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Hepatocellular type II fibrinogen inclusions in a patient with severe COVID-19 and hepatitis

To the Editor:

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 7 after transfer, despite antithrombotic prophylaxis with standard-dose 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 factor VII deficiency, and D-dimers of 10,620 ng/ml (cut-off for venous thromboembolism, <500 ng/ml) (Table S1). 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 1,048 U/L on day 25 (Fig. 1), with AST of 870 U/L, alkaline phosphatase of 196 U/L and total bilirubin of 26 μ mol/L (reference range, <21 μ mol/L) (Table S1). Synthetic liver cell function was preserved (factor V 140%; reference range, 70–180%). 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 lymphoplasmacytic 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. 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 (HBsAg, 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.

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 writing, ALT has dropped to 384 U/L, with AST of 166 U/L, alkaline phosphatase of 323 U/L, and normal total bilirubin.

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 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 α , β 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

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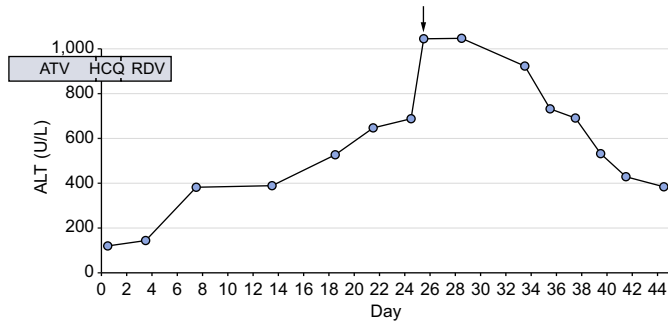


Fig. 1. Evolution of ALT over time. The arrow denotes the liver biopsy. ALT, alanine aminotransferase; ATZ, atazanavir; HCQ, hydroxychloroquine; RDV, remdesivir.

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. Mutations in fibrinogen genes cause congenital disorders that are typically associated with a-, hypo-, and/or dys-fibrinogenemia.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 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 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 that 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 inclusions⁹ 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

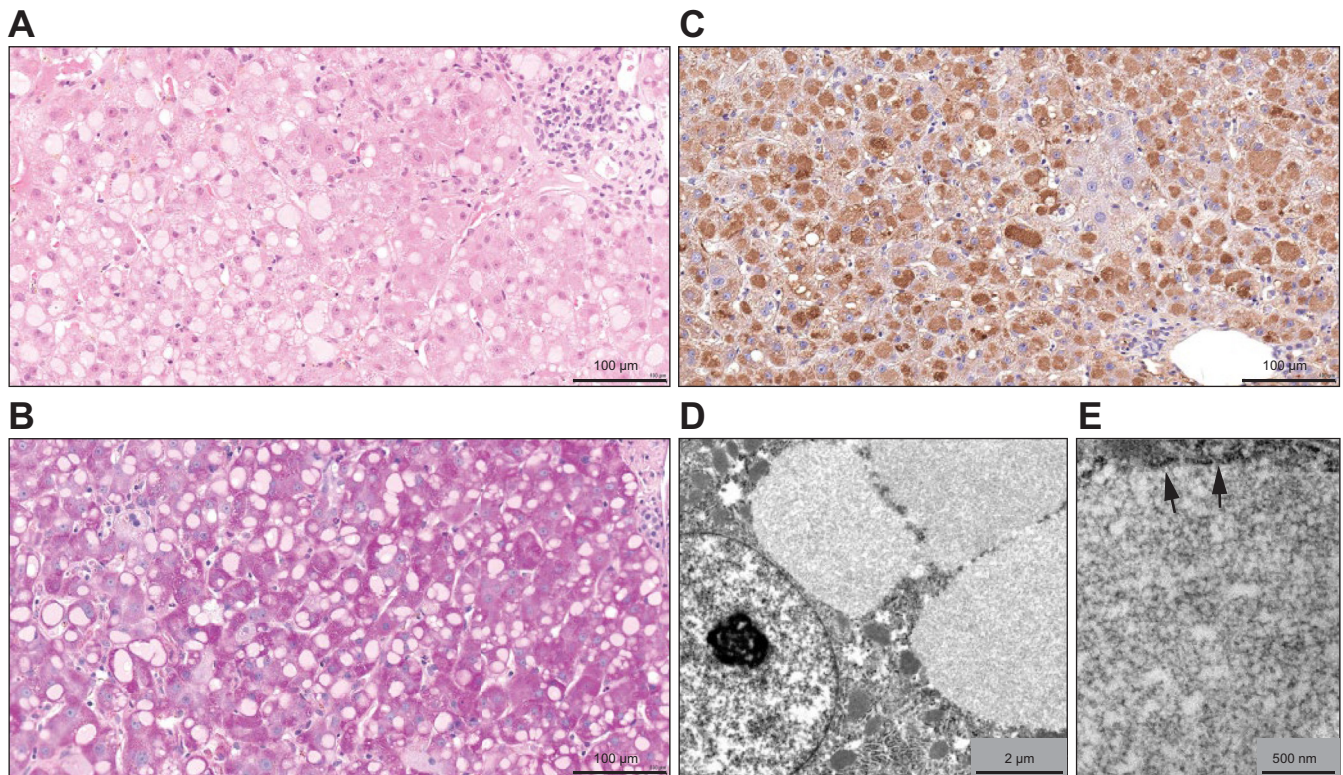


Fig. 2. Liver biopsy findings. Pale hyaline ground-glass inclusions are present in the cytoplasm of numerous hepatocytes (A, hematoxylin-eosin). They are negative for Periodic Acid Schiff staining (B), while exhibiting strong immunohistochemical reactivity for fibrinogen (C). At electron microscopy, they contain a faintly granular amorphous electron dense material (D, E) and appear as membrane-bound inclusions (E, arrowheads).

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 2 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 4 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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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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Authors' contributions

All authors were involved in the clinical management of the described patient. MF, DM, LA and CS wrote the manuscript. All authors revised the manuscript for important intellectual content.

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Supplementary data

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Extending the mode of action of triethylenetetramine (trientine): Autophagy besides copper chelation

To the Editor:

In this *Letter*, we aim to highlight a novel mode of action of triethylenetetramine (TETA), an FDA-approved copper-chelating agent used as a second-line treatment in patients affected by Wilson disease (WD). WD is a pathological condition characterized by an aberrant copper accumulation (linked to loss-of-function mutations in the gene coding for the export transporter ATP7B) that eventually causes hepatocyte poisoning and death, culminating in liver failure. The rationale for using TETA in WD is based on the ability of TETA to act as a mild copper chelator, reducing copper absorption in the gastrointestinal tract and favoring its elimination via feces and urine.^{1,2}

It has been previously demonstrated that copper overload can directly impair mitochondrial structure and dynamics, even at pre-pathological stages of the disease.^{3,4} The progressive accumulation of non-disposable copper-loaded mitochondria may explain a compensatory increase in cellular autophagy (which reportedly occurs in WD hepatocytes⁵), a pro-survival pathway that couples the bioenergetic demands of cells with the sequestration and subsequent lysosomal digestion of intracellular components.⁶ Consistently, blocking the autophagic process through the ablation of the essential autophagy gene *ATG7* precipitates the death of *ATP7B*^{-/-} hepatocytes challenged with copper, supporting the conclusion that autophagy is essential for cellular survival.⁵ Thus, insufficient autophagy may contribute to the initiation and progression of WD, in line with the observation that obesity, a state in which autophagy is inhibited due to the excess of nutrients, aggravates WD.⁷

Of note, a minimal amount of copper is required for the initiation of the autophagic cascade, as copper binds to and stimulates the kinase activity of the pro-autophagic kinases, Unc-51 like autophagy activating kinases 1 and 2 (ULK1, ULK2). Complete depletion of copper hence blocks autophagy.⁸ This sheds doubt on the long-term utility of copper chelation therapies for the treatment of WD. While the use of very high-affinity copper chelators⁹ can effectively attenuate the abnormally elevated copper burden, it is an open question whether copper

depletion below a threshold would not suppress autophagy, thus compromising the fitness of hepatocytes that depend on a constant autophagic flux, even at baseline.¹⁰

Driven by the fact that TETA displays a marked structural similarity to spermidine, a natural polyamine with widespread autophagy-dependent pro-health functions,¹¹ we decided to assess the effects of systemic TETA treatment in mice conditioned with high-calorie diets. Similar to spermidine,¹² treatment with TETA alleviated signs of metabolic syndrome

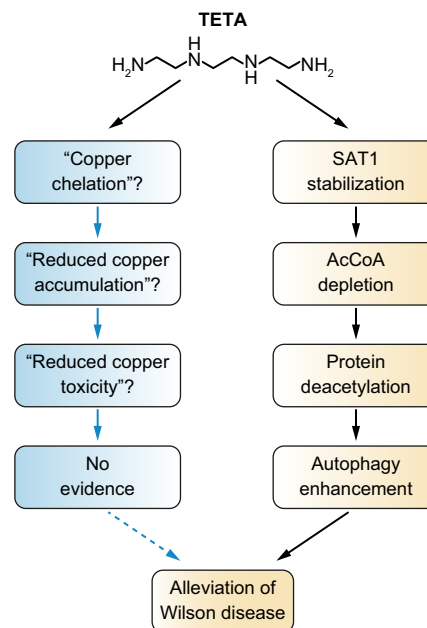


Fig. 1. Possible mode of action of TETA against Wilson disease. TETA is generally thought to act as a copper chelator (left side) that at the end avoids toxic copper accumulation in hepatocytes. However, this hypothesis is not entirely proven and, in mice, long-term TETA treatment fails to reduce the copper levels in internal organs. As an alternative (right side), TETA may act as an inducer of autophagy, thereby mediating hepatoprotective effects. In this scenario, TETA stabilized the enzyme spermidine acetyl transferase-1 (SAT1), thus increasing the consumption of acetyl coenzyme A (AcCoA), finally resulting in protein deacetylation and autophagy enhancement. Future investigation must determine whether autophagy induction alone (without copper chelation) may have beneficial effects on Wilson disease. TETA, triethylenetetramine. (This figure appears in color on the web.)

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