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SARS-CoV-2 persistence and non-protective immunity in infected haematological patients

We read with great interest the study by Fox *et al.*[1], reporting the outcomes of patients with SARS-CoV-2 receiving anti-cancer therapy. Data on COVID-19 in haematological patients remains limited indeed.[2,3] Clinicians should carefully weigh up the timing of elective therapies – leading to profound immunosuppression – with rapid proliferation of the patients' disease; curative options could improve prognosis. The European Hematology Association has recommended against prophylactic interruption of ongoing therapies; however, the exact intervals between a SARS-CoV-2 infection and therapy administration or allowed regimens remain unclear.[4,5]

On the other hand, it is currently unclear whether long-lasting sterilising immunity following SARS-CoV-2 infection is possible. Antibodies against the S1 domain of spike protein (S1), the respective receptor-binding domain (RBD) and the nucleocapsid protein (NP) have been detected in previously infected patients.[6] Cases of clear re-infection, as established by culture-based techniques, have not been documented at the moment; nonetheless, the role of detected antibodies which are present remains ambiguous.

In their study, Fox *et al.* have focused on the binary outcome of recovery/death in these patients.[1] As the authors clearly state, most patients present favourable outcomes despite their profound immunosuppression. However, the need for long-term follow-up could unveil a third outcome measure in this population, that of persistence. We hereby present the first case of a seroconverted SARS-CoV-2 patient with acute lymphoblastic leukaemia (ALL), presenting with a second episode of severe pneumonia shortly following chemotherapy, in a low prevalence setting.

Case presentation

A 35-year-old with a history of ALL was referred to our department on 26 March 2020 due to a positive SARS-CoV-2 PCR (polymerase chain reaction) test; at the time asymptomatic. He had previously received a cycle of R-hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, adriamycin, dexamethasone), including anti-CD20 monoclonal antibody (rituximab), 14 days prior to referral. On 8 April the patient presented with fever, hypoxaemia and bilateral infiltrates, indicative of pneumonia. A positive PCR test for SARS-CoV-2 established the diagnosis of COVID-19. The patient's condition and various regimen intolerances did not allow for any experimental therapeutic interventions, besides common antibiotics and oxygen supplementation. The patient followed an uncomplicated course, showing gradual improvement and decline in viral load (Fig 1). At the same time, SARS-CoV-2 antibody isotypes (IgG/IgA/IgM) against the N, S1 and RBD antigens were assessed by multiplex N-RBD-S1 assay (Protatonce Ltd), based on Luminex xMAP technology, and were found to be present, as shown in Table 1. The patient was then discharged to continue his treatment with a second R-hyper-CVAD cycle for his underlying disease, approximately one month after a negative PCR test on 25 May. On 2 July, the patient was readmitted with severe SARS-CoV-2 pneumonia, as confirmed by a positive PCR test for SARS-CoV-2,

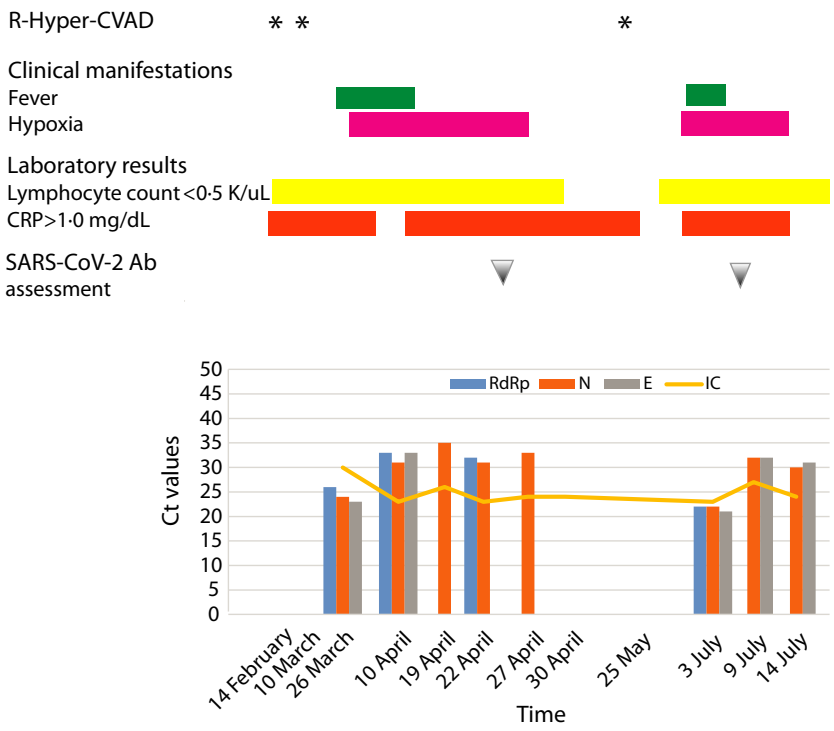

Fig 1. Timeline of hospital admissions and tests for SARS-CoV-2. Viral gene expression as inversely expressed by a number of Ct values, against the presence of an internal positive control (IC) (yellow line). Values below the IC critical cut-off denote detectable gene expression. Clustered bars indicate expression of RNA-dependent RNA polymerase (RdRp)(blue), nucleocapsid protein (N)(orange) and envelope (E)(grey). Colour blocks indicate the presence of fever (green), hypoxia (pink), lymphocyte count <0.5 K/μl (yellow) and CRP > 1 mg/dl (red). Clinical manifestations and laboratory signs of lower respiratory tract infection occur when viral gene expression appears to be below the IC critical threshold, denoting a positive result. Expression fades as time passes, until it disappears for one or more genes to indicate progressive viral clearance. Grey arrowheads and stars (*) indicate timing of antibody assessment and R-hyper-CVAD (rituximab, cyclophosphamide, vincristine, doxorubicin, adriamycin, dexamethasone) administration, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1. Antibody detection against different SARS-CoV-2 antigens.

Normalised median fluorescence intensity	Nucleoprotein	Spike S1	Spike RBD	Test interpretation
Cut-off Anti IgA-IgG-IgM	3·8	4·0	4·0	
1st admission serum pooled_Anti IgA-IgG-IgM	1·5	8·6	18·8	Positive
2nd admission serum pooled_Anti IgA-IgG-IgM	0·5	8·5	18·5	Positive
Cut-off Anti IgG	2·3	3·5	4·3	
1st admission serum_Anti IgG	1·1	11·9	56·9	Positive
2nd admission serum_Anti IgG	0·3	8·8	47·4	Positive
Cut-off Anti IgA	3·9	4·7	3·4	
1st admission serum_Anti IgA	4·7	2·0	11·0	Positive
2nd admission serum_Anti IgA	0·9	2·6	16·6	Negative
Cut-off Anti IgM	7·3	4·8	4·8	
1st admission serum_Anti IgM	2·5	9·7	8·6	Positive
2nd admission serum_Anti IgM	0·7	2·4	1·7	Negative

As per manufacturer interpretation rule (Protatonce Ltd), the patient presented positive anti-SARS-CoV-2 antibodies (green shading) against S1 and receptor-binding domain (RBD), but not nucleoprotein. First and second admission sampling was performed on 30 March and 4 July, respectively.

exhibiting high viral loads (Fig 1), but revealing an adequate IgG response against S1 and RBD (Table 1). Similar to the first admission, we exclusively followed supportive and antibiotic therapy, until the patient recovered and was discharged 25 days later, with negative PCR.

Discussion

We present the first case of a SARS-CoV-2 seroconverted haematological patient presenting with two consecutive episodes of severe COVID-19 pneumonia, following intense

intermediate chemotherapy. Our case raises two important issues: first, the possibility of re-infection with SARS-CoV-2, despite antibody presence; and second, that of possible viral persistence in immunocompromised patients.

A number of studies have previously reported evidence of SARS-CoV-2 're-activation'.[7] However, a false negative PCR test and a prolonged nucleic acid conversion, rather than recurrence, seems to be the case in these patients.[8] In our report, patients presented with typical clinical manifestations and detectable viral amplification while undergoing intense chemotherapy. Moreover, a gradual decrease in viral gene replication, reflecting decreasing viral activity, was noted in consecutive samples, in line with symptom resolution during both admissions. This finding, in the absence of antiviral or other COVID-19 related regimen administration, indicates primary self-mediated infection control, driven by immune reconstitution following courses of chemotherapy.

Even though antibodies were detected during both admissions, it is open to discussion whether specific anti-SARS-CoV-2 antibodies offer protection or whether a specific threshold is required. Antibodies against the S1 domain of spike protein, the RBD and the NP have been detected in previously infected patients.[6] Although the anti-NP and S1-generated antibodies show high sensitivity, specificity increases with RBD-specific antibodies.[9] The use of antigen combinations hereby exhibits improved performance and manages to discriminate between cases of cross-reactivity and/or cases of prior other coronavirus infections.[6] RBD-specific antibodies show greater potency to neutralise infection, but may not be enough to ensure viral clearance. It is possible that anti-NP presence is pivotal to confer immunity and also be thymus-dependent, as occurs in the paediatric population.[10] In the presence of impaired antigen presentation, due to the lack of B cells following rituximab administration, this could not be accomplished in our patient. Based on the knowledge of other corona viruses, we hypothesise that SARS-CoV-2 could evade an immune response in patients with a defective innate and adaptive humoral and cellular response, in combination with high viral loads, uncontrolled distal viral spread via exosome production and/or susceptible haplotypes.[11] Although coronaviruses are not known to undergo latency, the possibility of abortive or restrictive infections in combination with a hidden unknown reservoir, resulting in chronic infection, should be explored.

We argue that SARS-CoV-2 infection may show persistence in immunocompromised haematological hosts. A single similar case has recently been reported in an immunocompromised haematological patient with chronic leucocyte leukaemia; however, no antibodies were ever detected in this case, possibly due to immune impairment.[12] However, as shown herein, detectable antibodies may not be neutralising or confer immunity, and attending physicians should therefore be alert to symptom exacerbation, suggesting COVID-19 disease re-activation, especially during – or briefly following – times of chemotherapy administration. Comprehensive data on the management and outcome of patients with immune deficiencies remains scarce, even though a prolonged course of 10 days[13] or repeated courses of remdesivir administration have been reported.[12] A combination with other regimens – including convalescent plasma – has been utilised with favourable results; this cannot determine, however, whether it was immune reconstitution and spontaneous resolution, or our intervention which was responsible for the optimal outcomes.[12,14] Further studies in haematological patients are warranted.

Ethics statement

This study has been conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice, and approved by the local ethics committee and IRB.

Consent for publication

The patient reported on in this study signed an informed consent form to have his data anonymously analysed, utilised and published.

Availability of data and material

Data can be made available upon request, according to GDPR.

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Author contributions

KA, DZ, & ASy and AK were involved in the patient's SARS-CoV-2 and haematologic disease management, respectively; ASp carried out immunologic profile analysis; FP and LGA performed the patient's viral load and antibody measurements; MM and CG advised on the patient's management; KA co-ordinated the patient's management, drew figures and wrote the manuscript; CG, FP and ASp critically reviewed and corrected the manuscript. All authors contributed to the study's conception, design, and have seen and approved the manuscript.

Conflicts of interest

The authors have no competing interests.

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Developing the evidence base for the management of autoimmune haemolytic anaemia (AIHA): the UK experience

Recent data on the incidence and prevalence figures for autoimmune haemolytic anaemia (AIHA) from the Danish national register are 1·77/100,000 person-years and 17/100,000 persons.[1] Applied to the estimated mid-2019 United Kingdom (UK) population of 66,796,800,[2] this would translate to around 1200 cases/year and 11,000 affected individuals. Mortality directly attributed to AIHA and its treatment in 308 primary AIHA patients (median FU 33 months) has been estimated at 4%.[3] This paper aims to provide an update on approaches taken by a collaborative UK group to improve outcomes of patients with AIHA, and address research deficiencies which were highlighted in recent British Society of Haematology (BSH) guidelines.

Specifically, these guidelines demonstrate a number of limitations to determining best practice:

- A dearth of primary study data. Typically small retrospective series or case reports underpinned guidance on the use of immunosuppressive agents, and the larger case series describing outcome following splenectomy were over 50 years old.[4,5]
- Inconsistent terminology to define AIHA, disease severity and criteria for treatment response.[6]