Undetectable SARS-CoV-2 active adaptive immunity—post-vaccination or post-COVID-19 severe disease—after immunosuppressants use

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SUMMARY

Since the beginning of COVID-19 vaccination in New Jersey in December 2020, we have observed multiple cases of undetectable adaptive immunity, post-vaccination or post-COVID-19 infection, in patients using immunosuppressants. Here, we present three cases of patients using immunosuppressants: mycophenolate and tacrolimus for renal transplant; ocrelizumab for multiple sclerosis and rituximab for peripheral ulcerative keratitis. All three patients were admitted for acute respiratory distress syndrome (ARDS) from COVID-19 pneumonia; two patients reported having received full COVID-19 vaccination prior to admission and one unvaccinated patient required readmission. Our findings showed that these patients tested negative for SARS-CoV-2 IgM spike and CoV-2 IgG nucleocapsid antibodies. All three patients were treated with standard-of-care remdesivir, dexamethasone and convalescent plasma; two recovered successfully and one patient died from respiratory failure secondary to worsening ARDS from COVID-19 pneumonia. We highlight the challenges of treating immunosuppressed patients with COVID-19 pneumonia, in an era where dissemination of such information is paramount to helping doctors standardise and improve the quality of care for these patients.

BACKGROUND

As of October 2021, the global COVID-19 pandemic has totalled 234+ million infections and 4.7+ million deaths worldwide; the USA tops the list of most affected nations with 43+ million infections and more than 700 000 deaths.[1] The newly developed COVID-19 mRNA vaccines (Pfizer-BioNTech and Moderna) were approved by the Food and Drug Administration to be administered in the adult population as emergency use to prevent coronavirus infection and halt its continued spread. It was recommended that people at risk of severe disease were prioritised in getting the vaccine and that included those on immunosuppressive drugs for autoimmune disease, organ recipients and malignancies.[2] SARS-CoV-2 antibodies are induced following COVID-19 infection or vaccination. It typically takes 2 weeks after completion of vaccination or recent infection for our bodies to produce antibodies (adaptive immunity).[3–5] Since the beginning of COVID-19 vaccination in New Jersey in December 2020, we have observed multiple cases of diminished or absent adaptive immunity post-vaccination or post-COVID-19 infection while using immunosuppressants. Here we describe three cases of patients taking immunosuppressants: mycophenolate with tacrolimus, ocrelizumab, and rituximab and hospitalised with acute respiratory distress syndrome (ARDS) from COVID-19 pneumonia after COVID-19 vaccine or infection, and found to have undetectable antibody response.

CASE PRESENTATION
Case 1
A 70-year-old man with a medical history significant for end-stage renal failure with renal transplant (6 months ago) on prednisone, tacrolimus and mycophenolate; diastolic heart failure and insulin-dependent diabetes mellitus was hospitalised for ARDS due to COVID-19 pneumonia. He had completed Moderna COVID-19 vaccine series 1 month prior to diagnosis. However, antibody testing was negative for SARS-CoV-2 IgM spike and CoV-2 IgG nucleocapsid.

Initial treatment included bamlanivimab and etesevimab infusion, convalescent plasma (CP), remdesivir, dexamethasone, oxygen via nasal cannula and apixaban. With regard to his renal transplant, he was resumed on tacrolimus and half the dose of his mycophenolate.

Eventually, he was started on broad-spectrum antibiotics and received a second dose of CP. His clinical condition continued to worsen, requiring admission to the intensive care unit and mechanical ventilation (see figures 1 and 2).

Case 2
A 69-year-old woman with a medical history significant for hypertension, heart failure with reduced ejection fraction (35%) and multiple sclerosis (MS) on ocrelizumab every 6 months (last dose 4 months ago) was hospitalised for ARDS due to COVID-19 pneumonia.

She had completed Pfizer vaccine series 4 months prior to diagnosis. However, antibody testing was negative for SARS-CoV-2 IgM spike and CoV-2 IgG nucleocapsid. She completed a course of treatment with remdesivir, dexamethasone and one dose of CP (see figures 3–5).
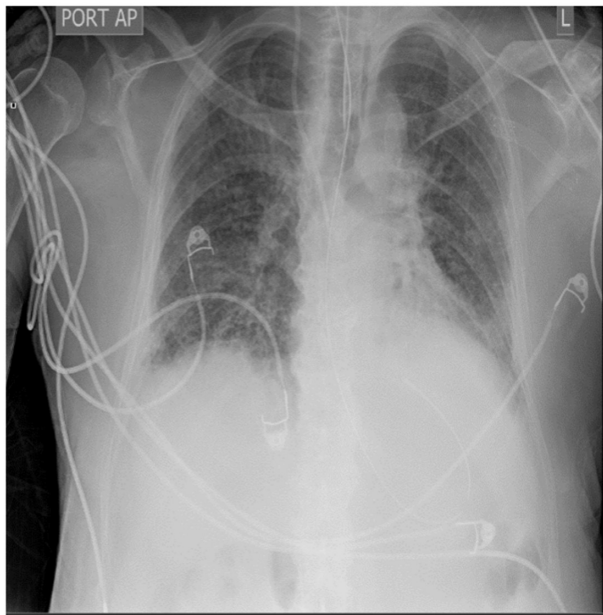
Case 3
The third case was a 45-year-old man with a medical history significant for controlled insulin-dependent diabetes mellitus and was legally blind from peripheral ulcerative keratitis (PUK) for which he received rituximab infusions every 6 months (last infusion 6 months ago). He was hospitalised for ARDS due

Figure 1 Chest X-ray showing diffuse bilateral mixed interstitial/alveolar opacities.

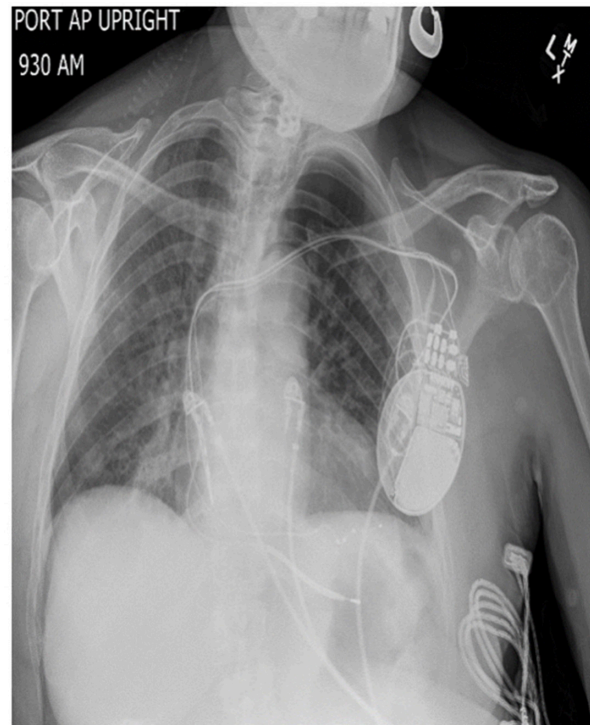**Figure 3** Chest X-ray showing multifocal mixed interstitial/airspace opacities within the lungs.

to COVID-19 pneumonia requiring oxygen via non-rebreather mask alternating with high-flow nasal cannula. He had not received the COVID-19 vaccine at diagnosis.

He received 8 days of remdesivir, 10 days of dexamethasone and 1 dose of CP. However, antibody testing was negative for SARS-CoV-2 IgM spike and CoV-2 IgG nucleocapsid. He clinically improved, maintaining oxygen saturation >95% on room air and was discharged home.

Two days after discharge, he was rehospitalised for high fevers, cough and dyspnoea and required oxygen via non-rebreather mask. Pulmonary embolism was ruled out with CT. At this time, COVID-19 PCR remained positive and the COVID-19 cycle threshold (Ct) was 15.5 and still with negative COVID-19 antibodies at day 19 of his symptoms. COVID-19 antibody testing was done repeatedly to assess for presence of a measurable immune response to COVID-19 infection given immunosuppression from rituximab. Decision was made to restart remdesivir for 5 more days, one dose of CP and 10 days of dexamethasone. He also completed 7 days of empirical broad-spectrum antibiotics for the possibility of hospital-acquired pneumonia. His COVID-19 antibody IgG spike turned positive on day 21 of symptoms. His clinical condition improved and was

discharged home on intranasal oxygen (see timeline on table 1 and figures 6 and 7).

OUTCOME AND FOLLOW-UP

Our 70-year-old male patient with renal transplant unfortunately died from severe COVID-19-related ARDS after a week course in the intensive care unit. Our 69-year-old female patient with MS on ocrelizumab continued to improve and was discharged home with oxygen; 2 weeks later at follow-up, she no longer required oxygen. At follow-up visit 2 weeks later, our 45-year-old male patient with PUK on rituximab also continued to improve, with significant improvement of airspace infiltrates on chest X-ray.

Figure 2 Timeline of patient renal transplant, vaccination, monoclonal antibody infusion, SARS-CoV-2 test and antibody results. This image was created by authors of this manuscript.
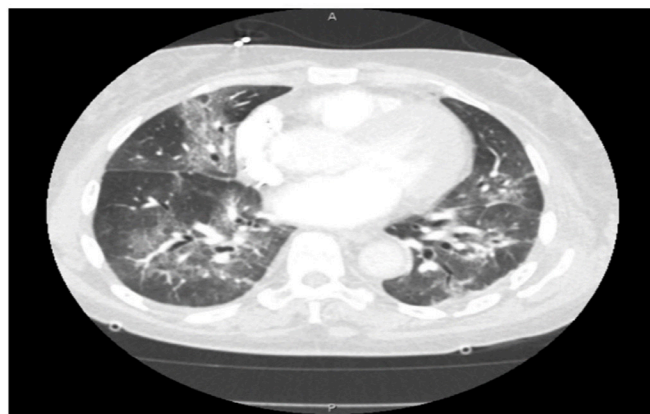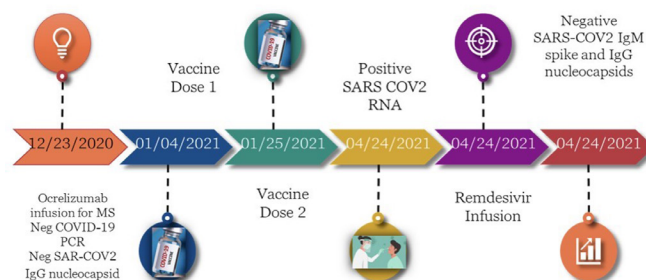

Figure 4 CT of the chest showing extensive ground-glass and interstitial opacities throughout the lungs, particularly within the right upper lobe.

Figure 5 Timeline of patient ocrelizumab infusion, vaccination, monoclonal antibody infusion, SARS-CoV-2 test and antibody results. This image was created by authors of this manuscript. MS, multiple sclerosis.

Figure 6 Chest radiograph showing multifocal consolidations.
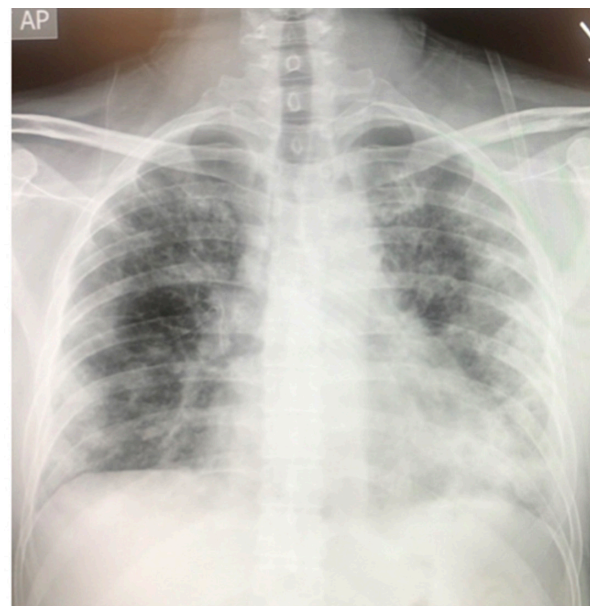
DISCUSSION

SARS-CoV-2 is an RNA virus that has become a major public health concern after the outbreak of the Middle East respiratory syndrome-CoV and severe acute respiratory syndrome-CoV in 2002 and 2012, respectively.[6] As of October 2021, the total number of COVID-19 cases had reached over 234+ million worldwide, with more than 4.7+ million confirmed deaths.[1]

In December 2020, the US Food and Drug Administration issued the first emergency use authorisation for the COVID-19 vaccine. The available vaccines included the Pfizer-BioNTech two-dose COVID-19 vaccine regimen given on days 0 and 21,[3] and the Moderna two-dose COVID-19 vaccine regimen given on days 0 and 28.[4] They both are messenger RNA vaccines that use mRNA delivered in a lipid nanoparticle to express a full-length spike protein and are given intramuscularly.[7 8] A phase I open-label trial demonstrated binding and neutralising antibody responses as well as CD4 cell responses to the vaccine.[9]

These vaccines have been proven to be safe and ~95% effective in preventing symptomatic COVID-19 with antibody formation measured from 7 days after the second dose of Pfizer,[3] and 14 days after the second dose of Moderna.[4] In addition, previous studies reveal protective antibodies are formed ~15 days after a confirmed COVID-19 infection.[5] Despite the role of the vaccines, it had been proposed that patients on immunosuppressants may not fully benefit from the vaccine and are still at a high risk of developing severe disease.[10]

We used two antibody tests for detection of antibodies, Elecsys Anti-SARS-CoV-2 S assays for the detection of SARS-CoV-2 IgM and IgG spike antibodies, and the SARS-CoV-2 IgG reagent kit for the detection of IgG nucleocapsid.

The Elecsys Anti-SARS-CoV-2 S assays used with Cobas E analysers is an electrochemiluminescence immunoassay used for qualitative and semiquantitative detection of antibodies to SARS-CoV-2 spike protein receptor-binding domain. It aids in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent infection. The Elecsys Anti-SARS-CoV-2 immunoassay demonstrated high sensitivity (99.5%) and specificity (99.80%) at ≥14 days post-PCR confirmation.[11–13]

The SARS-CoV-2 IgG reagent kit used with Architect is a chemiluminescent microparticle immunoassay designed to detect IgG antibodies to the nucleocapsid protein of SARS-CoV-2 in persons who are suspected to have had COVID-19. This test thus remains positive after infection. The SARS-CoV-2 IgG assay exhibited 99.9% specificity and 100% sensitivity for detecting the IgG antibody in persons 17 days after symptoms began.[13–15]

In our first case, renal transplant recipients appear to be at particularly high risk of critical COVID-19 illness due to chronic immunosuppression and coexisting medical conditions.[16]

The ideal treatment for renal transplant recipients with COVID-19 remains uncertain at present with variable approach and outcomes.[17] Our approach was to decrease the dose of mycophenolate, eventually discontinuing the drug if the patient required intubation, while continuing tacrolimus and corticosteroid. Similar approach has been cited in the literature.[17 18] The rationale being to reduce immunosuppression and to counterbalance the systemic inflammatory reaction.[18] The immune response of renal transplant recipients, particularly the T cell immune response, is significantly suppressed due to the long-term use of immunosuppressive agents.[19] Without protective antibodies, these patients may have high viral replication and the standard dosage of monoclonal antibody may not be enough to prevent disease progression.

Table 1 Timeline of SARS-CoV-2 antibodies

		Index admission			Readmission				
		Day 7	Day 10	Day 11	Day 18	Day 19	Day 20	Day 21	Day 24
PCR	SARS-CoV-2 RNA screen (rapid)	Pos			Pos				
	SARS-CoV-2 RNA PCR					Pos			Pos
Antibodies	SARS-CoV-2 IgM spike			Neg		Neg	Neg	Neg	
	SARS-CoV-2 IgG spike						Neg	Pos	
	SARS-CoV-2 AB IgG, nucleocapsid		Neg			Neg	Neg		

AB, antibody; Neg, negative; Pos, positive.
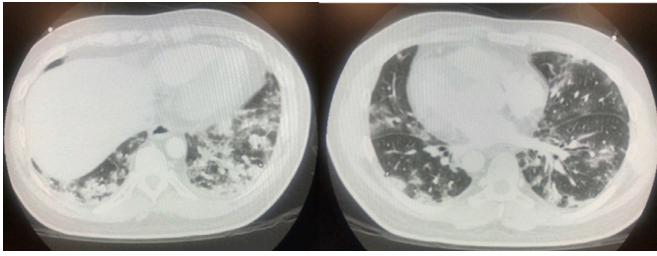
Figure 7 CT of the chest with multiple bilateral ground-glass opacities.

The first patient's first vaccine (Moderna) was on 14 February 2021, and the second vaccine was on 14 March 2021, which was also the day he became symptomatic (see figure 2).

He was tested at an urgent care facility on 19 March 2021 and found to be COVID-19 positive. He received bamlanivimab and estesevimab on 20 March 2021, however, symptoms persisted, and he was admitted on 31 March 2021, at which time antibody titres were checked. Therefore, the interval between vaccination and antibody titre verification is 45 days after the first vaccine dose and 17 days after the second dose. We suspect this patient became infected before he received the second dose of the vaccine and therefore may not have been able to mount a sufficient protective immune response. However, a US vaccine effectiveness study among healthcare providers found a single dose of Pfizer-BioNTech or Moderna COVID-19 vaccines to be 82% effective against symptomatic COVID-19 and two doses to be 94% effective.[20]

For the second case, antibody tests were also performed on the day of admission, 24 April 2021. She received her first vaccine dose (Pfizer) on 04 January 2021 and the second dose on 25 January 2021. Therefore, the interval between vaccination and antibody titre verification is 110 days after the first dose and 89 days after the second dose (see figure 5).

This patient received ocrelizumab for MS, which is a humanised anti-CD monoclonal antibody that works by inducing rapid and prolonged B cell depletion and results in impaired secondary humoral immune response to vaccination.[10] Its half life is 26 days, but recovery of B cells usually starts only 6–9 months after the completion of therapy and normal levels are obtained after 9–12 months. Its maintenance dose is administered as an infusion every 6 months.[21] CD20 is a cell-surface antigen mainly expressed by cells of the B cell lineage and a small subset of CD3^{+} T cells. These cells notably are responsible for antibody production, antigen presentation, secretion of pro-inflammatory cytokines and the increased expression of activation markers with production of pro-inflammatory cytokines such as TNFα, interleukin (IL)-1β or IL-17, respectively. By its effect on humoral immunity, it can dampen the humoral response to vaccines. The VELOCE trial in patients with MS showed attenuated but present humoral responses following pneumococcal, influenza and tetanus toxoid vaccinations upon initiation of ocrelizumab.[22]

In our case, the patient received the vaccine with no relation to the time frame of her infusion and thus far no guidelines have been recommended by the Centers for Disease Control and Prevention (CDC) regarding this category of patients. We approached her management with the available recommended treatment. She had an undetectable humoral response after mRNA SARS-CoV-2 vaccination, and this is concerning. However, it is possible she may have just run the course of the COVID-19 infection or had mounted an attenuated humoral

and possibly cellular immune response against COVID-19 giving her improvement in symptoms, but currently there is no standard way of assessing such immune response.

In the third case, our patient documents the use of rituximab which is also an anti-CD20 monoclonal antibody with selective B cell depletion. Its half life is 20.8 days and recovery of B cells is also prolonged usually in 6–9 months after the completion of therapy and normal levels are obtained after 9–12 months. As a result, rituximab may affect antiviral immunity, including the development of SARS-CoV-2 antibodies, risk of reinfection and impaired vaccine efficacy. However, anti-SARS-CoV-2 antibodies or immune complexes might potentially evoke monocyte or alveolar macrophage activation, thereby contributing to sustained secretion of pro-inflammatory cytokines and the development of pulmonary disease; and thus, rituximab may be thought to be beneficial in this way.[23] Some evidence has shown therapeutic efficacy of CP and plasma-based products in a subgroup of immunocompromised patients with iatrogenic B cell depletion.[24] As seen in our patient, there was some improvement after CP was given, with positive IgG spike antibodies noted after final dose. This demonstrates signs of measurable immunity. Interestingly, however, whether an IgG antibody response following infection or vaccination translates to protective immunity is still unknown.[25] The appropriate interpretation of results from SARS-CoV-2 IgG assays depends on a clear understanding of their performance characteristics and limitations. Robust IgG responses to both the spike protein found on the surface of virus particles and the nucleocapsid protein found inside the

Patient's perspective

The following is the patient's perspective of our 69-year-old female patient with multiple sclerosis on ocrelizumab.

I was expecting the COVID vaccine to protect me from getting the COVID infection, so I was surprised when I was diagnosed, and when my antibody was undetectable. I was afraid the infection would progress and my symptoms worsened. I am grateful there were other available treatments that helped me improve. However, I wonder whether I would need booster doses of the COVID vaccine to be able to develop antibodies, that may one day protect me from developing a severe infection.

Learning points

► Immunocompromised individuals may not mount a sufficient antibody response to the SARS-CoV-2 vaccine and are still at risk of serious infection.
► Post-vaccination antibody levels can be predictive of immunity. It may also help to determine persons who remain at high risk of severe infection, and those who may benefit from booster doses. However, there is no specific antibody cut-off level above which protection is guaranteed or below which a booster would be emphatically recommended.
► There are significant interindividual differences in the level and chronological appearance of antibodies in patients with COVID-19. The median seroconversion and the development of protective antibody after vaccination have been observed at approximately 2 weeks.
► Failure to develop detectable levels of antibodies in response to the SARS-CoV-2 vaccines may not indicate absence of vaccine efficacy.

virus particle occur following SARS-CoV-2 infection. There are conflicting reports as to the relative sensitivities of spike-based versus nucleocapsid-based IgG assays, particularly during the first 14 days after disease onset.[26–29]

Further studies are needed to assess the benefit of this treatment modality. The use of COVID-19 Ct time in this case was beneficial in that it prompted further treatment with CP, remdesivir and steroids, which resulted in improved outcome.

The presence of the SARS-CoV-2 viral genome in its own is not sufficient proof of infectivity and caution is needed in evaluation of the infectivity of samples.[30] Data suggest that lower Ct values may be associated with worse outcomes and that Ct values may be useful in predicting the clinical course and prognosis of patients with COVID-19. Further studies are warranted to confirm clinical value.[31] Availability of different SARS-CoV-2-specific Reverse Transcription-PCR kits with different sensitivities makes the definition of a general cut-off level for the Ct value challenging.[32]

In conclusion, compared with the general population, immunogenicity of COVID-19 vaccines appears to be lower in immunosuppressed individuals, and vaccine efficacy is uncertain in these patients. Nevertheless, the potential for severe COVID-19 in this population likely outweighs the uncertainties.[32] At this time, antibody testing is not recommended to determine response to vaccination, therefore it is of paramount importance that we determine the precise immune correlates of protection, as early intervention in these patients could mean the difference between life and death.[33]

Prospectively, further studies are needed to determine if temporary reduction in immunosuppression before vaccination, booster doses after the completed vaccination series or complementary therapy such as pre-exposure exogenous antibody are helpful to these patients.

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