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BRIEF DEFINITIVE REPORT

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Liver histopathology in severe COVID 19 respiratory failure is suggestive of vascular alterations

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Abstract

SARS2-CoV-2 breakout in Italy caused a huge number of severely ill patients with a serious increase in mortality. Although lungs seem to be the main target of the infection, very few information are available about liver involvement, possibly evocating a systemic disease. Post-mortem wedge liver biopsies from 48 patients died from severe pulmonary COVID-19 disease with respiratory failure were collected from two main hospitals in northern Italy. No patient had clinical symptoms of liver disease or signs of liver failure before and during hospitalization; for each of them liver function tests were available. All liver samples showed minimal inflammation features. Histological pictures compatible with vascular alterations were observed, characterized by increase in number of portal vein branches associated with lumen massive dilatation, partial or complete luminal thrombosis of portal and sinusoidal vessels, fibrosis of portal tract, focally markedly enlarged and fibrotic. SARS-CoV-2 was found in 15 of 22 samples tested by in situ hybridization method. Our preliminary results confirm the clinical impression that liver failure is not a main concern and this organ is not the target of significant inflammatory damage. Histopathological findings are highly suggestive for marked derangement of intrahepatic blood vessel network secondary to systemic changes induced by virus that could target not only lung parenchyma but also cardiovascular system, coagulation cascade and endothelial layer of blood vessels. It still remains unclear if the mentioned changes are directly related to virus infection or if SARS-CoV-2 triggers a series of reactions leading to striking vascular alterations.

KEYWORDS

liver histopathology, liver morphology in COVID 19 disease, SARS-Cov-2 infection and liver biopsy

1 | INTRODUCTION

SARS-Cov-2 infection was first diagnosed in December 2019 in Wuhan, China, and then it spread all around the world. On 11th March 2020 the World Health Organization declares a worldwide pandemic status. The large majority of patients develop a mild self-resolving infection, but in some cases patients present pneumonia and the most severe of them progress to a systemic disease with multiple organ dysfunction.

Bergamo, a city located in north of Italy not far from Milan, became quickly the epicenter of the Italian COVID-19 outbreak. Up to the end of March 2020 more than 9000 people were infected and at least 2500 persons died. Even the area of Milan registered a high number of infected people.

To our knowledge, this is the first report of a large series of liver histopathology findings from COVID-19 patients died for respiratory failure. Significant liver clinical involvement has never been observed, neither liver pathology in COVID-19 disease nor clinical-pathological correlations have been reported.

2 | METHODS

Wedge liver sample was obtained post-mortem in 48 SARS-CoV-2-positive patients. Testing for HCV antibodies were negative in all patients; one patient was HBsAg positive/ HBV DNA negative.

Main demographical and comorbidity data are summarize in Table 1.

The autopsies were performed after a median time of 6 hours from death in Airborne-Infection-Isolation autopsy rooms and the medical staff used the correct Personal Protection Equipment (PPE), according to "Engineering control and PPE recommendations for autopsies". Only skilled pathologists (AS, AG, PZ, LC, AP and MN) were enrolled to perform post-mortem procedures.

Sampling procedure was partial autopsy limited to lungs, heart and liver in 30 patients (Papa Giovanni XXIII Hospital Bergamo) and a complete autopsy in 18 cases excluding brain (Luigi Sacco Hospital Milan). No patient had previous history of liver disease or portal hypertension nor developed clinical signs or symptoms of liver failure during hospital stay.

A median of two tissue blocks were taken random from each liver as macroscopic aspect was normal; the size of all the blocks obtained was comparable. Tissues were fixed in 10% buffered formalin for >48 hours and embedded in paraffin. Three-µm paraffin sections were stained with by Haematoxylin and Eosin.

Each wedge liver sample contained at least 20 portal fields; histological examination was performed blindly by experienced

TABLE 1 Demographics data and comorbidity findings in 48 patients

Male/Female (ratio)	22/8 (2.75:1)
Age (y) mean (range)	71 (32-86)
No comorbidity	6/45 (13.3%)
Hypertension	24/45 (53.3%)
Cardiovascular Disease (different than hypertension)	17/45 (37.8%)
Diabetes	13/45 (28.9%)
Obesity	7/45 (15.6%)
Kidney disease	10/45 (22.2%)
Pulmonary disease	5/45 (11.1%)
Information not available	10/45 (22.2%)

TABLE 2 Main histopathological findings in liver wedge biopsies

	All patients n = 48 (100%)
Portal vein parietal fibrosis (phlebosclerosis)	
Absent	19(39%)
Focal (up to 25% of the portal tracts)	14 (29%)
Multifocal (25%-75% of the portal tracts)	11 (22%)
Diffuse (>75% of the portal tracts)	4 (8%)
Herniated portal vein in periportal parenchyma	
Absent	12 (25%)
Focal (up to 25% of the portal tracts)	18 (37%)
Multifocal (25%-75% of the portal tracts)	13 (27%)
Diffuse (>75% of the portal tracts)	5 (10%)
Periportal abnormal vessels	
Focal (up to 25% of the portal tracts/ parenchyma)	27 (56%)
Multifocal (25%-75% of the portal tracts/parenchyma)	18 (37%)
Diffuse (>75% of the portal tracts/ parenchyma)	3 (6%)
Fibrosis	
Absent	11(24%)
Portal fibrosis	29 (60%)
Incomplete fibrous septa	8 (16%)
Lobular inflammation	
Absent	24 (50%)
Mild	23 (48%)
Moderate	1 (2%)
Severe	0 (0%)
Portal inflammation	
Absent	16 (33%)
Mild	32 (66%)
Moderate	0 (0%)
Severe	0 (0%)
Vascular thrombosis	
Partial portal	24 (50%)
Complete portal	11 (23%)
Incomplete sinusoidal	7 (14%)
Complete sinusoidal	6 (12%)
Parenchymal confluent necrosis	
Absent	30 (65%)
Mild (<25% of the lobule)	5 (11%)
Moderate (25%-50% of the lobular)	7 (15%)
Severe (>50% of the lobule)	6 (12%)
Steatosis	
Absent	22 (46%)

(Continues)

TABLE 2 (Continued)

	All patients n = 48 (100%)
Small droplets (% of affected hepatocytes)	3 (5,5,20% of liver cells) (6%)
Large droplets (%)	1 (20% of liver cells) (2%)
Mixed small and large droplets (%)	22 (46%) (50,50,50,40,20,10,30,10,10,10,20,50,10,20,30 25, 30, 10, 20, 60, 20, 15% of liver cells)

pathologists confident with liver histopathology (AS, LL, RR, AP and MN) and questionable reports were reviewed jointly. Supplementary immunohistochemical searches for immunophenotyping of inflammatory cells (CD3, CD20) or for easier detection of endothelial layer and vessel conformation (CD34, factor VIII, D2-40) were obtained in 20 cases. Actin smooth muscle antibody (SMA) was applied to all samples to study framework of muscular layer of portal veins and pericytes activation. Details of antibodies used are reported in Table S1.

SARS-CoV-2 RNA was searched in 22 samples by RNAscope® 2.5 LSx Assays Leica Biosystems' BOND RX System, using a method of in situ hybridization (ISH) to visualize single RNA molecules per cell in formalin-fixed, paraffin-embedded tissue mounted on slides.

Laboratory findings were obtained from clinical charts: we considered the highest value of P p-thrombin time (PT, expressed as international normalized ratio -INR-), D-dimer, aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GGT) and bilirubin reached during the hospital stay.

Results are expressed as ratio with the upper reference levels (fold-x-ULR); for platelet count and for Albumin the lowest value was selected. As a result of very short hospital stay, few data are lacking for some patients.

3 | RESULTS

Main histological findings obtained from liver biopsies are summarized in Table 2.

Lobular architecture was well preserved in all the samples; inflammatory infiltrate, when present, was mainly represented by scattered portal and lobular lymphocytes (predominantly CD4 T lymphocytes) with variable degree of portal vein endotheliitis

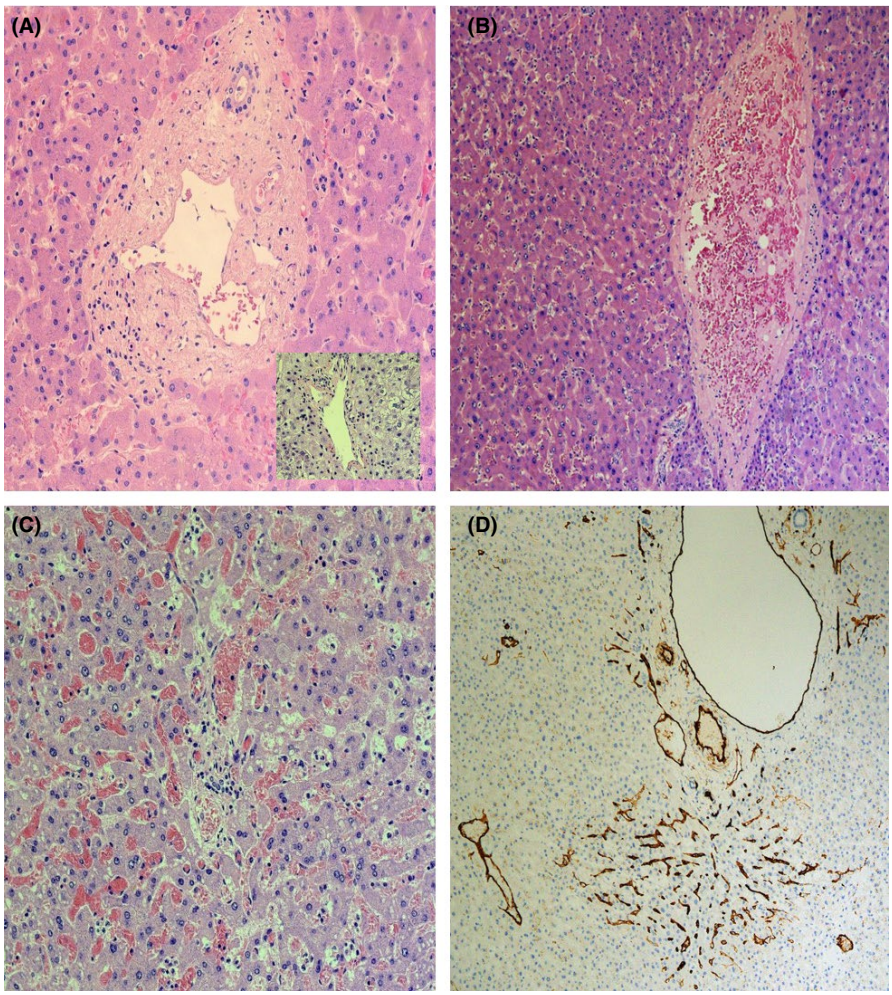

FIGURE 1 A: Regular liver architecture with scattered portal and lobular lymphocytes, moderate portal fibrosis; widened portal vein with fibrotic walls; bile duct without significant histological alteration. Compare normal portal tract in cadaveric liver donor (inset) (H&E, 100X); B: diffuse alterations of intrahepatic vascular structures characterized by severe dilatation and complete luminal thrombosis (H&E, 100X); C: portal vein and periportal sinusoids occlusive thrombosis (H&E, 100X); D: CD34 expression in abnormal portal vein branches endothelium and diffuse network of sinusoids in all parts of the lobule (100X)

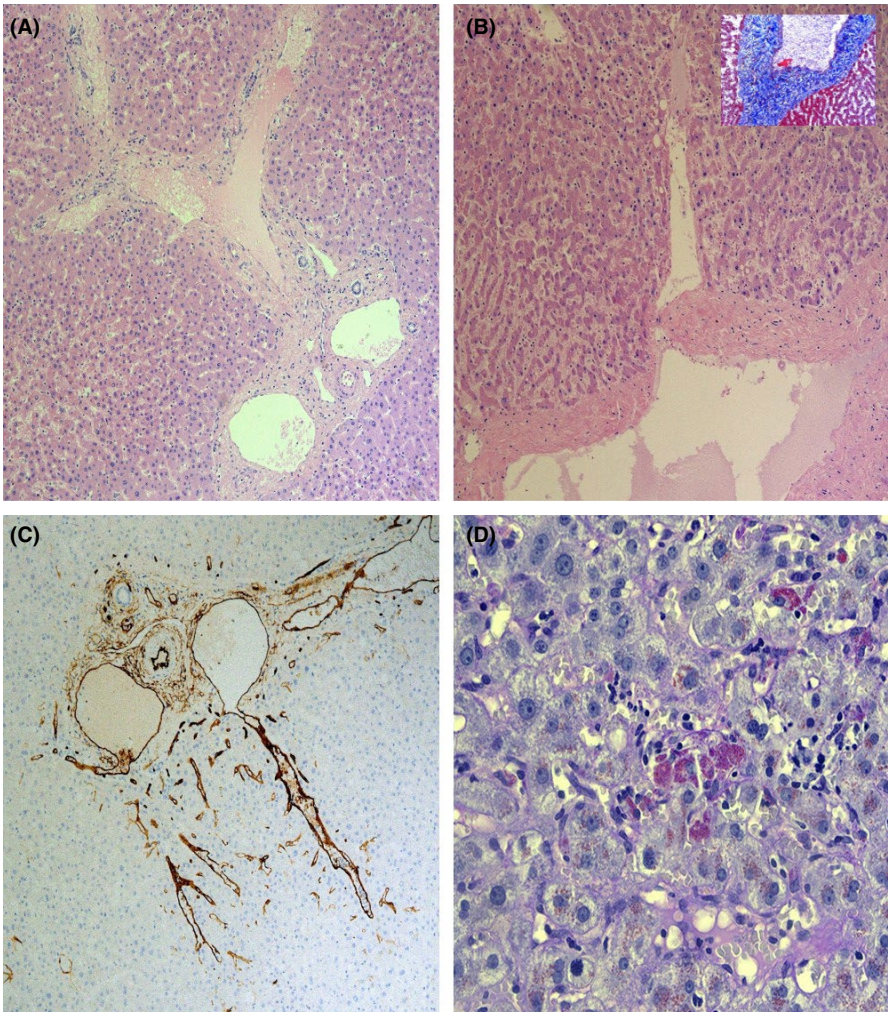

FIGURE 2 A: Roughly enlarged portal field with proliferations of portal veins and luminal severe dilatation (H&E, 100X); B: severe portal vein wall fibrosclerosis (H&E, 100X), highlighted by trichrome stain (inset) (100X); C: portal veins showing lumen focally herniated in periportal liver parenchyma and completely coated by hepatocytes (CD34, 100X); D: activated Kupffer cells with large cytoplasm containing necrotic debris (PAS diastase, 100X)

and only mild/moderate portal fibrosis was detected; biliary intrahepatic tree did not show any significant histological alteration (Figure 1A).

The definitely emerging morphological features were characterized by diffuse alterations of intrahepatic vascular structures (portal branches and sinusoids) and variable degree of partial/complete luminal thrombosis (Figure 1B,C); CD 34 decorated abnormally sinusoids in all lobular zones (Figure 1D). Central veins did not show significant alteration of lumen calibre and of wall configuration.

Majority of portal fields showed an increased number of portal veins associated with luminal severe dilatation (Figure 2A) and wall fibrosis (Figure 2B); in a large number of portal veins the lumen focally herniated in periportal liver parenchyma and completely circumscribed by liver cells plates (Figure 2C). Kupffer cells were found extremely activated with large cytoplasm containing necrotic debris (Figure 2D).

In order to exclude post-mortem cloths, only vessels with clearly enlarged lumen occupied by red cells mixed with lymphocytes and granulocytes were considered affected by thrombotic process. Questionable cases were reviewed jointly by two pathologists and diagnosis of thrombosis reported only if complete agreement was met (Figure 3A). Steatosis (large droplets, small droplets, mixed large and small droplets) was observed in more than 50% of samples; all 18 obese patients showed liver steatosis, which was also found in 14 overweight subjects (BMI >25, <30).

Smooth muscle layer of portal vein lamina media was fragmented (Figure 3B), partially lost and occasionally infiltrated by inflammatory cells lymphocytes, likewise overlying endothelium (Figure 3C). SMA antibody also decorated a large number of perivascular-activated pericytes within portal vein walls and in adventitial areas; this peculiar cells are not usually detected in routine setting.

Immunohistochemical search for C4d, performed in all samples, was completely negative.

SARS-CoV-2 was detected in 15/22 tested samples inside blood cloths or within endothelial cells cytoplasm.

All the patients but one showed some abnormality of liver function tests as summarized in Figure 4. The abnormality rate was as follows: AST 40/41 (97.6%), ALT 25/41 (61.0%), GGT 20/26 (76.9%) and albumin 18/22 (81.8%). Three patients experienced ALT level > 10xN and their liver morphology was characterized by confluent hemorrhagic necrosis (Figure 3D).

A total of 25/26 (96%) patients had a very high value of D-dimer values ≥500 ng/dL; the distribution of PT-INR values and platelet count are summarized in Figure 4 (bottom).

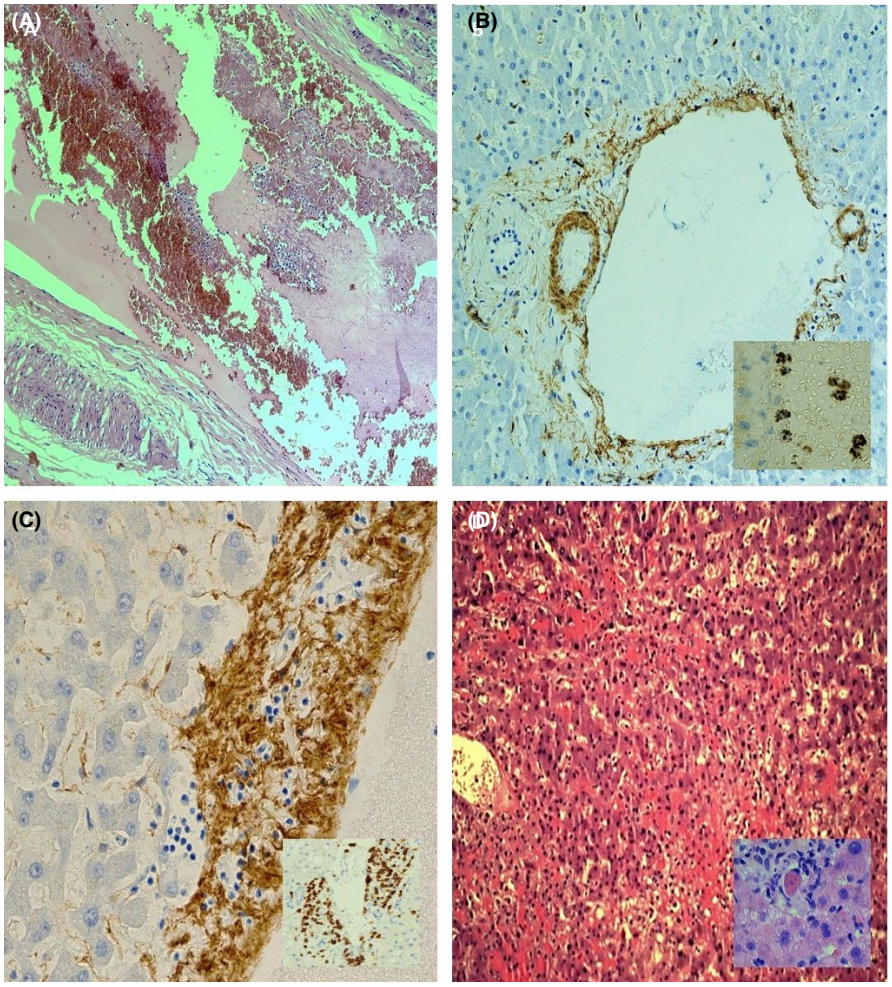

FIGURE 3 A: Example of genuine thrombosis: portal branch with clearly enlarged lumen obliterated by red cells mixed and stratified with lymphocytes and granulocytes (H&E, 100X); B: smooth muscle layer of portal vein lamina media extremely irregular fragmented (SMA, 100X); SAR-CoV-2 virions are demonstrated within vessel lumen and on endothelial cells (ISH) (inset); C: medium layer of a portal vein, partially lost and infiltrated by inflammatory cells lymphocytes, also attaching endothelial layer (SMA, 400X); CD3-positive lymphocytes attack endothelium and medium vessel layer (inset); D: severe confluent haemorrhagic necrosis in a patient with elevation of ALT > 10 N (H&E, 100X); inset showing liver necrosis by apoptosis (H&E, 400X)

4 | DISCUSSION

Morphological studies concerning description and interpretation of liver parenchymal changes induced or related to SARS-CoV-2 infection are completely lacking. By now only one post-mortem report is available in Chinese language and focused mainly on lung destructing lesions[1]; a review paper quoted a single case of liver post-mortem biopsy, pointing out bile duct damage as main histological feature.[2] Some authors stressed peculiar severe alterations of clotting parameters induced by viral infection.[3] Lung histopathological injury induced by COVID-19 infection has been extensively investigate in the largest group of subjects till now described, confirming severe thrombotic disturbances.[4]

Knowledge of liver pathology is totally blank and there are no comparisons available at present time among laboratory findings and liver histology.

Since outbreak of this pandemic in Lombardy (Italy), we had the unique option to collect a large series of liver samples of patients dead with SARS-CoV-2 infection: furthermore laboratory tests were available allowing a comparison between morphology and biochemical findings. Liver histology examination demonstrated that this organ is not involved in any significant inflammatory response to SARS-Cov-2 virus detected by ISH in tissue and does not develop any significant clinical and histological bile duct damage, in disagreement with a previous single report[2]; our data strongly support the clinical impression that liver failure is not a main issue in management of COVID-19-affected patients, as previously pointed out.[5]

All our morphological findings are consistent with a vascular-related damage caused by impaired blood flow, generating lesions similar to histological picture observed in hepatopulmonary syndrome and in obliterative portal venopathy.[6-9] Moreover diffuse network of sinusoids decorated by CD34 additionally suggests a disturbed circulation of blood within the liver.[10]

A presumable physiopathological explanation could be an increased blood flow within the liver, sometimes related to heart distress, and/or thrombotic phenomena in portal and sinusoidal vessels which modify intrahepatic blood circulation. High D-dimer levels in blood, previously described a risk factor in COVID 19 disease,[11] were evident in most patients and support this hypothesis along with histological changes in lungs of patients dead from COVID-19 infection which display huge number of thrombotic medium and small calibre branches of pulmonary arteries infection.[4]

The abnormal high levels of transaminases observed in three patients could be explained by extensive vascular portal and sinusoidal thrombosis, leading to confluent parenchymal necrosis and liver cells accelerated apoptosis. To support this hypothesis, we underline that cases of obliterative portal venopathy[6,9] were associated with

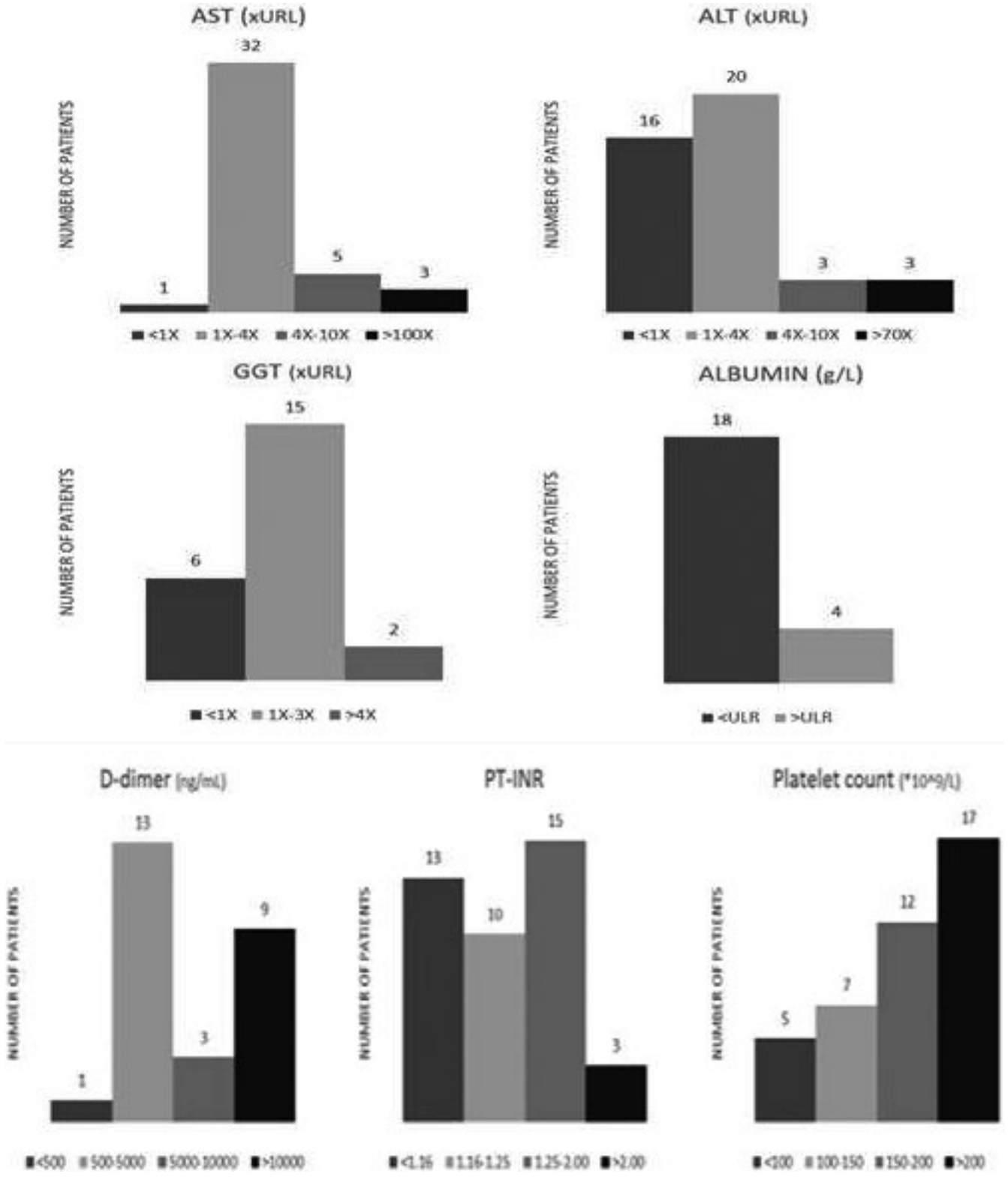

FIGURE 4 Biochemical changes in liver function tests (AST, ALT, GGT [fold-x-URL], albumin g/l) (top), PT-INR, D-Dimer and platelets (bottom). Data are not available for all patients as a result of a very short time of hospital stay. (URL: Upper Reference Limit)

congenital or acquired anomalies of some factors activated during clotting cascade. Another putative mechanism could be an immunological attack to endothelial layer, recently reported about lungs.[12]

The histological findings strengthen the hypothesis that the derangement of coagulation process or impairment of blood circulation or endothelial damage could be main triggers mechanism in pathogenesis of COVID-19 damage, not only within the liver but probably also in other solid organs. This theory fits really well with some recently published observations by a British group focusing the main rule of clotting system in COVID-19 liver damage.[5]

It is questionable if the injury needs strictly the ongoing presence of the SARS-Cov-2 virus in tissues or if the reaction targeting endothelium and vessels walls is induced by virus but it is afterwards self-maintaining; further investigations are in progress about this item.

Our findings concerning detection of SARS-CoV-2 virus in the liver by ISH method are really intriguing but at present insufficient to support any definite conclusion; we anyway support the hypothesis that viral infection wages a series of pathological processes that proceed disconnect from virus presence.

Another main point of interest concerns the evidence in all samples of massive pericytes activation. Active involvement in the recruitment of inflammatory cells in liver injury by pericytes is a well-known mechanism; inflammatory process may induce myofibroblast-like cells transformation of pericytes generating quick and abundant amounts of extracellular matrix proteins and finally leading to vessel wall fibrosis.[13]

A crucial question is if any previous chronic asymptomatic condition such as phlebosclerosis noted in variable degree of severity in all biopsies could be a hidden predisposing factor to liver damage partially related to the advanced age of the patients. This precondition may be exacerbated by a further inflammatory injury and pericytes activation induced by viral infection and clearly evident in our material.

A promising field of great interest will involve the study of endothelial cell alterations induced by virus infection and its interactions with clotting process. Further studies are required to reach more detailed information on this item.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Autoptic exams and histopathological examination: AS, AG, LL, DM, PZ, LC, RR, EL and AP. Paper writing: AS, MN, MGA, GP and MS. Laboratory testing and critical results evaluation: GP, MS and MG.

ETHICAL APPROVAL

This work is based exclusively on routine file autoptic material and is ethical approval exempt to use anonymous data. The study followed the Italian general rules used for research related to scientific purposes (official regulations n.72-26 /03/2012).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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