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Research Note

Clinical, virologic and immunologic features of a mild case of SARS-CoV-2 reinfection

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ABSTRACT

Objectives: To report a case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reinfection 6 months after the first infection in a young healthy female physician. Both episodes led to mild coronavirus disease 2019 (COVID-19).

Methods: SARS-CoV-2 infections were detected by real-time reverse transcriptase PCR (RT-PCR) on nasopharyngeal specimens. Reinfection was confirmed by whole-genome sequencing. Kinetics of total anti-S receptor binding domain immunoglobulins (Ig anti–S RBD), anti-nucleoprotein (anti-N) and neutralizing antibodies were determined in serial serum samples retrieved during both infection episodes. Memory B-cell responses were assessed at day 12 after reinfection.

Results: Whole-genome sequencing identified two different SARS-CoV-2 genomes both belonging to clade 20A, with only one nonsynonymous mutation in the spike protein and clustered with viruses circulating in Geneva (Switzerland) at the time of each of the corresponding episodes. Seroconversion was documented with low levels of total Ig anti–S RBD and anti-N antibodies at 1 month after the first infection, whereas neutralizing antibodies quickly declined after the first episode and then were boosted by the reinfection, with high titres detectable 4 days after symptom onset. A strong memory B-cell response was detected at day 12 after onset of symptoms during reinfection, indicating that the first episode elicited cellular memory responses.

Conclusions: Rapid decline of neutralizing antibodies may put medical personnel at risk of reinfection, as shown in this case. However, reinfection leads to a significant boosting of previous immune responses. Larger cohorts of reinfected subjects with detailed descriptions of their immune responses are needed to define correlates of protection and their duration after infection. **Pauline Vetter, Clin Microbiol Infect 2021;27:791.e1–791.e4**

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reinfection has been described as soon as 2 months after an initial coronavirus disease 2019 (COVID-19) episode [1]. Reinfection events in immunocompetent [2,3] or immunosuppressed [4] patients have been observed in a limited numbers of individuals so far and are often incompletely documented. Such cases can provide information on the determinants of the quality and duration of the protective immune response elicited after SARS-CoV-2 primary infection and the risks associated with the emergence of new mutations.

Here we report a case of SARS-CoV-2 reinfection in a young healthy physician, leading to a second episode of mild COVID-19.

Methods

Samples taken via nasopharyngeal swabs (NPSs) were collected using 3 mL universal transport medium tubes and tested for SARS-CoV-2 by real-time reverse transcriptase PCR (RT-PCR) using the Cobas 6800 SARS-CoV-2 RT-PCR assay (Roche, Basel, Switzerland). Virus loads were estimated from the cycle threshold (Ct) values retrieved for the *E* gene, as previously described [5]. Respiratory viruses were tested using an in-house RT-PCR panel during reinfection. Viral genome sequences of the first and the subsequent infections were recovered using a whole-genome sequencing approach (Microsynth, Balgach, Switzerland). In addition, Sanger sequencing was performed on both specimens to complete uncovered regions in the *S* gene. Immunoglobulins were measured using commercially available kits (Elecsys Anti-SARS-CoV-2 anti-N and Elecsys Anti-SARS-CoV-2 anti-S, Roche, Rotkreuz, Switzerland; and ELISA IgG S1, Euroimmun AG, Lübeck, Germany). Neutralizing antibody titres were assessed by a plaque reduction neutralization assay using serially diluted sera. Memory B cell (MBC) responses were assessed in peripheral blood mononuclear cells. Supplementary Methods are available in the online version of the article. Written informed consent was obtained from the patient.

Results

On 10 April 2020, a 36-year-old asymptomatic female physician tested positive for SARS-CoV-2 (viral load of 1.26E+05 copies/mL) on a NPS collected during an active nosocomial outbreak investigation (Fig. 1(A)). Two days later, she developed asthenia and headache lasting for 2 weeks, and experienced slight memory loss and difficulties concentrating upon resuming work. Independent of this episode, she was enrolled into a longitudinal cohort study assessing the seroprevalence of COVID-19 in healthcare workers (HCWs) at our institution, for which sequential serum sampling was performed starting 1 week before the first episode. Anti-S1 IgG was negative 7 days before the first infection; seroconversion was confirmed at day 14 and 1 month after infection with anti-S1 IgG and total immunoglobulins directed against the nucleoprotein (anti-N Ig) and against the receptor-binding domain of the spike protein (anti–S RBD Ig) at low but increasing titres over time (Fig. 1(B)).

On 15 October 2020, another nosocomial cluster was identified, and she was tested again as part of hospital surveillance. She was asymptomatic and RT-PCR results were negative. On 30 October, while still working in a COVID-19 ward, she developed asthenia, followed by shivering, rhinorrhoea, anosmia, arthralgia, headache and exertional dyspnoea. The next day, the RT-PCR result was positive (viral load of 2.94E+07 copies/mL) and was confirmed by a second NPS taken 4 days later, showing a rapid decline in virus load (2.25E+04 copies/mL) [6]. All symptoms resolved within 10 days. No viral coinfection was detected at that time. During the second episode, serologies retrieved on 4, 12 and 35 days after symptom onset showed a high reactive antibody titre with the quantitative anti–S RBD assay as soon as 4 days after symptom onset (Fig. 1(A) and (B)) and remained at a similar level for up to 1 month. Anti-N Ig levels had already increased from moderate to high levels after first infection and remained high after reinfection.

Low titres of neutralizing antibodies were observed at day 14 after diagnosis after the first infection and already showed a slight decline at 1 month. In contrast, a high titre was already detectable 4 days after the second onset of symptoms, and titres further increased during the second week and stayed elevated at 1 month (Fig. 1(B)).

A total of 96.8% and 98.8% genome coverage was obtained by whole-genome sequencing for viral genome sequences obtained in specimens collected during the first (EPI_ISL_708381) and second (EPI_ISL_708380) episodes respectively. Because the *S* genes were not fully covered in both specimens, a complementary Sanger sequencing approach was used. Although both viral sequences belong to the 20A clade (https://nextstrain.org/sars-cov-2/), sequencing revealed that the two viruses recovered 6 months apart were different (>99.9% bp identity) and clustered with viruses circulating locally in the hospital clusters during each of the corresponding episodes (Supplementary Fig. S1). The spike protein differed by only one mutation, S477N, observed during the second infection (Supplementary Table S1 and Supplementary Fig. S2), which emerged in Europe during summer 2020 [7].

MBC responses were assessed 12 days after onset of symptoms (Supplementary Fig. S3). High frequencies of anti-S1 and anti-N MBCs were detected (anti-S1 1.68% and anti-N 0.88% of IgG-producing MBCs), comparable to responses seen months after SARS-CoV-2 infections.

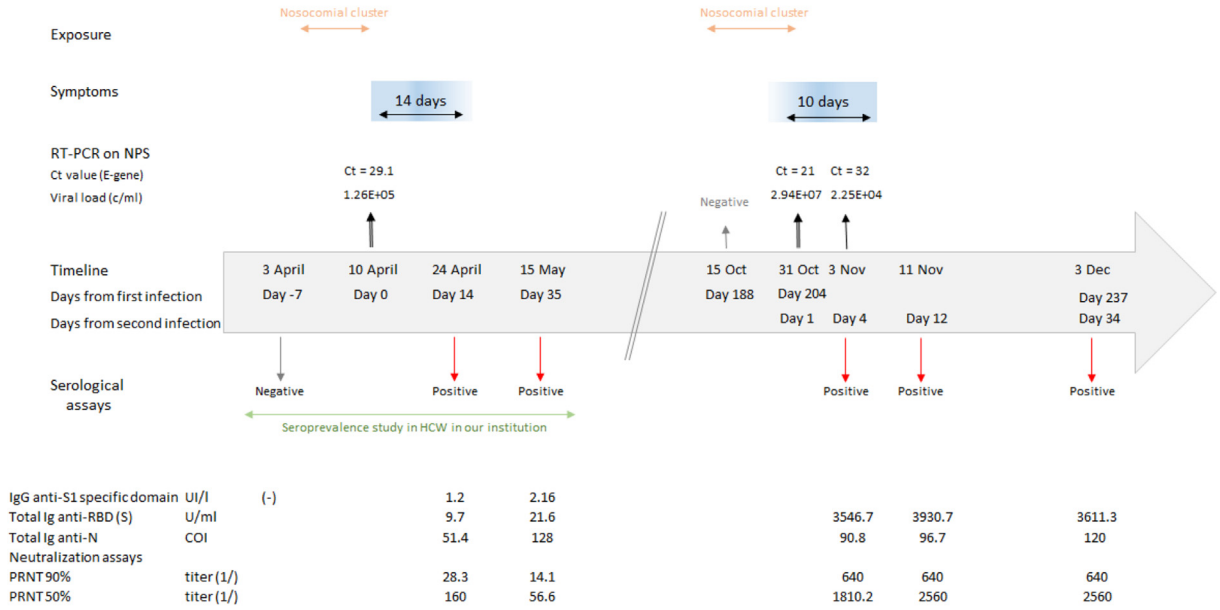
Discussion

Clinical, immunologic and virologic investigations allowed documentation of a SARS-CoV-2 reinfection with a different virus in a young, healthy physician. This reinfection led to a second mild episode, which quickly and fully resolved. Anti–S RBD antibody titres reached only low levels, while neutralizing antibody titres were already declining 1 month after the first infection. The SARS-CoV-2–specific B-cell responses elicited after the first episode were immediately boosted by the reinfection, which was attested by the rapid antibody titre increases and the strong MBC responses. A rapid increase of neutralizing antibodies has also been described in a young male subject with an asymptomatic reinfection [8]. Here, the high titres of neutralizing antibodies observed early after the second infection correlated with an early and strong reactivation of the memory B-cell response as soon as day 12 after reinfection.

We suppose that the mechanism leading to reinfection is a loss of protection elicited after the first episode, as is known to be the case for other human coronaviruses [9]. The only mutation found in the spike region between the two episodes has not been associated with reduced neutralization by human convalescent sera [10]. *In vitro,* this mutation may lead to monoclonal antibody neutralization resistance, but whether this is relevant for human reinfection remains to be studied. Of note, it was not possible to assess whether the reinfection may have been the consequence of exposure to a high viral inoculum.

As per local recommendations, HCWs are asked to get tested when displaying symptoms compatible with COVID-19. In our institution, more than 700 HCWs were infected in the community or hospital setting during the first wave of COVID-19 from February to May 2020, and around another 2000 HCWs have been infected since September 2020. Among them, this is the only reinfection that has been well documented within the last 9 months. Three more cases at least 6 months from the first episode are under investigation on the basis of

A



IgG anti-S1 specific domain	UI/l	(-)		1.2	2.16			
Total Ig anti-RBD (S)	U/ml			9.7	21.6	3546.7	3930.7	3611.3
Total Ig anti-N	COI			51.4	128	90.8	96.7	120
Neutralization assays								
PRNT 90%	titer (1/)			28.3	14.1	640	640	640
PRNT 50%	titer (1/)			160	56.6	1810.2	2560	2560

B

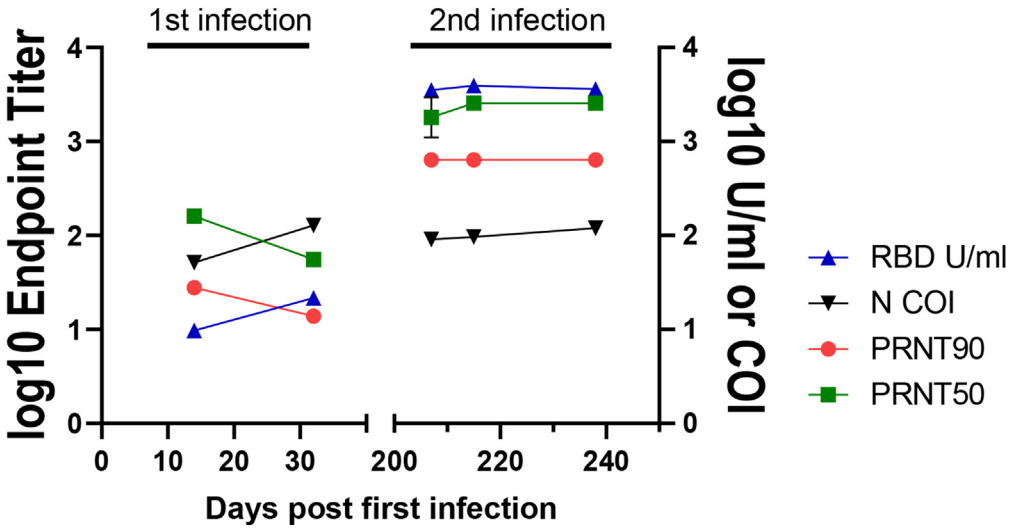


Fig. 1. (A) Schematic representation of clinical course of patient with serologic and virologic results. Day 0 indicates day of diagnosis when asymptomatic or day of onset of symptoms. (B) Kinetics of serologic responses. Neutralizing endpoint titres 50% (green squares) and 90% (red dots) plaque reduction are shown (PRNT50 and PRNT90 respectively) for five different sampling time points during first and second infection. Blue triangles indicate anti–S RBD titres (U/mL); black triangles, anti-NCP titres (COI). Abbreviations: c/mL, copies per mL of specimen; COI, cutoff index; Ct, cycle threshold; HCW, healthcare worker; N, nucleocapsid protein/nucleoprotein; NPS, nasopharyngeal swab; PRNT, plaque reduction neutralization test; RBD, receptor binding domain; S, spike.

clinical, epidemiologic and laboratory data [11], all leading to a second mild COVID-19; and like many other cases reported in the literature, these cases are imperfectly documented. With only a few reports in the literature, SARS-CoV-2 reinfection seems to be rare within the first 6 months after COVID-19 [1,4,12,13].

On the basis of seasonal coronaviruses immunity, we can expect that reinfection will be increasingly common, with longer time intervals since the first SARS-CoV-2 infection, following a progressive decline of protective antibody titres [14]. Correlates of protection, including protective levels of neutralizing antibodies, remain to be determined. In addition to generating MBCs, SARS-CoV-2 elicits T-cell responses, even after paucisymptomatic disease [15]. The latter may occur without a detectable antibody response and may also confer some protection against reinfection, or at least severe COVID-19.

Conclusions

A rapid decline in neutralizing antibodies may put HCWs at risk of reinfection, as shown in this case. Reinfection, however, leads to

significant boosting of previous immune responses. Larger cohorts of reinfected subjects with detailed descriptions of their immune responses are needed to define correlates of protection and their duration after infection.

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Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.02.010.

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