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CLINICAL LETTER

WILEY

SARS-CoV-2 infection in a X-linked agammaglobulinemia adolescent: An immunological approach to treatment

To the Editor,

A very limited amount of data is present in the literature on SARS-CoV-2 infection in X-linked agammaglobulinemia (XLA) patients.[1] Moreover, it remains unclear the role of vaccination against SARS-CoV-2 in these subjects.[2]

IL-6 is the main cause of the cytokine storm[3] that characterized severe COVID-19; notably, the lack of IL-6 production by macrophages due to the absence of Bruton tyrosine kinase (BTK) that characterized XLA patients was suggested to be responsible for the mild-to-moderate clinical course of infection seen in these patients.[4]

Specific guidelines dealing with the particularities of treatment for COVID-19 in XLA patients are few and unclear. Ponsford et al.[5] suggest an individualized step-by-step clinical risk stratification in vaccinated XLA subjects in order to schedule additional vaccine boosters and possibly to estimate postexposure clinical disease severity. In acute SARS-CoV-2 infection, the administration of monoclonal antibodies and antiviral therapies was suggested, if humoral and T-cell-mediated immune responses are impaired, in order to prevent the risk of severe disease. However, an early treatment for patients with mild symptoms within the first few days of the disease may not be feasible.

Here, we present a case report of a SARS-CoV-2-infected XLA 12-year-old boy; XLA diagnosis was made at 6 years old after lung recurrent infections, which resulted in bilateral bronchiectasis with preserved respiratory function. Since then, he has been regularly treated with replacement immunoglobulins every 15 days. He was vaccinated with three doses of BNT162b2 mRNA vaccine; the last dose has been administrated 2 months before SARS-CoV-2 infection (Figure 1).

He developed mild symptoms such as rhinitis, cough, headache, and sore throat. On day 2, he tested positive at RT-PCR for SARS-CoV-2 strain BA.2 (B.1.1.529.2). He recovered from symptoms on day 7, and he tested negative at RT-PCR on day 15.

We investigated his immune response to guide the therapeutic approach. We performed immunological analysis during acute infection (AI) and after 1-month post-COVID-19 diagnosis (PI).

On day 5, we performed routine blood analysis to assess complete blood count, hepatorenal function, and inflammatory markers, which all resulted within normal ranges. We evaluated lymphocyte subsets by AQUIOS CL® flow cytometer (Beckman Coulter) using fresh blood samples. A customized antibodies mix was used for this clinical purpose (Beckman Coulter). We evaluated the percentage of T lymphocytes (CD3+/CD4+; CD3+/CD8+) and B lymphocytes (CD19+).

The B-cell compartment, as expected, was compromised (CD19+ 4/μL, 0.2%), whereas T cells were in the normal range according to age (CD3+ 1727/μL, 88.2%; CD4+ 1231/μL, 62%; CD8+ 342/μL, 17.2%).

The decision was made to treat our patient with Xevudy (Sotrovimab) monoclonal antibodies (500 mg in 8 mL). The drug administration took place on the fifth day after the molecular diagnosis of the infection. No side effects were reported. We did not use the antiviral drug because of the mild clinical course.

On plasma collected before monoclonal antibody infusion (day 5) (AI), we evaluated SARS-CoV-2 neutralization antibody activity (NTA) against SARS-CoV-2 B.1 (EU) and B.1.1.529 (Omicron) variants by virus neutralization assay as previously described (doi:10.3390/ijms232214341). Positive NTA threshold was put at a dilution of 1:20. No NTA was found against Omicron, whereas low level against EU (1:20) variant was detected. It is reasonable to assume that, at AI, the neutralizing activity detected, albeit low, is attributable to the immunoglobulin replacement therapy.

SARS-CoV-2-specific cell-mediated immune responses upon the stimulation of peripheral blood mononuclear cells (PBMCs) were also analyzed. Cells were stimulated with a pool of peptides covering the sequence of the spike of SARS-CoV-2 virus (BEI Resources Repository at NIH) and stained for flow cytometry analysis. The following anti-human antibodies were used: CD45 Krome Orange, CD4 PC5.5, CD8 PC7, CD45RA FITC, CCR7 PE, CD107a FITC, HLADRII PE, CD20 PC7, and IFNγ APC. Samples acquisition was performed on a CytoFLEX™ flow cytometer system equipped with CytExpert software (Beckman Coulter), and data were analyzed using Kaluza software, version 2.1.1. (Beckman Coulter).

No significant differences in CD4+ and CD8+ T effector memory (CD4+/CCR7-/CD45RA-, CD8+/CCR7-/CD45RA-) and central memory (CD4+/CCR7+/CD45RA-, CD8+/CCR7+/CD45RA-) lymphocytes were observed in unstimulated compared with SARS-CoV-2-specific cells at AI (Table 1 and Figure 2). In contrast with these data, SARS-CoV-2-specific IFNγ-producing CD8+ T lymphocytes were increased compared with the unstimulated condition.

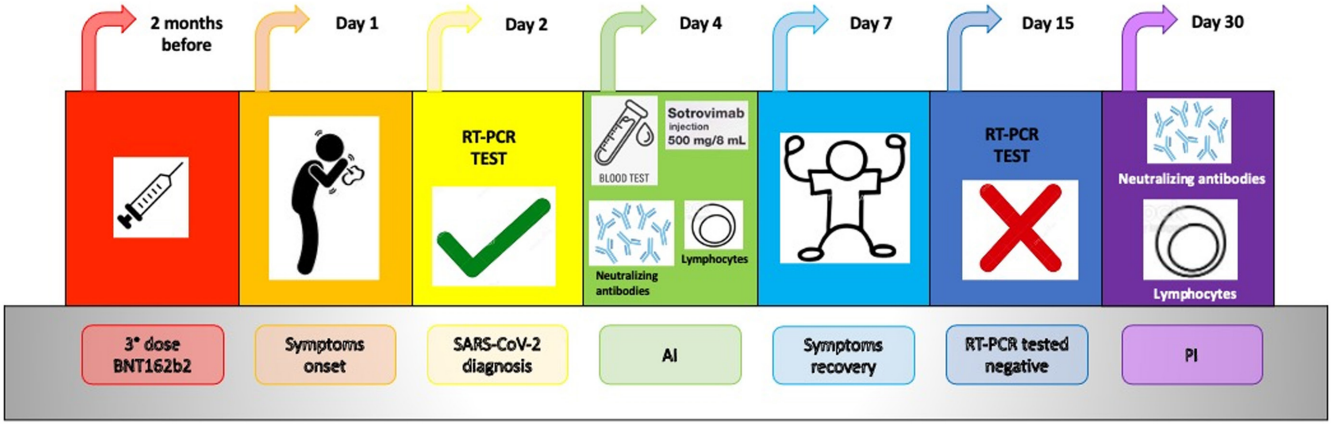

FIGURE 1 Timeline.

TABLE 1 Percentage of positive T cells in unstimulated condition and upon SARS-CoV-2 specific stimulation evaluated by flow cytometry.

	ACUTE INFECTION (AI)		POSTINFECTION (PI)		Fold change PI vs AI in stimulated
	Unstimulated	SARS-CoV-2-stimulated	Unstimulated	SARS-CoV-2-stimulated	
CD8+IFNγ+	0.99	6.57	0.04	0.17	0.12
CD8+CD107a+	13.3	8.44	1.31	3.79	0.45
CD8+HLADRII+	5.79	6.78	1.33	2.6	0.38
CD4+NAÏVE	75.07	78.05	80.24	75.42	0.97
CD4+EM	8.32	7.77	5.03	6.1	0.79
CD4+CM	8.56	7.61	10.91	15	1.97
CD4+EMRA	8.04	6.56	3.81	3.48	0.53
CD8+NAÏVE	53.29	56.89	62.67	57.31	1.01
CD8+EM	11.05	9.42	10.28	11.52	1.22
CD8+CM	8.05	8.82	14.07	19.73	2.24
CD8+EMRA	27.61	24.87	12.98	11.44	0.46

Note: The ratio of postinfection over acute infection (fold change over acute infection) is also reported.
Abbreviations: CM, central memory; EM, effector memory; EMRA, effector memory re-expressing CD45RA.

We determined IL-6 concentration in plasma at AI by a commercial ELISA kit (Invitrogen), and we compared the result with the data obtained in a group of 6 SARS-CoV-2 infected age-matched children reporting mild symptoms (data not shown). A low concentration of IL-6 (0.36 pg/mL) was found in plasma at AI, ranking among those who produced the least IL-6 in the control group (range 0.52–8.87 pg/mL, mean 3.9 pg/mL).

At PI, no clinical sequelae were found. On plasma collected 1-month postinfection, a high level of NTA was found both against EU (1:640) and Omicron (1:60) variants, as a consequence of the monoclonal antibodies' infusion. A moderate increment of SARS-CoV-2-specific IFNγ-producing CD8+ T lymphocyte and degranulating CTL was detected when compared to the unstimulated condition (Table 1). Notably, a robust increment in CD4+ and CD8+ central memory T lymphocytes (CCR7+/CD45RA-) was detected at PI compared with AI (Table 1 and Figure 2).

An initial consideration, which is based on the lack of the NTA in acute infection and the observation that virus-specific memory T cell was only seen postinfection, is that the booster dose of COVID-19 vaccine was unable to trigger a relevant immunological protection in this patient.

As previously reported, the absence of BTK could have a role in mitigating symptoms by impairing IL-6 production and consequently the cytokine storm.[4] Our results reinforce this suggestion, as the plasma concentration IL-6 in our patient was always marginal, thus potentially contributing to reduce the risk of developing more severe symptoms. However, this could be not enough to justify the clinical course of COVID-19 in our patient, as there are case reports of severe cases up to fatal outcomes in XLA patients.[6]

Omicron infection is known to cause a mild-to-moderate form of COVID-19 in the general population especially in children and adolescents, with significantly lower hospitalization and mortality rates, compared with the infections supported by previous VOCs.[7] This could be a determining factor in the mild clinical presentation we have observed in our XLA patient.

It is known that the fatal outcome and severe COVID-19 in general population are related to an impaired T-cell-mediated response.[8] A robust amount of SARS-CoV-2-specific IFNγ-producing CD8+ T

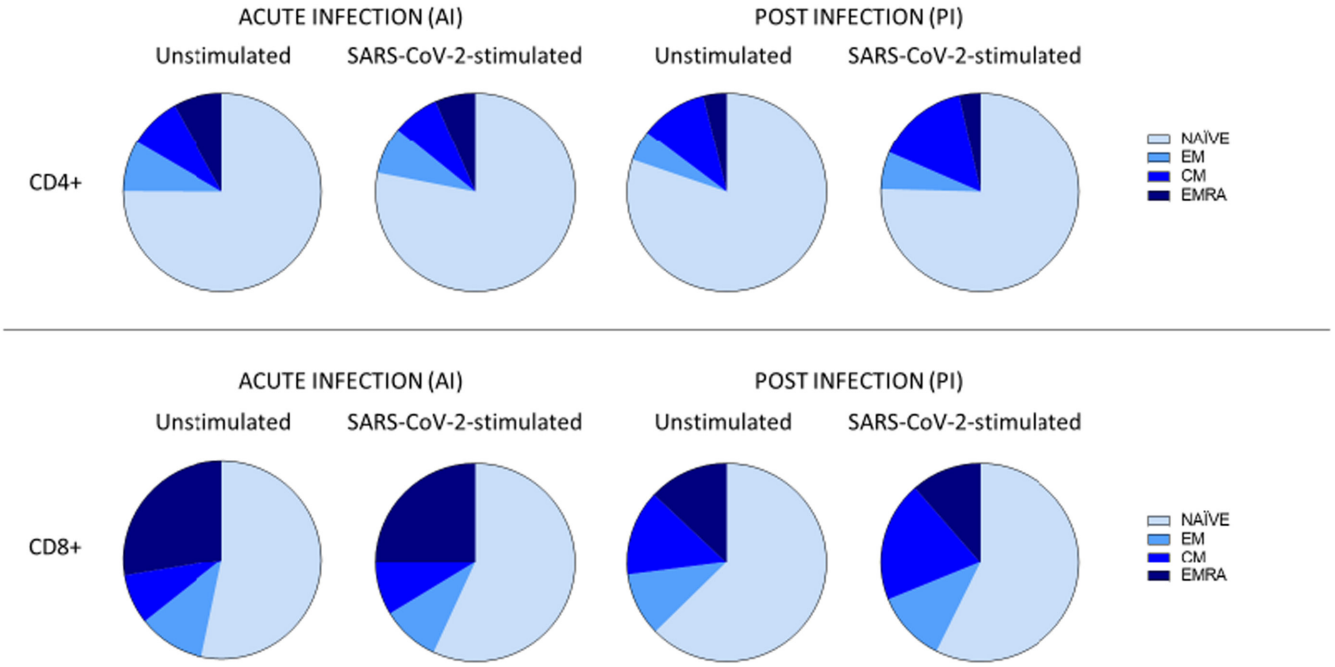

FIGURE 2 Evaluation of the T-cell subsets. Comparison between AI and PI in the percentage of CD4+ and CD8+ T-lymphocyte memory subsets.

lymphocytes and degranulating CTL was seen in our patients both in AI and PI, possibly explaining the mild clinical picture.

Results herein suggest that the use of monoclonal antibodies was helpful in this case; on the contrary, the decision of avoiding antivirals was supported by the a posteriori observation that a good T-cell-mediated antiviral response was present in this patient.

Ponsford et al.[5] state that the patient's immunological status is the best predictor of the risk of severe disease, thus being the driver to determine the best therapeutic approach. Unfortunately, this is not feasible, as evaluation of virus-specific T-cell responses is rarely performed due to associated technical challenges.

Therefore, we suggest that the best choice in these patients is to use monoclonal antibodies, reserving antiviral therapy only for cases with important comorbidities, and a compromised clinical picture at the beginning of the infection. Because of the low prevalence of this genetic condition, it will not be possible to perform randomized clinical trials to determine the best therapeutic approach to COVID-19 infection in XLA patients; retrospective observational studies and case reports will be the only tool allowing us to reach this goal.

AUTHOR CONTRIBUTIONS

Marta Stracuzzi: Conceptualization; methodology; investigation; formal analysis; writing – original draft; data curation. **Claudia Vanetti:** Conceptualization; methodology; data curation; investigation; formal analysis; writing – original draft. **Mario Clerici:** Writing – review and editing. **Gian Vincenzo Zuccotti:** Writing – review and editing. **Daria Trabattoni:** Conceptualization; methodology; data curation; investigation; formal analysis; writing – original draft. **Vania Giacomet:** Conceptualization; methodology; data curation; investigation; formal analysis; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the corresponding author, MS. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the coordinating center in Milan (protocol code 0034645 of 08/11/2020).

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

PEER REVIEW

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