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Letter to the Editor

Negative SARS-CoV-2 antibodies, T-cell response and virus neutralization following full vaccination in a renal transplant recipient: a call for vigilance

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To the Editor

Coronavirus disease 2019 (COVID-19) has overwhelmed the world since the end of 2019. In recent months, great optimism has resulted from an increasing number of available vaccines showing protection rates against COVID-19 of up to 95% [1]. However, the vaccines have not been tested in a number of frail patients, including organ-transplant recipients.

We followed two completely stable patients on haemodialysis. Patient 1 was a 43-year-old female who had been on haemodialysis for 2½ years. She was not a transplant recipient and did not receive immunosuppressive therapy, but received levothyroxine, antihypertensive medication, phosphate binders, darbepoetin α and vitamins. Complete blood cell counts were normal before vaccination. Creatinine was 613 $\mu\text{mol/L}$ (reference 45–90 $\mu\text{mol/L}$) and blood urea nitrogen was 13.3 mmol/L (reference 2.6–6.4 mmol/L). Patient 2 was a 58-year-old male who had been on haemodialysis for half a year

and had had three renal transplantations, the latest in 2011. He received mycophenolic acid 360 mg plus tacrolimus 3 mg daily (not prednisolone). He also received antihypertensive medication, phosphate binders, allopurinol, darbepoetin α and vitamins. Complete blood cell counts were normal apart from a slightly elevated white blood cell count of 11.0×10^9 cells/L (reference $3.5\text{--}8.8 \times 10^9$ cells/L) because of crystal synovitis. The creatinine was 777 $\mu\text{mol/L}$ (reference 60–105 $\mu\text{mol/L}$) and blood urea nitrogen 18.4 mmol/L (reference 3.5–8.1 mmol/L). Both patients were followed from the start of vaccination with the Pfizer–BioNTech COVID-19 vaccine to 4 weeks after the second dose. Both patients had mild local reactions after vaccination. Antibodies were measured 1–3 times a week, T-cell response 1 and 4 weeks after the second vaccination, and virus neutralization before and 4 weeks after the second vaccination.

Total Ig and IgG were measured with the Vitros Immunodiagnostic Products Anti-SARS-CoV-2 Ig total and IgG assay (Ortho Clinical Diagnostics, Cedex, France) which is a commercial immunoassay using the spike protein S1 antigen [2]. Results were reported as signal sample/cut-off (S/CO), with S/CO values < 1 interpreted as non-reactive and ≥ 1 interpreted as reactive.

T-cell responses were measured with an interferon- γ release assay (QuantiFERON SARS-CoV-2, QIAGEN, Germantown, MD, USA) [3]. In brief, blood collection tubes coated with Ag1 and Ag2—which are SARS-CoV-2 spike peptides for specific stimulation of CD4+ and CD8+ T cells—were used, with subsequent measuring of interferon- γ release by ELISA.

Finally, a plaque-reduction neutralization test (PRNT) adapted from a previously published protocol was performed [4]. In brief, patient EDTA-plasma before vaccination and 4 weeks after the second vaccination were aliquoted into a two-fold serial dilution with 15–45 PFU/mL of SARS-CoV-2 in complete viral culture media: Dulbecco's modified Eagle medium (DMEM), 2% heat-inactivated foetal bovine serum (FBS), 1% penicillin, streptomycin

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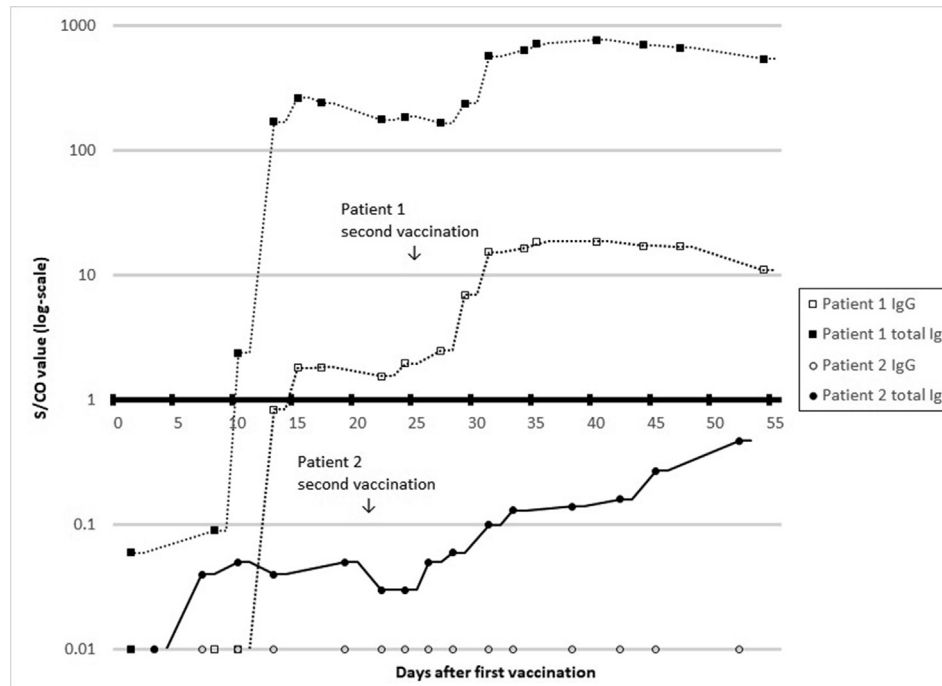


Fig. 1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody (total Ig and IgG) response from first vaccination to 4 weeks after the second vaccination from patients 1 and 2 (renal transplant recipients). Antibodies were measured as signal sample/cut-off (S/CO) with an S/CO value of ≥ 1 as reactive indicated by the bold horizontal line. Observe that the Y-axis is a log scale.

and amphotericin B in a concentration of 2.5 mg/L. The virus–plasma mixtures were incubated for 1 h at 37°C under 5% CO₂. The mixtures were placed onto a monolayer of Vero-E6 cells (ATCC® CRL-1586™) in six-well microtitre plates and incubated for another hour. Overlay medium with 1% agarose was added and the plates were incubated for 3 days. After fixation and staining, plaques were counted. The highest plasma dilution enabling a >90% plaque reduction was defined as the titre. Titres >1:10 were defined as neutralizing [5]. The SARS-CoV-2 antibody response is shown in Fig. 1.

Patient 1 had reactive total Ig and IgG antibodies (≥ 1) after approximately 2 weeks, and interferon- γ release could be detected 1 week and 4 weeks after the second vaccination. Furthermore, the patient had an increase in the titre of neutralizing SARS-CoV-2 antibodies from <1:4 before vaccination to 1:32 4 weeks after the second vaccination in the PRNT.

Patients 2 did not have reactive Ig or IgG antibodies (<1) 4 weeks after the second vaccination, and interferon- γ release could not be detected 1 week or 4 weeks after the second vaccination. The PRNT did not show any change in the titre of neutralizing SARS-CoV-2 antibodies as it remained <1:4 after vaccination.

The antibody assay for this study uses the spike protein as target and consequently it should be able to detect an antibody response to the Pfizer–BioNTech COVID-19 vaccine, which induces production of spike proteins. This is the same target used for the SARS-CoV-2 QuantiFERON test. The validity of the results is corroborated by the antibody and T-cell response of patient 1.

The most likely explanation to the lack of response in patient 2 is the effect of the immunosuppressive therapy, although other factors such as age, sex and renal failure could also influence the

response. From this report it is not possible to determine whether the effect is caused by mycophenolic acid, tacrolimus, or the combination. However, similar effects have been demonstrated with influenza vaccines, especially if patients received mycophenolic acid. In a recent study by Boyarsky et al. it was shown that older age, antimetabolite therapy (e.g. mycophenolic acid) and vaccination with the Pfizer–BioNTech vaccine, versus the Moderna vaccine, was associated with a lack of antibody response after a single dose of vaccine in solid-organ transplant recipients [5].

To our knowledge, no studies or reports so far have demonstrated convincing protection against SARS-CoV-2 infection in vaccinated organ-transplant recipients. Although the observations from this report cannot be considered any kind of proof that organ transplant recipients receiving immunosuppressive therapy will not respond to SARS-CoV-2 vaccines in general, it does call for vigilance. The case indicates a possible need to monitor the group of transplant recipients closely and uphold protective precautions until vaccine studies can demonstrate protection against COVID-19. If this is not the case, it is imperative that new strategies are explored fast, such as repeating vaccination with the same vaccine, to try to further boost immunity, or possibly change to another vaccine with another principle, such as the viral vector vaccines [1].

Author contributions

All authors have made substantial contributions to the work and all participated in writing (review and editing). Conceptualization and supervision: USJ, ISJ and CB. Investigation: USJ, RMP, DST and TEA. Formal analysis: RMP, DST, TEA, USJ, ISJ and CB. Writing of original draft: USJ.

Transparency declaration

All authors have stated that they have no conflicts of interest to declare. Written informed consent was obtained from both patients. The study was supported by the The Novo Nordisk Foundation, grant number NNF20SA0062931.

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