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Case Report

Secondary hemophagocytic lymphohistiocytosis and severe liver injury induced by hepatic SARS-CoV-2 infection unmasking Wilson's disease: Balancing immunosuppression



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ABSTRACT

A 21-year-old woman was hospitalized due to coronavirus disease 2019 (COVID-19)-associated respiratory and hepatic impairment concomitant with severe hemolytic anemia. Upon diagnosis of secondary hemophagocytic lymphohistiocytosis, immunosuppression with anakinra and steroids was started, leading to a hepatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and viremia. Subsequent liver biopsy revealed virus particles in hepatocytes by electron microscopy and SARS-CoV-2 virus could be isolated and cultured. Immunosuppression was stopped and convalescent donor plasma given. In the differential diagnosis, an acute crisis of Wilson's disease was raised by laboratory and genetic testing. This case highlights the complexity of balancing immunosuppression to control hyperinflammation versus systemic SARS-CoV-2 dissemination.

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Case presentation

On March 27, 2020, a 21-year-old female patient with known comorbidities complained of nausea, loss of appetite, and a frontal headache (illness day 1). The following day she noticed limb and back pain, a dry cough, and scleral icterus, as well as discoloration of the feces and dark urine. On day 3, she was hospitalized with fever (39 °C) and painless icterus. Abdominal

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imaging revealed hepatosplenomegaly and sludge in the gallbladder without cholestasis or signs of cholecystitis. She was started on ceftriaxone and metronidazole. The next day she developed diarrhea, emesis, and acute renal failure (inactive urine sediment). Examination of a stool sample showed no evidence of pathogenic bacteria. Laboratory work-up revealed a progressive hemolytic, macrocytic anemia without vitamin B12 or folate deficiency. Peripheral blood smear showed no spherocytes or schistocytes. A direct Coombs test as well as Hanta virus and leptospirosis testing were negative. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA testing of an oropharyngeal sample was positive. As her condition deteriorated, she was transferred to the University Hospital Regensburg on day 5.

Diagnostic work-up showed coronavirus disease 2019 (COVID-19) typical infiltrates in a thoracic computed tomography scan and respiratory insufficiency (SpO₂ 92% with 6 L/min of oxygen). A peripheral blood smear showed thrombocytopenia and activated lymphocytes likely associated with COVID-19 (Lüke et al., 2020). Ferritin was massively elevated, while ceruloplasmin was severely depressed, hinting at possible Wilson's disease. Thus a 24-h urine collection and copper analysis was initiated. Liver function tests were elevated, combined with a severe impairment of liver synthesis parameters (**Supplementary Material Table S1**). Auto-immune hepatitis was ruled out by serological tests. Additional clinical examination revealed no abnormalities apart from peripheral edema (in particular, no Kayser–Fleischer rings).

A bone marrow aspirate showed hemophagocytosis (**Supplementary Material Figure S1A, B**). **The HScore yielded 273 points, representing a >99% probability of** secondary hemophagocytic lymphohistiocytosis (sHLH) (Fardet et al., 2014; Mehta et al., 2020). Genetic testing revealed no clinically relevant sequence variants or copy number variations in the coding regions and flanking splice sites of the four genes currently associated with familial hemophagocytic lymphohistiocytosis: *PRF1*, *UNC13D*, *STX11*, *STXB2*. As elevated ferritin was found to be a predictor of fatality in a retrospective study suggesting mortality in COVID-19 is partly caused by virus-induced hyperinflammation (Ruan et al., 2020) and these patients likely benefit from immunosuppression (Mehta et al., 2020), anakinra (an interleukin-1 β antagonist) at a dose of 100 mg subcutaneously per day was initiated on day 6, resulting in mild clinical and laboratory improvements (Figure 1). It was decided to prescribe anakinra, because it has a short half-life and is therefore easy to adjust to the patient's clinical course. On day 9, leukopenia was detected and anakinra was stopped. On day 10, the patient deteriorated clinically, developed orthostatic hypotension, and central venous oxygen saturation dropped. On suspicion of worsening sHLH, anakinra was restarted together with 250 mg of prednisolone.

Over the next 2 days, the patient developed a fever, and plasma ferritin, transaminase, and lactate levels rose. Blood and urine cultures, as well as peripheral blood PCR screening for systemic viral infections including cytomegalovirus, adenovirus, Epstein–Barr virus, parvovirus B19, and human herpesvirus 6, and HIV/hepatitis A–E serology were negative. Both the antibiotic regimen and the immunosuppressive therapy were intensified (Figure 1). Oral zinc supplementation was started as a therapeutic option with minimal side effects for suspected Wilson's disease. Over the next 2 days, the fever disappeared but liver function (**Supplementary Material Table S1, Fig. 1**) and the patient's clinical condition worsened. A transjugular liver biopsy and further microbiological work-up were performed. Coronavirus viremia (10^3 SARS-CoV-2 RNA copies/mL) and an increase in respiratory tract virus load accompanied by worsening respiratory failure were detected. The liver biopsy showed 10^7 SARS-CoV-2 RNA copies per 10^6 cells of liver tissue (Figure 1C). Histology revealed signs of hepatitis, cholestasis, and periportal fibrosis (**Supplementary Material**

Figure S1C, D), and virus-like particles in hepatocytes were detected by electron microscopy (**Supplementary Material Figure S1E,F**). The absence of typical findings of autoimmune inflammation or copper in the liver biopsy made immune-mediated inflammation or toxic drug effects less likely (Zhang et al., 2020). Furthermore, SARS-CoV-2 was isolated and sequenced directly from liver tissue. A recent report by Wang et al. showed infection of hepatocytes by electron microscopy in only two postmortem cases (Wang et al., 2020).

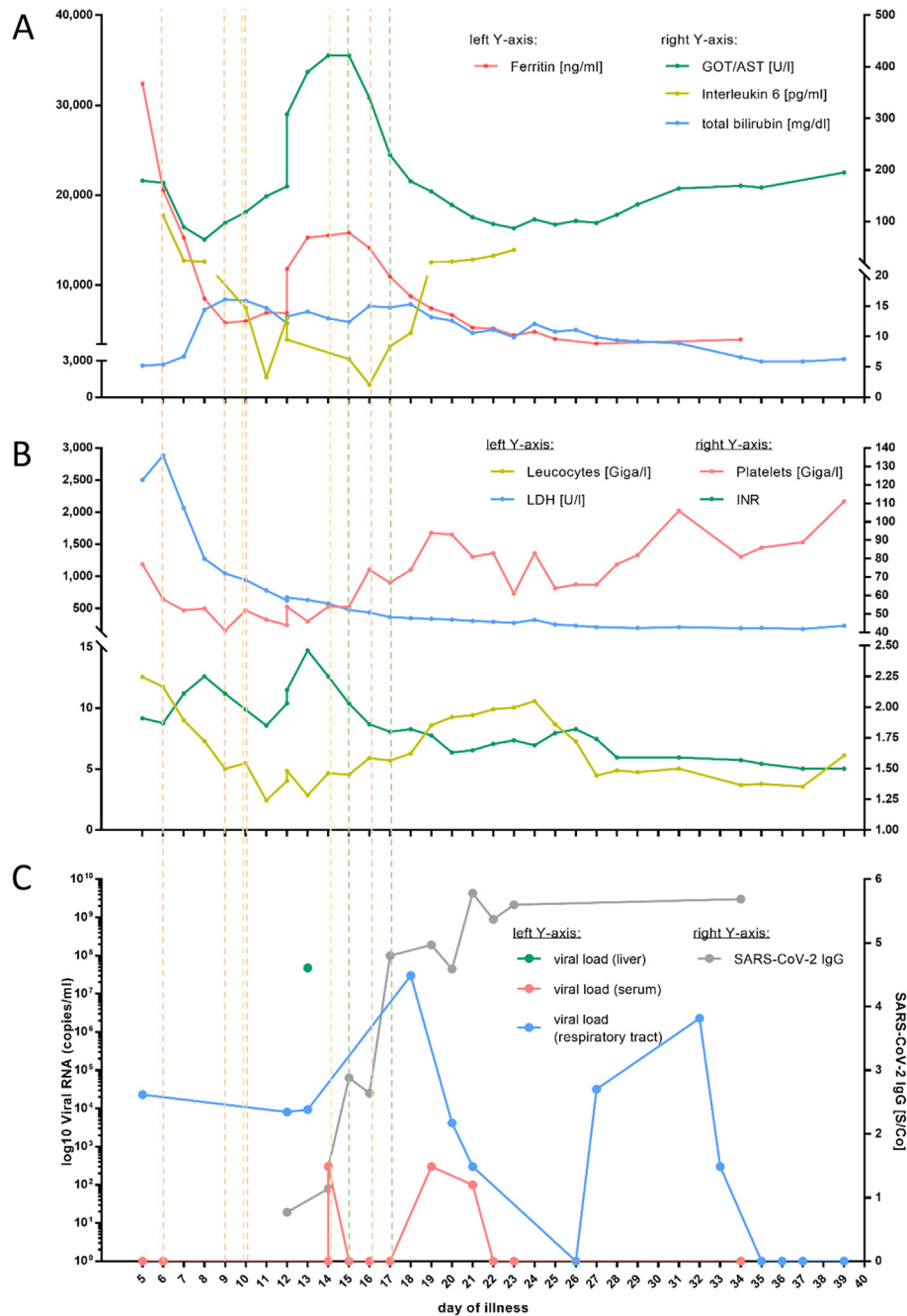
Immunosuppressive therapy was stopped on day 15 because of the systemic virus infection. Since only borderline SARS-CoV-2 IgG antibodies were present (Figure 1C), three doses of convalescent plasma were administered. Consecutively, the patient's clinical status, hematological function, and liver function improved, ferritin levels dropped, and SARS-CoV-2 IgG antibody levels rose (Figure 1C). Virus levels in the serum and respiratory tract also dropped. There were no signs of recurrence of sHLH.

The results of two repeated 24-h urine collections showed a markedly increased copper excretion (**Supplementary Material Table S1**). The findings of Coombs-negative hemolytic anemia, decreased ceruloplasmin, and markedly elevated urine copper excretion corroborated the diagnosis of Wilson's disease (5 points on the Leipzig scoring system). With the absence of Kayser–Fleischer corneal rings and rhodanine negativity of hepatocytes in the liver biopsy, which may be due to sampling variation, genetic testing revealed two heterozygous mutations in *ATP7B* (c.3443 T > C and c.3659C > T). A chelating agent was started and the patient could be discharged on day 40. On day 69, the patient presented to the outpatient clinic showing marked improvements in her clinical condition and laboratory parameters.

Discussion

While this patient presenting with SARS-CoV-2-associated sHLH might have had beneficial effects of early stage immunosuppression by anakinra reducing hyperinflammation, the addition of high-dose prednisolone and the intensification of anakinra may have led to systemic spreading of the virus and aggravation of SARS-CoV-2 hepatitis. Therefore, the positive effects of immunosuppression have to be carefully balanced with potential deleterious impairment of the immune system leading to prolongation of virus replication, systemic dissemination, and potentially life-threatening secondary infections, especially pulmonary mycosis or fatal hepatitis. Interestingly, the RECOVERY trial, as well as a meta-analysis, reported that the administration of systemic corticosteroids in moderate doses, compared with usual care or placebo, was associated with lower 28-day mortality in critically ill patients (Horby et al., 2020; Sterne et al., 2020). Concerning SARS-CoV-2 clearance in patients treated with corticosteroids, several studies have reported conflicting data, perhaps due to the different doses of steroids used (Schout et al., 2020). While higher doses of steroids seem to be associated with prolonged viral shedding (Li et al., 2020b), current COVID-19 treatment guidelines do not state a recommendation on monitoring SARS-CoV-2 viral load in critical COVID patients receiving immunosuppressive therapies (COVID-19 Treatment Guidelines Panel, Coronavirus Disease 2019 (COVID-19) Treatment Guidelines, National Institutes of Health).

With barely detectable SARS-CoV-2 antibody titers, we initiated the intravenous application of convalescent plasma (compassionate use). Afterwards, a rapid increase in SARS-CoV-2 IgG antibodies combined with an improvement in the patient's condition was detected. Of note, after the discontinuation of prednisolone, a slight increase in the SARS-CoV-2 IgG titer had already been observed before convalescent plasma application (Figure 1C). The pronounced increase after convalescent plasma treatment might have been facilitated by virus inactivation/opsonization. While



Anakinra 100mg	1	1	1	1	1	2	4	4	2	No immunosuppression					
Prednisolon 250mg				1	1	1	1								
Convalescent plasma									2		1				
Ceftriaxon g/d	2	2													
Meropenem g/d			3	3	3	3	3	3	3		3	3	3	3	3
Vancomycin g/d						1.5	1	1	1	1.5	1	1	1	1.5	1.5

Figure 1. Timeline of laboratory values (panels A and B), viral RNA load in the respiratory tract/serum/liver, titer of SARS-CoV-2 IgG (panel C), and immunosuppressive therapy/application of convalescent plasma. Medication: CP = convalescent plasma. Vancomycin according to plasma levels (goal 15–20 µg/ml). (Abbreviations: GOT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; INR, international normalized ratio.).

initial reports have described the effectiveness of convalescent plasma leading to an increase in the levels of neutralizing antibodies, as in the patient case reported here, a recently published randomized trial showed no statistically significant improvement in the time to clinical improvement (Li et al., 2020a).

In summary, this case highlights the complex differential diagnostic process for identifying the underlying causative events: sHLH, hepatic and systemic SARS-CoV-2 infection likely induced by immunosuppression, and Wilson’s disease, with two rare diseases in one patient. This appears to be the first report showing the

detection of SARS-CoV-2 in hepatocytes by electron microscopy, as well as virus isolation from a diagnostic liver biopsy in a convalescent patient. Another key aspect of this case is that when immunosuppression is applied, (systemic) virus replication has to be monitored closely and any deterioration in organ function under immunosuppression has to raise the suspicion of direct organ infection, warranting further diagnostic work-up, for instance by biopsy.

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Ethical approval

The study was approved by the Ethics Committee of the University Regensburg (ethics statement No. 20-1785-101) and written informed consent was obtained from the patient.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ijid.2020.12.047>.

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