of thromboembolic events have been reported among the about five million European people given the ChAdOx1 nCoV-19 AstraZeneca vaccine. According with the EMA's safety committee, in a report of March 18, 2021, the incidence of thromboembolic events occurring in vaccinated people was considered not to be higher than that observed in the general population. Such a comparison is not correct, since this syndrome is a new one because a thrombotic phenotype is present along with thrombocytopenia. Consequently, the benefits of the AstraZeneca vaccine were considered to outweigh the risks, despite possible links to rare blood clots associated with a low platelet count (EMA, 18 March 2021). Moreover, in the debate on these adverse events, two major points were underlined: i) the difficulty of distinguishing a causal effect from a coincidence[5]; ii) the strict association of COVID-19 disease with blood clotting, ending with thousands of deaths caused by blood clotting triggered by SARS-CoV-2 infection[6,7]. Further reports from EMA, evidenced that all vaccines currently being rolled out in European countries will be carefully monitored for possible thrombotic events. Moreover, EMA asked AstraZeneca to update the information sheet about a possible occurrence of a thrombotic events, although very rare[8].

On April 9th, 2021, five patients presenting with thrombosis in unusual sites and thrombocytopenia within ten days after receiving the first immunization with the ChAdOx1 nCoV-19 adenoviral vector vaccine were reported[3]. The patients were 32 to 54 years of age, four females and one male, and developed pulmonary, abdominal, portal and intracranial thromboembolic events. Laboratory tests showed, in all patients, a low platelet count, associated with elevated levels of D-dimer and high levels of IgG antibodies to PF4-polyanion complexes. Two patients recovered, whereas in the other three a fatal outcome occurred. Collectively, these data induced the authors to propose that these cases might represent a new syndrome similar to autoimmune heparin-induced thrombocytopenia, triggered by vaccination with ChAdOx1 nCoV-19. The definition of Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT) has been proposed for this new syndrome, mainly occurring in healthy young adults, with a female prevalence, presenting 7 to 10 days after receiving the first dose of the ChAdOx1 nCoV-19 vaccine[9].

Three younger women, ranging from 22 up to 46 years of age, were diagnosed with VITT following vaccination with COVID-19 vaccine AstraZeneca. All three women presented between 7 and 17 days after first vaccination, with mild to moderate thrombocytopenia, associated with intracranial venous sinus thrombosis (IVST). The following pathogenesis of the syndrome was hypothesized by the authors: the formation of antibodies against PF4 might be the trigger, causing thrombus formation followed by platelet consumption[10].

After these reports, concerns were raised over possible thrombotic events following immunization with the AstraZeneca vaccine. In the UK, by March 31, 2021, 79 cases of thrombosis occurring after the first dose of the AstraZeneca vaccine have been reported[11]. 51 cases were in women, 28 in men, 60 patients survived and 19 were fatal. In this cohort, the highest risk group was represented by young women taking hormonal contraceptives. This finding confirmed previous data on the association between oral contraceptives and the increased risk of fatal pulmonary embolism[12]. In a communication on 14th April 2021, six cases of

severe thrombosis have been reported following the administration of the first dose of the Johnson & Johnson (Janssen) COVID-19 vaccine (CDC 2021). The rarity of these adverse reactions, including thrombosis, after covid-19 vaccination induced to do not stop vaccination efforts, the only tool useful for preventing death due to COVID-19 disease[13].

Regarding the pathogenesis of the VITT syndrome, a novel mechanism has been reported in 23 patients presenting with thrombosis and thrombocytopenia after receiving the first dose of the AstraZeneca COVID-19 vaccine. In 22 out of 23 subjects, testing for antibodies to platelet factor 4 (PF4) was positive, suggesting that VITT should be considered a PF4-dependent syndrome[14]several vaccines have been developed and millions of doses delivered. Reporting of adverse events is a critical postmarketing activity.\nMETHODS: We report findings in 23 patients who presented with thrombosis and thrombocytopenia 6 to 24 days after receiving the first dose of the ChAdOx1 nCoV-19 vaccine (AstraZeneca).

Here we describe the vaccine-induced syndrome characterized by severe thrombosis and thrombocytopenia found in a patient, with fatal outcome, who underwent autopsy in our hospital.

Case Report

A 58-year-old male patient presented to ER, 13 days after receiving the first immunization with ChAdOx1 nCoV-19 vaccine (AstraZeneca), with abdominal pain, diarrhea and vomitus. Laboratory tests revealed a severe isolated thrombocytopenia (platelet count 28 x 10^9/L, decreasing to 17 x 10^9/L within 24 hours) and low fibrinogen serum levels [(101 mg/dl decreasing to 82 mg/dl the day after (NV: 180-350)]. Prothrombin Time and PTT resulted to be prolonged up to 3.77 INR (reference range 0.8-1.2 INR) and 62 sec (reference range the respectively the day after the admission to the Hospital). Very high D-Dimers levels were found at the admission to the Hospital (39.000 ug/L, reference range 0-200 ug/L). Hb was very low (6.8 g/dl) while a huge hepatic cytolysis (AST and ALT were found to be 9931 and 8.076 IU/L respectively) concomitantly with a severe renal failure (Creatinine 3.40 m/dl) were recorded. CT scan showed thrombosis of the portal vein and of the splenic vein. In addition, thrombosis was evidenced in several branches of the superior mesenteric vein. The patient quickly developed a multiple organ failure, till death, three days after the hospital admission.

At autopsy, deep thrombosis was found in multiple organs. In addition, a hemorrhagic necrosis of the gastrointestinal tract was detected, associated with thrombosis of the mesenteric veins. Microthrombi were also found in the capillaries of the choroid plexuses.

At histology, specimens obtained from both lungs showed a similar pattern. Interalveolar septa appeared thickened, with micro-thrombi in the lumen of dilated capillary vessels (Figure 1A). Oedema was also focally observed in the alveolar lumen, associated with cellular debris, originating a picture similar to that found in the early exudative phase of ARDS (Figure 1A). Interalveolar septa also showed vascular proliferation, giving rise to larger septa formed by two or three capillaries (Figure 1A). Fibrin thrombi were also detected in the lumen of pulmonary veins (Figure 1B). Occasionally, voluminous fibrin thrombi were observed inside septal capillaries, which appeared enlarged (Figure 1C). Large thrombi were also observed in the lumen of pulmonary arteries (Figure 1D). Thrombi were detected in both kidneys. Microthrombi were easily found in the capillaries of glomeruli (Figure 2A) as well as in the lumen of afferent and efferent arteries (Figure 2B). In the heart, microthrombi were observed in veins and capillaries, both in the subepicardial fat and scattered in the deep myocardium (Figure 3). In the liver, voluminous fibrin thrombi were diffusely observed in the lumen of the branches of the portal vein. Moreover, microthrombi were detected in the lumen of dilatated sinusoids (Figure 4A). Occasionally, portal vein branches appeared extremely dilated, extending out of the portal spaces, with voluminous fibrin thrombi in the lumen (Figure 4B). Microthrombi were also found in the vasa vasorum of the arterial wall of abdominal aorta (Figure 5). In the brain, microthrombi were particularly frequent in the capillaries of choroid plexuses (Figure 6). Rare fibrin thrombi were also detected in the cortical vessels. The intestinal wall showed diffuse hemorrhagic necrosis, extending from the mucosa to the serosa, associated with marked congestion of the venous vessels (Figure 7).

Discussion

The case here reported fulfills most of the criteria requested for the diagnosis of vaccine-induced immune thrombotic thrombocytopenia (VITT)[3]. The patient, a 58-year-old male, presented 13 days after receiving the first immunization with

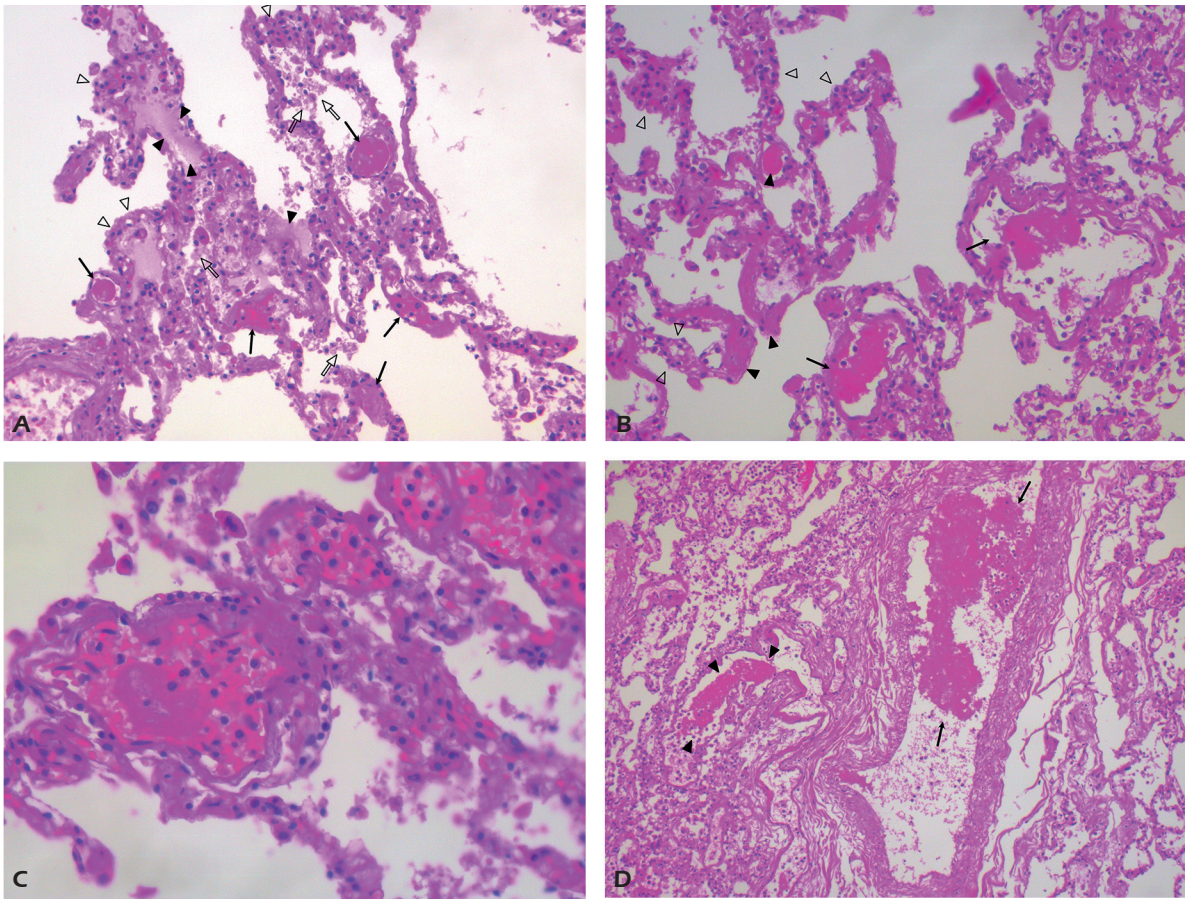

Figure 1. Lung: **A,** H&E 20x. At low power, alveolar spaces show variability in size, with enlarged alveoli adjacent to collapsed alveoli. Microthrombi are easily detected in the alveolar capillaries (arrows). Alveolar oedema is focally observed (arrowheads). Cellular debris are found in the alveolar lumen (open arrows). Some septa are thickened, due to vascular proliferation (open arrowheads). **B,** H&E 20x. Fibrin thrombi are observed in the lumen of some pulmonary veins (arrows). Multiple microthrombi are also found in the lumen of septal capillaries (arrowheads). Vascular proliferation is detected in the interalveolar septa, giving rise to solid vascular nests (open arrowheads). **C,** H&E 40x. Voluminous fibrin thrombus inside the lumen of an enlarged septal capillary. **D,** H&E 10x. Voluminous fibrin thrombus in the lumen of a pulmonary artery (arrow) and of a pulmonary vein (arrowheads). Cellular debris are detected inside the alveolar spaces (open arrowheads).

ChAdOx1 nCoV-19 vaccine (AstraZeneca). Laboratory tests revealed a severe isolated thrombocytopenia and low fibrinogen serum levels concomitantly with very high D-dimer levels. CT scan showed thrombosis of the portal vein, of the splenic vein. In addition, thrombosis was evidenced in several branches of the superior mesenteric vein. Moreover, at autopsy, we found diffuse and severe thrombosis in unusual sites, including lungs, liver, heart, aortic vasa vasorum, kidneys and choroid plexuses. In all these sites, thrombosis was mainly restricted to veins and capillaries, even though rare thrombi were also detected in branches of the pulmonary arteries further confirming that a pulmonary thrombosis may occur both during the COVID-19 infection and also in other clinical conditions[15,16]it has generally been believed that pulmonary embolism (PE. Moreover, a diffuse hemorrhagic infarct of the intestinal tract was observed, related to the diffuse thrombosis of the mesenteric veins. These findings, associated with the previous COVID-19 vaccination, support the diagnosis of VITT.

On April 2021, the New England Journal of Medicine published three studies[3,4,14]several vaccines have been developed and millions of doses delivered. Reporting of adverse events is a critical post-marketing activity.\nMETHODS: We report findings in 23 patients who presented with thrombosis and thrombocytopenia 6 to 24 days after receiving

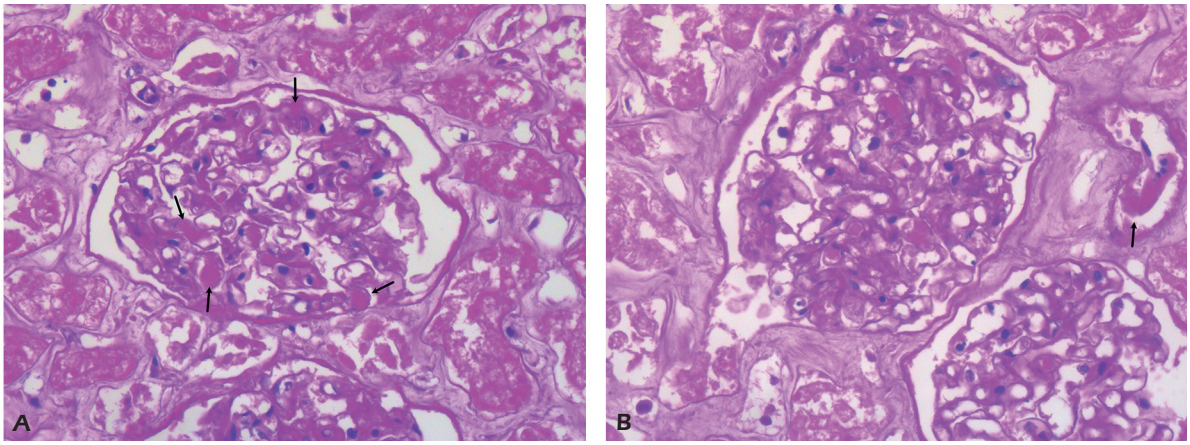

Figure 2. Kidney. **A,** H&E 40x. An enlarged glomerulus, with a reduced capsular space. Multiple microthrombi are observed in the lumen of glomerular capillaries (arrows). **B,** H&E 40x. Microthrombi are also found in the lumen of afferent and efferent arteries (arrow).

the first dose of the ChAdOx1 nCoV-19 vaccine (AstraZeneca concerning VITT whose general outcomes have been already cited in this article.

However, for a better and a more precise comprehension of the disease we put together these reports which included a total of 38 patients of whom 16/23 died (69.5%). The onset of the disease occurred between the 6th and 24th day after vaccination with the AstraZeneca vaccine. The characteristics of the patients studied show that the female gender and young age is prevalent but 12/39 were men (30.7%) while age ranged between 21 and 77 years. Not only young women, therefore, underwent VITT as it has been stated by media in the last months. Looking at the different case studies, it can be seen that cerebral vein thrombosis (CVT) is present in 26 patients (66%). The platelet count is very variable in that it ranged

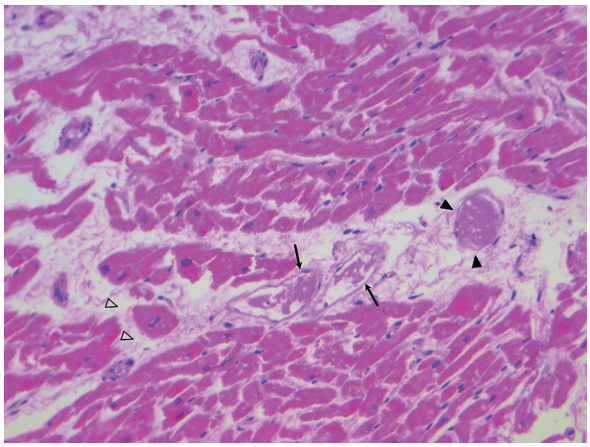

Figure 3. Heart. H&E 20x. Microthrombi are observed in the lumen of small veins (arrows) and capillaries (arrowheads) inside the myocardium. Myocardiocytes appear dissociated by interstitial oedema (open arrowheads).

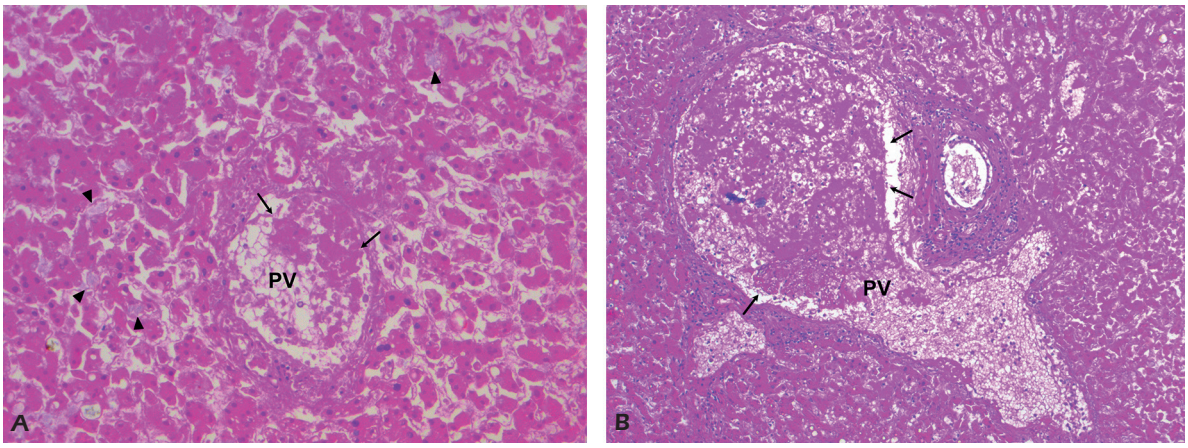

Figure 4. Liver. **A,** H&E 20x. A fibrin thrombus is observed in the lumen of a portal vein (PV). Microthrombi are also present in the sinusoidal lumen (arrowheads). **B,** H&E 10x. A markedly enlarged portal vein (PV), extending out of the portal space, containing a voluminous thrombus (arrows). Sinusoids appear enlarged and their lumen is occupied by a fibrinous network (arrowheads).

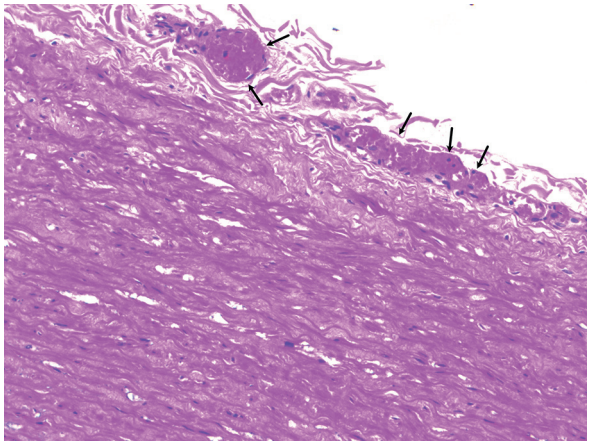

Figure 5. Section of the aortic wall. H&E 20x. In the adventitia, microthrombi are observed in the lumen of the vasa vasorum (arrows).

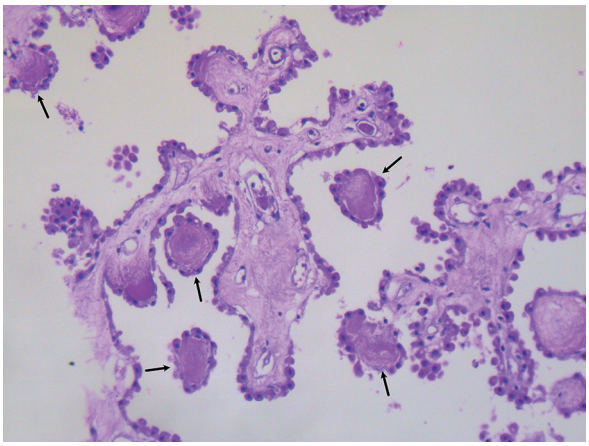

Figure 6. H&E 20x. Choroid plexus showing multiple microthrombi (arrows) in the lumen of capillaries.

from 7,000 up to 141,000 x 10^9/L. The very high values of D-Dimer, as the authors rightly note, are similar to those we in general note in terminal cancer patients especially when the disease is plurimetastatic. Fibrinogen levels are also very often reduced and close to the critical threshold of 100 mg/dl or even lower in several cases. Abnormalities of PT and PTT were then noted, especially the former. Another important point is the dosage of heparin-Pf4 antibodies, found positive in all but two patients in whom they have not been dosed. In 23 patients (59%) the ISTH criteria for Disseminated Intravascular Coagulation (OVERT DIC)[17] were present as in our case report. In particular, DIC appeared to have a prothrombotic phenotype along with an important secondary fibrinolytic response. In the patient here described, the widespread fibrin deposition, in both macro and microvascular vasculature, can explain why fibrinolysis was so greatly enhanced. A limit of this case report is the lack of the anti-Pf4 antibodies assay which, unfortunately, was not carried out. However, as it happened in two of the 38 cases described above, the lack of a dosage of the anti-Pf4 antibodies did not exclude a strong suspect of VITT. As Scully et al[14] suggested in their case series, the presence of D-Dimer >2000 ng/ml, low or normal fibrinogen level and no alternative diagnosis, the probability of being in front of a VITT is extremely high. On April 20, 2021, the International Society of Hemostasis and Thrombosis (ISTH) published in its web site similar criteria for the diagnosis of VITT. Among these criteria, they added that, if the anti-Pf4 antibodies assay is not available, a D-Dimer test of more than 4000 ng/ml is enough for both a VITT diagnosis and treatment with high dose Immunoglobulin, corticosteroids and a non-heparin anticoagulant such as fondaparinux, argatroban or a direct oral anticoagulant (rivaroxaban, apixaban).

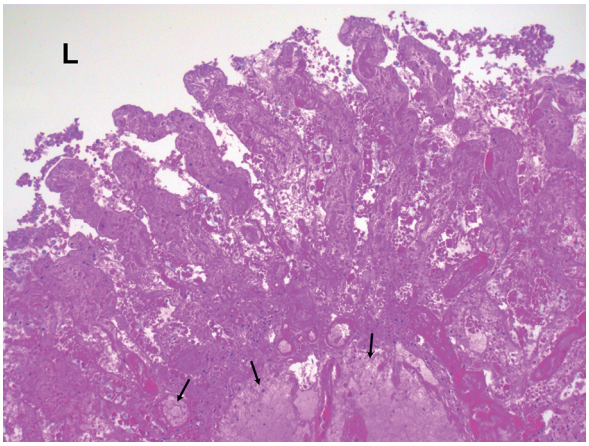

Figure 7. H&E 10x. Intestinal mucosa (ileum) shows severe architectural changes, due to diffuse hemorrhagic necrosis involving the entire intestinal wall. Submucosal vessels (arrows) show marked congestion. L = intestinal lumen.

It is worth noting that, in the present case, some differences were found, compared to previous reports of VITT. The most important finding regards the presence of multiple microthrombi in both choroid plexuses, in the absence of severe thrombotic or hemorrhagic events in the brain. In particular, we did not find thrombosis of the intracranial venous sinus, as

previously reported[10]. An accurate histological analysis of multiple samples of the central nervous system revealed rare thrombi in the cortical veins, a finding previously reported in VITT patients[3]. However, this could be arguably explained with the tissue variability for the representation of some target molecules that could locally enhance the pro-thrombotic activity by determining a spectrum of variation within the pro-thrombotic topographic cascade[18].

Conclusions

In our patient we believe that the majority of data necessary for a VITT final diagnosis were present, as very recently defined[5]. All clinical features of VITT were present in our case: deep vein thrombosis, pulmonary, portal vein, hepatic vein, mesenteric thrombosis and thrombocytopenia. The finding of cerebral thrombosis restricted to some districts, including choroid plexuses, is a new finding in VITT, that characterizes our case. The case here presented shows a diffuse and widespread thrombosis, indicating a very aggressive VITT.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgments

A written informed consent for publication of relevant data has been sought from patient's next of kin.

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