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## Immunological characteristics of severe acute hepatitis of unknown origin in a child post SARS-CoV-2 infection

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### ABSTRACT

Recent studies have reported that pediatric acute liver failure of unknown origin is immune-mediated, with CD8<sup>+</sup> T cells playing a key role. Moreover, investigation of superantigen-mediated T-cell activation by the SARS-CoV-2 spike protein in pediatric severe acute hepatitis is needed in the context of the proposed mechanism of multi-system inflammatory syndrome in children (MIS-C). We investigated the immunological characteristics of a Japanese pediatric patient with severe acute hepatitis post SARS-CoV-2 infection. The patient demonstrated autoimmune hepatitis-like liver histology with CD8<sup>+</sup> lymphocyte-predominant infiltration. There was Th1-type immune skewing, including remarkable peripheral CD8<sup>+</sup> T-cell activation and a skewed T cell receptor repertoire. We also found elevated plasma levels of the anti-SARS-CoV-2 spike-specific IgG antibody, and the titer peaked after treatment, as seen with MIS-C. These findings support that immunological activation involving SARS-CoV-2 spike protein plays a crucial role in a pediatric patient with acute severe hepatitis post SARS-CoV-2 infection.

### 1. Introduction

Pediatric acute liver failure is a rare and severe condition in which a child with no known history of liver disease rapidly develops synthetic liver dysfunction. In 43% of cases, no actual cause is identified. Therefore, there is a need to elucidate its pathophysiology [1]. The liver injury in these patients is thought to be immune-mediated, with CD8<sup>+</sup> T cells playing a key role [2]. Moreover, a skewed T-cell receptor repertoire of intrahepatic lymphocytes has been reported, suggesting T-cell activation may be antigen-driven [2]. Recently, it has been reported that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection could be one of the possible causes of severe acute hepatitis of unknown origin in children [3,4].

After the coronavirus disease-2019 (COVID-19) pandemic, autoimmune hepatitis-like conditions have been described following SARS-CoV-2 infection or vaccination [5]. Although their pathophysiology remains unclear, a recent study has demonstrated the contribution of

activated CD8<sup>+</sup> T cells with enrichment of SARS-CoV-2 spike protein-specific CD8<sup>+</sup> T cells [5]. Moreover, another study showed that most pediatric patients with acute hepatitis following SARS-CoV-2 infection were asymptomatic or mild COVID-19 patients who developed hepatitis suddenly 2–6 weeks later [6]. The fact is reminiscent of the similarity with the multisystem inflammatory syndrome in children (MIS-C), which occurs 2–6 weeks after SARS-CoV-2 infection. In MIS-C, SARS-CoV-2 persistence in the gastrointestinal tract can increase gut permeability, allowing the superantigen motif of the SARS-CoV-2 spike protein, which resembles to Staphylococcal enterotoxin B [7], to traffic across mucosal barriers and into the bloodstream, triggering widespread T-cell activation [8]. This superantigen-mediated T-cell activation has been proposed as a causal mechanism of MIS-C. This hypothesis may also apply to the pathophysiology of acute hepatitis of unknown origin in children post SARS-CoV-2 infection and investigation is required [9].

In this study, we report a case report of a pediatric patient with severe acute hepatitis of unknown origin five weeks post SARS-CoV-2

**Abbreviations:** COVID-19, coronavirus disease-2019; IL, Interleukin; MIS-C, multisystem inflammatory syndrome in children; MCP-1, monocyte chemoattractant protein-1; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

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infection who demonstrated autoimmune hepatitis-like liver histology and Th1-type immune skewing, including remarkable peripheral CD8<sup>+</sup> T-cell activation and a skewed T cell receptor repertoire, as well as elevated levels of the anti-SARS-CoV-2 spike-specific IgG antibody. This case provides insights into the pathophysiology of severe acute hepatitis of unknown origin in children, as well as hepatitis following SARS-CoV-2 infection.

## 2. Methods and results

A previously healthy 10-year-old Japanese boy was transferred to our hospital with acute hepatitis of unknown origin following SARS-CoV-2 infection. The patient's initial presentation included jaundice and abdominal pain five weeks after acquiring self-limiting COVID-19. The abdominal pain occurred every few days and relieved after defecation. There was no diarrhea, fever, rash, redness of pharynx, conjunctivitis, or other symptoms of Kawasaki disease, MIS-C, or adenoviral infection. There was no prior history of COVID-19 vaccination. Blood tests at the primary care center revealed a high transaminase level; the patient was transferred to our hospital three weeks after the onset of hepatitis due to gradual laboratory data exacerbation. The patient had not received any treatment other than fluid replacement before transfer. On arrival, the patient had a good general constitution and tested negative for SARS-CoV-2 from nasopharyngeal swab using polymerase chain reaction (PCR) analysis. The patient denied having consumed any drugs, had no allergies, and had a negative recent history of travel and animal contact. A clinical examination revealed jaundice with normal vital signs and neurological findings. An abdominal examination revealed no tenderness as well as normal liver and spleen. However, abdominal ultrasound and computed tomography revealed a periportal collar, gallbladder collapse, and gallbladder wall thickening, but no evidence of mechanical obstruction of the bile duct, portal vein, or hepatic vein. The laboratory investigation results indicated acute hepatitis (total bilirubin, 18.6 mg/dL; direct bilirubin, 12.6 mg/dL; AST, 1429 U/L; ALT, 1784 U/L; LDH, 382 U/L; GGT, 54 U/L; bile acids, 283 μmol/L; and platelet, 51,000 /μL). Lymphopenia (208 /μL) and high soluble interleukin (IL)-2 receptor (1513 U/mL) and ferritin levels (915.3 ng/mL) were also observed, suggesting an immune-mediated pathological condition. The alkaline phosphatase, albumin, PT-INR, and C-reactive protein (CRP) results were all within their normal biological reference intervals. We excluded MIS-C or hemophagocytic syndrome because no fever was consistently observed, the general condition was maintained, and no increase in CRP was observed. Serology and PCR testing were used to rule out viral hepatitis A, B, C, and E, and infections caused by cytomegalovirus, Epstein-Barr virus, herpes simplex virus, varicella zoster virus, enterovirus, adenovirus, and SARS-CoV-2, along with Wilson's disease. Both SARS-CoV-2 and adenovirus PCRs from stool were negative as well. Furthermore, autoimmune serology revealed no evidence of hyperglobulinemia and negative anti-nuclear antibodies or anti-LKM-1 antibodies, following which a liver biopsy was performed to identify the etiology of acute hepatitis. The patient was subsequently put on steroid pulse therapy, to which he demonstrated sensitivity (Supplementary fig. 1).

The histological assessment of liver biopsy revealed lymphocyte-predominant infiltration in the portal area and hepatic lobules. The inflammation in the portal area was mild, but irregular enlargement of the portal field and interface hepatitis were present. The hepatic lobules had rosette morphologies, acidophilic bodies, and spotty necrosis. The fibrosis in the portal area was mild. Inflammatory cells were predominantly composed of CD8<sup>+</sup> lymphocytes with abundant histiocytes (Fig. 1A and Supplementary fig. 2). Knodell score, histology activity index score, was 4 (periportal necrosis: 1, intralobular degeneration and focal necrosis: 1, portal inflammation: 1, fibrosis: 1). In addition, METAVIR fibrosis score and activity score were F2 and A1, respectively. SARS-CoV-2 PCR from liver tissue was negative (Supplementary fig. 3).

Peripheral immunophenotyping was performed as described in our

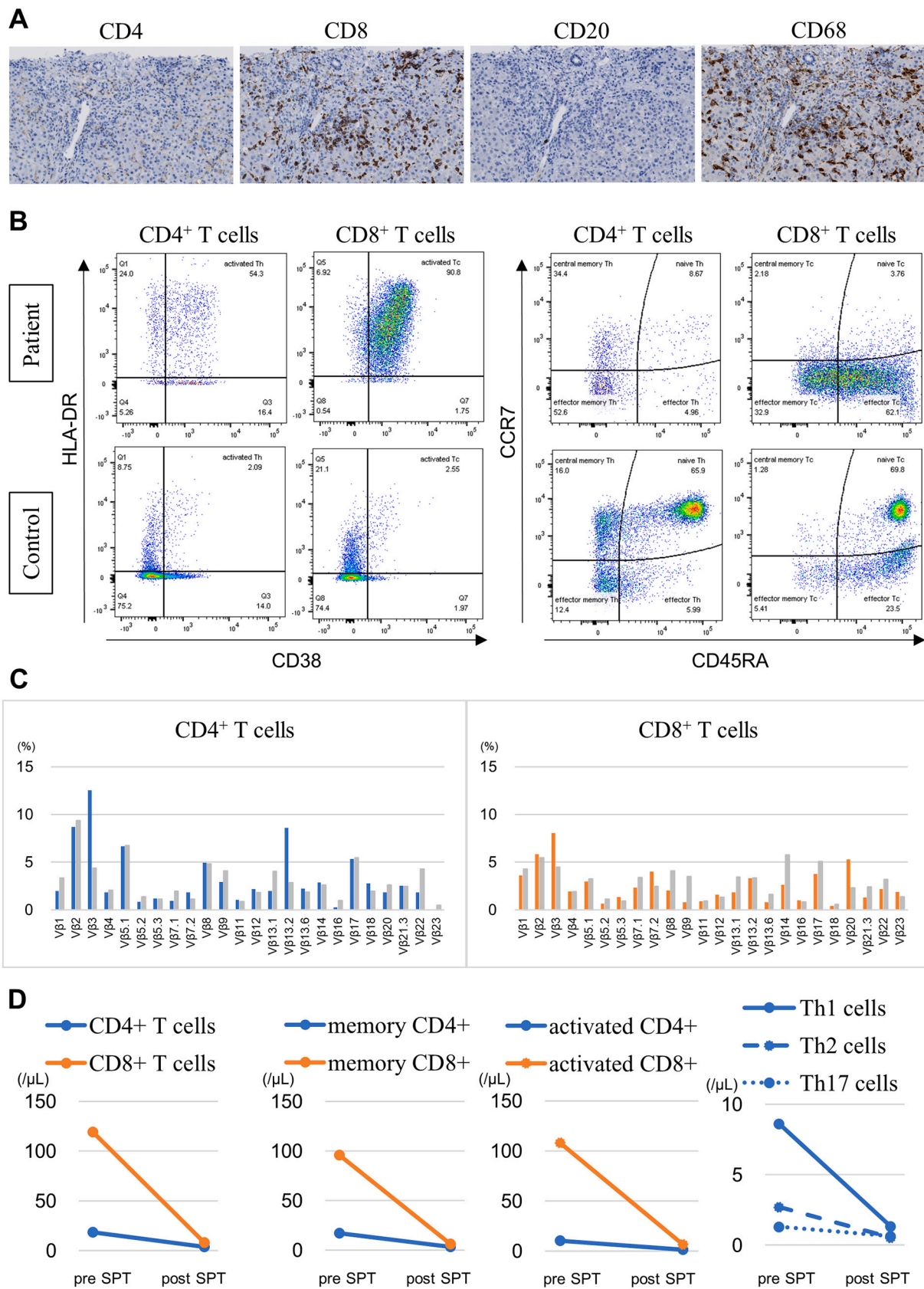
previous MIS-C report [10], and revealed remarkable T-cell activation (HLA-DR<sup>+</sup> CD38<sup>+</sup>), especially of CD8<sup>+</sup> T cells, along with marked lymphopenia (Fig. 1B left and Supplementary fig. 1A). The majority were differentiated memory (CD45RO<sup>+</sup>) or effector T cells (CD45RA<sup>+</sup> CCR7<sup>-</sup>), with naïve T cells (CD45RA<sup>+</sup> CCR7<sup>+</sup>) being depleted (Fig. 1B right). Furthermore, expansion of Vβ3<sup>+</sup> T cells, which are target of Staphylococcal enterotoxin B, was observed in both CD4<sup>+</sup> and CD8<sup>+</sup> T cells (Fig. 1C), as well as an increase in the number of activated and memory/effector phenotype of Vβ3-expressing T cells (80.3% and 89.2%, respectively). Memory CD4<sup>+</sup> T-cell subsets were skewed toward T-helper (h)1 cells (CD4<sup>+</sup> CD45RO<sup>+</sup> CXCR3<sup>+</sup> CCR6<sup>-</sup>), as was CD8<sup>+</sup> T-cell activation, which decreased rapidly post steroid pulse therapy (Fig. 1D). In line with these findings, IL-6, IL-8, IL-10, monocyte chemoattractant protein-1 (MCP-1), IL-12/IL-23p40, CXCL9, CXCL10, and IL-18 levels were elevated, but not IL-1β (Fig. 2A). Interestingly, the anti-SARS-CoV-2 spike-specific IgG antibody titer peaked after treatment, 64 days after acquiring COVID-19, and 29 days after the onset of hepatitis (Fig. 2B), similar to the behavior seen with MIS-C [7,9]. This was consistent with changes in total IgG levels.

## 3. Discussion

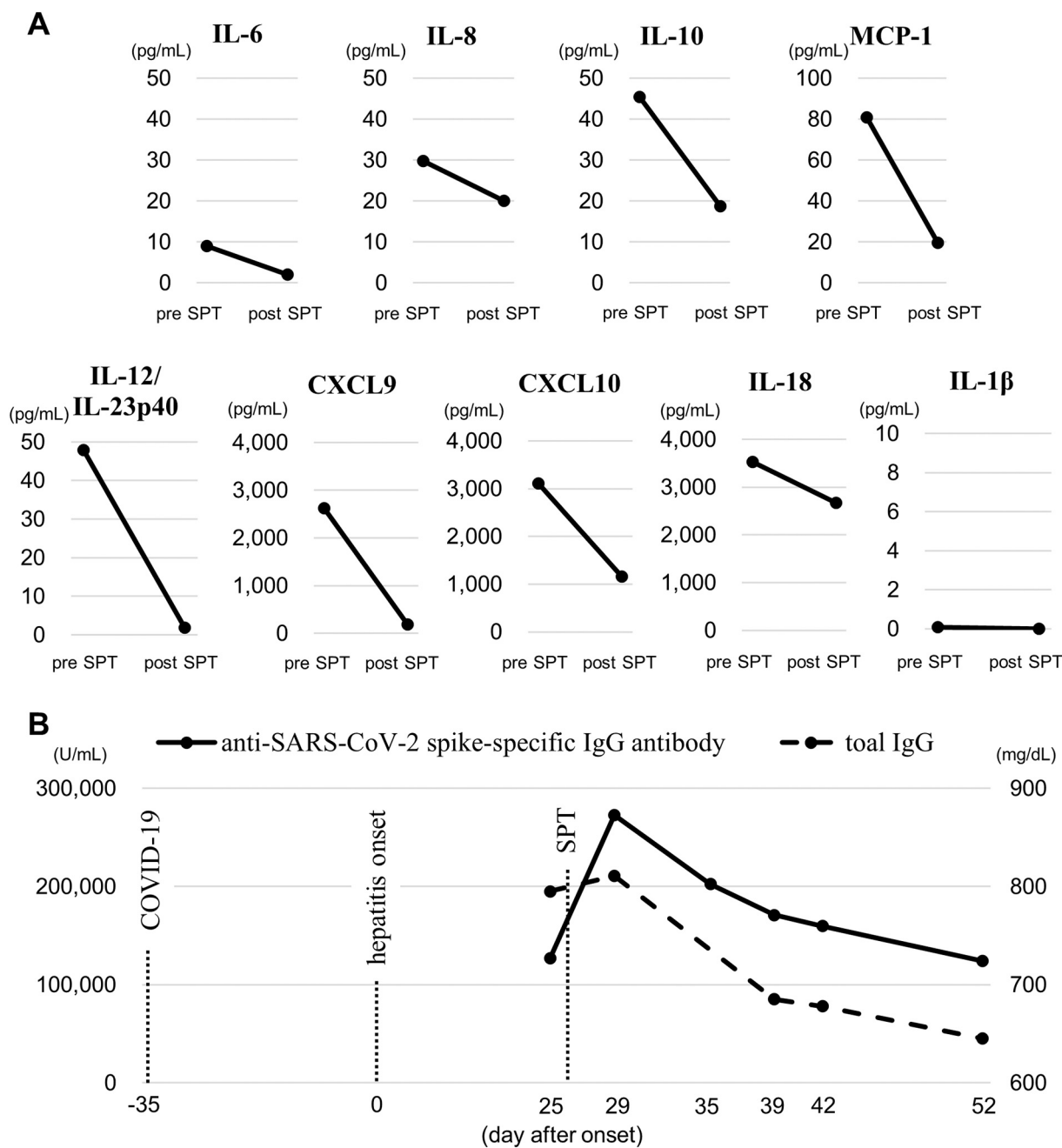
We investigated the immunological characteristics of a Japanese patient with severe acute hepatitis of unknown origin post-SARS-CoV-2 infection. T-cell activation was widespread, especially of CD8<sup>+</sup> T cells, with evidence of Th1-type immune skewing and the expansion of Vβ3-expressing cells, suggesting superantigen-mediated T-cell activation. Elevated plasma IL-12/IL-23p40, CXCL9, and CXCL10 further support the involvement of CD8<sup>+</sup> T cells. We also discovered high serum levels of the anti-SARS-CoV-2 spike-specific IgG antibody. The titer increased following treatment, implying that the pretreatment immune profile reflected ongoing exposure to the SARS-CoV-2 spike protein. Although this is a single case study, such dynamic and characteristic immunological change supports that immunological activation involving SARS-CoV-2 spike protein plays a crucial role in pediatric patients with acute hepatitis post SARS-CoV-2 infection, similar to MIS-C.

The World Health Organization (WHO) reported a recent outbreak of severe acute hepatitis in children of unknown origin on April 15, 2022, in Disease Outbreak News [11]. Although a link between human adenovirus infection and SARS-CoV-2 infection has been suspected, the exact cause is still unknown [3,4]. Despite the fact that MIS-C is characterized by T-cell activation with the expansion of Vβ21.3 [10,12], the patient had expansion of Vβ3 rather than Vβ21.3. It cannot be ruled out that this may be a non-specific finding. However, the number of MIS-C cases rapidly decreased after the Omicron variant spread [13]. The current outbreak of severe hepatitis in children of unknown origin, on the other hand, may be associated with the Omicron variant [14]. These evidences lead us to speculate that the evolving SARS-CoV-2 spike protein may alter its target. Nonetheless, further research is needed on the association between the functional role of SARS-CoV-2 spike protein as a superantigen and severe acute hepatitis in children.

We also found a characteristic liver histology of predominant CD8<sup>+</sup> lymphocytes infiltration, which is consistent with indeterminate acute liver failure in children [2]. Furthermore, although the patient did not have autoimmune hepatitis serologically, autoimmune-hepatitis-like histological findings, such as interface hepatitis, rosette morphologies, and steroid sensitivity, were consistent with those of other patients with liver injury post SARS-CoV-2 vaccination [5,15]. Moreover, CD8<sup>+</sup> lymphocyte infiltration with peripheral CD8<sup>+</sup> T-cell activation and an increase in plasma levels of IL-18, CXCL9, but not IL-1β, shared considerable similarities with the findings of mouse autoimmune hepatitis models [16]. These findings back previous reports that SARS-CoV-2 infection is associated with the autoimmune hepatitis-like condition and suggest that it may be partially responsible for severe acute hepatitis of unknown origin in children.



**Fig. 1.** Liver histological and peripheral immunophenotyping. (A) Immunostaining of liver histology. (B) Representative T-cell subsets prior to steroid pulse therapy (SPT). Remarkable T-cell activation (HLA-DR<sup>+</sup> CD38<sup>+</sup>) and depleted naïve T cells (CD45RA<sup>+</sup> CCR7<sup>+</sup>) are depicted. The peripheral blood from a 13-year-old boy with a remission period of ulcerative was used as a control. (C) T cell receptor repertoires prior to SPT. The Gray bar represents the reference value. The expansion of Vβ3 is observed. (D) Changes in each T-cell subsets before SPT (pre SPT) and the day after the end of SPT (post SPT). T-cell subsets were skewed toward CD8<sup>+</sup> T cells and Th1 cells, which decreased rapidly post SPT.



**Fig. 2.** Peripheral plasma assay. (A) Changes in plasma cytokine and chemokine levels before steroid pulse therapy (pre SPT) and the day after the end of SPT (post SPT). The elevated cytokine and chemokine levels were decreased post SPT. They were measured by a Cytometric Bead Array (Becton Dickinson, Franklin Lakes, NJ, USA) except for IL-18. IL-18 was measured on a commercial assay (LSI Medience Corporation, Tokyo, Japan). (B) Changes in anti-SARS-CoV-2 spike-specific IgG antibody. Total IgG levels are included in the graph as comparison. The titer peaked after treatment, 64 days after acquiring COVID-19, and 29 days after hepatitis onset. They were measured using a human SARS-CoV-2 spike IgG ELISA kit (Thermo Fisher Scientific, Waltham, MA, USA).

**4. Conclusion**

We described the case of a pediatric patient with severe acute hepatitis of unknown origin post SARS-CoV-2 infection who demonstrated autoimmune hepatitis-like liver histology with inflammatory cells predominantly composed of CD8<sup>+</sup> lymphocytes. There was Th1-type immune skewing, including remarkable peripheral CD8<sup>+</sup> T-cell activation and a skewed T cell receptor repertoire. The patient also had elevated plasma levels of the anti-SARS-CoV-2 spike-specific IgG antibody, and the titer peaked after treatment, as seen with MIS-C. These findings support that immunological activation involving SARS-CoV-2 spike protein plays a crucial role in a pediatric patient with acute hepatitis

following SARS-CoV-2 infection in the context of the pathological hypothesis of MIS-C. However, further prospective studies to support these findings are warranted.

**Ethics statement**

Immunologic analyses were conducted in the context of a research project (H29-310) approved by the hospital ethics committee. Written informed consent was obtained from the patient and patient’s parents.

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## Authors' contributions

KI, TT, ST, KY, and MT, clinical care of the patient; AM and KI, drafting of the manuscript; KA and DM, histopathological evaluation; AM, KI and HT, immunological data analysis and interpretation. DM and HT, critical revision of the manuscript; All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

## Declaration of Competing Interest

The authors declare no conflict of interest in relation to this manuscript.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clim.2022.109138>.

## References

- [1] J.E. Squires, E.M. Alonso, S.H. Ibrahim, V. Kasper, M. Kehar, M. Martinez, et al., North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition Position Paper on the Diagnosis and Management of Pediatric Acute Liver Failure 74, 2022, pp. 138–158.
- [2] C.A. Chapin, H. Melin-Aldana, P.A. Kreiger, T. Burn, K. Neighbors, S.A. Taylor, et al., Activated CD8 T-cell hepatitis in children with indeterminate acute liver failure: results from a multicenter cohort, *J. Pediatr. Gastroenterol. Nutr.* 71 (2020) 713–719.
- [3] M.M. Mücke, S. Zeuzem, The recent outbreak of acute severe hepatitis in children of unknown origin - what is known so far, *J. Hepatol.* 77 (2022) 237–242.
- [4] E. Lurz, D. Lenz, P. Bufler, A. Fichtner, S. Henning, M. Jankofsky, et al., Letter to the editor - a pediatric hepatologists' perspective, *J. Hepatol.* (2022), <https://doi.org/10.1016/j.jhep.2022.05.039>.
- [5] T. Boettler, B. Csernalabics, H. Salié, H. Luxenburger, L. Wischer, E.S. Alizei, et al., SARS-CoV-2 vaccination can elicit a CD8 T-cell dominant hepatitis, *J. Hepatol.* (2022), <https://doi.org/10.1016/j.jhep.2022.03.040>.
- [6] R.K. Ratho, A.A. Asati, N. Mishra, A. Jain, S.K. Rawat, COVID-19 associated hepatitis in children (CAH-C) during the second wave of SARS-CoV-2 infections in Central India: is it a complication or transient phenomenon, *medRxiv* (2022), <https://doi.org/10.1101/2021.07.23.21260716>.
- [7] M.H. Cheng, S. Zhang, R.A. Porritt, M. Noval Rivas, L. Paschold, E. Willscher, et al., Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation, *Proc. Natl. Acad. Sci. U. S. A.* 117 (2020) 25254–25262.
- [8] L.M. Yonker, T. Gilboa, A.F. Ogata, Y. Senussi, R. Lazarovits, B.P. Boribong, et al., Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier, *J. Clin. Invest.* 131 (2021), e149633.
- [9] P. Brodin, M. Arditi, Severe acute hepatitis in children: investigate SARS-CoV-2 superantigens, *Lancet Gastroenterol. Hepatol.* 7 (2022) 594–595.
- [10] A. Morita, S. Hosaka, K. Imagawa, T. Ishiodori, Y. Nozaki, T. Murakami, et al., Time course of peripheral immunophenotypes of multisystem inflammatory syndrome in children, *Clin. Immunol.* 236 (2022), 108955.
- [11] World Health Organization, Acute hepatitis of unknown aetiology – the United Kingdom of Great Britain and Northern Ireland, Available at: <https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON368>, 2022. Accessed April 15, 2022.
- [12] M. Moreews, K. Le Gouge, S. Khaldi-Plassart, R. Pescarmona, A.L. Mathieu, C. Malcus, et al., Polyclonal expansion of TCR Vbeta 21.3(+) CD4(+) and CD8(+) T cells is a hallmark of multisystem inflammatory syndrome in children, *Sci. Immunol.* 2021 6 (2021), eabh1516.
- [13] Centers for Disease Control and Prevention, Daily MIS-C Cases and COVID-10 Cases Reported to CDC (7-Day Moving Average), Available at: <https://covid.cdc.gov/covid-data-tracker/#mis-national-surveillance>, 2022. Accessed May 28, 2022.
- [14] H. Nishiura, S.M. Jung, K. Hayashi, High population burden of omicron variant (B.1.1.529) is associated with the emergence of severe hepatitis of unknown etiology in children, *Int. J. Infect. Dis.* 122 (2022) 30–32.
- [15] C. Efe, A.V. Kulkarni, B.T. Beretta-Piccoli, B. Margo, A.F. Stättermayer, M. Cengiz, et al., Liver injury after SARS-CoV-2 vaccination: features of immune-mediated hepatitis, role of corticosteroid therapy and outcome, *Hepatology* (2022), <https://doi.org/10.1002/hep.32572>.
- [16] A. Ikeda, N. Aoki, M. Kido, S. Iwamoto, H. Nishimura, R. Maruoka, et al., Progression of autoimmune hepatitis is mediated by IL-18-producing dendritic cells and hepatic CXCL9 expression in mice, *Hepatology* 60 (2014) 224–236.