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ACTA PÆDIATRICA NURTURING THE CHILD WILEY

BRIEF REPORT

Iatrogenic immunosuppression can lead to prolonged viral shedding and absent immune response to COVID-19

In the era of the COVID-19 pandemic, paediatricians need to remember that immunocompromised children might experience prolonged viral shedding and impaired immunological response to SARS-CoV-2. We report a girl diagnosed with an unresectable angiosarcoma of the liver who underwent liver transplantation in June 2019. The basic immunosuppression protocol consisted of tacrolimus and prednisolone. Additionally, she was on adjuvant treatment with trametinib, a mitogen-activated protein kinase (MEK) inhibitor.

Eight months post-transplantation, she was diagnosed with intestinal Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) confirmed by biopsies. She was treated with weekly doses of rituximab during four consecutive weeks with clinical improvement and a decrease in EBV DNA serum levels to undetectable within three weeks' post-treatment.

Two months after completing rituximab treatment, our patient developed fever and was diagnosed with COVID-19 by a positive SARS-CoV-2 PCR test (nasal swab). She developed mild symptoms including fluctuating fever and tiredness, but no upper-airway symptoms and maintained normal oxygen saturation and respiratory rate. Liver function tests increased to a maximum of AST 282 IU/mL, ALT 324 IU/mL, bilirubin 16 μmol/L, and the PK/INR was normal. Fluctuating CRP, transaminases and bilirubin levels were noted in the following weeks (Figure S1). The patient had clinically recovered from COVID-19 two weeks after the onset of infection. However, she subsequently experienced three recurrent episodes of fever and mild upper-airway symptoms. During a period of 3 months from the first day of symptoms, eight PCR tests for SARS-CoV-2 were positive. The viral load, as determined by cycle threshold (Ct values) of positive PCR tests, dropped with time from a maximum of 13 at the onset of symptoms, to 35 3 months later. Eventually, 3 months after the first positive PCR test, she tested negative for SARS-CoV-2. Repeated testing for SARS-CoV-2 IgG remained negative (Figure S1).

In October 2020, she again developed upper-airway symptoms and fever. Serum levels of ALT, AST and CRP were elevated, and a nasal swab was positive for SARS-CoV-2, adenovirus and rhinovirus. She recovered clinically within a few days, and repeated nucleic acid detection tests for SARS-CoV-2 in nasal and throat swabs as well as in serum and stool were negative.

The SARS-CoV-2 PCR test taken in October was positive on two different test platforms (targeting the viral envelope and the RNA polymerase; NeuMoDx®) and thus considered true positive, with a Ct value of 33.2, indicating very low number of viral copies and a low risk of infectivity. The viral load was too low to allow for viral isolation and sequencing. We were therefore unable to determine whether the positive PCR test corresponded to an actual *de novo* SARS-CoV-2 infection with a new strain or a reactivation of her former infection.

To elucidate this, a thorough immunological investigation was carried out. A peripheral lymphocyte profile revealed very low number of CD20+ B-lymphocytes, 0.07 × 10^9/L (reference interval; 0.2–1.6 × 10^9/L) and CD19+ B-lymphocytes, 0.08 × 10^9/L (reference interval; 0.2–1.6 × 10^9/L). However, the number of CD3+ and CD4+ T-lymphocytes was normal at 0.73 × 10^9/L (reference interval; 0.3–2.0 × 10^9/L). The number of NK cells (CD16/+CD56+) was also reduced at 0.05 × 10^9/L (reference interval; 0.09–0.9 × 10^9/L). Flow-cytometric assay of specific cell-mediated response in activated whole blood (FASCIA) analysis displayed neither CD8+ or CD4+ response towards SARS-CoV-2 nor any other tested antigens (ie pneumococci, tetanus, influenza, PPD, candida or varicella). She demonstrated only weak reactions towards mitogens and superantigens, indicating a broad T-cell suppression. Her peripheral immunoglobulin levels were normal. Testing for vaccine immunity demonstrated good antibody levels towards microbial agents that she had previously been vaccinated against.

The outcome of COVID-19 in liver-transplanted adults and children is comparable to outcome in immunocompetent patients, indicating that the immunocompromised state alone does not predict a more severe illness or higher mortality, possibly since the inflammatory response is blunted.[1,2] The majority of healthy individuals develop SARS-CoV-2 antibodies within 3 weeks after symptom onset, but the antibody response can be delayed with prolonged viral shedding in immunocompromised patients.[3]

Our patient was treated with rituximab shortly before acquiring COVID-19. Rituximab is a potent B-cell depletion drug targeting CD20+ B cells. B-cell function is impaired for 6–12 months after treatment, which is consistent with the finding of low CD 20+ B-cell levels 8 months after rituximab treatment. Adults on rituximab treatment who acquire COVID-19 often remain seronegative with prolonged viral shedding and have a more severe disease outcome.[4] The recent rituximab treatment most probably diminished our patient's ability to mount an antibody response and clear the virus.

The patient was treated with tacrolimus and low-dose prednisolone. Tacrolimus is a calcineurin inhibitor that impairs cytotoxic T-cell function and indirectly affects B-cell function and antibody production by suppression of T helper cells. Tacrolimus and prednisolone treatment in transplanted patients do not lead to higher mortality or worse clinical outcome of COVID-19.[2]

Additionally, the adjuvant cancer treatment with trametinib, a cytotoxic MEK-inhibitor, is known to significantly reduce T-cell proliferation, cytokine production and antigen-specific expansion of both CD4+ and CD 8+ cells, but has no or only mild impact on T-cell viability.[5] Hence, the patient's immunosuppressive and cancer treatment seem to have resulted in an immunocompromised state with normal levels of CD 4+ and CD 8+ cells, whereas antigen (including SARS-CoV-2) specific T-cell proliferation was undetectable, which impaired clearance of SARS-CoV-2.

Heavy immunosuppression including combined B- and T-cell deficiency impaired the patient´s ability to clear SARS-CoV-2, resulting in prolonged viral shedding and PCR positivity where viral load was finally suppressed to undetectable levels but infection probably not completely cleared. This implies future risk of recurrent viral reactivation. It is quite likely that immunocompromised patients with reduced B- and T-cell functions will not respond adequately to SARS-CoV-2 vaccines. These patients need to be closely monitored during the COVID-19 pandemic.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

1. Goss MB, Galván NTN, Ruan W, et al. The pediatric solid organ transplant experience with COVID-19: an initial multi-center, multi-organ case series. Pediatr Transplantat. 2021;25:e13868.
2. Nicastro E, Verdoni L, Bettini LR, et al. COVID-19 in immunosuppressed children. Front Pediatr. 2021;9:629240. https://doi.org/10.3389/fped.2021.629240
3. Burbelo PD, Riedo FX, Morishima C, et al. Detection of nucleocapsid antibody to SARS-CoV-2 is more sensitive than antibody to spike protein in COVID-19 patients. J infect Dis. 2020;222:206-213.
4. Loarce-Martos J, García-Fernández A, López-Gutiérrez F, et al. High rates of severe disease and death due to SARS-CoV-2 infection in rheumatic disease patients treated with rituximab: a descriptive study. Rheumatol Int. 2020;40:2015-2021. https://doi.org/10.1007/s00296-020-04699-x
5. Vella LJ, Pasam A, Dimopoulos N, et al. MEK inhibition, alone or in combination with BRAF inhibition, affects multiple functions of isolated normal human lymphocytes and dendritic cells. Cancer Immunol Res. 2014;2:351-360. https://doi.org/10.1158/2326-6066.CIR-13-0181

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