

Unique Hepatic Manifestations of COVID-19 Induced Immune Dysregulation in Children Corresponding Author: Julia Kleinhenz, MD, University of Chicago Comer Children's Hospital; Division of Gastroenterology, Hepatology & Nutrition. Email: julia.kleinhenz@uchospitals.edu

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Abstract

The two cases we present are the first to demonstrate novel manifestations of COVID-19- related interaction between the liver and the immune system in pediatric patients. Written informed consent was obtained from the parent/guardian to publish this report in accordance with the journal's patient consent policy.

Unique Hepatic Manifestations of COVID-19 Induced Immune Dysregulation in Children

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Conflicts of Interest

The authors whose names are listed immediately above certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Introduction

COVID-19 was originally identified as a respiratory illness with emerging literature highlighting extrapulmonary manifestations ranging from neurological to dermatological, inclusive of gastrointestinal and hepatobiliary complications. In adult literature, between 10 and 58% of hospitalized patients with COVID-19 have elevated liver enzymes, predominantly transaminitis with aspartate aminotransferase (AST) higher than alanine aminotransferase (ALT). Transaminitis greater than five times the upper limit of normal is uncommon, as are significant elevations in alkaline phosphatase. COVID-19 associated liver injury (known as COVALI) is postulated to result from direct virus-mediated hepatocyte damage via angiotensin converting enzyme-2 (ACE-2) receptor, ischemic/hypoxic bystander injury, cytokine-driven and immune-mediated damage, or antiviral drug-related liver injury. COVALI in adult studies is typically mild and self-limiting.

Medical literature on liver injury associated with COVID-19 in children is more limited and spans from single center cohorts to case reports, covering both respiratory COVID-19 as well as the unique multi-system inflammatory syndrome (MIS-C). A study of 291 patients (220 with COVID-19 and 71 with MIS-C) had an overall rate of elevated ALT of 36%, 31% in COVID-19 cases and 51% in MIS-C cases. Severe liver injury, denoted as ALT > 200 U/L, was noted in 8% of children with COVID-19 and 4% with MIS-C with one child with MIS-C and acute liver failure that recovered (*Perez et al. 2021*). No matter straightforward COVID-19 or more severe development of MIS-C, those with elevated ALT had more severe disease with higher rates of multisystem organ dysfunction, respiratory failure, and longer hospitalization as well as ICU stay (*Perez et al. 2021*).

Few case reports have described pediatric patients presenting with acute liver failure in the context of acute COVID-19 infection. While recovery with native liver was the more common outcome, at least one case had a fatal outcome (*Sgouropoulou et al. 2021*).

Consent

Written informed consent was obtained from the patient's parent/guardian to publish this report in accordance with the journal's patient consent policy.

Case Presentations

In this brief report, we present two cases of pediatric patients who had unique presentations of immune dysregulation targeting mainly the liver post COVID-19 infection. The first developed COVID-19-associated acute liver failure and aplastic anemia necessitating a liver transplant, while the other developed phospholipid syndrome presenting as acute portal vein thrombosis and hepatic abscess formation.

Case 1

A previously healthy ex-full-term two-year-old female presented with a two-day history of abdominal pain, non-bloody, non-bilious emesis, and jaundice. Four months prior to presentation, both the patient and her

unvaccinated parents tested positive for SARS-CoV-2 via RT-PCR nasal swab. They all had mild cough and runny nose that resolved over a ten day period. Three days prior to her presentation, she developed cough, congestion, jaundice and tactile fever.

The patient's medical history was significant for minor viral upper respiratory infections. She had never been hospitalized nor had surgery, did not take daily medications or endorse any allergies. The family history was negative for liver diseases.

The patient's vital signs on initial presentation were within appropriate limits for age. She had scleral icterus, generalized jaundiced skin, and multiple bilateral lower extremity bruises each measuring 2 to 3 cm in diameter and concentrated at the mid-shins. Her lung examination was normal. She had no hepatomegaly nor splenomegaly, and the rest of her physical exam was otherwise unremarkable.

The patient's initial diagnostic assessment was notable for transaminitis with ALT: 1,902 U/L, AST: 2,158 U/L, leukopenia $4.9 \times 10^3/\mu\text{L}$, thrombocytopenia: $75 \times 10^3/\mu\text{L}$, and a prolonged INR at 1.4. Her respiratory viral panel was positive for SARS-CoV-2 and Parainfluenza 3.

Her abdominal ultrasound demonstrated an echogenic liver parenchyma with no focal lesions.

Liver biopsy showed acute hepatitis with patchy hepatic necrosis and dropout. Her complete blood count stayed stable but her transaminitis rose alongside INR. INR peaked at 5.4 on the ninth day of admission despite Vitamin K infusions. Ammonia level went up to 250 $\mu\text{g/dL}$ and she started manifesting hepatic encephalopathy. A second liver biopsy was performed on day 10 showing massive necrosis. Immunostains revealed a predominant population of CD8+ T-cells that were present in both the lobules and portal tracts. There were fewer CD4+ T-cells and CD20+ B-cells (which were primarily located within the portal tracts). In addition, the CD163 immunostain highlighted a significant infiltrate of macrophages within the lobules. She was urgently listed for a liver transplant as status 1A.

On the 12th day of admission, the patient received an orthotopic left lateral liver transplant.

The patient tolerated surgery well and made significant progress in her post-transplant course. However, her anemia and thrombocytopenia worsened to hemoglobin $< 5.6 \text{ g/dL}$ and platelets $< 10 \times 10^3/\mu\text{L}$ requiring serial transfusions of packed red blood cells and platelets. She also developed leukopenia and neutropenia with absolute neutrophil count (ANC) below 500. A bone marrow biopsy confirmed the diagnosis of idiopathic aplastic anemia.

She was treated per aplastic anemia protocol with intravenous immunoglobulin (IVIG), anti-thymocyte globulin (ATG), methylprednisolone. Her immunosuppression regimen was switched from tacrolimus to cyclosporine.

At three months post-treatment for aplastic anemia, her hemoglobin stabilized in the 9-11g/dL range, platelets ranged between $100 - 120 \times 10^3/\mu\text{L}$, and her liver chemistry panel remained within normal ranges.

Case 2

A 9-year-old female with a past medical history of nutritional iron deficiency anemia presented to the emergency department with a three week history of subjective fevers, diarrhea, three kilogram weight loss, and abdominal pain. Upon initial presentation, she was febrile, tachycardic, and had right upper quadrant abdominal pain.

Initial lab work was significant for leukocytosis of $23.4 \times 10^3/\mu\text{L}$, CRP: 152 mg/L and ESR: 47 mm/hr. She had a negative SARS-CoV-2 via RT-PCR nasal swab and a positive anti-SARS-CoV-2 N antibodies. The evaluation for autoimmune, viral infections, acetaminophen level were negative. D-dimer was elevated at 8.5ug/ml, fibrinogen: 424 mg/dl, ferritin: 617 ng/mL. Her liver chemistry panel had normal transaminases but a low albumin at 2.3mg/dL. Abdominal computed tomography (CT) was performed and showed scattered clusters of low-attenuation areas in both liver lobes and opacification of the right and left portal vein, suggestive of possible thrombosis.

Further imaging with MRI MRCP with and without contrast revealed a 1.4 cm inflammatory focus in the periphery of the right hepatic lobe concerning for an abscess as well as diffuse right portal vein thrombosis. Vancomycin and ceftriaxone were initiated and a 7 fr drain was placed by the interventional radiology team in the right-sided liver abscess. Twenty cc of frank pus was drained, which subsequently grew *Streptococcus constellatus*. Attempt at intervening on the focus on the left lobe did not yield drainable fluid collection. Immune deficiency workup was performed and negative, including a negative CGD test (Dihydrorhodamine Flow Cytometric Test).

Hypercoagulation studies were performed to evaluate the underlying cause of thrombosis, and she was found to have an abnormal LA/APS studies concerning for antiphospholipid syndrome. Anti-phos Serine/PT IGM antibodies were positive. Her Cardiolipin and beta 2 glycoprotein antibodies were negative. DRVVT, aPTT, and dilute prothrombin initial clotting times were all prolonged and continued to be prolonged after mixing tests. The DRVVT screen was elevated at 58.8 seconds (normal range is less than 40.1 seconds), PTTLA screen was elevated at 53.3 seconds (normal range is less than 42.6 seconds), and the Dilute Prothrombin Time Screen was elevated at 71.8 seconds (normal range 46.9 seconds). These results eliminated the possibility of coagulation factor deficiency and were consistent with the presence of factor inhibitors, such as those present in anti-phospholipid syndrome.

The patient was discharged on anticoagulation therapy with low molecular weight heparin and oral amoxicillin/clavulanate to follow up outpatient for repeat testing of LA/APS panel, abdominal imaging and vascular Doppler. The only notable trigger for new-onset antiphospholipid syndrome in this patient was past COVID-19 infection.

Discussion

Pediatric Acute Liver Failure & Immune Dysregulation

Acute liver failure (ALF) is a fast accelerating process with high morbidity and mortality. In recent years, more research has gone into investigating the immune component of ALF, especially in indeterminate-pediatric acute liver failure (I-PALF) where most common etiologies of ALF including viral, vascular, metabolic, drugs/toxins are ruled out and no definitive precipitating cause could be found. I-PALF accounts for over 30% of cases of PALF. Recent studies have revealed evidence towards immune-mediated processes in I-PALF. In addition, I-PALF patients are more likely to develop aplastic anemia, which by itself is a disease process also mediated by immune dysregulation

(*Xing et al. 2014*).

The Pediatric Acute Liver Failure (PALF) Group reported on an interferon-gamma (IFN- γ) -related inflammatory activity in patients with PALF that is associated with survival with native liver. In contrast, interleukin 6 (IL-6) and IL-8 producing pathways were associated with patients demise (*Xing et al. 2014*). Other groups have found a group of CD8+ T-cells in patients with I-PALF, proposing this could be a histologic biomarker for PALF due to immune dysregulation. This study was multicenter, included a cohort of 37 I-PALF and 18 PALF patients with known etiologies. Both cohorts had their liver tissue stained for T-cells, B-cell, macrophages, perforin and tissue resident-memory T cells. CD8+ immunohistochemical staining was found significantly abundant (82%) in the I-PALF group compared to the known diagnosis group (7%) with p-value less than 0.0001. In addition, CD8+ cells were positive for perforin (effector function) and were flagged as CD103-positive (resident memory phenotype).

Liver tissue from other processes such as autoimmune hepatitis can also demonstrate ranges of CD8+ dense staining, thus CD8+ staining should not be considered as pathognomonic of I-PALF, but rather a sign of liver injury. These findings have led to the use of the newer nomenclature “activated CD8 T-cell hepatitis.”

Another defining feature of activated I-PALF is age factor. Patients affected by I-PALF are younger than those with established diagnoses, inferring that the developing immune system is susceptible to this kind of immune-mediated injury (*Narkewicz et al. 2018*). Vodovotz et al. dived into the immune pathways segregating cohorts of patients by age: infant, toddler, young childhood, older childhood and adolescent. They found an

age-associated maturity of the immune system that flips patients to a differential of inflammatory pathways, which could shed light on outcomes of patients with PALF (Vodovotz *et al.* 2020).

Pediatric Acute Liver Failure & Aplastic Anemia

Aplastic anemia is most commonly seen with infections such as infectious hepatitis, EBV, CMV, parvovirus B19, and HIV. However, there is a correlation between I-PALF and increased risk of developing aplastic anemia (AA) and early signs of bone marrow dysfunction. There are similarities between AA and I-PALF as they are more immune-driven processes involving CD8+ T-cells and high levels of inflammatory cytokines. AA is a well-described condition that may develop concomitantly with acute hepatitis (hepatitis-associated aplastic anemia (HAAA)) or following liver transplant in patients with fulminant liver failure. The first report of AA after ALF was in 1987 by Stock *et al.* Further cases have described it primarily in pediatric patients with I-PALF (Itterbeek *et al.* 2002). Mechanistic studies of HAAA have described immunologic dysregulation as the main pathogenesis: activated CD8+ T cells are cytotoxic to myelopoietic bone marrow cells, T-cell clones are formed early on in acute hepatitis, these clones attack similar target antigens including hepatocytes and myeloid cells (Ikawa *et al.* 2013).

Pediatric Acute Liver Failure & Antiphospholipid Syndrome

The antiphospholipid syndrome (APS) is an entity on its own (also known as primary APS) and is also associated with systemic lupus erythematosus (SLE). Prevalence of APS in SLE is approximately 25% (Singh *et al.* 2013). The pathophysiology of this syndrome remains unclear as is the why/when of antiphospholipid antibodies (aPL) generation. A combination of genetic and environmental factors play a role. Known triggers are commonly viral and bacterial infections. Trauma, surgery, immune abnormalities, anticoagulation withdrawal, parasitic and fungal infections, along with some malignancies have also been connected to the etiology of this condition.

APS is defined by presence of aPL and a vascular thrombosis and/or complication of pregnancy. The thrombotic episodes range from superficial thrombophlebitis to myocardial infarction, stroke, and catastrophic APS (CAPS). CAPS may develop in less than 1% of APS patients and involves multiple blood clots that develop over a short time frame. These clots impair microcirculation and lead to multisystem organ injury most commonly in brain, lungs, and kidneys.

More recently, research has shown a coagulopathy associated with COVID-19, which suggests an immune-mediated pathway reminiscent of APS and the severe form of CAPS (Serrano *et al.* 2021). Xiao *et al.* reported COVID patients with multiple aPL positivities had a higher incidence of cerebral infarction compared to patients who were negative (Xiao *et al.* 2020). Complement activation has been linked to COVID-19 and contributes to APS pathogenesis (in murine models); the association between the two needs further investigation (Fischetti *et al.* 2005).

Conclusion

The two cases we present are the first to demonstrate novel manifestations of COVID-19- related interaction between the liver and the immune system in pediatric patients. While activated CD8+T cell hepatitis and hepatitis-associated aplastic anemia (HAAA) are defined and well-reported on in the literature, we herein report the first association with the novel COVID-19 infection. We also report another manifestation of immune dysregulation post COVID infection that could potentially represent an atypical manifestation of MIS-C in the form of antiphospholipid (APL) syndrome leading to portal vein thrombosis.

These two pediatric cases shed light on the integral role that COVID plays in the initiation of a pro-inflammatory state that may serve as a trigger for the propagation of unregulated inflammatory cascade and immune dysregulation. It will be important to keep COVID- related activated CD8+ T-cell hepatitis in the PALF differential as well as COVID- related APL and thrombotic events, especially as pediatric case rates continue to increase. While COVID-19 infections are often reported as ‘mild’ in pediatric patients, it is important to keep rare and severe post-COVID complications in mind, both as clinicians as well as community members who are integral in promoting vaccination efforts.

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