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Response to SARS-CoV-2 Initial Series and Additional Dose Vaccine in Patients with Predominant Antibody Deficiency

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Title: Response to SARS-CoV-2 Initial Series and Additional Dose Vaccine in Patients with Predominant Antibody Deficiency

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Abstract:

Background: SARS-CoV-2 infection in patients with predominant antibody deficiency (PAD) is associated with high morbidity, yet data regarding response to SARS-CoV-2 immunization in PAD patients, including additional dose vaccine, is limited.

Objective: We sought to characterize antibody response to SARS-CoV-2 vaccine in PAD patients and define correlates of vaccine response.

Methods: We assessed levels and function of anti-SARS-CoV-2 antibodies in 62 PAD patients compared to matched healthy controls at baseline, at 4-6 weeks following initial series immunization - a single dose of Ad26.COV2.S (Janssen) or two doses of BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna) - and at 4-6 weeks following additional dose immunization, if received.

Results: Following initial series SARS-CoV-2 vaccination, PAD patients had lower mean anti-spike antibody levels compared to matched healthy controls (140.1 U/mL vs. 547.3 U/mL; p=0.02). Patients with secondary PAD (e.g., use of B-cell depletion therapy) and patients with severe primary PAD (e.g., common variable immunodeficiency with autoinflammatory complications) had the lowest mean anti-spike antibody levels. Immune correlates of a low anti-spike antibody response included low CD4+ helper T cells, low CD19+ total B cells, and low class-switched memory (CD27+IgD/M-) B cells. Additionally, a low (< 100 U/mL) anti-spike antibody response was associated with prior exposure to B-cell depletion therapy, both at any
time in the past (OR 5.5; CI 1.5-20.4; p=0.01) and proximal to vaccination (OR 36.4; CI 1.7-791.9; p=0.02). Additional dose immunization with an mRNA vaccine in a subset of 31 PAD patients increased mean anti-spike antibody levels (76.3 U/mL pre- to 1065 U/mL post-
additional dose; p<0.0001).

Conclusions: Patients with secondary and severe primary PAD, characterized by low helper T cells, low B cells, and/or low class-switched memory B cells, were at risk for low antibody response to SARS-CoV-2 immunization, which improved following additional dose vaccination in the majority of patients.

Highlights Box:

1. What is already known about this topic?
   • SARS-CoV-2 infection in patients with predominant antibody deficiency is associated with high morbidity; however, an understanding of the response to SARS-CoV-2 immunization in these patients is limited.

2. What does this article add to our knowledge?
   • Patients with secondary and severe primary PAD, characterized by low B cells, low helper T cells, and/or low class-switched memory B cells, had low antibody response to SARS-CoV-2 immunization, which improved following additional dose vaccination.

3. How does this study impact current management guidelines?
   • These data identify patient factors associated with low response to SARS-CoV-2 vaccination and support recommendations regarding additional doses of COVID-19 vaccines in patients with moderate/severe forms of immune deficiency.
Key words: SARS-CoV-2, COVID-19, vaccine response, humoral immunodeficiency, predominant antibody deficiency, common variable immunodeficiency, CVID, hypogammaglobulinemia, specific antibody deficiency, IgG subclass deficiency, anti-spike antibody, anti-nucleocapsid antibody, neutralization assay, additional dose.

Abbreviations: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), combined immunodeficiency (CID), coronavirus disease 2019 (COVID-19), common variable immunodeficiency (CVID), predominant antibody deficiency (PAD), immunoglobulin G subclass deficiency (IgG SD), specific antibody deficiency (SAD), subcutaneous immunoglobulin (SCIG), intravenous immunoglobulin (IVIG)
Introduction:

With the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, there has been an unparalleled rapid development of vaccines. This includes the use of mRNA vaccines, including BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), and adenoviral vector vaccines, including Ad26.COV2.S (Janssen). However, patients with underlying immune deficiencies including predominant antibody deficiency (PAD) were excluded from the clinical trials assessing SARS-CoV-2 vaccine efficacy (1-3), and data regarding the response to vaccination in patients across the clinical spectrum of PAD are limited to case series (4-7).

In studies to date of coronavirus disease 2019 (COVID-19) among patients with immunodeficiency, there has been demonstration of high morbidity and mortality relative to the general population (8). In one study, 63% of immunodeficient patients with COVID-19 required hospitalization, with a case-fatality rate of approximately 10% (9). Additionally, severe COVID-19 disease has been associated with specific primary and/or secondarily acquired defects in the underlying immune signaling pathways that are critical in the defense against viral pathogens (10, 11). Among patients with primary antibody deficiencies, there are limited data to suggest worse outcomes among patients with common variable immunodeficiency (CVID) as compared to agammaglobulinemia (12). However, data regarding the severity of COVID-19 infection in patients with immunodeficiency varies widely by patient demographic (9, 13-15). Together, these data suggest underlying immunophenotypic correlates of both risk for and protection against naturally acquired SARS-CoV-2. Whether underlying immunophenotypic factors also determine response to the novel SARS-CoV-2 vaccines is largely unknown.
Patients with PAD demonstrate increased susceptibility to infections and impaired vaccine responses. The response to vaccination can be used as a correlate of the immune system’s ability to fight natural infections and is a component of the diagnosis for several types of PAD disorders (16). While application of vaccines and interpretation of antibody responses can be complex, there are guidelines regarding the interpretation of vaccine responses in patients with immunodeficiency such as for the pneumococcal polysaccharide and tetanus toxoid vaccines (17). However, given the recent development of SARS-CoV-2 vaccines, the response of patients with PAD has not been fully elucidated.

To better understand the immunogenicity of the SARS-CoV-2 vaccines in patients across the clinical spectrum of PAD, we evaluated anti-SARS-CoV-2 antibody levels and neutralization capacity following both initial course of vaccination as well as in those who received an additional dose vaccination.

Methods:

This study was performed at Mass General Brigham under an Institutional Review Board-approved protocol (#2021P002414). Antibody response to the SARS-CoV-2 vaccine in patients with known PAD was evaluated. Inclusion criteria were: Adult PAD patients longitudinally followed at Mass General Brigham who underwent initial series SARS-CoV-2 vaccination between December 16, 2020 and June 9, 2021 as well as PAD patients who had clinically obtained testing during this same time period. Patients who received additional SARS-CoV-2 vaccines after their primary series were assessed longitudinally. Exclusion criteria were:
PAD patients with any prior positive polymerase chain reaction (PCR) testing for SARS-CoV-2. PAD diagnoses were confirmed by manual chart review by a clinical immunologist and met consensus definitions (16, 18, 19). Patients with any confounding variables at the time of immunodeficiency diagnosis (e.g., clonal lymphocyte population or ongoing immunosuppression without potential for discontinuation) were considered secondary PAD. Patients with primary PAD were further subclassified as mild (IgG subclass deficiency, specific antibody deficiency (SAD), and primary hypogammaglobulinemia), moderate (uncomplicated common variable immunodeficiency (CVID), defined as an absence of co-occurring autoinflammatory clinical features (20)), and severe (complicated PAD that encompassed the diagnoses of activated PI3K-delta syndrome (APDS), TACI deficiency, NFKB1 deficiency, and complicated CVID/SAD (CVIDc/SADc), defined as a presence of co-occurring autoinflammatory clinical features (20) but without a known genetic etiology). We evaluated demographic information and clinical characteristics including type of PAD, vaccine type received, previous genetic testing if performed/available, and previous immune testing performed including native antibody levels, native antibody responses to vaccines (e.g. pneumovax23, *Hemophilus influenza* B [HIB], tetanus, diphtheria and pertussis [Tdap]), peripheral blood lymphocyte counts, and T-cell functional studies, as available. We evaluated previous and current treatment regimens with a focus on immunoglobulin replacement type, if received, and other immunosuppressants or biologics received in the past or in close proximity to vaccination (defined as six months prior to one month post immunization).

Serologic assays were performed through Massachusetts General Pathology Laboratory using the Roche Elecsys Anti-SARS-CoV-2 S-antibody test (evaluating antibodies to the SARS-CoV-2 spike (S) protein receptor binding domain; ‘anti-spike antibody’) and the Roche Elecsys
Anti-SARS-CoV-2 N-antibody test (evaluating antibodies to the SARS-CoV-2 nucleocapsid domain ‘anti-nucleocapsid antibody’). These tests are semiquantitative and have been correlated with neutralizing immunity (21, 22). The Roche S-antibody assay reports results in absorbance units per mL (U/mL) with values ≥ 0.8 U/mL considered reactive (23). We further delineated a minimum threshold protective anti-spike antibody response as ≥ 100 U/mL, which has been correlated with a detectable level of pseudovirus neutralization in healthy control subjects (24).

The Roche N-antibody assay reports a cutoff index (COI) with values ≥ 1.00 COI considered reactive.

Neutralization was measured using a SARS-CoV-2 pseudovirus neutralization assay that has been previously described (25). Briefly, lentiviral particles encoding both luciferase and ZsGreen reporter genes were pseudotyped with SARS-CoV-2 spike protein and produced in 293T cells, titered using ZsGreen expression by flow cytometry and used in an automated neutralization assay with 50–250 infectious units of pseudovirus co-incubated with three-fold serial dilutions of serum for 1 hour. Neutralization was determined on 293T-ACE2 cells.

Percent neutralization was determined by subtracting background luminescence measured in cell control wells (cells only) from sample wells and dividing by virus control wells (virus and cells only). pNT50 values were calculated by taking the inverse of the 50% inhibitory concentration.

Quantitative detection of total (IgA/M/G) and individual isotype (IgG, IgA or IgM) antibodies to SARS-CoV-2 receptor binding domain (RBD) was performed by enzyme linked immunosorbent assay (ELISA) as previously described (24, 26).

PAD participants were matched on age (+/-10 years) and time from the most recent vaccination (+/- 14 days) at a ratio of 1:1 to healthy controls. The control population was healthy, ambulatory adults sampled in August 2020 or early 2021, consented under IRB protocols.
(2020P001081 and 2020P002274), as described previously (24). The comparator cohort of 62 healthy control volunteers had anti-spike and anti-nucleocapsid antibodies to SARS-CoV-2 evaluated on identical Roche Elecsys platforms through the Massachusetts General Pathology Laboratory.

Repeated measures ANOVA for continuous variables and conditional logistic regression for categorical variables were utilized for matched participants and those who received additional vaccination after the initial series. One-way ANOVA with Tukey’s post-hoc correction, and simple logistic regression were used to compare SARS-CoV-2 antibody levels among subgroups of patients with different PAD types. To account for extreme heteroscedasticity, all antibody responses to SARS-CoV-2 vaccine were reported as geometric means (+/-95%CI), and log transformations were used to transform all antibody measures before statistical analyses to estimate p-values. Statistical analyses were completed with SAS 9.4 (SAS Institute, Cary, NC) and Prism Version 7.01 (Reston, VA); a two-tailed p value of <0.05 was considered significant.

Results:
Antibody response to the SARS-CoV-2 vaccine is lower among patients with PAD compared to healthy controls.

101 individuals with PAD met criteria for this study (Figure E1). 62 of the 101 met criteria for case-control matching based on age (+/- 10 years) and time from the most recent vaccination (+/- 14 days) at a ratio of 1:1 case to control, and these 62 patients were used for all subsequent analyses (Table 1). The mean age of the 62 patient PAD cohort was 52.5 years. There were 43 women (69.4%) and 19 men (30.6%). There were no statistically significant differences between PAD and healthy control groups in terms of age, gender, and time between
blood draw and the most recent vaccination. The PAD group consisted of more non-Hispanic white patients (95.2%) than the control population (p<0.01). Among both groups, most participants had received the mRNA-1273 (Moderna) vaccine (PAD: 53.2% vs. healthy control: 54.8%), followed by the BNT162b2 (Pfizer) vaccine (PAD: 40.3% vs. healthy control: 24.9%), followed by the Ad26.COV2.S (Janssen) vaccine (PAD: 6.5% vs. healthy control: 20.9%) (p=0.05).

We observed significantly lower mean anti-spine antibody levels in PAD patients compared to matched healthy controls following initial series SARS-CoV-2 vaccination (anti-spine antibody level for all vaccine types 140.1 vs. 547.3 U/mL; p=0.02) (Table 2, Fig. 1A). The odds of mounting a protective anti-spine antibody response ≥ 100 U/mL were 2.5 times higher in healthy controls than those diagnosed with PAD. In evaluating response to the specific SARS-CoV-2 vaccine received, PAD patients had significantly lower antibody responses compared to matched healthy controls following immunization with mRNA-1273 (Moderna) and Ad26.COV2.S (Janssen) (Table 2, Fig. 1B). Overall, SARS-CoV-2 anti-spine antibody titers were significantly higher in PAD patients who had received either mRNA vaccine platform as compared to the Ad26.COV2.S (Janssen) vaccine (Fig. 1C).

Antibody response to SARS-CoV-2 vaccine is lower among PAD patients with secondary and severe primary immunodeficiency.

To determine whether anti-spine antibody responses correlated with clinical diagnosis, we subcategorized our PAD cohort (Table 3). Ten patients met criteria for secondary PAD due to the presence of a potentially confounding immunosuppressive variable at the time of immune deficiency diagnosis. 52 patients had no confounding variables at the time of diagnosis and met
criteria for primary PAD. We further subcategorized primary PAD patients by underlying degree of humoral immune dysfunction. Immunologic testing including native immunoglobulin levels (prior to immunoglobulin replacement therapy), antibody titers to T cell-dependent and T cell-independent immunizations, peripheral lymphocyte flow cytometry (including analysis of B cell and T cell maturation), and T cell functional testing of T cell receptor, mitogen, and antigen stimuli for this cohort are detailed in Table E1. Primary PAD participants were classified as mild (IgG subclass deficiency, SAD, and primary hypogammaglobulinemia; n=12), moderate (CVID without autoinflammatory clinical features, ‘CVID’; n=21), or severe (complicated PAD encompassing the diagnoses of APDS, TACI deficiency, NFKB1 deficiency, and complicated CVID/SAD with autoinflammatory clinical features but without a known genetic etiology ‘CVIDc/SADc’; n=19).

Patients with secondary PAD had significantly lower mean anti-spike antibody levels compared to patients with primary PAD (13.4 U/mL vs. 219.8; p=0.02) (Table 4, Fig. 2A). We additionally analyzed pseudovirus neutralization in post-immunization serum available in 37 PAD patients and SARS-CoV-2 anti-RBD-specific antibody levels, including IgG, IgA, and IgM, in post-immunization serum available in 36 PAD patients. Overall, we observed linear correlations between anti-spike antibody levels and pseudovirus neutralization function and anti-RBD antibody levels, respectively, among PAD patients (Fig. 3). Similar to the observed difference in anti-spike antibody levels, patients with secondary PAD trended towards lower SARS-CoV-2 vaccine response by pseudovirus neutralization and total anti-RBD antibody levels compared to patients with primary PAD (Table 4, Fig. 2B,C).

Among primary PAD patients, those classified with severe disease had significantly lower mean anti-spike antibody levels compared to those classified with moderate disease and those
classified with mild disease (severe: 35.7 vs. moderate: 321.8 vs. mild: 2003, U/mL; p=0.001)
(Table 4, Fig. 2A). There was no statistically significant difference in anti-spike antibody
responses further delineated by sub-categorized clinical entity within the mild and severe disease
subtypes (Fig. 2A). Analysis of pseudovirus neutralization showed a similar trend, with
significantly lower mean neutralization function in severe as compared to mild PAD patients
(severe: 43.4 vs. mild: 389, pNT50; p=0.01) (Table 4, Fig. 2B). Finally, analysis of anti-RBD-
specific antibody responses showed a similar trend, with significantly lower mean anti-RBD
antibodies observed in severe as compared to mild PAD patients (severe: 0.3 vs. mild: 25.6, IgT
in U/mL; p=0.01) (Table 4, Fig. 2B,C). An exception was the IgM-specific anti-RBD response,
which was not statistically different between mild, moderate, and severe primary PAD groups.

Immunophenotypic risk factors for low anti-spike antibody response among patients with PAD.
Within our PAD cohort, we analyzed underlying immunophenotypic correlates of a
severely low antibody response to SARS-CoV-2 immunization, defined as an anti-spike antibody
level < 100 U/mL. PAD patients with anti-spike antibody levels < 100 U/mL had lower native
antibody levels, including IgG, IgA, and IgM, and lower native HIB vaccine levels (Table 5). In
addition, PAD patients with anti-spike antibody levels < 100 U/mL had lower absolute
circulating counts of total CD3+ T cells, CD4+ helper T cells, and total CD19+ B cells. Finally,
PAD patients with anti-spike antibody levels < 100 U/mL had demonstration of impaired B-cell-
maturation. Specifically, patients with <5% memory (CD27+ as % CD19+) B cells in circulation
and <2% class-switched memory (CD27+IgM/D- as % CD19+) B cells in circulation were at an
increased risk of having an anti-spike antibody level < 100 U/mL (OR 9.7; 95% CI 1.9-49.9;
p<0.01 and OR 2.3; 95%CI 0.6-9; p<0.01, respectively).
Passive transfer of anti-spike antibodies in patients receiving immunoglobulin replacement therapy occurred at a low level.

Despite exclusion in this study of PAD patients with any prior positive PCR testing for SARS-CoV-2, mean anti-nucleocapsid antibody levels were higher in PAD patients actively receiving intravenous immunoglobulin (IVIG) therapy (1.15 COI), compared to subcutaneous immunoglobulin (SCIG) therapy (0.19 COI), compared to no replacement immunoglobulin therapy (0.09 COI) (p=0.047; Table E2). These data were consistent with low levels of passively transferred anti-SARS-CoV-2 antibodies in immunoglobulin replacement products as previously described (27).

To determine whether passive antibody transfer could be confounding the anti-spike antibody analysis, we analyzed anti-spike antibodies at pre-vaccination timepoints in PAD patients on replacement immunoglobulin therapy, as available (n=16) (Fig. 4A). At the median time of final blood draw for this study, detection of passively transferred anti-spike antibodies was extremely low (anti-spike antibody levels > 1 U/mL, > 10 U/mL, and > 100 U/mL were seen in 82.7%, 37.8%, and 0.0% of unvaccinated PAD subjects receiving immunoglobulin replacement, respectively). These data suggested limited confounding of passively received anti-spike antibodies, particularly using a threshold response anti-spike antibody level ≥ 100 U/mL.

Moreover, PAD patients who mounted an anti-spike level ≥ 100 U/mL did not differ by immunoglobulin treatment status or dose per body weight (mg/kg) per month of immunoglobin therapy, if received (Fig. 4B). Instead, as noted above, differences in response to vaccine did correlate with underlying immunophenotypes including lower native immunoglobulin levels,
lower native HIB vaccine titer, lower absolute CD3+ T cells, CD4+ T cells, and CD19+ B cells, and lower absolute and percent class-switched memory B cells in circulation (Figure 4C).

Immunosuppression associated with low anti-spike antibody response among patients with PAD.

PAD patients who received any immunosuppression in the one month prior to SARS-CoV-2 immunization had a lower mean anti-spike antibody response (30.1 vs. 276.5 U/mL; p=0.02) (Table 6). Receiving any immunosuppression in the one month following SARS-CoV-2 immunization also trended towards a lower mean anti-spike antibody level among PAD patients (39.1 vs. 258.4 U/mL; p=0.07). Finally, PAD patients who had any previous use of a B-cell depletion agent (e.g., rituximab) had a lower mean anti-spike antibody response (11.6 vs. 289.8 U/mL; p<0.01) and an increased odds of mounting an anti-spike antibody response < 100 U/mL (OR 5.5; CI 1.5-20.4; p=0.01). This association became more pronounced when accounting for patients who received a B-cell depletion agent proximal to the time of immunization, which we defined as between 6 months prior to one month post-initial immunization. Specifically, PAD patients who had proximal use of a B-cell depletion agent had a lower mean anti-spike antibody response (0.67 vs. 308.8 U/mL; p<0.01) and an increased odds of mounting an anti-spike antibody response < 100 U/mL (OR 36.4; CI 1.7-791.9; p=0.02).

Response to additional dose SARS-CoV-2 vaccine among PAD patients.

31 of the initial 62 PAD patients received an additional dose of SARS-CoV-2 vaccine beyond their initial series, with follow-up anti-SARS-CoV-2 antibody testing performed. The majority (90.3%) received one additional mRNA vaccine dose following initial series mRNA immunization. In contrast, three patients (9.7%) received additional mRNA vaccine doses
following initial series Ad26.COV2.S (Janssen) immunization. Additional dose SARS-CoV-2 vaccine in the 31-patient PAD cohort significantly increased mean anti-spike antibody levels (76.3 U/mL pre- to 1065 U/mL post-additional dose; p<0.0001) (Table 7, Fig. 5A). Overall, additional dose SARS-CoV-2 vaccine in PAD subjects improved anti-spike antibodies to the level of their matched healthy controls following primary series immunization. The fold increase in anti-spike antibodies following additional dose SARS-CoV-2 vaccine was similar across risk factors that included clinical diagnosis (e.g., secondary PAD and severe primary PAD), initial receipt of the Ad26.COV2.S (Janssen) vaccine, severe immunophenotype (e.g., <2% class-switched memory B cells), and secondary immunosuppression (e.g., use of a B-cell depletion agent). The observed increase in anti-spike antibodies following additional series immunization was statistically significant for patients with moderate and severe primary PAD, specifically (Fig. 5B). Six patients (19.4%) had a persistently low (< 100 U/mL) anti-spike antibodies following additional dose SARS-CoV-2 vaccine. Analysis of the variables associated with low anti-spike antibodies following initial series immunization (Tables 5, 6) identified that the only persistent correlation was recent use of a B-cell depleting therapy in the 6 months preceding to the one month following additional dose vaccine (OR 23; CI 2.5-213.7; p=0.006).

Discussion:

This is the largest case-to-control matched immunodeficiency patient cohort evaluating response to SARS-CoV-2 vaccination to date and the first study evaluating additional vaccine doses in patients with PAD. As SARS-CoV-2 infection in patients with PAD is associated with
high morbidity, an improved understanding of the effectiveness of SARS-CoV-2 immunization in immunodeficient patients is critical.

In this study, we found that approximately 60% of PAD patients were able to develop anti-spike antibody responses ≥ 100 U/mL following initial series SARS-CoV-2 vaccination. However, compared to healthy controls matched for age and time from immunization, PAD patients had significantly lower mean anti-spike antibody levels. Underlying PAD diagnosis and immunophenotypic markers of disease severity correlated with response to vaccination. Specifically, anti-spike antibody levels were lowest in those patients with secondary and severe primary PAD (such as complicated CVID) as compared to those with mild PAD (such as IgG subclass deficiency, SAD, and primary hypogammaglobulinemia). Certain immunophenotypic markers correlated with a lower anti-spike antibody response, including low native antibody levels (IgG, IgA, and IgM), low native IgG antibodies for HIB, low CD4+ helper T cells, low CD19+ total B cells, and low class-switched memory (CD27+IgD/M-) B cells. These clinical diagnostic and immunophenotypic risk factors may help clinicians to better stratify their patients with predominant antibody deficiency in terms of identifying those patients at highest risk for a low antibody response following SARS-CoV-2 immunization.

Many patients with PAD will require secondary immunosuppression to manage autoimmune and/or autoinflammatory disease co-morbidities (28). Here secondary immunosuppression, and in particular use of a B-cell depleting agent, most frequently rituximab, was associated with a decreased humoral immune response to SARS-CoV-2 vaccination. Prior B-cell depleting agent use correlated with severely low (< 100 U/mL) mean anti-spike antibody levels in this study. These data are consistent with prior reports of lower SARS-CoV-2 vaccine
antibody responses following B-cell depleting therapy in other immunodeficient patient demographics (4, 29), and suggest that this patient population should maintain increased precautions and vigilance regarding potential COVID-19 exposures. Overall, these data highlight the unique risk for patients with primary immunodeficiency related to SARS-CoV-2 vaccination, both the potential for diminished immune response due to a congenital immunodeficiency – and the potential for diminished immune response related to use of secondary immunosuppression.

In PAD patients who received an additional SARS-CoV-2 vaccine, specifically one or more additional mRNA vaccine doses, anti-spike antibody levels increased significantly. These data support recommendations from the Center for Disease Control regarding additional doses of COVID-19 vaccines both as a part of the primary series and then as booster doses in patients with moderate/severe forms of immune deficiency (30). Increased anti-spike antibody levels following additional dose immunization were observed even in PAD patients with an ‘at risk’ immunophenotype for poor response to initial series immunization (e.g., low class-switched memory (CD27+IgM/D-) B cells). These data suggest a significant benefit to additional dose vaccination in these patients with moderate to severe immune deficiency phenotypes. This additional dose vaccine increased anti-spike antibodies to the level of matched controls following initial series immunization. However, the optimal timing and number of vaccine doses needed to prevent or mitigate disease in patients with PAD requires future studies. Additionally, there are no data yet addressing the SARS-CoV-2 vaccine memory response in the PAD patient demographic. Trends toward different isotype anti-RBD antibody responses between PAD diagnoses may suggest that specific PAD patients are more predisposed to short-lived antibody responses following SARS-CoV-2 vaccine. Data from patients with CVID have suggested an
extra-follicular or incomplete germinal center response to SARS-CoV-2 vaccination, yielding a marked reduction in RBD-specific B cells (31). However, a dedicated follow-up study of the SARS-CoV-2 humoral immune response over time in PAD patients is needed to address the question of vaccine durability in this patient demographic. Finally, secondary immunosuppression, specifically recent B-cell depletion therapy, was the only persistent risk factor for a low (<100 U/mL) anti-spike antibody response following additional dose immunization. These data suggest that additional doses of immunization may not be an adequate strategy in this particular patient demographic and consideration of alternate options such as tixagevimab/cilgavimab (Evusheld) for prophylaxis may be warranted.

A limitation to this study includes potential confounding from immunoglobulin replacement that may contain antibodies to SARS-CoV-2 (27). Our analysis demonstrated that no patients on immunoglobulin replacement had pre-vaccine anti-spike antibodies that met criteria for minimal threshold response to vaccination, which was defined in our study as ≥ 100 U/mL. These data suggest that passive antibody transfer from immunoglobulin replacement therapy occurred, but at very low levels, at the time of this analysis. These data also highlight the importance of effective vaccine counseling in this patient demographic, given that at the time of this study, immunoglobulin replacement alone did not confer large amounts of antibodies against SARS-CoV-2. It is expected that confounding from passive transfer of SARS-CoV-2 antibodies in immunoglobulin replacement will increase over time, thus making future studies more challenging. Other potential limitations include that this analysis was performed using data from a large, but single healthcare system, so these findings may not be generalizable to other settings. Additionally, there may be sampling bias in that differences may exist between those patients who consented to be a part of this study versus those who did not. We found that SARS-CoV-2
anti-spike antibody levels trended towards higher in PAD patients who had received the mRNA-1273 (Moderna) vaccine as compared to the other vaccine platforms. However, this was a retrospective analysis and patients were not assigned to vaccination platforms and therefore, there may be selection bias in the patients that chose specific vaccines.

Additional studies are needed to characterize the immune response of PAD patients following vaccination. In addition to the antibody responses analyzed here, T-cell-response to vaccination may provide important cellular immune protection against severe infection, which we are unable to assess with this serologic data. T cells may confer long-lasting immune memory against coronavirus, and this has been reported in SARS-CoV-1 survivors (32). Additionally, even in the absence of neutralizing antibodies, there have been reports of cellular immune response without seroconversion (33). Longitudinal studies are needed to evaluate the duration of response to vaccine to determine the optimal vaccination strategy as antibody responses can wane over time (17).

Our data provide new insights into the immune response to SARS-CoV-2 vaccination in patients with PAD. Patients with secondary and severe primary PAD developed lower antibody responses to SARS-CoV-2 vaccination, which improved following additional dose immunization for SARS-CoV-2. Certain immunophenotypic risk factors were associated with low response to vaccine (including low native antibody levels (IgG, IgA, and IgM), low native IgG antibodies for HIB, low CD4+ helper T cells, low CD19+ total B cells, and low class-switched memory (CD27+IgD/M-) B cells), however, following additional dose vaccination, these patients reached anti-spike antibody levels comparable to their matched healthy control population after initial series vaccination. This highlights the importance of careful monitoring in this particular subset
of patients with a moderate to severe immune deficiency phenotype, and also underscores the importance of additional and booster dose vaccination in this patient population. Given the high morbidity from COVID-19 infection in this population, strategies to improve host immunity using booster vaccination should be considered in addition to maintaining precautions regarding COVID-19 infection.
### Table 1. Demographic characteristics of cases and controls

|                  | PAD (n=62) | Healthy Control (n=62) | p-value  |
|------------------|------------|------------------------|----------|
| Age (years, mean) | 52.5       | 52.6                   | 0.93     |
| Gender (%F)       | 69.4       | 56.5                   | 0.14     |
| Non-Hispanic White (%) | 95.2   | 61.1                   | <0.01    |
| Missing           | -          | 8                      |          |
| Vaccine (% (n))   |            |                        |          |
| mRNA-1273 (Moderna) | 53.2 (33) | 54.8 (34)               |          |
| BNT162b2 (Pfizer) | 40.3 (25)  | 24.9 (15)               |          |
| Ad26.COV2.S (Janssen) | 6.5 (4)  | 20.9 (13)               | 0.05     |
| Time from most recent vaccination to blood draw (days) | 36.6 | 35.1 | 0.16 |

### Table 2. Anti-spike antibody levels in PAD patients compared to matched healthy controls

|                  | PAD (n=62) | Healthy Control (n=62) | p-value  |
|------------------|------------|------------------------|----------|
| Anti-spike antibody (U/mL) (geometric mean (95%CI)) | 140.1 (59.2-331.5) | 547.3 (280.2-1069.0) | 0.02     |
| Anti-spike antibody (%) |            |                        |          |
| Odds Ratio (95%CI) |            |                        |          |
| <100               | 59.7       | 79.0                   | 0.03     |
| 100-1000           | 40.3       | 21.0                   |          |
| Odds Ratio <100 (95%CI) | 38.7 | 2.5 (1.1-5.7) |          |
| Odds Ratio 100-1000 v <100 (95%CI) | 21.0 | 2.5 (0.95-6.8) | 0.06 |
| Odds Ratio >1000 v <100 (95%CI) | Ref. | 2.5 (1.02-6) | 0.046 |
| Anti-spike antibody (U/mL) (geometric mean (95%CI)) | 305.2 (87.4-1065) | 1905 (988.9-3669) | 0.03     |
| mRNA-1273 (Moderna) | 106.9 (33.9-337.3) | 258 (49.3-1349) | 0.22     |
| BNT162b2 (Pfizer) | 1.2 (0.1-13.6) | 50.0 (15.2-164.7) | 0.03     |
| Ad26.COV2.S (Janssen) |            |                        |          |

### Table 3. Subcategorization of PAD cases

| PAD Diagnosis | Primary (n=52) | Secondary (n=10) | p-value  |
|---------------|----------------|-----------------|----------|
|               | Mild (n=12) | Moderate (n=21) | Severe (n=19) |  |
| Clinical Entities | - IgG SD (3) | - SAD (5) | - PHG (4) | Complicated PAD (19): - APDS (4) - TACI def (3) - NFKB1 def (1) - CVIDc/SADc (gene not known) (11) | Dx confounded by: - clonal suppression (3) - immunosuppression (7) |
| PID Genetic Testing | Yes (% (n)) | 33.3 (4) | 42.8 (9) | 84.2 (16) | 0.005 | 10.0 (1) | 0.0074 |
| Pathogenic variant (% (n)) | 0.0 (0) | 0.0 (0) | 42.1 (8): - PIK3CD (3) - TNFRSF13B (3) - PIK3AP1 (1) - NFKB1 (1) | <0.0001 | 0.0 (0) | 0.19 |
| IgR | Yes (% (n)) | 33.3 (4) | 85.7 (18) | 84.2 (16) | 0.0012 | 40.0 (4) | 0.041 |
| Immunosuppression (ever) | Yes (% (n)) | 75.0 (9) | 90.5 (19) | 78.9 (15) | 0.47 | 90.0 (9) | 0.57 |
| Intermittent prednisone or Plaquenil only (% (n)) | 66.7 (8) | 52.4 (11) | 15.8 (3) | 0.0082 | 0.0 (0) | 0.01 |
Table 4. Antibody response to SARS-CoV-2 vaccine in PAD patients by clinical subtype

|                     | Primary PAD (n=52) | All Primary PAD (n=52) | Secondary PAD (n=10) | p-value |
|---------------------|--------------------|------------------------|----------------------|---------|
|                     | Mild (n=12)        | Moderate (n=21)        | Severe (n=19)        | (total) | (total) |         |         |
| Anti-spike antibody (U/mL) (geometric mean (95%CI), n) | 2003 (677.1-5922) | 321.8 (81-1279)       | 35.7 (7.7-166.2)     | 0.001   | 219.8 (90.1-536.2) | 13.4 (1.1-170.8) | 0.02   |
| Neutralization (pNT50) (geometric mean (95%CI), n) | 389 (103.8-1458) | 90.2 (35.3-230.1)     | 43.4 (16.6-113.7)    | 0.01    | 96.6 (52.0-179.3)  | 30.9 (6.2-154.4)  | 0.17   |
| Anti-RBD antibody (IgT, U/mL) (geometric mean (95%CI), n) | 25.6 (8.4-78.2)  | 7.1 (1.1-47.5)        | 0.3 (0.02-4.9)       | 0.01    | 3.0 (0.8-11.3)     | 0.1 (0.0-58.5)    | 0.09   |

Table 5. Antibody response to SARS-CoV-2 vaccine in PAD patients by underlying immunophenotype

| Native Immunoglobulin Levels (mean, mg/dL) | Anti-spike antibody <100 U/mL (n=25) | Anti-spike antibody >/=100 U/mL (n=37) | p-value |
|-------------------------------------------|--------------------------------------|---------------------------------------|---------|
| IgG                                       | 443                                  | 678                                   | <0.01   |
| IgA                                       | 45                                   | 171                                   | <0.01   |
| IgM                                       | 60                                   | 103                                   | 0.03    |
| IgG1                                      | 311                                  | 392                                   | 0.07    |
| IgG2                                      | 146                                  | 182                                   | 0.36    |
| IgG3                                      | 32                                   | 34                                    | 0.93    |
| IgG4                                      | 13                                   | 16                                    | 0.19    |
| Missing (n)                               | (3-16)                               | (7-12)                                |         |
| IgG Antibody Levels (mean)                |                                      |                                       |         |
| S. Pneumoniae (% > 1.3 µg/mL)             | 47                                   | 56                                    | 0.29    |
| H. Influenzae (mg/L)                      | 0.3                                  | 1.6                                   | <0.01   |
| Tetanus (IU/mL)                           | 0.98                                 | 1.4                                   | 0.42    |
| Diphtheria (IU/mL)                        | 0.28                                 | 0.25                                  | 0.85    |
| Missing (n)                               | (10-13)                              | (7-16)                                |         |
| Flow cytometry (mean, absolute count of cells/µL) |                      |                                       |         |
| CD3+ | 986 | 1287 | 0.04 |
| CD4+ | 559 | 810  | 0.02 |
| CD8+ | 356 | 416  | 0.3  |
| CD3+CD16+56+ | 190 | 200 | 0.71 |
| CD45+CD4RA | 234 | 348 | 0.05 |
| CD19+CD27+IgM/IgD- | 4 | 12 | <0.01 |
| CD19+CD27+IgM/IgD+ | 26 | 33 | 0.26 |

### Severity Markers

- **<20% CD4+CD45RA+ (% CD4+)**
  - Ref. 1 (0.16-6.1) 0.64
- **<5% CD19+CD27+ (% CD19+)**
  - Ref. 9.7 (1.9-49.9) <0.01
- **<10% CD19+CD27+ (% CD19+)**
  - Ref. 2.3 (0.6-9) 0.22
- **<2% CD19+CD27+IgM/IgD- (% CD19+)**
  - Ref. 11 (2-60) <0.01

### Table 6. Antibody response to SARS-CoV-2 vaccine in PAD patients by secondary immunosuppression

| Immune suppression (ever) | Anti-spinal antibody (U/mL) (geometric mean (95%CI)) | p-value | Anti-spinal antibody <100 U/mL (OR (95%CI)) | p-value |
|---------------------------|----------------------------------------------------|---------|---------------------------------------------|---------|
| Yes                       | 142 (55.3-367.8)                                    | 0.91    | 0.35 (0.08-1.6)                             | 0.18    |
| No                        | 123.9 (9.9-1,543)                                   |         |                                             |         |

| Immune suppression (<1 month prior) | Anti-spinal antibody (U/mL) (geometric mean (95%CI)) | p-value | Anti-spinal antibody <100 U/mL (OR (95%CI)) | p-value |
|------------------------------------|----------------------------------------------------|---------|---------------------------------------------|---------|
| Yes                                | 30.1 (5.6-162.4)                                   | 0.02    | 1.5 (0.5-4.5)                               | 0.45    |
| No                                 | 276.5 (104.9-728.9)                                |         |                                             |         |

| Immune suppression (<1 month post) | Anti-spinal antibody (U/mL) (geometric mean (95%CI)) | p-value | Anti-spinal antibody <100 U/mL (OR (95%CI)) | p-value |
|------------------------------------|----------------------------------------------------|---------|---------------------------------------------|---------|
| Yes                                | 39.1 (6-253.1)                                     | 0.07    | 1.4 (0.4-4.6)                               | 0.61    |
| No                                 | 258.4 (87.1-766.3)                                 |         |                                             |         |

| B cell Depletion Therapy | Anti-spinal antibody (U/mL) (geometric mean (95%CI)) | p-value | Anti-spinal antibody <100 U/mL (OR (95%CI)) | p-value |
|--------------------------|----------------------------------------------------|---------|---------------------------------------------|---------|
| Ever | 11.6 (1.3-94.7) | <0.01 | 5.5 (1.5-20.4) | 0.01 |
| Recent (<6 months prior to <1 month post) | 289.8 (121.9-688.7) |         |                                             |         |

### Table 7. Response to additional dose SARS-CoV-2 vaccine in PAD patients

| Clinical Subtype | Initial Series Vaccine anti-spinal antibody (U/mL) | Additional Dose Vaccine anti-spinal antibody (U/mL) | Fold change | p-value |
|------------------|----------------------------------------------------|---------------------------------------------------|-------------|---------|
|                  | Geometric mean (95% CI)                            | Geometric mean (95% CI)                           |             |         |
|                  | Geometric mean (95% CI)                            | Geometric mean (95% CI)                           |             |         |
|                  | Geometric mean (95% CI)                            | Geometric mean (95% CI)                           |             |         |
|                  | Geometric mean (95% CI)                            | Geometric mean (95% CI)                           |             |         |
|                  | Geometric mean (95% CI)                            | Geometric mean (95% CI)                           |             |         |
|                  | Geometric mean (95% CI)                            | Geometric mean (95% CI)                           |             |         |
|                  | Geometric mean (95% CI)                            | Geometric mean (95% CI)                           |             |         |
|                  | Geometric mean (95% CI)                            | Geometric mean (95% CI)                           |             |         |

- Clinical Subtype
  - All PAD (n=31) 76.3 (22.5-259) 1065 (395-2,871) 14-fold <0.0001
| Type of Vaccination Series | Janssen + mRNA (n=3) | mRNA + mRNA (n=28) |
|----------------------------|----------------------|---------------------|
| Secondary (n=4)            | 10.5                 | 102.3               |
| Primary (n=27)             | (0.07-1.603)         | (27.6-379.2)        |
|                            | 124.1                | 1464                |
|                            | (0.2-69.396)         | (565-3,795)         |
| Mild (n=5)                 | 786.9                | 9188                |
| Moderate (n=11)            | 277.5                | 2441                |
| Severe (n=11)              | 14.9                 | 381.1               |
|                            | (49.9-12,414)        | (4558-18,522)       |
|                            | (34.0-2,251)         | (507.6-11,742)      |
|                            | (1.9-119.3)          | (78.8-1,844)        |
| Immunophenotype            |                      |                     |
| <20% CD45RA+ (%CD4+)       | 38.6                 | 15.0                |
| >/=<20% CD45RA+ (%CD4+)    | 136.6                | (0.06-25,476)       |
|                            | (32.9-565.6)         | (72.6-63,064)       |
|                            | 15.5                 | (549.1-4,399)       |
| >2% CD27+IgM/IgD- (%CD19+) | 66.6                 | 1,337               |
| >/= 2% CD27+IgM/IgD- (%CD19+) | 1,289             | (14.0-316.9)        |
|                            | (428.7-3,877)        | (464.8-3,848)       |
|                            | 6,759                | (2,282-20,019)      |
| IgG <500 mg/dL             | 16.5                 | 286.6               |
| IgG >/=500 mg/dL           | 167.7                | (2.0-135.2)         |
|                            | (27.1-1,040)         | (40.6-2,025)        |
|                            | 2,694                | (950.6-7,637)       |
| B cell Depletion Therapy   |                      |                     |
| Ever                       |                      |                     |
| Yes                        | 12.7                 | 248.2               |
| No                         | 159.2                | (19.7-3,127)        |
| Recent (<6months prior to <1month post) |            | (715.4-5,220)      |
| Yes                        | 0.89                 | 26.6                |
| No                         | 222.5                | (2.1-335.1)         |
|                            | (71.1-696.2)         | (1,168-5,712)       |
Figure legends:

**Figure 1.** SARS-CoV-2 anti-spike antibody levels (U/mL), shown in log scale and compared between matched healthy controls (grey squares; n=62) and patients with PAD (red circles; n=62). Shown by all vaccine types (A) and by specific initial series SARS-CoV-2 vaccine type received (B, C). Symbols represent unique individuals, bars represent geometric means (+/- 95%CI) of total indicated patients (n), and shading represents the assay lower limit of reactivity. * p<0.05, ** p<0.01.

**Figure 2.** SARS-CoV-2 anti-spike antibody levels (U/mL) (A), SARS-CoV-2 pseudovirus neutralization values (pNT50) (B), and SARS-CoV-2 anti-RBD antibody titers (U/mL) (C), shown in log scale and compared between PAD diagnoses as indicated. Symbols represent unique individuals, bars represent geometric means (+/-95%CI) of total indicated patients (n), and shading represents the assay lower limit of detection (LLD) or reactivity, respectively. * p<0.05, ** p<0.01.

**Figure 3.** SARS-CoV-2 anti-spike antibody level (U/mL) correlates linearly with pseudovirus neutralization function (pNT50) (A) and total anti-RBD antibody level (U/mL) (B) in patients with PAD. Linear regression analysis from 37 (A) and 36 (B) PAD patients with correlation coefficients (r²) and significance (p) shown. Shaded area represents 95% confidence limits.

**Figure 4.** Evaluation of timing of SARS-CoV-2 anti-spike antibody testing in relation to potential for passive antibody transfer (A). Detection of threshold anti-spike antibodies (>1 U/mL, >10 U/mL, or >100 U/mL) in vaccine-naïve PAD patients receiving immunoglobulin replacement therapy (IVIG/SCIG). Data are shown as percent positive by Kaplan-Meier curve.
with symbols indicating events over a period of 276 days following emergency use authorization (EUA) of the Pfizer vaccine (top) with corresponding dates of blood draw for all PAD patients included in this study shown as median (+/-IQR) days (bottom). Threshold vaccine response, defined in the study as an anti-spike antibody level ≥100 U/mL, shown in relation to immunoglobulin replacement therapy (B) and underlying immunophenotype (C). Symbols represent unique individuals, bars represent means (+/-SD) of total indicated patients (n). * p<0.05, *** p<0.001.

Figure 5. SARS-CoV-2 anti-spike antibody titers (U/mL), shown in log scale and compared between initial series SARS-CoV-2 vaccination (red circles) and additional dose SARS-CoV-2 vaccination (green circles). Data are shown for the total boosted PAD cohort (n=31) and compared to their matched healthy controls (n=31) (A) and by PAD diagnosis (B). Symbols (A) represent unique individuals and bars represent geometric means (+/-95%CI) of total indicated patients (n). Symbols (B) represent geometric means (+/-95%CI) of total indicated patients (n). Shading represents the assay lower limit of reactivity. ** p<0.001, **** p<0.0001, ns=not significant.
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SARS-CoV-2 anti-RBD Antibody (U/mL)

\[ r^2 = 0.82 \]

\[ p < 0.0001 \]

\[ r^2 = 0.56 \]

\[ p < 0.01 \]
**A**

Unvaccinated patients on IVIG/SCIG with threshold anti-Spike Antibody (%)

TIME (days from Pfizer EUA)

Blood drawn for anti-Spike Antibody

initial series (n=62)

boost (n=31)

**B**

p=0.85

Patients on IgR (%)

SARS-CoV-2 anti-Spike Antibody (U/mL)

p=0.95

Replacement Immunoglobulin Dose (mg/kg/month)

SARS-CoV-2 anti-Spike Antibody (U/mL)

**C**

p=0.05

CD4+ T cells (cells/uL)

SARS-CoV-2 anti-Spike Antibody (U/mL)

p=0.001

CD19+ B cells (cells/uL)

SARS-CoV-2 anti-Spike Antibody (U/mL)

p=0.001

Switched Memory B cells (% CD19+)

SARS-CoV-2 anti-Spike Antibody (U/mL)

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Unvaccinated patients on IVIG/SCIG with threshold anti-Spike Antibody (%)

- Black triangle: >/= 1 U/mL
- Diamond: >/= 10 U/mL
- Red square: >/= 100 U/mL

TIME (days from Pfizer EUA)

Blood drawn for anti-Spike Antibody

initial series (n=62)

boost (n=31)

---

Patients on IgR (%)

SARS-CoV-2 anti-Spike Antibody (U/mL)

- Gray: IVIG
- Light blue: SCIG
- Black: None

---

Replacement Immunoglobulin Dose (mg/kg/month)

SARS-CoV-2 anti-Spike Antibody (U/mL)

---

CD4+ T cells (cells/uL)

SARS-CoV-2 anti-Spike Antibody (U/mL)

---

CD19+ B cells (cells/uL)

SARS-CoV-2 anti-Spike Antibody (U/mL)

---

Switched Memory B cells (% CD19+)

SARS-CoV-2 anti-Spike Antibody (U/mL)
A

SARS-CoV-2 anti-Spike Antibody (U/mL)

HC (n=3)

PAD initial series (n=3)

PAD additional series (n=3)

reactive (≥0.8 U/mL)

threshold response (≥100 U/mL)

B

SARS-CoV-2 anti-Spike Antibody (U/mL)

initial series

additional series

p=0.14

p=0.05

reactive (≥0.8 U/mL)

threshold response (≥100 U/mL)
A

all vaccine types

SARS-CoV-2 anti-Spike Antibody (U/mL)

HC (n=62)  PAD (n=62)

threshold response (≥100 U/mL)

reactive (≥0.8 U/mL)

B

mRNA-1273  BNT162b2  Ad26.COV2.S

SARS-CoV-2 anti-Spike Antibody (U/mL)

HC (n=34)  PAD (n=33)  HC (n=15)  PAD (n=25)  HC (n=13)  PAD (n=4)

threshold response (≥100 U/mL)

reactive (≥0.8 U/mL)

p=0.22

C

PAD (n=62)

SARS-CoV-2 anti-Spike Antibody (U/mL)

mRNA-1273 (n=33)  BNT162b2 (n=25)  Ad26.COV2.S (n=4)

threshold response (≥100 U/mL)

reactive (≥0.8 U/mL)