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Thrombotic sinusoiditis and local diffuse intrasinusoidal coagulation in the liver of subjects affected by COVID-19: the evidence from histology and scanning electron microscopy

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Abstract. – OBJECTIVE: Liver injury has been reported in patients with COVID-19. This condition is characterized by severe outcome and could be related with the ability of SARS-CoV-2 to activate cytotoxic T cells. The purpose of this study is to show the histological and scanning electron microscopy features of liver involvement in COVID-19 to characterize the liver changes caused by the activation of multiple molecular pathways following this infection.

PATIENTS AND METHODS: Liver biopsies from 4 patients (3 post-mortems and 1 in vivo) with COVID-19 were analyzed with histology and by scanning electron microscopy.

RESULTS: The liver changes showed significant heterogeneity. The first case showed ground glass hepatocytes and scattered fibrin aggregates in the sinusoidal lumen. The second evidenced intra-sinusoidal thrombi. The third was characterized by sinusoidal dilatation, atrophy of hepatocytes, Disse's spaces dilatation and intra-sinusoidal aggregates of fibrin and red blood cells. The fourth case exhibited diffuse fibrin aggregates in the dilated Disse spaces and microthrombi in the sinusoidal lumen.

CONCLUSIONS: In COVID-19-related liver injury, a large spectrum of pathological changes was observed. The most peculiar features were very mild inflammation, intra-sinusoidal changes, including sinusoidal dilatation, thrombotic sinusoiditis and diffuse intra-sinusoidal fibrin deposition. These findings suggested that a thrombotic sinusoiditis followed by a local diffuse intra-vascular (intra-sinusoidal) coagulation could be the typical features of the SARS-CoV-2-related liver injury.

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Key Words:
SARS-CoV-2, COVID-19, Liver injury, Thrombosis, Sinusoiditis.

Introduction

Coronavirus Disease 2019 (COVID-19) has caused one of the most important global pandemics of the last century. On March 28th, 2021, 127,233,141 confirmed cases of COVID-19 with 2,789,941 deaths have been reported in the world. Most studies on COVID-19 have been focused on lungs as the main organ involved in the disease. Recently cardiovascular[1] and liver pathology have been introduced in the spectrum of SARS-CoV-2-related pathological changes[2]. In previous years, liver involvement has been described in patients infected by two coronaviruses. One is responsible for the severe acute respiratory syndrome (SARS)[3], the other one for the Middle East respiratory syndrome (MERS)[4]. Recent data on liver involvement in patients with COVID-19 evidenced abnormal liver tests in 75% of affected subjects. Liver injury has been reported in more than 20% of hospitalized patients. This observation was particularly associated with the progression toward severe pneumonia[5]. A more recent review[6] reported that the incidence of liver injury ranges from 15% up to 53% in survivors, and up to 78% in death patients.

Increased serum levels of liver enzymes have been more frequently reported in males[7], elderly patients and subjects with higher body mass index[8]. Patients presenting with severe COVID-19, admitted to intensive care units, were reported to show higher rates of liver dysfunction than subjects with less severe disease[9]. As a result, liver injury might be more prevalent in severe than in mild cases of COVID-19[10]. Patients with liver involvement displayed abnormal serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl-transferase (GGT)[5,10]. Moreover, elevated bilirubin levels[11] and decreased albumin serum levels have been reported in COVID-19 patients[12]. Regarding prognostic value of liver involvement in COVID-19 patients, no difference has been reported between survivors and non-survivors[13]. Recently, an electron microscopy study showed the presence of SARS-CoV-2 in liver cells[14]. Liver injury has been characterized by severe outcome[15]. Clinically significant acute liver[16] injury appears uncommon in COVID-19 patients. SARS-CoV-2 activated cytotoxic T cells might be the cause of liver derangement in some patients.

The purpose of this study is to present the histological features of liver involvement in 4 patients with COVID-19, associated with scanning electron microscopy findings, in order to better characterize the liver changes caused by the activation of multiple molecular pathways following this infection[17].

Patients and Methods

Liver biopsies of four COVID-19 patients were collected during the COVID-19 pandemic at the Pathology Unit of the University Hospital of Cagliari. After being formalin-fixed and paraffin embedded, biopsies were cut to four-micron-thick sections. The sections were stained with hematoxylin & eosin, silver stain and immune-stained for keratin 7. Then, a detailed histological report was described, including the most important elementary lesions of each case.

Some 6-7 μm thick slices were dedicated to scanning electron microscopy (SEM) observation. The freshly cut slices were mounted on the microscope slide. These slices were deparaffinized by immersion in xylene for 48 hours. Subsequently, they were re-hydrated in descending ethanol scales. They were treated with a solution of 1% osmium and 1.25% ferrocyanide for 1 hour in a humid chamber. After numerous washes in PBS (Phosphate Buffered Saline), the slides were dehydrated in ascending ethanol scales. The slides were dried at critical point in CO_2. Then, they were 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, https://www.zeiss.com/microscopy/int/about-us.html).

Results

Case 1

A 68-year-old man was admitted to a COVID hospital with a respiratory insufficiency. The molecular nasopharyngeal swab for SARS-CoV-2 infection tested positive. Transaminase serum levels were 2-3 times than normal values. Thus, a liver biopsy was performed. At histology, the liver architecture was preserved since no fibrous septa were evidenced. Most of the hepatocytes

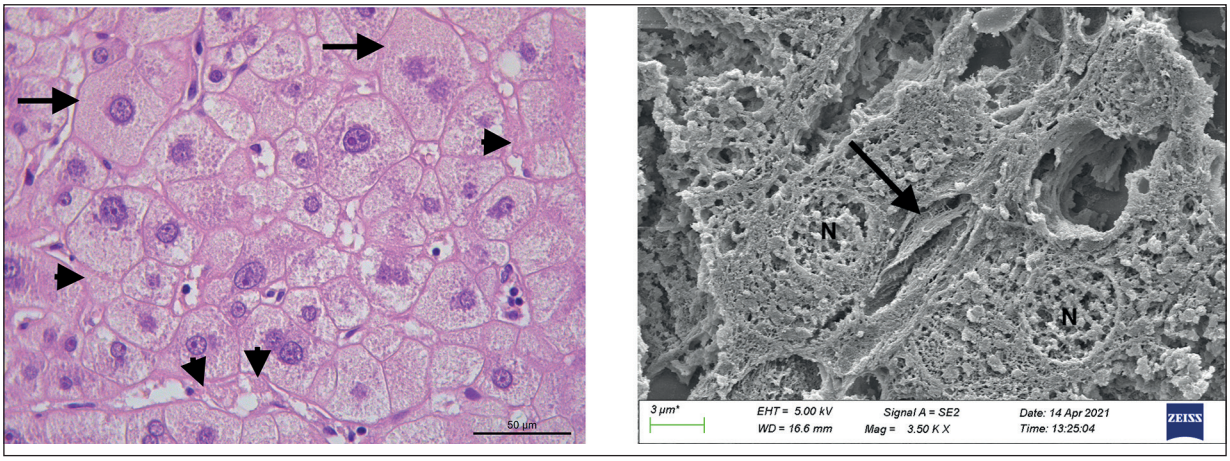

Figure 1. Liver biopsy of patient 1. **A,** At histology, hepatocytes are enlarged, with abundant granular cytoplasm, focally showing ground-glass appearance (arrows). Focally, in the lumen of dilated sinusoids, a fibrillary network is detected (arrowheads). H&E, original magnification: ×400. **B,** At SEM, fibrillary aggregates of fibrin are found inside the sinusoidal lumen (Arrow); hepatocyte nucleus (N). Original magnification: ×3.500.

showed a granular cytoplasm, with increased mitochondria. Several hepatocytes also showed a typical ground-glass appearance. Then, occasionally dilated sinusoids with fibrillar material inside the sinusoidal lumen were observed (Figure 1A). By scanning electron microscopy (SEM), fibrin aggregates were frequently observed in the sinusoidal lumen (Figure 1B).

Case 2

A 63-year-old man, after some days of dry cough and fever, developed a progressive respiratory failure. The patient died at home before hospitalization. Post-mortem molecular nasopharyngeal swab tested positive for SARS-CoV-2. At autopsy, lungs were heavy (right lung 1,100 g; left lung 920 g), reddish in color, diffusely edematous and exhibited a firm consistency. At histology, liver architecture was preserved. Likewise, no significant inflammatory changes were detected in portal tracts. Foci of macro- and micro-vesicular steatosis was observed in the hepatocytes. The most relevant changes were detected inside the sinusoids. These appeared dilated, with the lumen occupied by a fibrillar material, red blood cells, and scattered lympho-

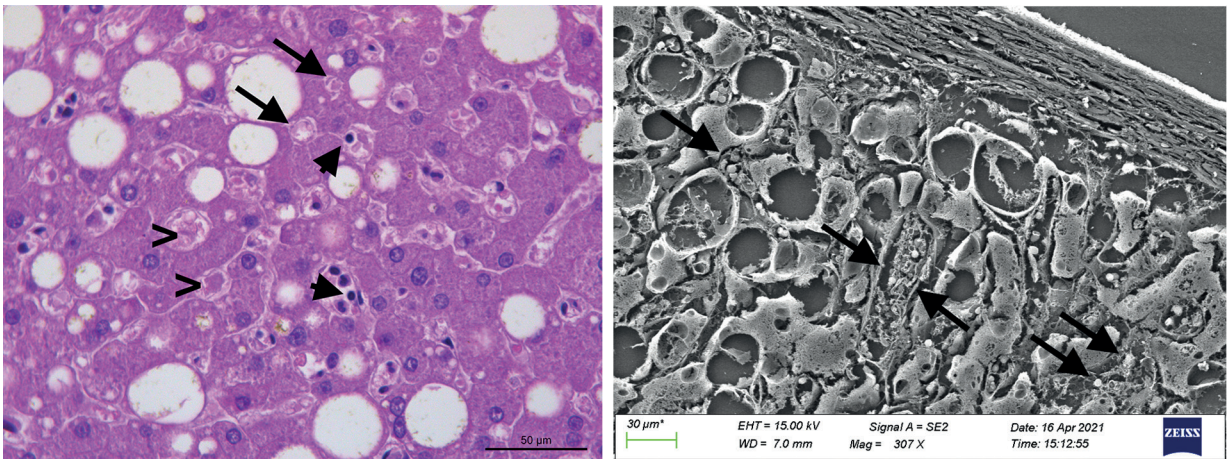

Figure 2. Liver of patient 2. **A,** At histology, hepatocytes show macro-micro-vesicular steatosis. Sinusoids are markedly enlarged, containing abundant fibrillary aggregates (Arrows), red blood cells and lymphocytes (Arrowheads). Some intrasinusoidal thrombi are also found (Open Arrows). **B,** At SEM, markedly enlarged sinusoids were occupied by a fibrin network, trapping red blood cells and lymphocytes (Arrows).

cytes. Microthrombi were focally found inside the sinusoidal lumen (Figure 2A). At SEM, sinusoids were confirmed as the main target of SARS-CoV-2 liver involvement. Sinusoids appeared larger and occupied by red blood cells and lymphocytes, surrounded by a fibrillary network, suggestive for fibrin (Figure 2B).

Case 3

A 56-year-old male died because of an acute respiratory failure. In order to better explain and detail the cause of the death the post-mortem autopsy was performed.

At autopsy, a liver specimen was obtained. The histological picture was characterized by marked and diffuse sinusoidal dilatation, associated with atrophy of the hepatocytic trabeculae. The sinusoidal lumen was occupied by eosinophilic fibrillar material of probable fibrin origin. Microthrombi were easily detected inside the sinusoidal lumen, in all the acinar zones (Figure 3A). Scanning electron microscopy confirmed the marked sinusoidal dilatation, occasionally evolving towards peliosis-like pattern (Figure 3B). Dilated sinusoids were occupied by a fibrin network, inside which red blood cells were frequently found. Intra-sinusoidal microthrombi were also observed. These findings were suggestive for a thrombotic sinusoiditis complicated by a local disseminated intravascular coagulation (CID) (Figure 3b). At higher power view, by Scanning electron microscopy it was possible to better evidence the fibrin network inside the lumen of dilated sinusoids (Figure 3C).

Case 4

A young woman with comorbidities was admitted to the hospital with respiratory insufficiency. Molecular nasopharyngeal swab confirmed the infection by SARS-CoV-2. At autopsy, a liver specimen was obtained. The histological picture showed a preserved architecture. A focal inflammatory lymphocytic infiltrate was found inside the acinus, associated with mild steatosis (Figure 4A). The most relevant changes were observed in the sinusoidal lumen, in which fibrin and red blood cells were found (Figure 4A). Disse's spaces appeared dilated, often occupied by fibrillar material. At scanning electron microscopy, the dilated sinusoidal lumen was occupied by a fibrin network, with trapped red blood cells. This feature gave rise to a thrombotic sinusoiditis pattern (Figure 4B). Fibrin aggregates frequently were found to extend into dilated Disse's spaces (Figure 4B). Microthrom-

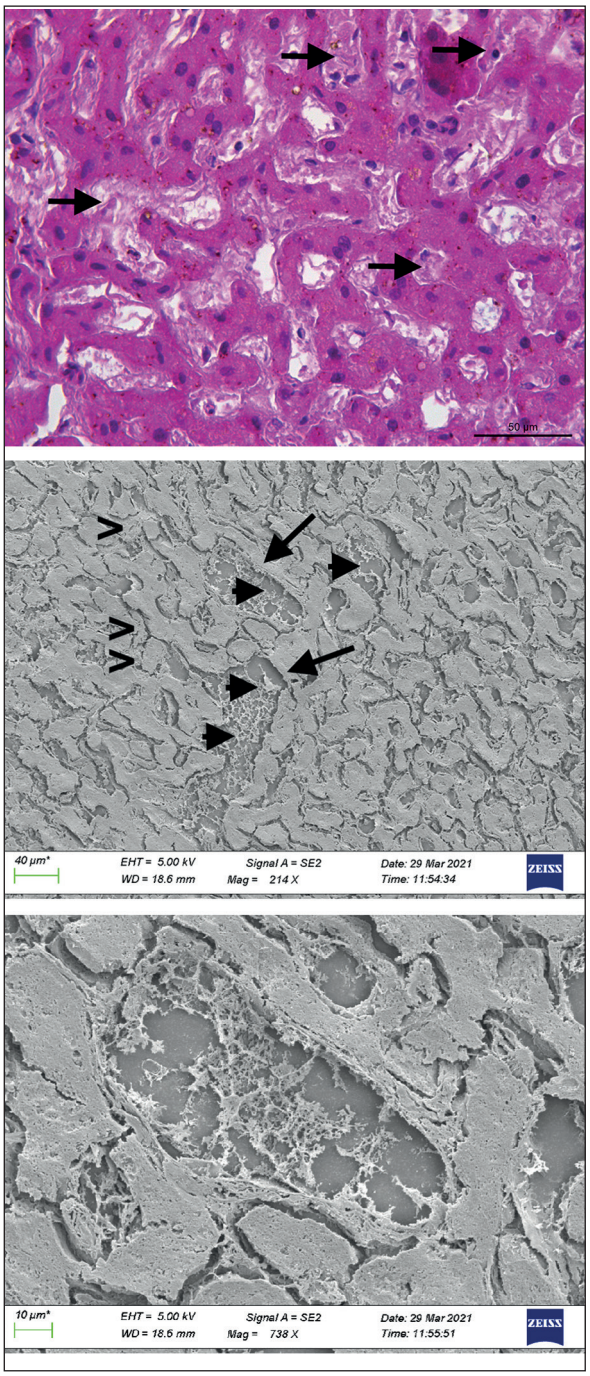

Figure 3. Liver of patient 3. **A,** The histological picture is characterized by a marked sinusoidal dilatation. Sinusoids contain abundant fibrillary material, highly suggestive for fibrin deposition, with the tendency to give rise to microthrombi (Arrows). **B,** SEM confirms the relevance of sinusoidal dilatation, focally originating a peliosis-like pattern (Arrows). Dilated sinusois contain a fibrin network (Arrowheads). Scattered intrasinusoidal microthrombi are also observed (Open Arrows). Original magnification: ×214. **C,** At high power, the fibrin network inside the sinusoidal lumen is more evident. Original magnificagtion: ×738.

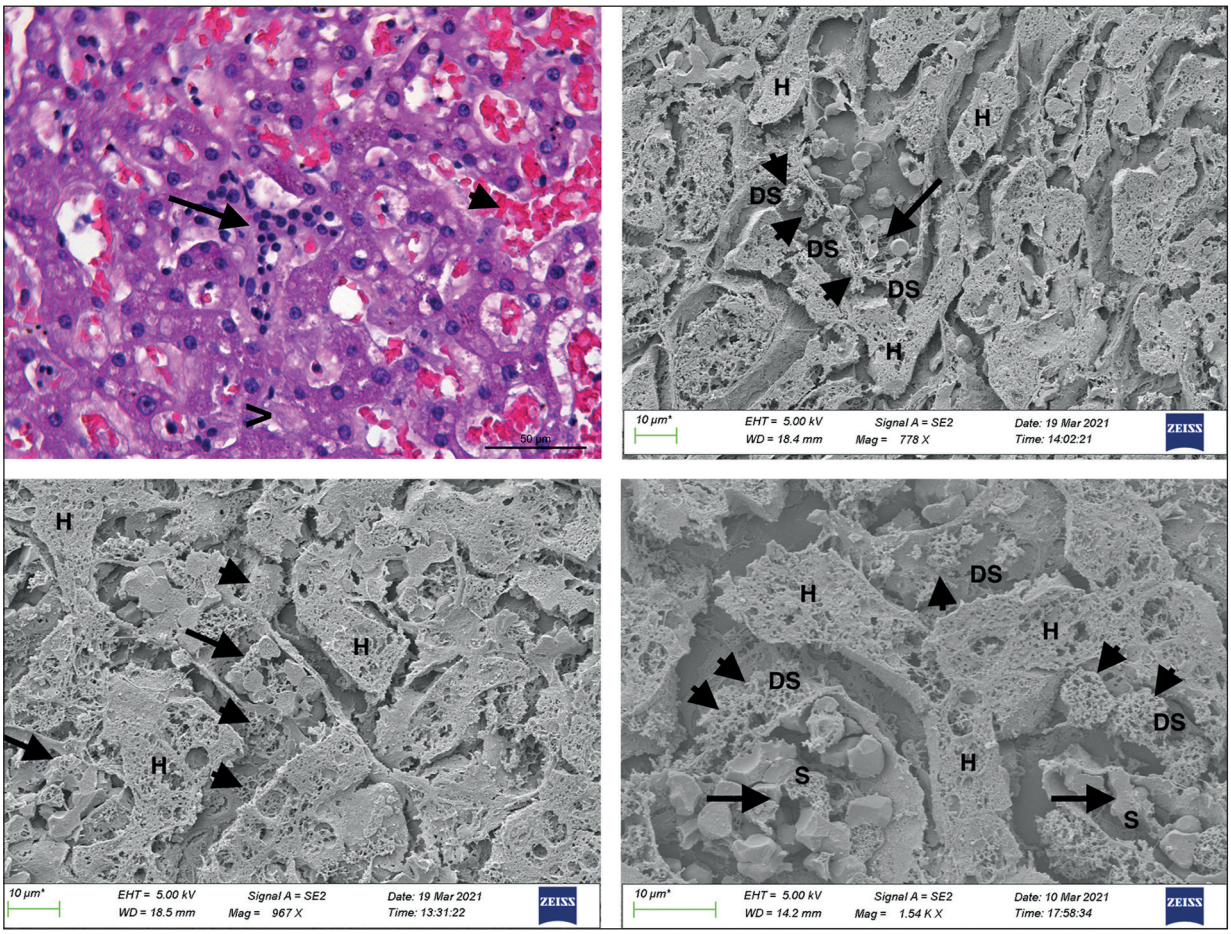

Figure 4. Liver of patient 4. **A,** At histology, sinusoidal dilatation, associated with scattered lymphocytes (Arrow) and red blood cells (Arrowhead) characterize the biopsy. Scattered microthrombi (Open Arrow) are also found. **B,** At SEM, Sinusoidal dilatation was associated with atrophy of hepatocytes (H). Red blood cells, enveloped by a fibrin network (Arrows) are observed in the sinusoidal lumen. Small globular fibrin aggregates (Arrowheads) are frequently observed in dilated Disse spaces (DS). Original magnification: ×778. **C,** Dilated sinusoids with microthrombi (Arrows) and dilated Disse spaces with globular aggregates of fibrin (Arrowheads) characterize the SEM pattern at higher power. H=hepatocytes. Original magnification: ×967. **D,** Atrophic hepatocytes (H), enlarged Disse spaces (DS) containing fibrin deposits (Arrowheads) and microthrombi (Arrows) inside the lumen of dilated sinusoids (S).

bi, mainly formed by fibrin and red blood cells, were regularly detected inside the sinusoidal lumen (Figure 4C). At higher power, it was possible to better characterize microthrombi inside the dilated sinusoids. Microthrombi were associated with the fibrin deposition inside delated Disse's spaces (Figure 4D).

Discussion

Intensive care teams, radiologists and pulmonologists have been the point of the medical spear in battling the COVID-19 pandemic in the world over the past year. This fact was due to the ability of SARS-CoV-2 to cause the acute respiratory distress syndrome, often fatal in older patients[18]. However, another group of specialists is becoming more engaged in the battle against SARS-CoV-2 infection: hepatologists.

The lifestyle changes were forced upon the world by the COVID-19 pandemic and by the multiple restrictions. As demonstrated by the literature[19], a surge in liver disease has been predicted in the months and years to come. Excessive caloric intake coupled with reduced physical activity and exercise are the well-known triggers of the development of non-alcoholic fatty liver disease (NAFLD). This is the most common chronic liver disease in Europe and USA[20].

In addition, SARS-CoV-2 can infect and damage liver tissue directly[14]. In this scenario, con-

flicting results have been reported regarding liver histology in patients with COVID-19. In the report of two COVID-19 autopsies, a 77-year-old man showed centrilobular steatosis, whereas a 42-year-old man showed liver cirrhosis, probably related to concurrent previous pathologies[21]. Steatosis has also been reported by Xu et al[22] as the main liver change associated with COVID-19[15]. Nevertheless, it should be taken into consideration that micro vesicular steatosis is a common finding in patients dying from sepsis of other origins.

The first open question on the relationship between liver injury and SARS-CoV-2 infection regards the clinical significance of the detection of high serum levels of transaminases in patients with severe COVID-19. Is it a real sign of liver involvement? Or instead, a "bystander hepatitis"? The latter has been occasionally observed to be responsible for the rise of transaminase serum level in other systemic viral infections[23].

Another open question regards the doubt that liver impairment might be mainly due to non-viral factors. Liver damage might also be secondary to the administration of several drugs used to treat subjects with severe disease. These drugs may include antibiotics, antivirals and steroids[24].

In particular, treatment with lopinavir/ritonavir has been associated with abnormal liver function tests[24]. A SARS-CoV-2 related reactivation of a pre-existing liver disorder, including autoimmune liver disease, should be also considered when dealing with an increase in transaminase serum levels in patients with COVID-19[25]. Furthermore, it is unknown the possible chronic consequences that SARS-CoV-2 infection might trigger in the liver[17]. The dysregulation of the renin-angiotensin system might activate stellate liver cells into myofibroblasts, favoring the development of liver fibrosis[26].

Liver changes of the four cases here described show a previously unreported marked variability of the elementary lesions detectable in the liver of patients affected by SARS-CoV-2 infection. Previous reports were mainly focused on micro vesicular steatosis as the main hepatocellular change[21]. Contrasting with that, liver injury in our patients changed significantly from one case to the next, often in the absence of a significant degree of steatosis. Ground glass hepatocytes characterized the first case, associated with the presence of scattered fibrin aggregates in the sinusoidal lumen. Intra-sinusoidal thrombi were the main feature of the second case. Sinusoidal dilatation with atrophy of hepatocytes, Disse's spaces dilatation and intra-sinusoidal aggregates of fibrin and red blood cells were the main changes found in the third case. The fourth case was characterized by the finding of focal fibrin aggregates and microthrombi in the sinusoidal lumen.

Our findings suggest the existence of a large spectrum of pathological changes in COVID-19-related liver injury. Alike of what has been seen in lung injury, COVID-19-related liver disease carries out the peculiar and specific features of SARS-CoV-2 infection. First, the inflammatory component elicited by this coronavirus infection is very mild in the liver, as previously reported by Sonzogni et al[27] in a large series of COVID-19 patients. Second, in our patients, sinusoids appear to be the site of the major changes in this infection. Intra-sinusoidal changes, including thrombi formed by a fibrin network with trapped red blood cells, represented the most peculiar feature observed in our cases. Other features were sinusoidal dilatation and activation of Kupffer cells. These findings taken together, indicated a thrombotic sinusoiditis followed by a local diffuse intravascular (intra-sinusoidal) coagulation as the typical features of the SARS-CoV-2-related liver injury. Our results confirm the hypothesis of a derangement of the intrahepatic blood vessel network as a marker of liver disease due to SARS-CoV-2[27]. The relevant sinusoidal damage, observed in 3 out of 4 cases in our study, are not in agreement with other reports. However, the 3 cases characterized by sinusoidal damage were the ones who died for the COVID-19. For this reason, we may suggest that the sinusoidal damage could be a marker of severe disease. Vascular pathology, including sinusoidal microthrombi, was infrequent, being seen in 15% of liver from patients with COVID-19[28]. We should also underline how in our study, the occurrence of severe sinusoidal changes was restricted to the more severe cases, in which SARS-CoV-2 infection led to death. On the contrary, in the liver biopsy of the patient who survived, sinusoidal fibrin deposition was mild and focal. These data taken together could induce to consider severe thrombotic sinusoiditis as an important negative prognostic factor in COVID-19 patients.

As for the mechanisms underlying the insurgence in the liver of these vascular changes, multiple hypotheses have been proposed according to the multiple molecular pathways triggered by SARS-CoV-2[17]. A virus-induced cytopathic effect might be responsible for the endothelial damage in the hepatic sinusoids. As a result, the

local thrombotic process is triggered, ending with a local disseminated intravascular coagulation (DIC). This is alike of what has been proposed for pulmonary thrombosis both in the course of COVID-19 infection[29] and other clinical conditions[30]. DIC is induced by monocytes which expose the tissue factor, the trigger of blood coagulation, on their membrane. Thus, monocytes release cytokines such as Interleukin 6 and Tumor Necrosing Factor. All these factors can provoke endothelial damage, which is able to expose the tissue factor. Tissue factor induces further activation of blood coagulation and thrombosis[31]. Blood coagulation is an ancestral system which works along with the immune system for trapping both bacteria and viruses. These mechanisms can explain why thrombosis appears to be a defensive response against foreign infective attacks[32].

Liver damage might be also related to the interaction between intrahepatic cytotoxic T cells and Kupffer cells[10]. Hypoxia and shock are related to the insurgence of acute respiratory distress syndrome (ARDS) induced by SARS-CoV-2. Hypoxia and shock might also cause hepatic ischemia and hypoxia-reperfusion dysfunction[33]. Indirect involvement of the sinusoidal endothelium by systemic inflammation, iatrogenic causes and ventilation might be added to the list[34]. The findings of this study seem to be similar to those of the Veno-Occlusion Disease (VOD). Though, the setting of VOD is quite different since in general it occurs after bone marrow transplantation[35].

Sinusoidal thrombosis is thought to be the outcome of an endothelial damage due to cytoreductive drugs on one hand, but also to a direct damage induced by cytokines on the other[36]. All these features can explain why liver may be the target of several and different injuries. These injuries also include SARS-CoV-2 infection.

Conclusions

In conclusion, in 3 out of 4 cases in the liver we found the morphological changes suggestive of a prothrombotic phenotype induced by SARS-CoV-2 infection, as described by Marongiu et al[37]. Interestingly, in our study, the higher blood coagulation activation was in the sinusoids of the 3 patients who died for the disease. The less blood coagulation activation was observed in the survivor patient (Case 1). These findings confirm previous reports indicating that a severe liver disease is associated with a severe outcome and with a poor prognosis. The local hepatic diffuse intravascular coagulation (DIC) mainly occurred in the sinusoidal lumen and in the Disse's spaces, giving rise to a liver injury with a peculiar pattern, that appears characteristic of COVID-19-related liver injury.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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