Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection 85 (2022) e56–e58

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letter to the Editor

Reactivation/relapse of SARS-CoV-2 in a child following haematopoietic stem cell transplantation, confirmed by whole genome sequencing, following apparent viral clearance

To the Editor,

We read with interest the article by Chappell et al concluding that immunocompromised children and young people are at no increased risk of severe COVID-19.[1] This prospective cohort study was carried out over a year and identified 38 cases of SARS-CoV-2 infection (1527 patient cohort) with only 4 patients needing hospital admission with no severe disease. Although this is a small cohort of positive patients, the study did not report any cases of relapse during the time period. COVID-19 relapse (also described as reactivation, recrudescence or recurrence) is commonly defined as the clinical recurrence of symptoms compatible with COVID-19 accompanied by a positive test for SARS-CoV-2 within 90 days of primary infection.[2] Confirmed relapse requires demonstration of the same strain via whole genome sequencing (WGS). Defining clinical relapse in children is challenging due to asymptomatic COVID-19 and evidence of reactivation of SARS-CoV-2 virus in the paediatric population is limited. We report a case following haematopoietic stem cell transplantation (HSCT) in the paediatric setting and discuss the implications for clinical and infection control practice.

A four-month-old male was diagnosed with infant B-cell acute lymphoblastic leukaemia (B-ALL) in September 2020. He was stratified as high risk, and HSCT was planned following conventional chemotherapy. He was diagnosed with asymptomatic COVID-19 in December 2020 and therefore transplantation was postponed. Leukaemic relapse occurred in January 2021 and re-induction chemotherapy commenced. He remained asymptomatic from COVID-19, but SARS-CoV-2 initially continued to be detectable by RT-qPCR in his stool and nasopharyngeal samples (see Fig. 1). Cycle threshold (CT) levels varied in both stool and respiratory samples becoming progressively higher (indicating lower viral loads); it was not clear whether this represented viable virus or remnant non-viable genetic material. Once further remission was attained, it was decided to undergo matched unrelated donor HSCT in March 2021. Prior to HSCT he had had two negative respiratory, and one negative stool samples for SARS-CoV-2. Two days after stem cell infusion, SARS-CoV-2 was detected in a nasopharyngeal aspirate at a cycle threshold of 19, corresponding with a high viral load. Subsequent stool samples also showed CT values in similar ranges (Fig. 1). He had an episode of confirmed bacterial neutropenic sepsis, relating to mucositis, which was treated with broad spectrum antibiotics. He completed transplantation with no complication from SARS-CoV-2 and engrafted with 100% donor haematopoiesis and no evidence of graft versus host disease (GVHD). In May 2021 he was diagnosed with relapsed bone marrow and central nervous system disease. He had episodes of bacterial sepsis and invasive fungal disease but, despite remaining SARS-CoV-2 positive for a further 4 months, he did not require treatment for COVID-19. Due to progressive disease, he underwent CAR-T therapy and a second HSCT but subsequently died from progressive disease at the age of 19 months. Despite further conditioning and immunosuppression, further relapse of SARS-CoV-2 did not occur, suggesting the engrafted HSCT provided sufficient immune response to clear the SARS-CoV2 infection. WGS was undertaken on two respiratory samples taken in December 2020 (first positive sample) and June 2021 (after HSCT during neutropenia and prolonged positivity). WGS confirmed both isolates belonged to the B.1.1.7 lineage of SARS-CoV-2 (alpha variant) with a difference of 5 base pairs in the consensus sequences (Fig. 2), suggesting relapse rather than re-infection.

Our case highlights the importance of WGS in differentiating reactivation versus reinfection in patients who have prolonged interaction with the healthcare environment. Healthcare associated transmission of SARS-CoV-2 is well documented and has implications for infection control practice. Sequencing can inform infection control practice by providing granular data to understand complex transmission events.[3] We hypothesise that replicant competent virus persisting in our patient is the most likely explanation for the prolonged detection of SARS-CoV-2 virus in respiratory and stool samples. Profound immunosuppression during HSCT conditioning allowed viral reactivation despite negative stool and respiratory sample testing prior to transplantation. We did not submit samples for viral culture however there is evidence that lower CT values are generally associated with infectious and culturable virus.[4] There are suggestions that recovering patients with higher CT values can be considered non-infectious with relaxation of infection prevention and control precautions. The exact cut-off of CT values relating to viable virus is not established and would depend on multiple factors including testing platform and sample type. SARS-CoV-2 PCR testing on all sample types in our institution was performed in-house using one-step RT-qPCR targeting the N gene (N2 primers) and Takara mastermix as previously described.[5] These findings challenge the assumption that high CT values do not represent replicant competent virus especially where a patient's immunity is compromised. Instead, this case highlights the need to evaluate high CT values with reference to the patient specific scenario. We also highlight the potential utility of SARS-CoV-2 stool testing for assessing infection risk in an immunocompromised patient. It is established that SARS-CoV-2 can replicate in and be cultured from the gut.[6-8] Of note, our patient shed virus at CT values between 26–32 in the stools for over one month following negative respiratory samples. Since immunocompromised patients have been found to shed virus for prolonged periods, including in the stool, this poses considerable potential infection control risks in a

https://doi.org/10.1016/j.jinf.2022.05.043

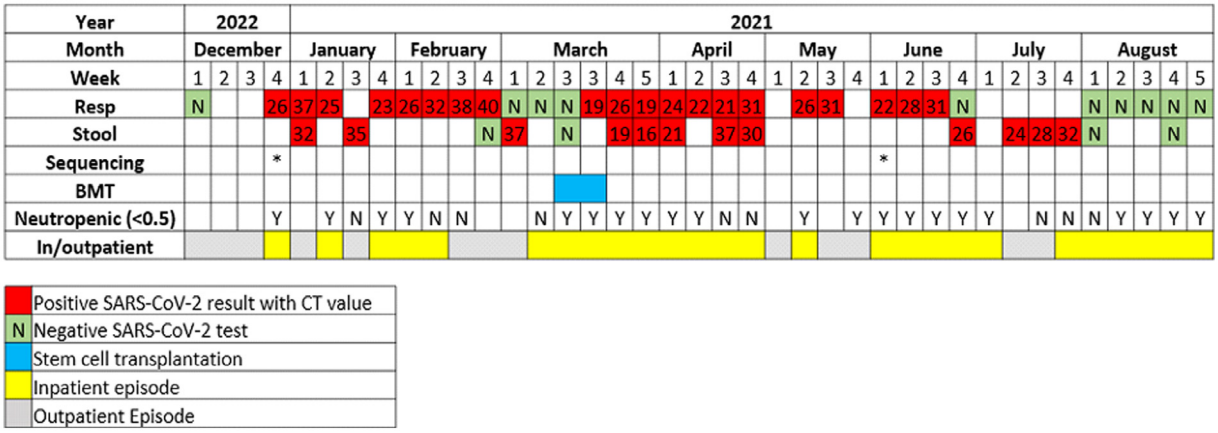

Fig. 1. Timeline comparing RT-PCR positivity in different sample types, sequencing, neutropenic episodes, and location.

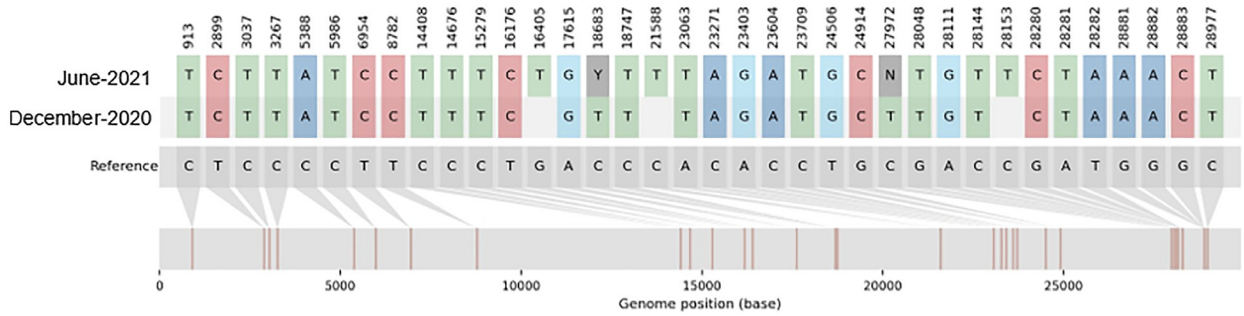

Fig. 2.. SNIPIT plot demonstrating the 5 nucleotide differences between the June 2021 sequence and the December 2021, comprising of 4 single nucleotide variants and 1 ambiguous base call. Variants were called against the Wuhan-Hu-1 reference sequence (NC_045512.2).

healthcare environment for which appropriate policies and mitigation strategies need to be in place.[9,10]

Whilst COVID-19 continues to warrant intensive infection control practices, we recommend that stool testing for SARS-CoV-2 should be considered in certain population groups with consideration for prolonged repeated testing. Despite the extensive global experience of COVID-19 our understanding of reactivation of SARS-CoV-2 virus in the immunosuppressed paediatric population is limited. This case report highlights some of the challenges involved in managing these complex patients.

Authors' contributions statement

J.H, E.G., H.D and J.B conceptualized the study. J.H. wrote the original draft and final manuscript. N.S. undertook formal analysis. All authors contributed to reviewing and editing the manuscript.

Funding

None.

Conflicts of Interest

None declared.

Acknowledgements

We would like to thank all healthcare workers who delivered clinical care to the patient and family during this time. The hard work of the Infection Control team to help mitigate the risk of COVID-19 across the Trust, and the microbiology department for the dedication and hard work implementing the comprehensive SARS-CoV-2 testing for the patients.

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