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Letter to the Editor

Serological profile of first SARS-CoV-2 reinfection cases detected within the SIREN study

A study published by Hanrath and colleagues¹ in this Journal found no SARS-CoV-2 reinfection cases between the first two waves of the pandemic in a cohort of healthcare workers. However, several SARS-CoV-2 reinfection cases have been reported during the second wave, although reinfection definitions are not consistent.^{2,3}

It is crucial to understand whether SARS-CoV-2 antibody titres could be used as a correlate of protection in assessment of disease susceptibility. In the SIREN study, a large national longitudinal cohort of more than 44,000 healthcare workers, participants are followed for at least 12 months using fortnightly symptom and exposure questionnaires and nucleic acid amplification testing (NAAT), with monthly antibody testing against SARS-CoV-2.⁴ Potential reinfections are flagged when meeting the following criteria: two positive RT-PCR tests at least 90 days apart (with no additional intervening positives) or a new RT-PCR positive test at least four weeks after a positive SARS-CoV-2 antibody test. Additional total antibody testing is performed at Public Health England laboratory using the semi-quantitative Elecsys Anti-SARS-CoV-2 nucleocapsid (N) protein assay and fully quantitative Elecsys Anti-SARS-CoV-2 spike (S) protein assay which targets the receptor binding domain (RBD) (Roche Diagnostics).⁵

We here describe two reinfection cases in which additional serological assays were performed: in-house recombinant SARS-CoV-2 IgG spike (S) protein RBD indirect ELISA,⁶ live virus microneutralisation using SARS-CoV-2 isolate England/02/2020,⁷ and pseudovirus neutralisation.⁸ Semi-automated multiplexed immuno-blotting assay was performed to detect RBD-, N-, S1-, S2- and S-specific IgG, IgA and IgM antibodies.⁸

Case 1

A 45-year-old female nurse, with history of asthma and treated breast cancer, was SARS-CoV-2 antibody positive on 7th August 2020. She reported COVID-19 symptoms in March 2020 (dry cough, fever, headache and myalgia, followed by anosmia and ageusia), however RT-PCR was not performed. On 10th October, during a nosocomial outbreak of SARS-CoV-2, she became SARS-CoV-2 PCR positive, however asymptomatic at the time of testing. Four days later, she reported headache followed by sore throat, myalgia, arthralgia, ageusia and a productive cough. She reported milder symptoms during the second episode.

SARS-CoV-2 was successfully cultured from the earliest of several samples taken between 10th to 23rd October. A phylogenetic analysis was undertaken to compare sequences derived from the PCR positive swabs with circulating SARS-CoV-2 strains in the UK, using cluster investigation and viral epidemiology tools (Pangolin

COVID-19 Lineage Assigner). Infection was due to SARS-CoV-2 lineage B.1.523 with exact concordance between all sequences obtained from the individual. Sequences segregated to the same lineage, within one or two SNPs as samples from 18 other individuals involved in the nosocomial outbreak.

Prior to reinfection, S binding antibodies (RBD ELISA and Roche S/RBD ECLIA) and neutralising antibodies (live virus and pseudovirus) were at or below the limit of detection but were boosted significantly following reinfection, with neutralising antibodies increased to high titres > 1:1000 33 days after reinfection (Fig. 1).

The immuno-blotting results (Fig. 2a) demonstrated N-specific IgG was clearly detectable at the time of reinfection, whereas the intensity of the S-specific band was weak, consistent with other serological results. IgM levels were undetectable. In contrast, all antigens except S2 were clearly detectable by IgA 30 days after reinfection.

Case 2

A 37-year-old female administrator had SARS-CoV-2 antibodies on 28th August 2020. She described COVID-19 symptoms in March 2020, (fever, shortness of breath, flu-like symptoms, anosmia and ageusia) that lasted 4 weeks, when no RT-PCR test was performed. A surveillance RT-PCR test undertaken on 06th October 2020 was positive, when she had coryzal symptoms and diarrhoea that lasted less than 24 h, with no other symptoms.

Genomic analysis identified from swabs on 6th and 9th October were identical and belonging to B.1.258.4 lineage. Serology demonstrated an increase in antibody reactivity in all assays following the second infection (Fig. 1). Prior to the reinfection, both N and S antibodies were detectable with low levels of neutralising antibodies (live virus and pseudovirus). However, within three days of reinfection, neutralising antibodies increased to a titre of >1:200.

Previous exposure to SARS-CoV-2 was evident from the immuno-blot (Fig. 2b). Thus, IgG and IgA specific to the Nucleocapsid protein, but not other antigens, could be detected prior to the onset of symptoms. Two weeks later, an increase in antibody responses was observed, against the N antigen, Spike antigen, RBD and S1 sub-domains. IgM was detected against N and S1, albeit weaker against the latter antigen.

The mechanisms of failure of immune protection from reinfection have not been clearly elucidated. In these two cases, an anamnestic antibody response was observed using virus neutralisation, antibody binding and immuno-blotting assays. All investigations showed an increase in antibody levels following the onset of symptoms in both cases and low or absent levels of neutralising antibodies at time of reinfection. This might be due to a lack of effective antibody response after the first infection or decrease in neutralising antibody titres over time, as observed in other studies.⁹

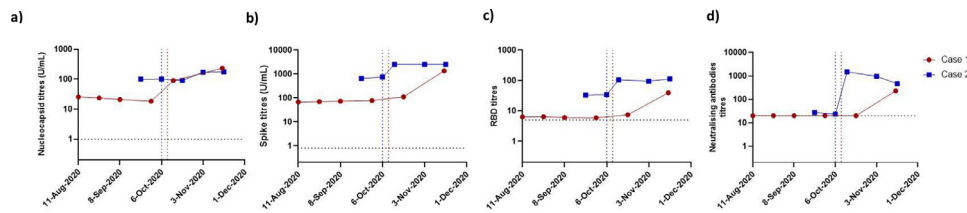


Fig. 1. Serological response in Case 1 and Case 2 against SARS-CoV-2, including anti-N, anti-S, anti-RBD and neutralising antibodies. Vertical dashlines represent the re-infection events for Case 1 (red) and Case 2 (blue). Horizontal dashline represents cutoff values. (a) Anti-SARS-CoV-2 nucleocapsid (N) protein assay (Roche Diagnostics - Cutoff ≥ 1.0 U/mL). (b) Fully quantitative Elecsys Anti-SARS-CoV-2 spike (S) protein assay (Roche Diagnostics - Cutoff ≥ 0.8 U/mL).⁵ (c) In-house recombinant SARS-CoV-2 IgG spike (S) protein receptor binding domain (RBD) indirect ELISA (Cutoff ≥ 5.0).⁶ (d) Neutralising antibodies were detected using a live virus microneutralisation assay, using England/2/2020 virus (Cutoff ≥ 20.0).⁷

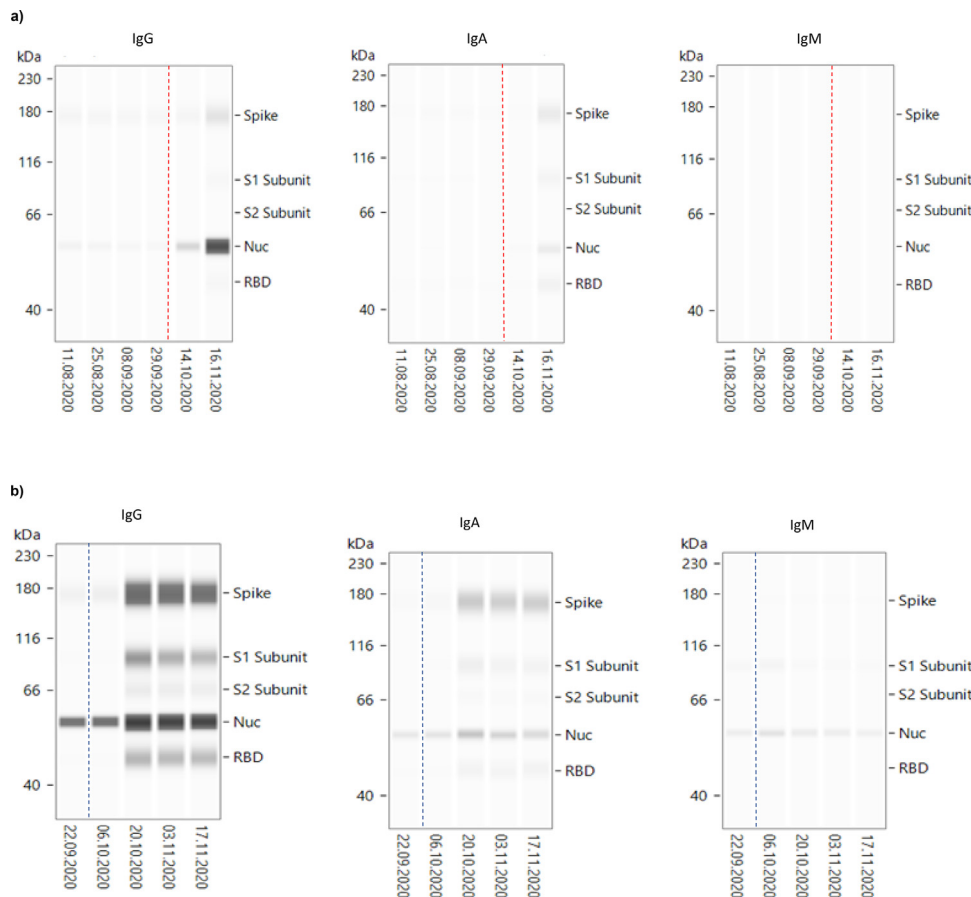


Fig. 2. Immuno-blotting of Case 1 (a) and Case 2 (b) plasma samples showing the reactivity of IgG (left), IgA (middle) and IgM (right) against the Spike, S1, S2, N and RBD antigens of SARS-CoV-2. Dashed lines represent the reinfection events.

Our data are consistent with the hypothesis that absence or low levels of neutralising antibody titres are likely correlate with a lack of protection against SARS-CoV-2 reinfection. There is strong evidence that neutralising antibodies play a critical protective role. Was estimated that neutralising antibody titres can offer an accurate prediction of immune protection, with neutralisation level for 50% protection being 54 U/mL, which equates to a titre of 1:10 or 1:30 in most virus neutralisation assays.¹⁰ The sera from both cases had virus neutralising antibody levels below this threshold at the time of SARS-CoV-2 reinfection.

There are some limitations in this study: no samples from the first infection episodes were available, thus no comparative genomic analysis of infecting viruses was possible. However, the sequences obtained in October were genetically distant from SARS-CoV-2 viruses from March 2020 but closely related to viruses circulating locally at time of reinfection. Secondly, symptoms from

the first infection episodes are subject to recall bias. Finally, our analysis was restricted to two cases; therefore, our hypothesis will require support from more extensive studies. Further analysis using a case-control design is essential to clarify the potential role of neutralising antibodies in SARS-CoV-2 reinfection.

Declarations of Competing Interest

None.

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