Open Forum Infectious Diseases

CORRESPONDENCE

## Clinical Considerations During Breakthrough Coronavirus Disease 2019 Infections in Vaccinated Individuals With Autoimmunity

To the Editor—The article by Peluso and colleagues [1] in *Open Forum Infectious Diseases* highlights the complexities of evaluating postvaccination severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immune responses in a patient with connective tissue disease. The authors identified suboptimal production of anti-spike receptor-binding domain (RBD) antibodies that lacked neutralization capacity after 3 doses of mRNA-1273 (Moderna) vaccine despite holding rituximab for over 6 months. This patient developed virus-specific T-cell responses, but due to the lack of established immune correlates of protection, the clinical implications of these results are unknown [1, 2].

This problem is complicated further by the wide variety of immunosuppressive conditions and immune-modulating therapies these patients receive, both of which significantly impact postvaccination immune responses. To highlight this point, we present a 55-year-old woman with well-controlled rheumatoid arthritis (RA) on tofacitinib, a Janus kinase (JAK) inhibitor, for >5 years. She presented with breakthrough coronavirus disease 2019 (COVID-19) despite receiving the 2-dose mRNA-1273 (Moderna) vaccination series and demonstrating a positive SARS-CoV-2 anti-spike (anti-S) antibody on the semiquantitative Elecsys-Roche antibody binding assay. Anti-nucleocapsid antibody by the Elecsys-Roche assay was negative, consistent with prior vaccination. She had received the mRNA-1273 vaccine 3 months prior while holding tofacitinib for 1 week after each injection. An RA flare at day 5 post–first injection necessitated a 1-week course of prednisone to manage symptoms.

On presentation, informed consent was obtained, and she enrolled in clinical trial NCT04401436. She had 3 days of chills, cough, and dyspnea on exertion. COVID-19 was diagnosed by nasal polymerase chain reaction and confirmed as the Alpha (B.1.1.7) variant by spike protein sequencing. Examination revealed a decrease in oxygen saturation with ambulation (approximately 92%–93%) but not at rest. She had elevated C-reactive protein (13.2 mg/L) but normal lymphocyte count and no infiltrate on chest radiograph. Due to her immunosuppressed condition with concern for high risk of severe disease, she was treated on day 3 of symptoms with bamlanivimab and etesevimab based on its emergency use authorization and had rapid improvement over the next 48 hours.

To further evaluate her vaccine-induced antibody responses, 2 serologic platforms were used to quantitatively assess SARS-CoV-2 anti-S antibodies in addition to the prior Elecsys-Roche antibody binding assay. These were all performed at presentation on a research basis and included the V-Plex meso-scale enzyme-linked immunosorbent assay (ELISA), which was used to measure quantitative anti-spike RBD immunoglobulin G (IgG), and the GenScript cPass, which is a surrogate viral neutralization test (sVNT) [3, 4]. Results were compared between our index patient and 4 age-matched control women (mean age, 58.5 years [range, 51–65 years]) who had also completed 2 doses of the mRNA-1273 vaccine. All controls were healthy with no history of COVID-19. Their postvaccine immune response was evaluated approximately 6 weeks after the second dose.

Quantitative anti-spike RBD IgG by the meso-scale ELISA identified a mean titer of 5488.2 IU/mL (range, 1709–10 149 IU/mL) in the 4 healthy controls, which was significantly greater than the 225.8 IU/mL titer in our index patient. The GenScript cPass assay is an sVNT that measures anti-S antibody–mediated inhibition of the interaction between the SARS-CoV-2 spike RBD and the angiotensin-converting enzyme 2 (ACE2) receptor. The result is reported as percentage signal inhibition and has been shown to detect neutralizing antibody titers [4]. At presentation, our index patient had 54% signal inhibition, which was approximately half the response found in the 4 vaccinated controls (mean, 96% [range, 95%–97%]). These results highlight notable differences in quantitative antibody responses despite qualitatively meeting criteria to be considered a positive result on both the Elecsys-Roche and GenScript cPass assays.

T-cell stimulation assays were also performed, as previously described [5], using SARS-CoV-2 spike protein lyophilized peptides, produced by Miltenyi Biotec [6], covering the immunodominant portions of the S1 and S2 domains. Three stimulation conditions were used, including peptides from the D614G spike protein, Alpha variant spike protein, and nucleocapsid protein. After stimulation with D614G spike peptides, the mean percentage of $CD4^+$/interferon gamma $(IFN\text{-}\gamma)^+$ in healthy controls was 0.542% (range, 0.40%–0.77%) compared to 0.093% in our index patient (Figure 1). Similar results were seen in $CD8^+IFN\text{-}\gamma^+$ cells with the mean percentage in healthy controls at 2.33% (range, 0.55%–4.0%) but 0.135% in our patient. In fact, the CD4 and CD8 IFN-γ+ T cells detected in our index patient were at levels similar to those observed in unvaccinated individuals.

Two months after recovering from acute COVID-19, repeat GenScript cPass

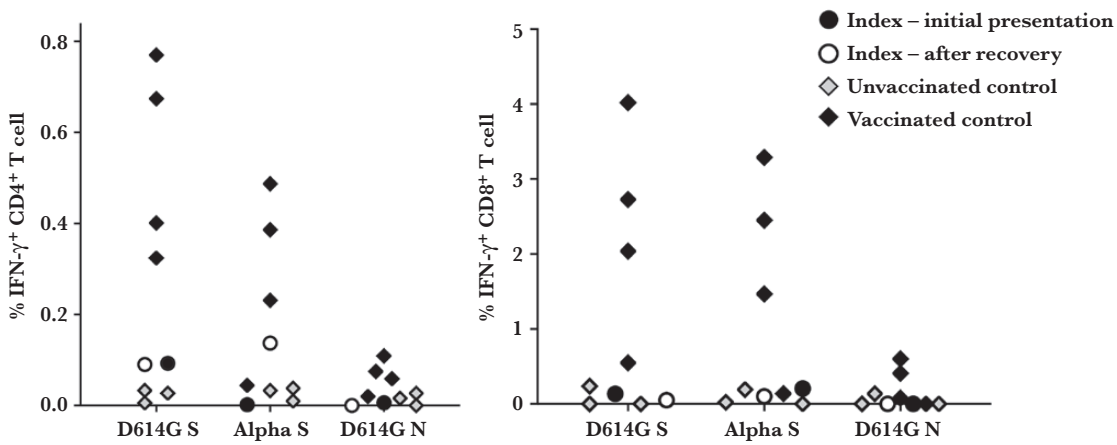


**Figure 1.** T-cell stimulation assay highlighting postvaccine T-cell responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens in healthy controls compared to our index patient. Results for the index patient are represented by circles (black, initial presentation; white, 2 months postrecovery from coronavirus disease 2019 [COVID-19]). Healthy controls are divided into unvaccinated responses (n = 3, gray diamonds) and vaccinated responses (n = 4, black diamonds). Index patient and healthy control cells were incubated overnight with SARS-CoV-2 antigens (D614G spike protein [D614G S]; Alpha variant spike protein [Alpha S]; nucleocapsid protein [D614G N]). Interferon gamma (IFN-γ)$^+$ CD4 and CD8 T cells were measured using flow cytometry. Percentage of IFN-γ$^+$ CD4 and CD8 T cells poststimulation with SARS-CoV-2 D614G spike and Alpha spike protein were consistently low in our index patient and resembled the level seen in unvaccinated controls. No significant T-cell responses were seen on stimulation with SARS-CoV-2 nucleocapsid protein, confirming that these results are due to vaccination and not prior COVID-19. T-cell stimulation assay was repeated at 2 months after recovery in the index patient, with a small increase noted only in CD4$^+$ T-cell response against Alpha S.

assay demonstrated boosting of signal inhibition to 78% in our patient, but this continued to remain lower than the mean signal inhibition of 96% found in our vaccinated controls. Repeat T-cell stimulation assay showed a small increase in the percentage of CD4$^+$IFN-γ$^+$ T cells when stimulated with Alpha spike peptides to 0.23% compared to 0.01% previously. Overall, the percentage of CD4 and CD8 IFN-γ$^+$ T cells continued to closely resemble unvaccinated controls.

Immunosuppressed populations have impaired responses to COVID-19 vaccinations. SARS-CoV-2 anti-S antibodies following mRNA vaccination are detectable in as few as 34% of transplant patients [7]. Higher rates of anti-S seropositivity are found in those with malignancies and chronic inflammatory diseases (88%–90%); however, anti-S titers in these patients are consistently lower than in healthy controls [8, 9]. Monitoring anti-S antibodies is often performed but the clinical utility of these results is unknown and routine testing is not recommended by the Food and Drug Administration (FDA) [10]. Antibody-mediated protection can vary depending on quantity produced and neutralizing capability. Additionally, T-cell responses are known to contribute to immune protection against SARS-CoV-2 [11, 12]. T cells are important in the immune response to COVID-19 in those with hematologic malignancies, and T-cell responses can remain effective during infection with SARS-CoV-2 variants [11, 13, 14].

At this time, little is known about vaccine-mediated protection in those with autoimmunity treated with a variety of immunomodulating agents. Immune correlates of protection remain largely undefined. Neutralizing antibodies correlate with protective immunity, but they are difficult to measure and standardize [2, 3]. Quantitative binding antibody levels are easier to standardize and compare across populations, but optimal cutoffs are still being determined [2]. Both strategies remain imperfect as they only measure circulating antibodies and evidence suggests that memory B cells play an important role even when antibody titers wane [15]. Cellular immunity is also contributing to vaccine-mediated immunity against SARS-CoV-2 [11]. However, specific T-cell immune responses, including long-lived memory populations, are difficult to quantify in a standardized fashion, decreasing their utility as a correlate for protection.

To complicate things further, our patient had a complex perivaccination course with holding of tofacitinib, RA disease flare, and subsequent brief corticosteroid treatment. It is challenging to decipher the aspects of her disease course that specifically impaired her antibody and T-cell–mediated vaccine responses, and this highlights another layer of complexity in evaluating immune correlates in people with underlying immune dysregulation. Immunomodulating agents can differentially impact antibody titers depending on their mechanism of action [9]. This is likely true for cellular immune function as well. Therefore, it remains unknown how the unique immunosuppressive regimen of our patient fully impacted her immune response against SARS-CoV-2.

For these reasons, there is still no known clinical utility gained by checking for SARS-CoV-2 antibodies after vaccination, and the FDA continues to recommend against their routine use [10]. Immunosuppressed patients should be treated early and aggressively when diagnosed with COVID-19. We argue that

the presence of SARS-CoV-2 anti-S antibodies could provide a false sense of reassurance in these populations and optimal treatment should follow currently established treatment guidelines.

Importantly, after recovery from COVID-19, our patient did have some improvement in antibody-mediated signal inhibition by the GenScript cPass assay; however, there was minimal change in SARS-CoV-2–specific T-cell responses after recovery. It is possible that JAK inhibition with tofacitinib could impact T-cell activation to a greater extent than antibody production. It is also unknown if specific immune responses to natural infection were limited by the administration of monoclonal antibodies.

These questions remain critical, especially as new SARS-CoV-2 variants, such as Delta, continue to emerge. The durability and efficacy of established antibody- and cell-mediated immune responses may vary further against these new variants. Well-controlled studies are essential to clarify the impact of these variables on vaccine responses in immunocompromised hosts. Continued study on immune correlates of protection in a diversity of immunocompromised states remains a high priority.

## Notes

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