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Predictors of seroconversion after coronavirus disease 2019 vaccination

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Seroconversion is the development of detectable and specific antibodies in the blood serum, because of either previous infection or vaccination. Seroconversion postvaccination has been studied extensively in both SARS-CoV-2 and other viral respiratory illnesses such as influenza. Factors such as age, previous vaccination, comorbidities, and immunocompromising conditions have been found to influence postvaccination seroconversion and the magnitude of response. Studies have also revealed that immunosuppressed groups can have inadequate responses to other vaccines such as diphtheria. Seroprotection refers to a particular threshold of antibody generation that is required to have an adequate degree of protection against infection. In influenza, for example, age is a considerable factor influencing the degree of seroprotection and quantitative antibody titers.

Although there is a high rate of seroconversion in general after vaccination against SARS-CoV-2, a minority of patients do not seroconvert even after multiple vaccine doses. We sought to better understand the importance of factors such as age, sex, underlying comorbid and immunocompromising conditions, the type of vaccine in seroconversion, and the magnitude of the antibody response post-vaccination against SARS-CoV-2.

For this study, we queried the Mayo Clinic COVID-19 database to investigate factors associated with a negative antispikes antibody response after completion of a SARS-CoV-2 vaccination series.

**Methods**

Demographic and clinical data were collected from patients who had a validated antibody assay against the SARS-CoV-2 spike protein at least 14 days after having 2 doses of mRNA vaccine (Pfizer or Moderna) or after 1 dose of viral vector vaccine (Janssen). More importantly, completion of vaccination did not include the third dose of mRNA vaccine or a dose of mRNA vaccine after the viral vector vaccine. We did not assay neutralizing antibody levels. Informed consent was not required. The electronic medical record was used to calculate the Charlson comorbidity index (severity-weighted sum of diseases). Groups with a negative (<0.4 U/mL) vs positive (≥0.4 U/mL) antispikes antibody responses were compared with χ² or Kruskal Wallis tests, as appropriate. Multivariable logistic regression was used to compare the odds of seroconversion failure by a set of patient characteristics (age, presence of immunodeficiency, transplant history, Charlson comorbidity index, and days from vaccine to antibody testing), stratified by sex. Odds ratios (OR) and 95% confidence intervals (CI) were reported. P values less than .05 were considered statistically significant. All analyses were performed using Statistical Analysis System version 9.4 (SAS Institute Inc, Cary, North Carolina).

**Results**

A total of 805 patients underwent SARS-CoV-2 spike protein assay testing between February 4, 2021 and May 18, 2021. All included patients underwent SARS-CoV-2 spike protein assay testing at least 14 days after completing their COVID-19 vaccination series (Table 1). A total of 307 patients received the 2-dose series of Pfizer mRNA vaccine, 221 patients had received the 2-dose series of Moderna mRNA vaccine, and 15 patients had received the single dose of the Janssen vaccine. The median age of patients was 67.1 years (range, 20.5-97.2 years) and 52.9% were women. Underlying comorbidities were typically noted in the cohort, including diabetes in 22.4%, chronic pulmonary disease in 33.5%, and history of transplantation in 26.6% of the patients. More importantly, this cohort of patients did not represent the general population.

Out of the 805 patients, 622 (77.3%) attained seroconversion as defined by a titer of greater than or equal to 0.4 U/mL, and 183 (22.7%) failed to seroconvert after vaccination against SARS-CoV-2. Table 1 compares the characteristics by seroconversion status. On univariate analysis, those who failed to seroconvert were more likely to be men (56.8% vs 44.2%; P = .003), have older age (median 68.5 vs 66.7 years; P = .02), have a previous history of transplant (34.4% vs 24.3%; P = .006), or have an immunocompromised state (73.2% vs 50.6%; P < .001). The comorbidity score, as measured by the severity-weighted Charlson index, was higher for those who failed to seroconvert (median 4 vs 3; P < .001).

Given the difference in seroconversion by sex, we investigated multivariable analyses stratified by sex. Among women, the odds of seroconversion failure were noted for age 50 years and older vs younger than 50 years (OR, 2.78; 95% CI, 1.19-6.51; P = .02), presence of immunodeficiency (OR, 2.18; 95% CI, 1.18-4.01; P = .01), adjusting for transplant history, Charlson comorbidity index, and days between vaccination and antibody testing. Among men, the presence of immunodeficiency was the strongest predictor for seroconversion failure (OR, 3.09; 95% CI, 1.77-5.38; P < .001), adjusted for age, transplant history, Charlson comorbidity index, and days between the vaccine and antibody testing (Table 2). The overlap among age 50 years and older, transplant history, presence of immunodeficiency, and cancer by sex is depicted in Figure 1.

We further analyzed the clinical characteristics of the 183 out of 805 (22.7%) patients who had a lack of seroconversion after COVID-19 vaccination. A total of 128 patients (69.9%) carried a diagnosis of cancer with the most common type being hematologic (n = 116 [90 hematological only + 26 with mixed], 90.6%), with leukemia (n = 82/116, 70.7%), lymphoma (n = 23/116, 19.8%) and paraproteinemias (n = 10/116, 8.6%) accounting for most of the cases. Remission status was available in 110 patients with cancer and the majority (87/110, 79.1%) were not in remission. Therapy with immunosuppressive drugs was noted in 93 (50.8%) of patients most (n = 83/93, 89.2%) receiving ongoing immunosuppressive therapy at the time of vaccination. Among 134 (73.2%) patients with a diagnosis of immunodeficiency, 110 (82.1%) had a primary immunodeficiency. Of note, 11 of these patients were previously reported in a publication by our group. Cancer (n = 128, 69.9%), B cell depletion therapy (n = 90/115, 78.3%), and immunosuppressant steroid use (n = 71/93, 76.3%) seemed to be the other common characteristics among vaccine nonresponders (Table 1).

**Discussion**

We present one of the largest cohorts analyzing the demographic and clinical characteristics associated with seroconversion after COVID-19 vaccination. Novel findings from our study include the finding that 22.7% of patients failed to seroconvert, with most of these patients either having cancer or B cell depletion therapies. It is important to note that our study did not evaluate the actual efficacy of COVID-19 vaccination. Male sex has emerged as a strong predictor of adverse COVID-19 outcomes.19,20 Our study found a similar association between seroconversion and sex, with an overrepresentation of men among those who failed to seroconvert. Many studies have found similarly higher rates of seroconversion and antispikes antibody levels in women compared with men.11–15 A recent publication reported that prevaccination estradiol levels in women correlated with the rate of seroconversion after an inactivated vaccine (BBIBP-CorV). Among elderly patients and individuals with chronic lymphocytic leukemia, women also had a higher seroconversion rate after COVID-19 vaccination.17–19 This sex dimorphism in the rates of seroconversion is also present after other COVID-19 vaccines that are not mRNA. For instance, studies from India using the ChAdOx1-nCOV and BBV-153 vaccines24 and from Chile using the CoronaVac vaccine25 also reported lower antibody responses in men compared with women.

Older age was a marked risk factor for a lack of seroconversion in our cohort, which is consistent with findings published by other groups.22,23 For instance, a study among health care workers in Israel
Table 1
Basic Demographic Characteristics of Patients in the Cohort (N = 805)

| Variable                          | Total (N = 805) | Seronegative (N = 183) | Seropositive (N = 622) | P value<sup>a</sup> |
|-----------------------------------|-----------------|------------------------|------------------------|---------------------|
| Age (y)                           | 67.1 (20.5-97.2) | 68.5 (26.4-92.0)       | 66.7 (20.5-97.2)       | .02                 |
| Male sex                          | 379 (47.1%)     | 104 (56.8%)            | 275 (44.2%)            | .003                |
| Type of vaccine                   |                 |                        |                        |                     |
| Pfizer                            | 307 (38.1%)     | 65 (35.5%)             | 242 (38.9%)            | .08                 |
| Moderna                           | 221 (27.5%)     | 56 (30.6%)             | 165 (26.3%)            |                     |
| Johnson & Johnson                 | 15 (1.9%)       | 7 (3.8%)               | 8 (1.3%)               |                     |
| Unknown                           | 262 (32.5%)     | 55 (30.1%)             | 207 (33.3%)            |                     |
| Race                              |                 |                        |                        | .32                 |
| White                             | 730 (91.9%)     | 172 (94.0%)            | 558 (91.3%)            |                     |
| African American                  | 20 (2.5%)       | 5 (2.7%)               | 15 (2.5%)              |                     |
| Asian                             | 26 (3.3%)       | 5 (2.7%)               | 21 (3.4%)              |                     |
| Other                             | 18 (2.3%)       | 1 (0.5%)               | 17 (2.8%)              |                     |
| Body mass index (kg/m<sup>2</sup>)| 26.7 (15.8-52.9)| 26.7 (16.2-44.3)       | 26.8 (15.8-52.9)       | .72                 |
| Charlson Comorbidity Index        | 3.0 (0.0-21.0)  | 4.0 (0.0-16.0)         | 3.0 (0.0-21.0)         | <.001               |
| Immunocompromised state           | 449 (55.8%)     | 134 (73.2%)            | 315 (50.6%)            | <.001               |
| Type of immunodeficiency, N       | 449             | 134                    | 315                    |                     |
| Primary immunodeficiency          | 305 (67.9%)     | 110 (82.1%)            | 195 (61.9%)            |                     |
| CVID                              | 28 (6.2%)       | 3 (2.2%)               | 25 (7.9%)              |                     |
| Non-CVID                          | 277 (61.7%)     | 107 (79.9%)            | 170 (54.0%)            |                     |
| Secondary immunodeficiency        | 144 (32.1%)     | 24 (17.9%)             | 120 (38.1%)            |                     |
| History of transplant (any)       | 214 (26.6%)     | 63 (34.4%)             | 151 (24.3%)            | .006                |
| Type of transplant, N             | 214             | 63                     | 151                    |                     |
| Stem cell                         | 85 (39.7%)      | 14 (22.2%)             | 71 (47.0%)             |                     |
| Lung                              | 34 (15.0%)      | 16 (25.4%)             | 18 (11.9%)             |                     |
| Kidney                            | 32 (15.0%)      | 12 (19.0%)             | 20 (13.2%)             |                     |
| Other<sup>b</sup>                 | 63 (29.4%)      | 21 (33.3%)             | 42 (27.8%)             |                     |
| Dialysis                          | 35 (4.3%)       | 12 (6.6%)              | 23 (3.7%)              | .10                 |
| Asthma                            | 122 (15.2%)     | 29 (15.8%)             | 93 (15.0%)             | .77                 |
| Congestive heart failure          | 129 (16.0%)     | 36 (19.7%)             | 93 (15.0%)             | .13                 |
| Chronic pulmonary disease         | 270 (33.5%)     | 73 (39.9%)             | 197 (31.7%)            | .04                 |
| Diabetes                          | 180 (22.4%)     | 49 (26.8%)             | 131 (21.1%)            | .10                 |
| Prediabetes                       | 8 (4.4%)        | 2 (12.0%)              | 6 (3.7%)               |                     |
| Connective tissue disease         | 106 (13.2%)     | 22 (12.0%)             | 84 (13.5%)             | .60                 |
| Time from last vaccine dose to antibody test (d) | 42 (14-119) | 40 (14-91) | 42 (14-119) | .02 |
| Antispike antibody response (U/mL): |           |                        |                        |                     |
| Negative (< 0.4)                  | 183 (22.7%)     | 183 (100.0%)           | —                      |                     |
| Mild responder (0.4-100)          | 128 (15.9%)     | —                      | 128 (20.6%)            |                     |
| Medium responder (101-250)        | 59 (7.3%)       | —                      | 59 (9.5%)              |                     |
| Significant responder (> 250)     | 435 (54%)       | —                      | 435 (69.9%)            |                     |
| Hypertension                      | 68 (37.2%)      | —                      | —                      |                     |
| Smoking                           | 71 (39.4%)      | —                      | —                      |                     |
| Cancer                            | 128 (69.9%)     | —                      | —                      |                     |
| Type of cancer (N = 128)          |                 |                        |                        |                     |
| Hematological                     | 90 (70.3%)      | —                      | —                      |                     |
| Carcinoma                         | 12 (9.4%)       | —                      | —                      |                     |
| Mixed                             | 26 (20.3%)      | —                      | —                      |                     |
| Type of hematological cancer (N = 116) |           |                        |                        |                     |
| Lymphoma                          | 23 (19.8%)      | —                      | —                      |                     |
| MDS/Myelofibrosis                 | 2 (1.7%)        | —                      | —                      |                     |
| Paraproteinemia                   | 10 (8.6%)       | —                      | —                      |                     |
| Leukemia                          | 82 (70.7%)      | —                      | —                      |                     |
| Other                             | 5 (4.3%)        | —                      | —                      |                     |

Abbreviations: CVID, common variable immunodeficiency; MDS, myelodysplastic syndrome.

NOTE. Data represent number (percentage) or median (range).

<sup>a</sup>P values were calculated using χ<sup>2</sup> or Kruskal-Wallis tests.

<sup>b</sup>Other types of transplants included heart, liver, pancreas, multiple organs, and others.

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Table 2
Multivariable Logistic Regression Results Predicting Odds of Seronegativity

| Variable                          | Women                                      | Men                                        |
|-----------------------------------|--------------------------------------------|--------------------------------------------|
|                                  | OR (95% CI)                               | P value         | OR (95% CI)                               | P value         |
| Age: 50+ vs <50 y                 | 2.78 (1.19-6.51)                           | .02            | 0.55 (0.25-1.23)                           | .15            |
| Immunodeficiency: yes vs no       | 2.18 (1.18-4.01)                           | .01            | 3.09 (1.77-5.38)                           | <.001           |
| Transplant history: yes vs no     | 1.63 (0.90-2.96)                           | .11            | 0.67 (0.39-1.17)                           | .16            |
| Charlson comorbidity index: OR for 1-level increase | 0.98 (0.91-1.05) | .51 | 1.05 (0.98-1.13) | .18 |
| Time from vaccine to antibody test: OR for 1-week increase | 0.92 (0.84-1.09) | .04 | 0.95 (0.88-1.02) | .17 |

Abbreviations: CI, confidence interval; OR, odds ratio.
The ORs are adjusted for all covariates included in the model.
Figure 1. Venn diagrams depicting the overlap among age 50 years and older, history of transplant, immunocompromised state, and cancer by sex among the 183 seronegative patients. The numbers illustrated represent the number of patients. One man and 1 woman had none of the characteristics mentioned above.

reported that lack of seroconversion after the first dose of the BNT162b2 (BioNTech Pfizer vaccine) mRNA COVID-19 vaccine was associated with older age and that SARS-CoV-2 antibody levels decreased with increasing age.22 Similarly, another study of Israeli health care workers found that, although all their participants seroconverted after COVID-19 vaccination, individuals younger than 50 years of age had higher SARS-CoV-2 antibody levels.23

History of transplantation is also a known risk factor for lack of seroconversion after COVID-19 vaccination. A retrospective study among 82 kidney transplant (KT) recipients found that the lack of seroconversion after 2-dose COVID-19 vaccination was associated with age 60 years and older (OR, 4.50; \( P = 0.02 \)) and the use of an anti-metabolite immunosuppressive agent, such as azathioprine or derivatives of mycophenolic acid (OR, 5.26; \( P = 0.004 \)).24 Other studies in similar groups of patients have confirmed this impaired COVID-19 vaccination–induced antibody response, such as a recent report stating that only 42% of KT recipients achieved seroconversion 8 weeks after the first vaccination.25 Interestingly, when comparing KT recipients to patients having dialysis, the authors found lower numbers of CD4-positive T cells producing cytokines in the former group. Notable risk factors of seroconversion failure included the number and type of immunosuppressive agents and the vaccine type, with lower seroconversion seen in patients who received the BNT162b2 vaccine.25 Others have found low rates of seroconversion after 2 doses of the BNT162b2 vaccine, with only 37.5% of KT recipients achieving seroconversion.26 Risk factors associated with seroconversion failure were older age (OR, 1.66), high-dose corticosteroids in the past year (OR, 1.3), triple immunosuppression (OR, 1.43), and use of mycophenolate (OR, 1.47).26

Similar findings have been reported in liver transplant (LT) recipients. One study compared the seroconversion rate among LT recipients, patients with cirrhosis, and healthy controls. Only 63% of LT recipients had seroconversion, compared with 97.9% of patients with cirrhosis and 100% of healthy controls.27 Among LT recipients, age older than 65 years (OR, 4.57) and arterial hypertension (OR, 2.50) were associated with no or low humoral response to COVID-19 vaccination. In contrast, calcineurin inhibitor monotherapy was associated with a lower likelihood of vaccine failure (OR, 0.36) when compared with other immunosuppressive therapies.27 Interestingly, other groups report similar seroconversion rates between pre-LT patients and healthy controls.28 Finally, other studies have evaluated the vaccine response in allogeneic hematopoietic stem cell transplant (HSCT) recipients. In one of these studies, the authors reported that 83% of the HSCT recipients had a positive antibody testing after the second dose (median interval of 35, range 18–77).29 In these individuals, factors associated with lack of seroconversion were a haploidentical transplant, recent HSCT, lymphopenia, and chemotheraphy or immunosuppressive therapy at the time of COVID-19 vaccination.

A prominent risk factor for lack of seroconversion is a history of primary immune deficiency. Our group has previously published a case series describing the efficacy of COVID-19 vaccination in patients with an underlying immune deficiency. Of the 11 patients included in this initial study, all but 1 patient seroconverted after COVID-19 vaccination.30 The only patient who did not respond had x-linked agammaglobulinemia. Notably, patients with an inborn error of immunity with severe COVID-19 tend to be younger and have higher rates of admission to the intensive care unit when compared with the general population.31 A study of the antibody response to COVID-19 vaccination among 81 patients with an inborn error of immunity revealed that a lower rate of seroconversion was observed among patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia (63%). In addition, the use of rituximab, less than 1000 CD3-positive T cells/µL, and less than 100 CD19-positive B cells/µL were associated with lower SARS-CoV-2 antispike immunoglobulin G levels in this cohort.32 Interestingly, others have noted that COVID-19 vaccination of patients with common variable immunodeficiency has provided the opportunity to study the effects of antibody deficiency on the immune responses to a novel antigen.33

Secondary immune deficiencies also need to be considered when assessing the antibody response to COVID-19 vaccination. For instance, a systematic review and meta-analysis among patients with immune-mediated inflammatory diseases revealed that individuals with rheumatoid arthritis and vasculitis had lower rates of seroconversion. Furthermore, rates of seroconversion were also lower in patients on anti- CD20 (rituximab) or anticytotoxic T lymphocyte–associated antigen (abatacept) therapies.34 Another study found that, after the first dose, 49% of patients with autoimmune disease vs 73% of the controls were seroconverted. After the second dose, both groups had a more than 80% rate of seroconversion, except for those patients on anti-CD20 treatment. In contrast, other studies in pediatric and adult patients with inflammatory bowel disease reported that biologic therapy use did not influence the rate of seroconversion.35,36

A history of malignancy was also a significant risk factor for lack of seroconversion. One study compared the antibody responses in 232 patients with cancer who were undergoing active treatment vs 261 age-matched healthy controls. After the first dose of the BNT162b2 vaccine, 29% of the patients with cancer achieved seroconversion vs 84% of the controls (\( P < .001 \)). Of the individuals who did not achieve
seroconversion after the second dose of the BNT162b2 vaccine, 29% had breast cancer and 74% were being treated with chemotherapy. 

Others have reported a lower rate of seroconversion in patients with hematologic malignancies (85%), particularly those who underwent anti-CD20 therapies (70%) and stem cell transplantation (73%). In contrast, those receiving immune checkpoint inhibitors or hormonal therapies had a high rate of seroconversion (> 97%). Finally, a study among 160 patients with hematologic malignancies found that active disease and B cell depleting therapies were associated with a lower rate of seroconversion, whereas a longer time from the last chemotherapy was associated with a higher rate of the humoral response.

Several limitations of our study need to be acknowledged. First, there is a selection bias in our cohort as providers most likely decided to check antibody levels in these individuals because of concerns for lack of seroconversion after COVID-19 vaccination. Second, even though we included patients from the 3 Mayo Clinic sites (Minnesota, Arizona, and Florida), our study was performed at a single academic institution. Third, our study did not include a longitudinal laboratory evaluation to identify the risk factors associated with a decline in the humoral response. Finally, we did not assay neutralizing antibody levels or assess the patients’ cell-mediated responses to the COVID-19 vaccines.

In conclusion, we have identified risk factors associated with lack of seroconversion after COVID-19 vaccination. These risk factors could be used to allocate health care resources and prioritize booster doses to those individuals with an absent or low humoral response to the vaccines.

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