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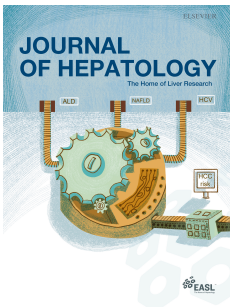

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Hepatocellular Type II Fibrinogen Inclusions in a Patient with Severe COVID-19 and Hepatitis

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A 62-year-old man without notable medical history was admitted to a regional hospital with high fever, cough and shortness of breath. Chest CT revealed bilateral ground-glass opacities consistent with viral pneumonia. SARS-CoV-2 infection was confirmed by positive RT-PCR on nasopharyngeal swab.

Initial treatment comprised atazanavir and ceftriaxone. Four days later, the patient's condition deteriorated and he required endotracheal intubation. He was then transferred to our tertiary care center for further management. Antimicrobial therapy was replaced by hydroxychloroquine and then remdesivir as well as by piperacillin-tazobactam (Fig. 1). Conventional lung protective ventilation with prone positioning was initiated. On day seven after transfer, despite antithrombotic prophylaxis with unfractionated heparin, bilateral segmental pulmonary embolism was diagnosed and therapeutic anticoagulation initiated. At that point, coagulation tests revealed slightly decreased prothrombin time (70%; reference range, 80-120%), explained by a mild constitutional isolated defect in factor VII, and D-dimers of 10,620 ng/ml (cut-off for venous thromboembolism, < 500 ng/ml) (Suppl. Table 1). By day 20, plasma fibrinogen rose to 7.1 g/l (reference range, 2.0-4.0 g/l). The further course was complicated by ventilator-associated pneumonia treated with cefepime and then meropenem as well as by critical illness polyneuropathy. PCR for SARS-CoV-2 was negative on bronchoalveolar lavage performed on day 21. A tracheotomy was performed on day 24.

In parallel, the patient developed hepatitis. On the day of admission to our center, transaminases were moderately elevated (alanine aminotransferase [ALT] 137 U/l [reference range, 11-60 U/l], aspartate aminotransferase [AST] 111 U/l [reference range, 14-50 U/l]), with only slightly elevated alkaline phosphatase (135 U/l; reference range, 36-108 U/l) and normal total bilirubin. Subsequent analyses revealed a progressive increase of ALT to a peak of 1048 U/l on day 25 (Fig. 1), with AST of 870 U/l, alkaline phosphatase of 196 U/l and total bilirubin of 26 µmol/l (reference range, < 21 µmol/l) (Suppl. Table 1). Synthetic liver cell function was preserved (factor V 140%). Antiviral medication and antibiotics had been stopped 20 days and 2 days prior to the peak of transaminases, respectively. The liver was normal on imaging, with patent portal and hepatic veins. Serologies and molecular testing for hepatitis B, C and E as well as herpes simplex, parvovirus B19, human herpesvirus 6, Epstein-Barr virus, and SARS-CoV-2 were negative. Blood PCR for cytomegalovirus (CMV) was positive at 50,800 copies/ml and ganciclovir at dose of 10 mg/kg/day was started, resulting in a drop of viremia to 2,100 copies/ml within 10 days.

Liver biopsy performed on day 25 revealed a mild lymphoplasmocytic infiltrate in the portal tracts, without interface hepatitis or fibrosis, together with a few apoptotic hepatocytes scattered throughout the lobules. The presence of some hepatocyte mitoses and of numerous ceroid macrophages indicated that hepatitis had been ongoing for a while already.

There was no evidence of endotheliitis or hemophagocytosis, and there was no sinusoidal fibrin deposition. The more striking histological feature was the presence of numerous ground-glass hepatocytes with weakly eosinophilic cytoplasmic inclusions of various size, showing round or reniform shape and sharp edges (Fig. 2A). Periodic Acid Schiff (PAS) stain was negative (Fig. 2B), as well as immunochemistry for hepatitis B surface antigen (not illustrated). The cytoplasmic inclusions strongly reacted with an anti-fibrinogen antibody (Fig. 2C), demonstrating that they were composed mainly of fibrinogen. They were also positive, in a patchy pattern, for C-reactive protein (not illustrated). Immunochemistry for CMV was negative; PCR for CMV in the tissue was only weakly positive (200 copies/ml). At electron microscopy, the inclusions contained a homogenous, moderately electron dense granular material (Fig. 2D). They were delineated, at least focally, by a membrane, arguing in favor of dilated endoplasmic reticulum (Fig. 2E). Hence, the morphological picture suggested hepatocellular type II fibrinogen inclusions (1).

Genetic analysis did not reveal any known mutations responsible for fibrinogen storage disease in exons 8 and 9 of the *FGG* gene.

In the following, the patient's condition progressively improved and he could be successfully weaned from mechanical ventilation on day 37. On day 44, at the time of this writing, ALT has dropped to 384 U/l, with AST of 166 U/l, alkaline phosphatase of 323 U/l and normal total bilirubin.

Discussion

In a patient with severe COVID-19, we describe an unusual form of liver disease, characterized by a ground-glass appearance of the hepatocytes resulting from the pathological cytoplasmic accumulation of fibrinogen. The differential diagnosis of ground-glass hepatocytes includes first the presence of hepatitis B surface antigen (HBsAg) in chronic hepatitis B infection that can be identified by specific immunohistochemistry (2). Then, most of the other types of ground glass inclusions are linked to the accumulation of abnormal glycogen granules and are therefore PAS positive (2). They are observed in Lafora's disease, type IV glycogenosis and cyanamide aversion therapy in alcoholic patients (2), and have also been more recently described as "polyglucosan-like" hepatocellular inclusions in patients under polypharmacotherapy (3). In our patient, the ground glass inclusions were PAS negative, which prompted us to think of the possibility of abnormal fibrinogen accumulation, confirmed by the strong immunohistochemical reaction with an anti-fibrinogen antibody, and by the electron microscopy feature of membrane-bound inclusions (1, 2).

Fibrinogen is a large, oligomeric glycoprotein complex produced in the liver and secreted into the blood. Fibrinogen Aα, Bβ and γ chains are encoded by the *FGA*, *FGB* and *FGG* genes, respectively. These are located on chromosome 4 and expressed almost exclusively in hepatocytes. Fibrinogen is converted by thrombin to fibrin, the most abundant component of a blood clot (4-5). Plasma fibrinogen levels are increased by mediators of the acute-phase inflammatory response, e.g. IL-6, or may be decreased as a result of consumption in disseminated intravascular coagulation. On the other side, mutations in fibrinogen genes cause congenital disorders that are typically associated with a-, hypo-, and/or dysfibrinogenemia (6). In rare cases, a few mutations clustered in exons 8-9 of *FGG* result in the intracellular accumulation of misfolded fibrinogen in hepatocytes, chronic liver disease of various severity, and hypofibrinogenemia (7). Of note, fibrinogen storage disease without hypofibrinogenemia, which corresponds to the clinical picture presented by our patient, has rarely been associated with acute infections in patients without any hereditary defect of fibrinogen (8-9).

Our patient presented very high plasma fibrinogen levels, making the presence of a known *FGG* mutation very unlikely, as confirmed by genetic testing. Increased fibrinogen production likely played a key role in the hypercoagulable state and pulmonary embolism (10). Increased fibrin formation and lysis can account for the very high levels of D-dimers as observed in our patient. This has been previously associated with worse outcomes in patients with COVID-19 (11-13).

Information on liver involvement in COVID-19 is limited to date (14-16). Elevated transaminases have been noted in up to 53% of patients with COVID-19. Liver injury in patients with SARS-CoV-2 infection may be caused by direct viral effects or indirectly by the systemic inflammatory response, drug toxicity, hemodynamic alterations or other factors. It appears to be more prevalent in severe as compared to mild cases of COVID-19.

Only few reports have assessed liver histology in COVID-19 (17-19). Observed lesions include sinusoidal dilatation, mild portal and lobular inflammation, microvesicular steatosis or patchy necrosis. To our knowledge, a manifestation similar to the one documented in our patient has not been reported to date in the setting of SARS-CoV-2 infection.

The histopathological substrate in our patient was an acute mostly lobular hepatitis with hepatocellular type II fibrinogen inclusions (9) associated with high plasmatic fibrinogen levels in a context of severe systemic inflammation. Based on the temporal relationships, none of the administered drugs could be unequivocally linked to ALT increase. However, it is possible that one of them or another as yet unidentified extrinsic or intrinsic factor impaired

fibrinogen secretion and contributed to intrahepatic accumulation as a "second hit". Of note, hydroxychloroquine impacts on lysosomal function, autophagy and the Golgi apparatus (20-21). Although our patient had been treated with hydroxychloroquine for only two days, one may speculate that this may have contributed to pathological hepatic accumulation of fibrinogen. A direct viral effect is less likely given the negative PCR results on nasopharyngeal swabs and bronchoalveolar lavage performed 10 days and four days prior to liver biopsy, respectively. In addition, SARS-CoV-2 does not appear to circulate systemically at relevant levels (22).

Experimental studies will have to confirm a cascade linking SARS-CoV-2-induced severe inflammation, hyperfibrinogenemia and an as yet unidentified additional factor impairing hepatic fibrinogen secretion with acquired fibrinogen storage disease and hepatitis.

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Legends to Figures

Figure 1. Liver biopsy findings. Pale hyaline ground-glass inclusions are present in the cytoplasm of numerous hepatocytes (A, hematoxylin-eosin). They are negative for Periodic Acid Schiff staining (B), while exhibiting strong immunohistochemical reactivity for fibrinogen (C). At electron microscopy, they contain a faintly granular amorphous electron dense material (D, E) and appear as membrane-bound inclusions (E, arrowheads).

Figure 2. Evolution of ALT over time. The arrow denotes the liver biopsy. ATZ, atazanavir; HCQ, hydroxychloroquine; RDV, remdesivir.

Supplementary Table 1. Evolution of laboratory parameters since transfer of the patient to our center (= day 0).

Parameters	Day 0	Day 7	Day 14	Day 21	Day 28	Day 34	Day 44
Hemoglobin (g/l)	104	93	85	87	84	84	87
White blood cells (G/l)	12.3	10.5	12.5	14.7	19.6	9.6	11.0
Lymphocytes (G/l)		1.03	0.63	1.32	1.76	0.38	1.36
Platelets (G/l)	123	84	232	318	196	212	256
D-dimers (ng/ml)	> 35,200	10,620	8,112	6,140	5,087	7,203	
Fibrinogen (g/l)			6.4 (day 18)	7.1	5.1	5.1	4.4 (day 39)
Prothrobin time (%)	55	70	60	55	50	50	50
Total bilirubin (µmol/l)	16	17	26	20	24	19	11
AST (U/l)	111	430	428	568	577	490	166
ALT (U/l)	137	382	426	646	1047	828	384
Alkaline phosphatase (U/l)	135			247	165	230	323
γ-GT (U/l)	54			224	190	209	287
Albumin (g/l)		21		19	22	22	
C-reactive protein (mg/l)	372	230	199	154	35	22	
Procalcitonin (µg/l)	28.5	4.8	4.0	3.0	2.3	3.2	
Ferritin (µg/L)	6114	6016	8285	7394	6279	4936