

Ultrastructural findings of lung injury due to Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT) following COVID-19 vaccination: a scanning electron microscopic study

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Abstract. Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare new syndrome occurring after the ChAdOx1 nCoV-19 vaccine immunization. Patients with VITT are characterized by a variable clinical presentation, likewise also the outcome of these patients is very variable.

Here we report the lung ultrastructural findings in the course of VITT of a 58-year-old male patient.

Alveoli were mainly dilated, irregular in shape, and occupied by a reticular network of fibrin, while interalveolar septa appeared thickened. The proliferation of small capillaries gave rise to plexiform structures and pulmonary capillary hemangiomatosis-like features. Near the alveoli occupied by a dense fibrin network, the medium-sized arteries showed a modified wall and an intraluminal thrombus.

This scenario looks quite similar to that found during COVID-19, where the lungs suffer from the attack of the antigen-antibodies complexes and the virus respectively. In both diseases, the final out-

come is a severe inflammation, activation of the haemostatic system and fibrinolysis.

Key Words:

VITT, Ultrastructural, Lung, Thrombocytopenia.

Introduction

Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a new definition for a very rare syndrome occurring from 5 to 48 days after receiving an immunization with the ChAdOx1 nCoV-19 vaccine¹. The incidence of VITT is low and significantly lower than the risk of serious outcomes associated with COVID-19². As a consequence, it has been suggested³ that the rare events of thrombosis,

typical of VITT, should not derail vaccination efforts to halt COVID-19 pandemic.

Patients with VITT are characterized by a variable clinical presentation, mainly including cerebral vein thrombosis, severe thrombocytopenia, and cerebral hemorrhage⁴. The clinical outcome of patients presenting with VITT is very changeable: some patients recover, whereas in others outcome may be fatal.

In the report of March 18th, 2021, of the EMA's safety committee, the incidence of thromboembolic events occurring in vaccinated people was considered not to be higher than that observed in the general population. As a consequence, 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). Some aspects of VITT are debated yet, including the difficulty of distinguishing a causal effect from a coincidence⁵. According with some authors, the AstraZeneca vaccine should not be linked to increased risk of blood clots⁶.

Laboratory tests in patients affected by VITT show a low platelet count, associated with elevated levels of D-dimer and high levels of IgG antibodies to platelet factor 4 (PF4)-polyanion complexes⁷. Taken together these data display how VITT might represent a syndrome similar to autoimmune heparin-induced thrombocytopenia, triggered by vaccination with ChAdOx1 nCoV-19⁸.

Regarding the pathogenesis of the syndrome, the formation of pathologic antibodies against PF4 might represent the trigger, causing thrombus formation followed by platelet consumption⁹.

In a previous paper from our group, we described the histological changes associated with VITT in multiple organs. Histology was characterized by thrombosis in multiple organs and tissues in unusual sites, including lungs, gut, liver, heart, choroid plexuses, aortic vasa vasorum and kidneys¹⁰. The diagnosis of VITT was performed, due to the presence of the majority of the pathological changes necessary for its definition: deep vein thrombosis, pulmonary embolism, portal vein thrombosis, hepatic vein thrombosis and mesenteric thrombosis⁵. Here, we describe the ultrastructural findings observed at scanning electron microscopy in the lungs of the same patient.

Case Report

A 58-year-old male patient presented, 13 days after receiving the first immunization with ChA-

dOx1 nCoV-19 vaccine (AstraZeneca), with abdominal pain, diarrhea, and vomitus. Clinical, laboratory, autoptic and histological data have been reported in a previous paper (Fanni et al²¹, 2021).

Materials and Methods

6-7 μ m thick slices were dedicated to SEM observation. The freshly cut slices mounted on the microscope slide were deparaffinized by immersion in xylene for 48 hours, subsequently re-hydrated in descending ethanol scales and treated with a solution of 1% osmium and 1.25% ferrocyanide for 1 hour in a humid chamber.

After numerous washes in Phosphate Buffered Saline (PBS), the slides were dehydrated in ascending ethanol scales, dried at critical point in CO₂, and then, mounted on aluminum support (stub) with double-sided tape. Finally, the samples were covered with a gold conductive film and observed at SEM (ZEISS Sigma 300).

Results

At panoramic view, lung parenchyma showed a modified architecture (Figure 1). Inter-alveolar septa appeared thickened and irregular in shape. Alveoli were collapsed, often appearing as thin fissures intermingled among septa. Some alveolar chambers were dilated and occupied by a reticular network of fibrin (arrows). In between zones of collapsed parenchyma, lung zones with a preserved architecture were observed (Figure 2). In these areas, alveoli appeared normal in size and structure. Alveolar chambers were empty and inter-alveolar septa were formed by a single capillary in which red blood cells might be identified (Figure 3). In zones of lung parenchyma with modified architecture, residual alveolar chambers often showed an irregular profile, due to the irregular expansion of the inter-alveolar septa. These appeared thickened, due to a tremendous proliferation of small capillaries giving rise to plexiform structures (Figure 4). Proliferating capillary structures originated pulmonary capillary hemangiomatosis-like features, inside which no pre-existing pulmonary structure were identifiable. At higher power, residual collapsed alveoli were identified inside the areas of capillary proliferation (Figure 5). Residual alveolar structures showed an irregular shape, due to the compression by the proliferating capillaries, extending from the inter-alveolar septa into the

Figure 1. Panoramic view of lung parenchyma shows modified architecture. Inter-alveolar septa are thickened and irregular in shape. Alveoli (A) are collapsed, appearing as thin fissures intermingled among septa (*). Some alveolar chambers are dilated and occupied by a reticular network of fibrin (F). X180.

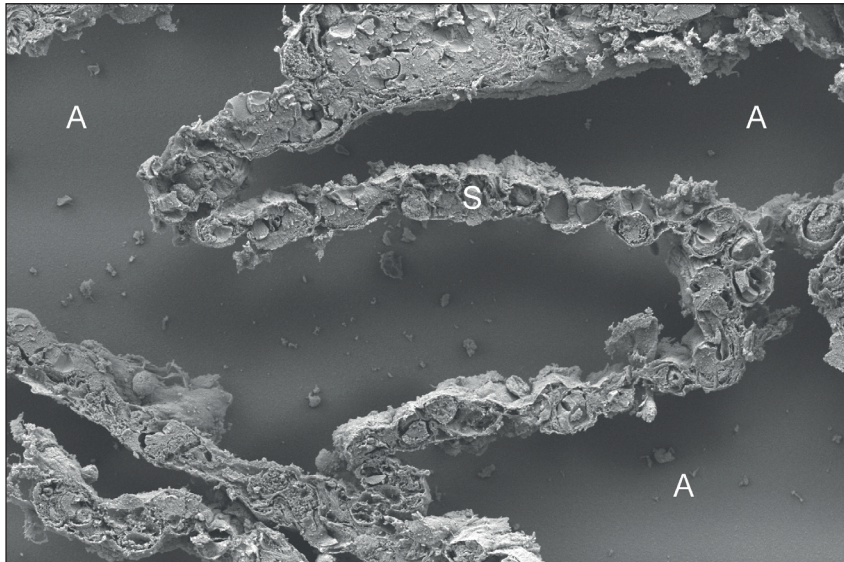
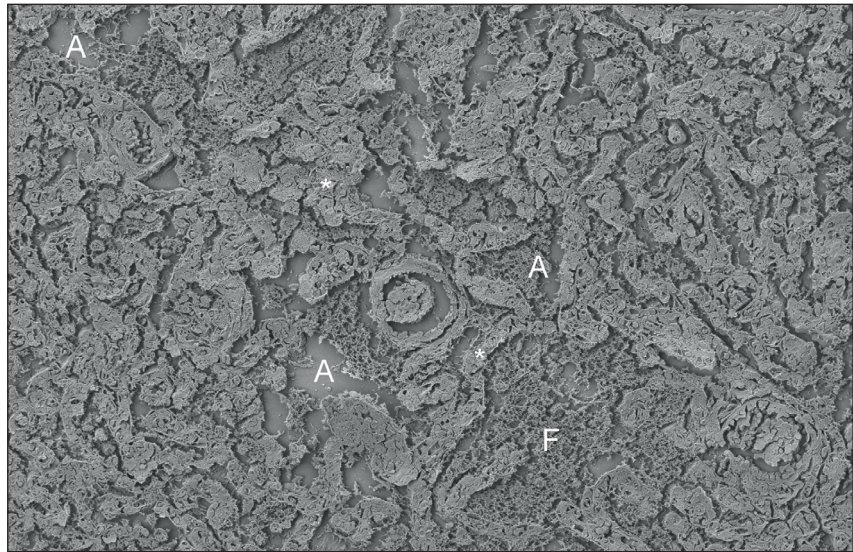


Figure 2. A pulmonary zone with a preserved architecture. In this area, alveoli (A) appear normal in size and structure. Alveolar chambers are empty and interalveolar septa (S) are formed by a single capillary. X 800.

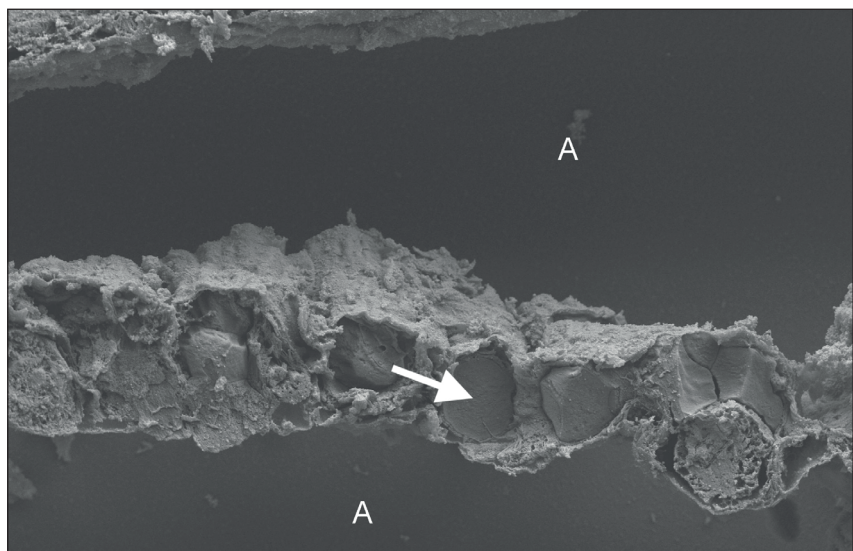


Figure 3. At higher power, a normal interalveolar septum, formed by a single capillary in which red blood cells (Arrow) may be identified (A=alveoli). X 2500.

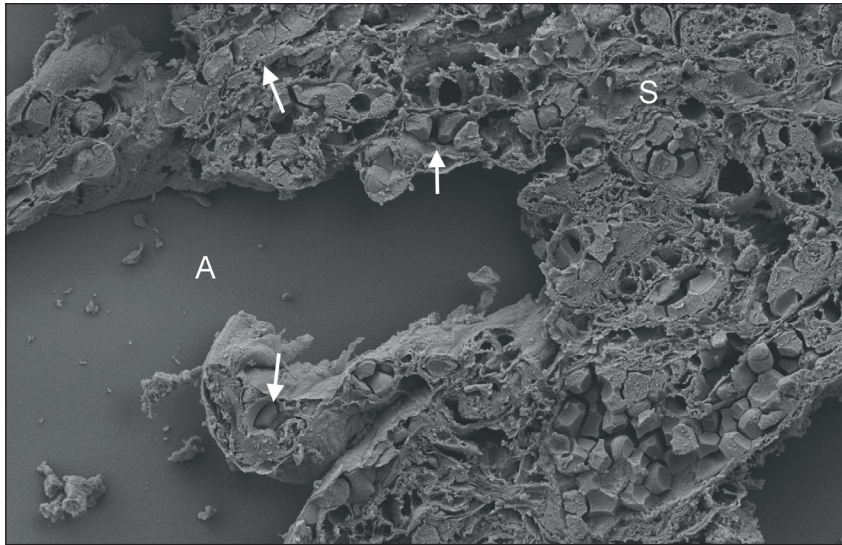


Figure 4. Zone of lung parenchyma with altered architecture. A residual alveolar chamber (A) shows an irregular profile, due to the expansion of the surrounding interalveolar septa (S). The proliferation of small capillaries gives rise to plexiform structures (Arrows). X 900.

Figure 5. At higher power, a collapsed alveolar chamber (A) is identified inside the areas of capillary proliferation. The residual alveolar structure shows an irregular shape, due to the compression by the proliferating capillaries (C) extending from the interalveolar septa into the alveolar chamber. X 4000.

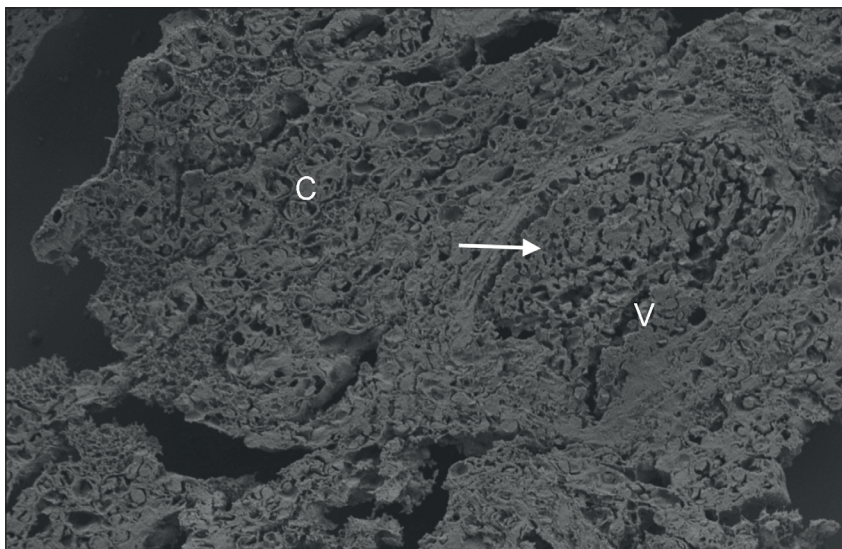
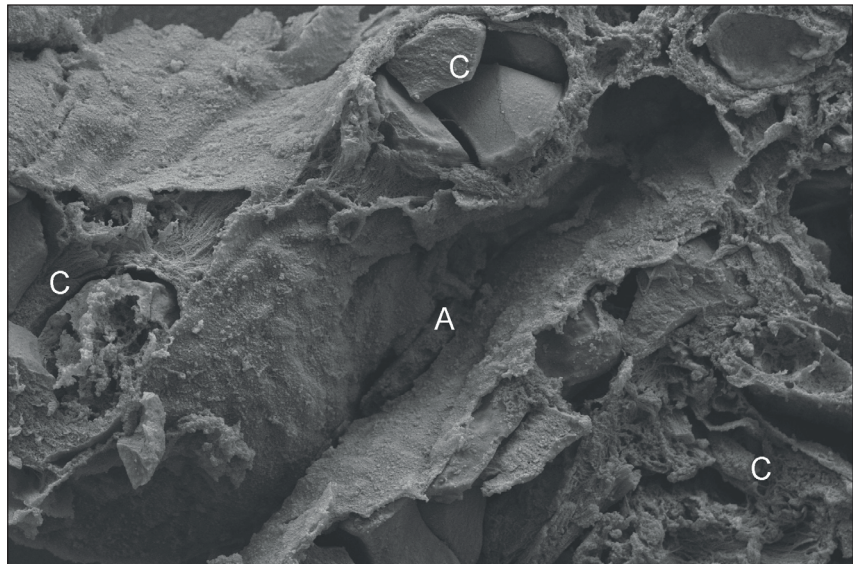


Figure 6. A large area of pulmonary parenchyma characterized by a completely modified architecture. Newly formed proliferating capillary structures (C) originate hemangioma-like pathological changes. The newly formed capillaries are often found to encircle large vessels, mainly arterial vessels (V), whose lumen is occupied by a thrombus (Arrow). X 360.

alveolar chambers. Large areas of the pulmonary parenchyma were characterized by a completely modified architecture, with severe pathological changes both in alveoli, septa, as well as in large vessels (Figure 6). These zones were characterized by a large number of newly formed capillary structures. The intensity of capillary proliferation originated frequent hemangioma-like pathological changes. The newly formed capillaries were often found to encircle medium-size vessels, mainly arterial vessels, whose lumen was occupied by a thrombus (Figure 6). Proliferation of new capillaries showed features of aggressivity against the arterial wall, which appeared irregular and thinner (Figure 6). At higher power, the consolidated areas were found to be formed by adjacent small

capillaries which encircled and infiltrated the pre-existing pulmonary vessels (Figure 7). The ability of proliferating capillaries to infiltrate the wall of the pre-existing alveoli was also evidenced (Figure 8). The coexistence of vascular and alveolar pathological changes was often observed in the same field (Figures 9 and 10). Medium-sized arteries with a modified wall and an intraluminal thrombus were frequently observed in close proximity to alveoli occupied by a dense fibrin network with inflammatory cells and red blood cells inside.

Discussion

Figure 7. At higher power, the solid areas are formed by adjacent small capillaries (C) which encircle and infiltrate the wall of pre-existing vessels (V) (Figure 7). X 760.

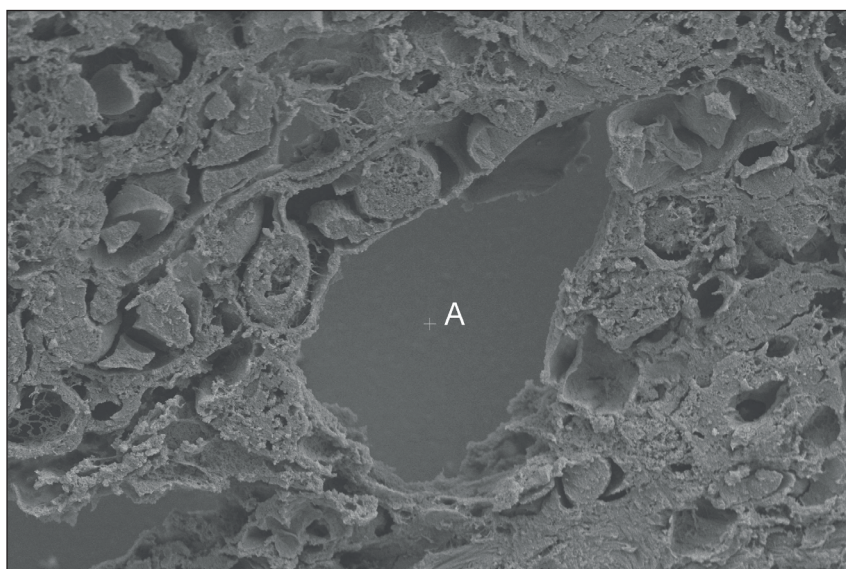
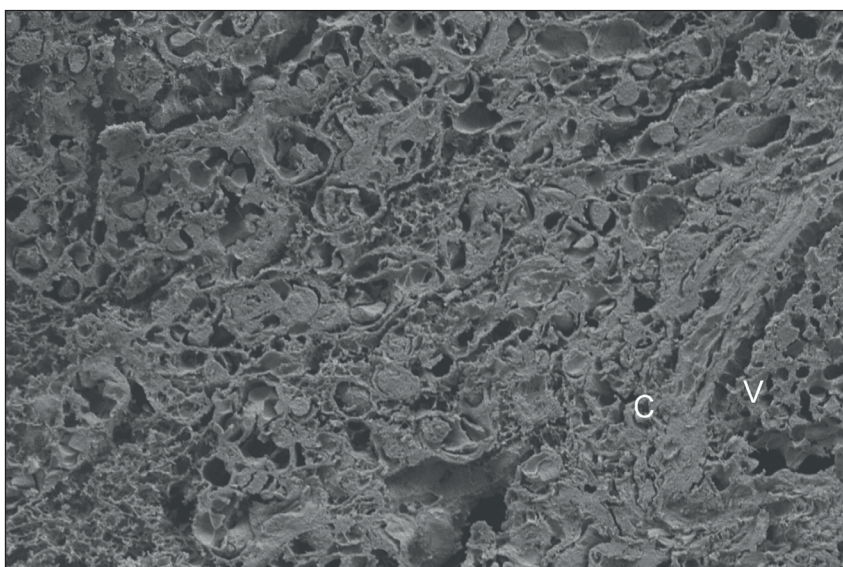


Figure 8. The ability of proliferating capillaries to infiltrate the wall of the pre-existing alveoli (A) is evidenced. The pulmonary architecture is completely modified by the vascular proliferation. X 1900.

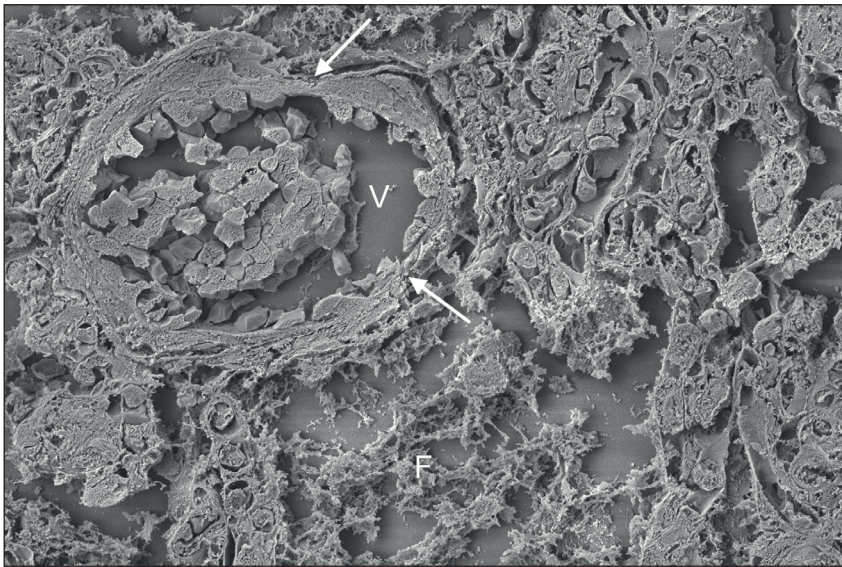


Figure 9. Coexisting vascular and alveolar pathological changes are found in the same field. A medium-sized vessel (V) with a modified wall (Arrows) and an intraluminal thrombus is observed in close proximity to an alveolar chamber occupied by a dense fibrin network (F). X 700.

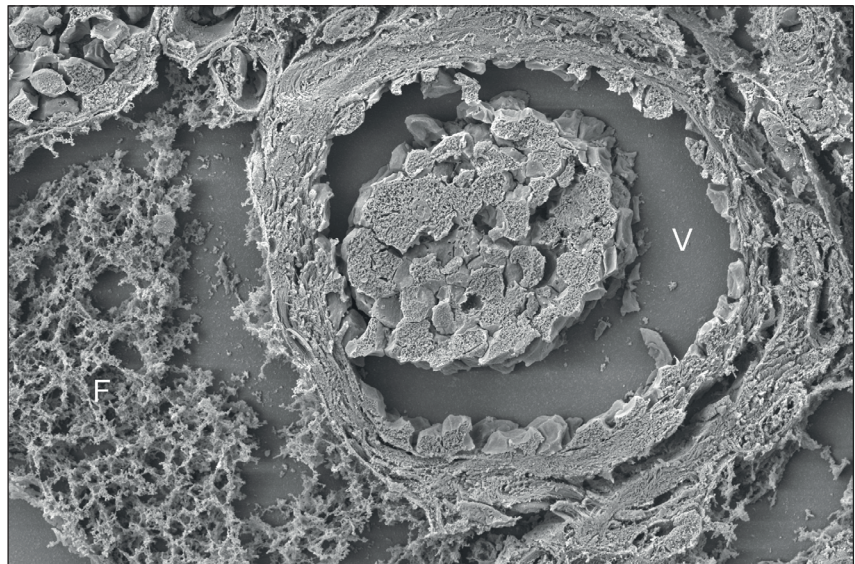


Figure 10. Dense fibrin deposition inside the alveolar chamber (F) and a thrombus inside the lumen of an adjacent vascular structure (V). X 1000.

The case here reported fulfills the majority of the criteria requested for the diagnosis of VITT¹¹ as previously reported¹⁰. This further evaluation was carried out by Scanning Electron Microscopy to obtain more detailed information on the pulmonary damage induced by Astra Zeneca vaccine. In Figure 4 a hyperplasia of the capillaries is shown in the interalveolar space which appears to be reduced. A pneumatic attempt is also evident to enter the interalveolar space mainly occupied by capillaries. This vascular structure also shows fibrin deposition in keeping with the concept that pulmonary thrombosis can occur during SARS-CoV2 infection¹². The interpretation of

this finding is that the capillary hyperplasia is a compensatory mechanism to counteract fibrin deposition occurring both in the capillaries and alveolar spaces. The latter, in fact, appear to be reduced because of the capillary hyperplasia and, in turn, try to expand themselves into the interalveolar spaces occupied by the vascular mesh. New pneumatic areas are, in fact, evident among the capillary net. These findings were evident in most parts of the lungs. The typical feature of the lung pathology has been showed in the Figures presented in the results section. In particular, a small artery has been shown to be in contact with an alveolar chamber which is occupied by fibrin

and red blood cells. What is evident is that the artery's lumen has inside a thrombus along with a significantly irregular wall. This characteristic abnormality is evident especially when the arterial wall is in contact with a fibrin deposit. At this site the anatomy of the arterial's wall appears to be damaged. It is possible that the fibrin deposition so closely related to the arterial wall can further damage the arterial vessel probably because a local activation of the fibrinolytic system may be crucial in also attacking the near structures. On the other hand, it is known that plasmin can induce the degradation of several components of the extracellular matrix (ECM) and basement membrane, such as collagen, vitronectin, laminin, fibronectin, and proteoglycans¹³. In particular, urokinase-mediated plasmin generation plays a role in the activation of several macrophage-derived metalloproteinases (MMP-3, -9, -12 and -13), triggering elastolysis and collagen lysis, resulting in media destruction and aneurysm formation¹⁴. If this mechanism is that really in cause in the course of SARS-CoV2 infection, a further negative impact on the arterial structure of the lungs is to be acknowledged. All these findings related to the anatomical structures of the lungs during VITT appear to be similar to those suggested to be present¹² and then found in

the pathology observation of the lungs' patients who suffered from COVID-19¹⁵. Therefore, the VITT syndrome belongs to the acute lung injuries which are characterized by extravascular and intravascular fibrin deposition as it was already stated many years ago¹⁶. Acute inflammation is the trigger of fibrin deposition both in the alveolar and vascular spaces because inflammation and blood coagulation/fibrinolysis are closely linked as they are two ancestral defensive systems¹⁷. However, an excessive immune response along with a secondary hypercoagulable state can be extremely dangerous, potentially being a fatal condition¹⁸. On the other hand, other clinical conditions, such as pneumonitis, chronic obstructive pulmonary disease, asthma and Gaucher's disease are to be considered among those in which pulmonary thrombosis has been recognized¹⁹. Finally, the pathogenesis of VITT is an example of how the immune system is closely linked to the hemostatic system. A Pf4-DNA fragment coming from the adenovirus vaccine is able to elicit an immune reaction in predisposed patients, hence inducing the activation of both platelets and monocytes which in turn lead to the thrombus formation²⁰. A schematic representation of the essential steps of the pathogenesis of VITT is depicted in the Figure 11.

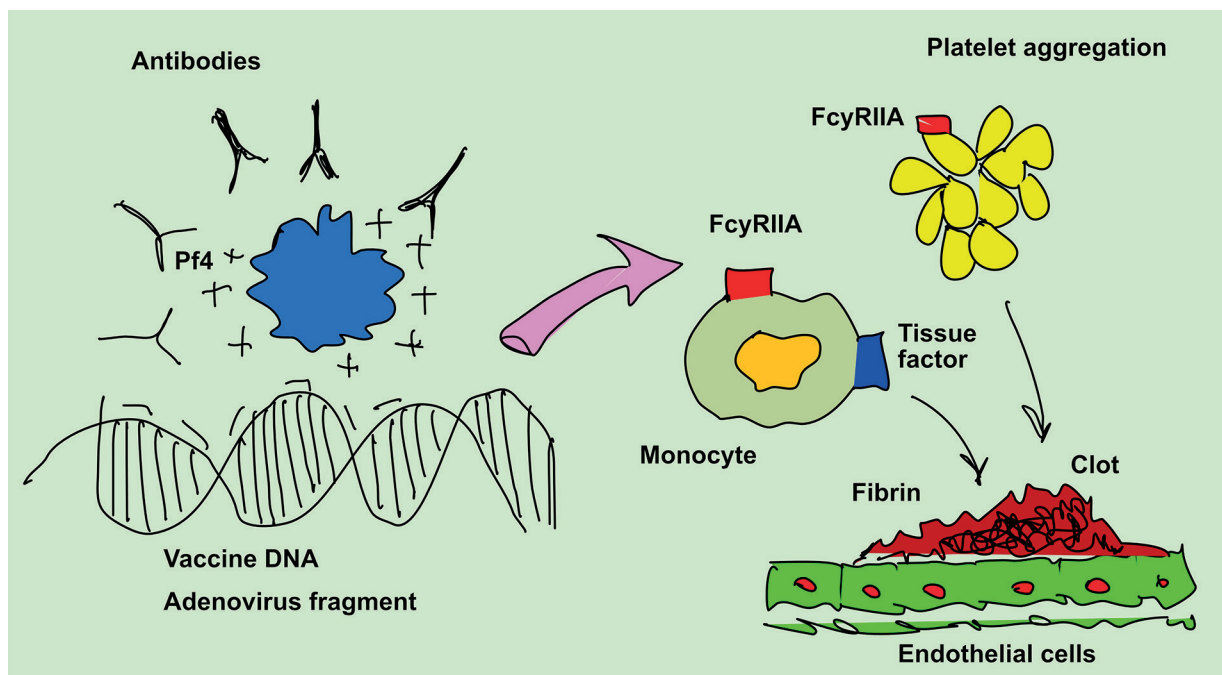


Figure 11. VITT pathogenesis: antibodies against DNA-Pf4 complexes induce platelets and monocytes activation via the the Fc γ RIIA receptor. Both Platelet aggregation and coagulation activation occur. The latter is triggered by tissue factor exposed by activated monocytes. Thrombosis occurs.

Conclusions

This report is the first, at least to our knowledge, which describes the ultrastructure characteristics of the pulmonary involvement in the course of VITT. The involvement of the arterial wall by the closely attached fibrin with a possible secondary damage is a new finding. The global scenario is quite similar to that found during COVID-19. In both the diseases the lungs suffer from the attack of the antigen-antibodies complexes and the virus respectively. The final outcome is a severe inflammation which in turn activates the hemostatic system and thus fibrinolysis. The latter may be the cause of a further damage to small arterial vessels so contributing to the abnormalities found in this report. Finally, it is worth noting that fibrin deposition is common in both the alveolar and vascular spaces.

Conflict of Interests

The authors declare that they have no conflict of interest.

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