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Poor Humoral Response in Solid Organ Transplant Recipients Following Complete mRNA SARS-CoV-2 Vaccination

To the Editor:

The rapid and widespread administration of vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) offers great promise for curbing the spread of infection and potentially reaching herd immunity. However, because immunocompromised individuals were excluded from the initial clinical trials of mRNA vaccinations, the humoral response in immunocompromised solid organ transplant (SOT) recipients that are completely vaccinated (2 doses of mRNA SARS-CoV-2 vaccines) is not fully characterized. Recent studies report that 15% and 54% of SOT participants had detectable antibody levels by serologic testing after the first and second dose of vaccine, respectively (1, 2). How quantitative antibody levels correlate with neutralization capacity and how antibody profiling varies between SOT recipients vs immunocompetent individuals and those who recovered from SARS-CoV-2 infection is not well understood.

We retrospectively studied 37 SOT recipients who received lung (n = 16), kidney (n = 6), heart (n = 13), liver (n = 1), or heart and lung (n = 1) transplants and had negative PCR tests for SARS-CoV-2, all of whom were on immunosuppressive medications (tacrolimus, cyclosporine, sirolimus, or everolimus). The SOT recipients had a male:female (M:F) ratio of 27:10 with a median age of 64 years [interquartile range (IQR) 50 to 69] and received 2 doses of mRNA vaccine (Pfizer or Moderna) within 21 days (median, IQR 19 to 25). In control groups, 10 immunocompetent individuals had a M:F ratio of 2:8 with a median age of 66 years (IQR 57 to 75) and received 2 doses of vaccine within 19 days (median, IQR 18 to 25). Individuals with past COVID-19 infection (n = 11) had a median age of 61 years (IQR 55 to 64), a M:F ratio of 8:3, and a median of 40 days since their first positive PCR test (Supplemental Table 1).

This study was conducted under an approved Institutional Review Board protocol at the University of Texas Southwestern Medical Center. Lithium heparin plasmas were stored at 4 °C after collection for up to 72 h and then at -80°C before analyses. Semiquantitative measurement of anti-SARS-CoV-2 spike protein antibody (anti-S IgG) was performed on an Abbott Alinity instrument (analytical measurement range: 50 to 50 000 AU/mL, maximum dilution 1:5). To assess the neutralizing capacity of plasma, the inhibitory effects on spike receptor-binding domain (RBD) and angiotensin converting enzyme 2 receptor interactions were quantified using an enzyme-linked immunosorbent assay (3).

Quantitative serological assays of anti-S IgG (cutoff value 50 AU/mL) revealed a positive response in 15 of 37 fully vaccinated SOT recipients. The seroconversion rate of SOT recipients (40.5%) was significantly lower than the 100% positive antibody response in fully vaccinated immunocompetent individuals and patients with past COVID-19 infection (Fig. 1) (4). In addition, the median value of anti-S IgG in SOT recipients (1.4 AU/mL) was approximately 10 000-fold lower than median values observed in vaccinated immunocompetent individuals (13 951.5 AU/mL) and patients with past infection (16 532.5 AU/mL) (*t*-test; $P < 0.005$) (Fig. 1). We next assessed plasma antibody function in SOT recipients. Neutralization assays against spike RBD proteins revealed that anti-S IgG titers were precisely correlated with capacity to neutralize spike RBD proteins (Pearson correlation R = 0.905 vs 0.62 in immunocompetent, 0.71 in past infection group).

Because SOT recipients have poorer seroconversion rates and lower anti-S IgG levels, they may still be at high risk for SARS-CoV-2 infection after complete vaccination. Through PCR screening, a COVID-19–positive SOT recipient was identified 13 days after complete vaccination (grouped as individuals with past infection). Notably, a serological study revealed 39 391.2 AU/mL of anti-S IgG 17 days after the positive PCR test (30 days after complete vaccination), a value significantly higher than that of most SOT recipients (36 of 37) but comparable with those of fully vaccinated immunocompetent or previously infected

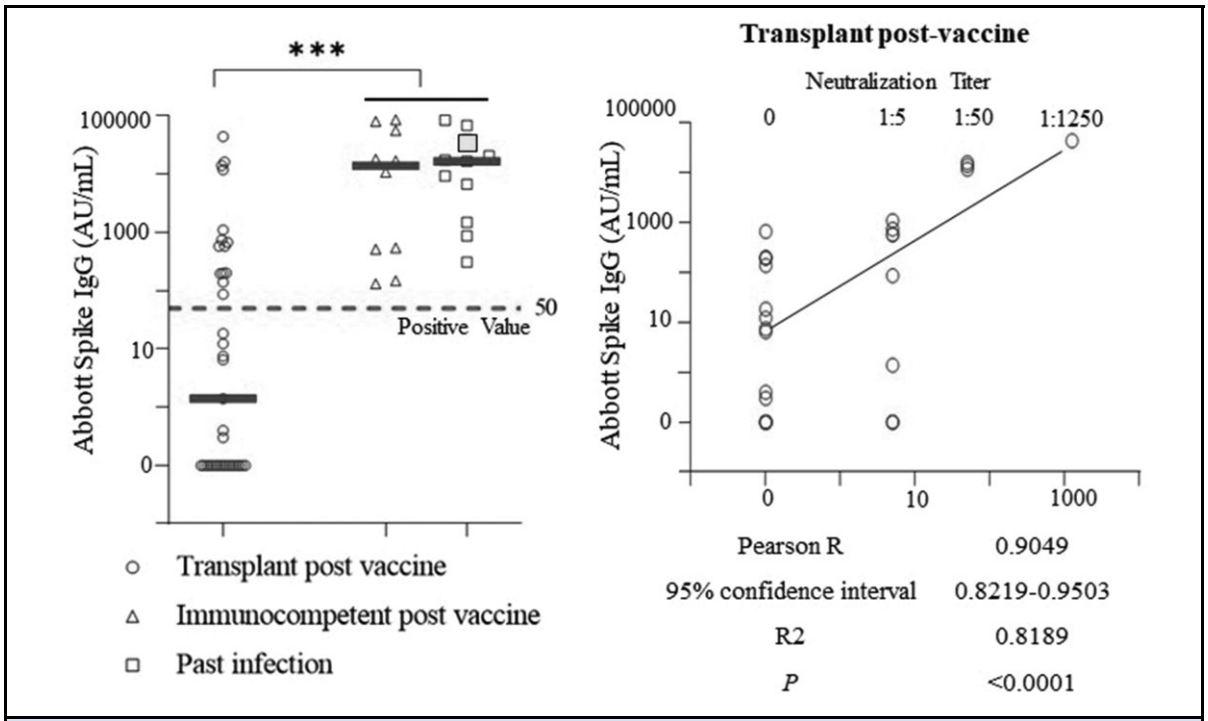

Fig. 1. Serological profiling of SOT recipients following complete vaccination (2 doses of mRNA vaccine).
Left. Anti-S IgG was positive in the plasma of 15 of 37 SOT recipients, with a seroconversion rate of 40.5%. In contrast, 100% seroconversion rates were seen in immunocompetent individuals with complete vaccination (n = 10) or past COVID-19 infection (n = 11). Spike IgG concentrations were plotted on a log scale on the *y*-axis with data points of zero manually plotted. The dashed green line indicates reported positive value of anti-S IgG. Solid red lines indicate the medians of individual study groups. The SOT recipient with prior complete vaccination and subsequent COVID-19 infection in the past infection group is highlighted in yellow. ***, *P*-value for student *t*-test <0.005 when comparing SOT to immunocompetent and past infection groups combined. Right. Pearson correlation analysis demonstrating correlation of anti-S IgG values in SOT recipients with their capacity to neutralize spike RBD proteins. Spike IgG concentrations and neutralization titers were plotted on a log scale with data points of zero manually plotted. The solid black line indicates the trend line of simple linear regression.

control groups (Fig. 1) (4). This finding indicates that subsequent infection may function as an additional stimulus to boost antibody response and induce higher levels of anti-S IgG in SOT recipients.

Taken together, our findings demonstrate that SOT recipients have significantly poorer antibody response to the currently employed (2 dose) mRNA vaccination in both seroconversion rate [40.5% in our study and 54% by other reports (2)] and a median level of anti-S IgG 10 000-fold lower than immunocompetent individuals. The unexpectedly high level of anti-S IgG in a fully vaccinated SOT recipient with subsequent COVID-19 infection suggests that SOT recipients may benefit from additional vaccine dose(s) to boost seroconversion rate and increase anti-S IgG production. Because anti-S-IgG level precisely correlated with neutralization capacity in SOT recipients (Fig. 1), antibody profiling can be monitored using automated quantitative assays with cutoffs corresponding to certain neutralization titers (5). To determine whether SOT recipients and other immunocompromised patients may benefit from additional vaccine dose(s), large-scale clinical trials are needed.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOT, solid organ transplant; IQR, interquartile range; COVID-19, coronavirus disease; anti-S IgG, anti-SARS-CoV-2 spike protein antibody; RBD, receptor-binding domain.

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Wide Variation in Threshold Cycle Values Clouds the Interpretation of SARS-CoV-2 Infectiousness

To the Editor:

There is continuing interest in using threshold cycle (Ct) values from real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays to determine infectiousness and timing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Demand for reporting Ct values has been justified to guide the administration of therapeutics, prognostication, identifying reinfection, or removing isolation precautions (1). However, there are fundamental flaws when interpreting a qualitative test in a quantitative manner where Ct values vary on a multitude of factors, including sample collection, specimen type, reagents used, extraction/PCR platform(s), PCR targets, and laboratory practices (e.g., transportation and storage).

We analyzed the correlation between E gene Ct values and duration of symptom onset from confirmed COVID-19 cases in Alberta, Canada (population 4.4 million). Data recorded from March to May 2020 from the Alberta Health Services Public Health and Alberta Precision Laboratory databases were linked. Symptom duration and status at the time of collection were determined during case investigation by Public Health using a standardized questionnaire. Cases with known symptomatology and tested with Ct values generated from Alberta Precision Laboratory's laboratory-developed E gene singleplex assay were included. Based on proficiency panels (published and unpublished), internal evaluations of our assay had similar sensitivity, specificity, and precision to United States Federal Drug Administration (FDA) approved assays (2, 3).

Oropharyngeal, nasopharyngeal, and nasal specimens for SARS-CoV-2 testing were collected in universal transport media (UTM-RT, COPAN Diagnostics or VTM, Yocon). Endotracheal tube aspirate specimens were collected in sterile containers. All specimens were stored at 4 °C prior to SARS-CoV-2 testing within approximately 72 h.

Of 7974 cases, 5756 met inclusion criteria. Oropharyngeal, nasopharyngeal, nasal, and endotracheal tube aspirate specimens made up 71.6%, 19.1%, 8.1%, and 1.1% of specimens, respectively. At the time of collection, 787 (13.7%) were asymptomatic, 92 (1.6%) presymptomatic (symptom onset within 2 days after collection), 3107 (54.0%) with symptom onset within 7 days, and 1770 (30.7%) with symptom onset >7 days. Excluding those tested on Day 1 of symptom onset, a linear positive correlation between days of symptoms and median Ct values was observed (coefficient of determination (R^2) = 0.970, $P < 0.001$, Fig. 1).

Despite a linear positive correlation between days of symptom onset and median Ct value, Ct values ranged widely, regardless of symptom onset or specimen type. For example, 25% of individuals with symptom onset ≤7 days had Ct values >29.1, indicating that a lower