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ORIGINAL RESEARCH

Trajectories of humoral and cellular immunity and responses to a third dose of mRNA vaccines against SARS-CoV-2 in patients with a history of anti-CD20 therapy

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ABSTRACT

Background The majority of patients with B-cell-depleting therapies show compromised vaccination-induced immune responses. Herein, we report on the trajectories of anti-SARS-CoV-2 immune responses in patients of the RituxiVac study compared with healthy volunteers and investigate the immunogenicity of a third vaccination in previously humoral non-responding patients.

Methods We investigated the humoral and cell-mediated immune response after SARS-CoV-2 messanger RNA vaccination in patients with a history with anti-CD20 therapies. Coprimary outcomes were antispike and SARS-CoV-2-stimulated interferon-γ concentrations in vaccine responders 4.3 months (median; IQR: 3.6–4.8 months) after first evaluation, and humoral and cell-mediated immunity (CMI) after a third vaccine dose in previous humoral non-responders. Immunity decay rates were compared using analysis of covariance in linear regression.

Results 5.6 months (IQR: 5.1–6.7) after the second vaccination, we detected antispike IgG in 88% (29/33) and CMI in 44% (14/32) of patients with a humoral response after two-dose vaccination compared with 92% (24/26) healthy volunteers with antispike IgG and 69% (11/16) with CMI 6.8 months after the second vaccination (IQR: 6.0–7.1). Decay rates of antibody concentrations were comparable between patients and controls (p=0.70). In two-dose non-responders, a third SARS-CoV-2 vaccine elicited humoral responses in 19% (6/32) and CMI in 32% (10/31) participants.

Conclusion This study reveals comparable immunity decay rates between patients with anti-CD20 treatments and healthy volunteers, but inefficient humoral or CMI after a third SARS-CoV-2 vaccine in most two-dose humoral non-responders calling for individually tailored vaccination strategies in this population.

Trial registration number NCT04877496; ClinicalTrials.gov number.

Key messages

What is already known about this subject?

- Patients receiving immunosuppression such as anti-CD20 B-cell-depleting therapies show decreased humoral and cellular immune responses to vaccines, including SARS-CoV-2 vaccines.

What does this study add?

- This study shows the longitudinal trajectory of antibody decay rates and reveals that these are comparable between patients with anti-CD20 treatments and healthy volunteers.
- Moreover, a third vaccine dose in humoral non-responders elicits humoral or cellular immunogenicity in a subset of patients with a history of anti-CD20 therapies.

How might this impact on clinical practice or further developments?

- Three SARS-CoV-2 vaccine doses are required but not sufficient for immunogenicity in some patients receiving anti-CD20 therapies, requiring close monitoring of vaccine-elicited immunogenicity in this population.
- Long-term data on breakthrough infections after SARS-CoV-2 vaccines are lacking in this population, but the present data suggest that, once an immune response is successfully established, the trajectories of immune responses in anti-CD20-treated patients appear like in the general population.

INTRODUCTION

COVID-19 has a deleterious effect in many patients, including those treated with

immunosuppressive drugs. Additionally, increasing evidence supports the finding that messanger RNA (mRNA)-based vaccines elicit inferior responses in immunocompromised patients, especially in those with anti-CD20 therapies.[1 2] In the initial RituxiVac study, we showed that in a mixed population of patients with kidney transplant, autoimmune disease or cancer, only 49% of patients produce a humoral immune response after mRNA vaccination against SARS-CoV-2.[3] Our recent meta-analysis revealed similar results by several studies, with some differences depending on the types of patient populations.[4]

Recent publications support the notion that patients with a weak humoral immune responses benefit from a third vaccine dose. Indeed, in approximately 30% of solid-organ transplant recipients seroconversion occurred on a third or fourth SARS-CoV-2 vaccination.[5–7] Individual case reports and one larger preprint article revealed increasing humoral and cell-mediated immunity (CMI) after a third dose of SARS-CoV-2 vaccination in two-dose humoral non-responders with a history of CD20 treatment.[8–10] At present, clinical outcome data are lacking to determine whether fully vaccinated but seronegative patients are at least partially protected from severe COVID-19. To add more complexity, even in healthy populations, the time frame of protection from severe COVID-19 by SARS-CoV-2 vaccines is under debate,[11] but much more so in the immunocompromised.[12] Additionally, it remains to be determined whether trajectories of SARS-CoV-2 antibodies and CMI of immunocompromised patients differ from those of healthy individuals.

This study represents a follow-up of patients with treatment history of anti-CD20 therapy from our initial RituxiVac cohort, in which both humoral and CMI responses to SARS-COV-2 mRNA vaccines had been assessed in an investigator-initiated, single-centre, case–control study (RituxiVac V.1.0).[3] We herein present the 6-month follow-up data on antibody and CMI trajectories in initial humoral responders comparing B-cell-depleted patients with healthy volunteers as well as data on immune responses after a third dose of mRNA SARS-CoV-2 vaccines in two-dose humoral non-responders.

METHODS

Study design

The RituxiVac study was an investigator-initiated, single centre, open-label, case–control study conducted at the Departments of Nephrology and Hypertension, Rheumatology and Immunology, Haematology, Neurology and Dermatology of the University Hospital in Bern, Switzerland. The study design was previously reported.[3] In brief, COVID-19-naïve patients with a treatment history of anti-CD20 drugs (rituximab or ocrelizumab) and completion of a two-dose series of SARS-CoV-2 mRNA vaccination ≥4 weeks earlier were enrolled between 26 April and 30 June 2021. Treatment data and indication for anti-CD20 therapies were collected, and age, sex and immunosuppressive comedication were further analysed. Official vaccination reports or self-reported vaccination dates and types were recorded. Furthermore, unmatched healthy volunteers were enrolled at least 4 weeks after completion of their two-dose mRNA vaccination. Patients and healthy volunteers aged less than 18 years or with prior SARS-CoV-2 infection were not eligible. All study participants were tested for the presence of antinucleocapsid antibodies, and those with positive results were excluded from the analysis.

All participants initially received either BNT162b2 mRNA COVID-19 vaccine (BioNTech/Pfizer, Comirnaty) or mRNA-1273 vaccine (COVID-19 VACCINE Moderna, Spikevax) as issued by the Swiss national COVID-19 vaccination programme. Starting on 2 August 2021, as per the guidelines of the Swiss federal authorities and independently of the present study, immunocompromised patients who were humoral non-responders after two vaccinations were invited to receive a third dose of BNT162b2 mRNA COVID-19 vaccine or mRNA-1273 vaccine. All RituxiVac participants and two additional patients were contacted by phone or during regular visits and invited to participate in the present follow-up study. This included an assessment either 4 weeks after the third vaccination for initial humoral non-responders or 6 months (±2 months) after the second vaccination in initial humoral responders.

This study was supported by Bern University Hospital. The funder had no influence on the design or conduct of this study and was not involved in data collection or analysis, in the writing of the manuscript or in the decision to submit for publication. The study was registered on clinicaltrials.gov and was performed in accordance with the principles of the Declaration of Helsinki. The authors assume responsibility for the accuracy and completeness of the data and analyses as well as for the fidelity of the study and this report to the protocol.

Study procedures

Baseline data collection

Study nurses and physicians completed a 20-item questionnaire for the follow-up study visit. Dates and types of administered vaccines (BNT162b2 mRNA COVID-19 vaccine or mRNA-1273 vaccine) were obtained from the existing study database, and additional data were retrieved from the official vaccination records when available or were self-reported by participants.

Blood collection and measurement of anti-SARS-CoV-2 S1-IgG and NC-IgG

Blood was collected in serum tubes or lithium heparin tubes. For the detection of SARS-CoV-2-specific antibodies, IgG antibodies targeting the SARS-CoV-2 S1 protein were measured by ELISA (Euroimmun AG, Lübeck, Germany) as previously reported.[13] In brief, Samples were diluted 1:100, and 100 µL of diluted samples, prediluted positive or negative controls and a prediluted calibrator were added for 1 hour at 37°C. After

washing three times, 100 µl of HRP-labelled secondary antihuman IgG was added for 30 min at 37°C, followed by three additional washes. Next, 100 µL of tetramethylbenzidine (TMB) solution was added for 20 min. The reaction was interrupted with 100 µL of 0.5M H_2SO_4, and the results were obtained by measurement at OD450-620 nm. Antibody results were expressed as ratio ($OD_{sample}/OD_{calibrator}$). A cut-off of >1.1 index s/c was applied for positive results as per the manufacturer's instructions. To allow an exclusion of the participants with previous COVID-19, an antinucleocapsid ECLIA test was used on a Cobas 8000 analyzer (Roche Diagnostics, Rotkreuz, Switzerland).[14] The cut-off was calculated by calibrator measurements and determined at ≥1.0 index s/c as per the manufacturer's instructions.

Measurements

SARS-CoV-2-stimulated interferon-γ release was measured in whole blood of some participants (n=79) using the QuantiFERON SARS-CoV-2 Starter Pack (Qiagen Cat No./ID: 626715) as described.[3] As per the manufacturer's protocol, blood from lithium heparin tubes was incubated with spike peptide pools or a mitogen for 16 hours. Next, interferon-γ ELISA (Qiagen Cat No./ID: 626410) was used for quantification of CMI, using a cut-off value of 0.15 IU/mL as reported before.[3 15]

Outcomes

The coprimary end points were the proportion of patients with a history of anti-CD20 therapies showing a persisting humoral or CMI against SARS-CoV-2 spike protein 6 months after completion of SARS-CoV-2 vaccination, in comparison to immunocompetent controls, and the detection of a humoral response or CMI against SARS-CoV-2 spike protein in two-dose humoral non-responders who received a third dose of SARS-CoV-2 mRNA vaccines. Humoral responses were defined as anti-SARS-CoV-2 S1 ≥1.1 (Index).[16]

Prespecified secondary end points were the rate of decline in anti-S1 IgG and CMI in patients with anti-CD20 therapy and healthy volunteers, and the effects of initial anti-S1 concentration and CMI after a two-dose vaccination schedule, demographic data, time since last treatment and cumulative dose of B-cell depleting agents, treatment indication or blood markers of immunocompetence on humoral responses to SARS-CoV-2 mRNA-based vaccines.

Statistical analysis

For this follow-up study, the initial RituxiVac study population was eligible without further power analysis or prescreening procedures. Statistical analyses were conducted using R software V.4.0.4.[17] Fisher's exact test was used for comparison of categorical variables. Wilcoxon rank sum test or t test was used for comparison of continuous variables as indicated. Linear regression analyses were performed using the lm function in R. Statistical significance was assumed at a two-tailed p<0.05. P values and 95% CIs are not adjusted for multiple testing.

RESULTS

Demographic and clinical characteristics of the participants

For the RituxiVac V.2.0 Study, 65 patients, including 33 two-dose humoral responders and 32 non-responders and 26 healthy controls, were assessed at a follow-up visit between 2 September and 10 November 2021, reflecting a follow-up rate of 63% for patients and 81% for volunteers (online supplemental figure 1). Patients with de novo antinucleocapsid antibodies at the follow-up visit were excluded (n=4 for patients, n=1 for volunteers). Demographic details, treatment history and vaccination data are presented in table 1. Anti-CD20 drugs included rituximab and ocrelizumab and were prescribed for autoimmune disease in 45 cases (69%), for malignancy in 5 cases (7.7%) and for ABO-incompatible kidney transplantation in 15 cases (23%). Overall, 20 patients (31%) received anti-CD20 treatment in the period after the second vaccination dose and before follow-up evaluation, with a median dose of 1 g (IQR: 0.5–1.0 g), among them 8 of the 33 patients receiving a third vaccine during follow-up (online supplemental table 1). Immunosuppressive comedication of patients was present in 41 patients (63%) and included corticosteroids within last 6 months in 32 cases (49%), calcineurin inhibitors in 17 cases (26%), antimetabolites in 21 cases (32%), methotrexate in 2 cases (3%), cytotoxic chemotherapy in 1 case (1%) or other immunosuppressive drugs in 3 cases (5%). No healthy volunteer was treated with immunosuppressive drugs.

Trajectories of immunity in humoral responders to two-dose SARS-CoV-2 mRNA vaccination

Serum samples of two-dose vaccine humoral responders were obtained a median of 6.8 months (IQR: 6.0–7.1) after the second vaccination and 4.6 months (4.4–5.0) after the initial assessment in volunteers versus 5.6 months (5.0–6.7) after vaccination and 3.9 months (3.5–4.0) after the first assessment in patients. We recorded a median spike S1 IgG level of 4.17 (3.1–5.8) Index s/c in volunteers and 3.7 (1.7–5.7) in the two-dose vaccine humoral responders with a history of anti-CD20 treatment (p=0.42) (figure 1A left panel, table 2). Anti-S1 IgG concentrations above the manufacturer's cut-off were present in 92% of volunteers and 88% of patients at follow-up. CMI was detectable in 44% (14/32) of patients compared with 69% (11/16) of healthy volunteers (figure 1B left panel, table 2).

In patients with a decline of anti-S1 IgG below detection cut-off, the initial concentration of anti-S1 was lower with 2.26 s/c (1.98–2.43) versus 7.14 s/c (4.89–8.55) in those with persisting antibodies (p<0.01). Moreover, patients with persisting antibodies tended to be younger at study enrollment and had lower rates of immunosuppressive comedication. Parameters of immunocompetence such as CD4, CD19 counts or total IgM were not associated with

Table 1 Baseline characteristics, vaccination history of patients and healthy controls, and anti-CD20 B-cell depletion history of patients in the study

	Baseline characteristics		
	Patients	Healthy volunteers	P value
	n=65	n=26	
Male sex (%)	33 (51%)	10 (38%)	0.3
Median age in years	66 (54, 73)	53 (45, 60)	0.002
Vaccine (BioNTech/Pfizer) (%)	41 (63%)	8 (31%)	0.005
Time between second vaccine dose and V2 Visit (months)	5.97 (5.23, 6.87)	6.75 (5.97, 7.16)	0.075
Time between third vaccine dose and V2 Visit (months)	1.15 (1.00, 1.31)	–	
Months between V1 and V2 visit (months)	3.90 (3.50, 4.43)	4.57 (4.35, 5.03)	0.004
Cumulative dose anti-CD20 (g)	3.00 (1.60, 5.00)	–	
Time between last anti-CD20 therapy and last vaccine dose (years)	1.26 (0.24, 3.68)	–	
Indication for anti-CD20 therapy			**<0.001**
Autoimmune disease	45 (69%)	–	
Haematological cancer	5 (7.7%)	–	
Kidney transplantation	15 (23%)	–	
Immunosuppressive co-medication			
Any	41 (63%)	–	
Any corticosteroids within last 6 months	32 (49%)	–	
Prednisolone equivalent >0 mg to 2.5 mg daily at follow-up	13 (20%)	–	
Prednisolone equivalent >2.5 mg to 5 mg daily at follow-up	10 (15%)	–	
Prednisolone equivalent >5 mg daily at follow-up	0 (0%)	–	
Calcineurin inhibitors	17 (26%)	–	
Antimetabolites	21 (32%)	–	
Methotrexate	2 (3%)	–	
Cytotoxic chemotherapy	1 (1%)	–	
Other	3 (5%)	–	

antibody persistence, neither was treatment history of rituximab (time since last dose and cumulative dose). In an analysis of covariance using linear regression models, anti-S1 IgG concentrations at follow-up depended on initial anti-S1 concentration (p<0.01) but were independent from anti-CD20 treatment status (p=0.8), indicating a similar rate of anti-S antibody clearance in both patients and healthy volunteers (online supplemental figure 2), (online supplemental table 2). Different indications for or additional doses of anti-CD20 therapies during follow-up did not significantly influence the antibody trajectories (online supplemental figures 3 and 4). In the present data set, presence of coimmunosuppression did not affect the persistence of anti-S1 antibodies (online supplemental figure 5).

Immune response to a third mRNA SARS-CoV-2 vaccine dose

Patients without an initial humoral response to a two-dose SARS-CoV-2 vaccine series were given a third dose of mRNA-1273 vaccine (n=8/32) or BNT162b2 mRNA COVID-19 vaccine (n=23/32) after a median period of 5.0 months (4.1–6.0) after their second vaccination dose. Assessments at follow-up revealed an anti-S1 IgG seroconversion in 19% (6/32) of these participants (figure 1A, right panel), and CMI in 32% (10/31) at follow-up (figure 1B, right panel). Three-dose humoral responders did not significantly differ from humoral non-responders with regards to demographic, clinical and immunocompetence parameters (online supplemental table 2). Third-dose humoral responders had higher CD19 counts at baseline (p=0.052) and received a lower cumulative anti-CD20 dose (p=0.13). Despite the very low patient numbers, we observed a tendency to higher initial anti-S in the three-dose vaccine humoral responders with 0.16 (0.14–0.29) s/c compared with non-responders with 0.12 (0.09–0.15) s/c (p=0.069). Different indications for or additional doses of anti-CD20 therapies during follow-up did not significantly influence the seroconversion rates after the SARS-CoV-2 third vaccine (online supplemental figures 3 and 4). Patients under coimmunosuppression tended to have higher immunogenicity of the third SARS-CoV-2 vaccine compared with those on anti-CD20 monotherapy, but this finding did not reach statistical significance (online supplemental figure 5). To summarise, overall only a fraction of patients with a

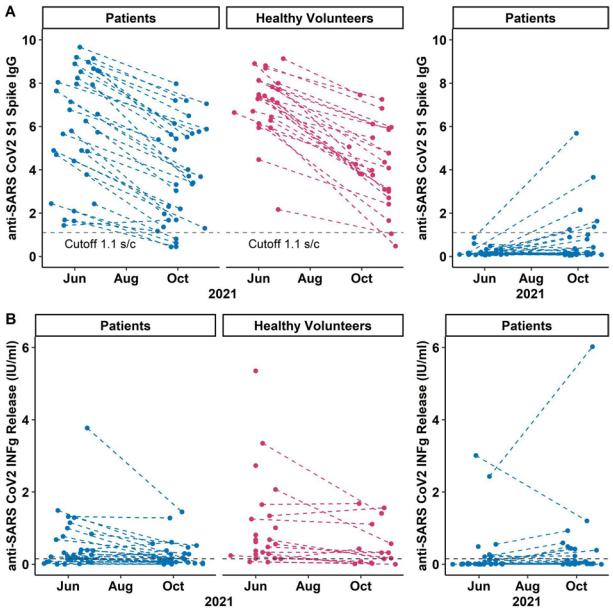

Figure 1 Humoral and cellular responses to anti-SARS CoV2 vaccines at follow-up. Anti-S1 Spike IgG levels at study visit 1 and 2 in patients and volunteers with two dose humoral response (A, left panel) and patients with a third dose vaccination (A, right panel). The dotted grey line denotes the cut-off anti-SARS-CoV-2 S1-IgG value of 1.1 (signal to cutoff ratio). Cell-mediated immunity was assessed at study visit 1 and 2 in volunteers and in patients with two dose humoral response (B, left panel) and patients with a third dose vaccination (B, right panel). The dotted grey line in B denotes the cut-off of 0.15 IU/mL. Each point represents one study visit. Intraindividual values are connected with dashed lines, the later one representing the follow-up visit after two vaccines (left panels), or the post-third vaccination visit (right panels).

history of anti-CD20 treatments presented seroconversion or CMI after a third SARS-CoV-2 vaccination.

DISCUSSION

Our longitudinal analysis of 91 participants of the initial investigator-initiated, single centre, open-label RituxiVac study[3] shows a comparable rate of decline of circulating antispike antibodies and CMI between patients with anti-CD20 therapies and healthy volunteers at 6 months of follow-up after a two-dose schedule of mRNA SARS-CoV-2 vaccines with 88% of patients and 92% of volunteers still presenting with detectable antibody levels and a surprising 44% of patients and 69% of volunteers displaying persisting CMI in the peripheral circulation. Moreover, we demonstrate that only a minority of patients with anti-CD20 therapies that were two-dose humoral non-responders developed antispike antibodies or CMI after a third SARS-CoV-2 vaccination. These findings may assist the design of future, more individualised vaccination strategies in this immunocompromised patient population.

The initial RituxiVac study[3] was finalised shortly before the Swiss Federal Office of Public Health allowed a third dose of SARS-CoV-2 vaccination in immunocompromised patients who did not show humoral responses to a two-dose series. Therefore, we converted the study design to a longitudinal observational study. Our main finding is, thus, that after a third dose of SARS-CoV-2 mRNA vaccines, only a subset of patients mounts a humoral immune or CMI response. Emerging reports by others have found a similar rate of around 25% de novo antispike seroconversion after a third dose of SARS-CoV-2 vaccines in patients on anti-CD20 therapies that were humoral non-responders after a two-dose vaccination scheme.[8–10 18–23] Several studies that used EliSpot or activation-induced markers to semiquantitatively assess CMI revealed higher rates of CMI than humoral responses after a third SARS-CoV-2 vaccine in patients with autoimmune diseases under anti-CD20 treatment.[8 19 24–26] These assays measure the fraction of circulating mononuclear cells or T cells that are activated by SARS-CoV-2 spike protein; by contrast, we here report a reduction of the overall spike protein-driven interferon-γ release in the whole blood of patients on anti-CD20 treatments, which may rather reflect the quantitative response in CMI. Others have further discriminated CMI after two SARS-CoV-2 vaccines in this patient population: vaccine-driven CD4 responses were more impaired than CD8+ cell responses, with severe impairment of circulating T follicular helper cell subpopulations in anti-CD20-treated patients, and vaccine-driven CD8 responses were absent in patients with low CD4 count.[19 27] Overall, CMI in anti-CD20-treated patients may be diminished or at least very heterogeneous after 2 or 3 SARS-CoV-2 vaccinations,

Table 2 Immune responses of patients and healthy volunteers at anti-SARS CoV2 S1 Spike IgG levels HumoHJumoral follow-up

	Anti-Spike IgG persistence		Third dose vaccine IgG response
	Patients, **N=33***	Healthy volunteers, **N=26***	Patients, **N=32***
S1 IgG positive	29/33 (88%)	24/26 (92%)	6/32 (19%)
Quantiferon positive	14/32 (44%)	11/16 (69%)	10/31 (32%)
Double positive	14 (32 (44%)	10/16 (62%)	2/31 (6.5%)
Double negative	4/32 (12%	1/16 (6.2%)	17/31 (55%)

Patients received two or three doses of mRNA vaccines, and healthy volunteers received two doses of mRNA vaccines against SARS-CoV-2
*n(N (%).

depending on patient population or comedication.[4] Together with the ubiquitously reported impaired humoral responses to SARS-CoV-2 vaccines in anti-CD20-treated patients, the consequence is undoubtedly an increased risk of severe breakthrough infections in this population, as reported from two potentially overlapping study populations.[28 29]

Antibodies and CMI in peripheral circulation decay at similar rates in patients with a history of anti-CD20 therapies compared with healthy volunteers, and the initial magnitude of immune response predicts persistence of anti-SARS-CoV-2 antibodies at a 6 months period. These data are in agreement with previous reports in the general population[30 31] and further support the current recommendation of the Swiss Federal Office of Public Health to provide all immunosuppressed patients access to a three-dose primary vaccination series.

Safety profiles of SARS-CoV-2 vaccines are good, including in patients with autoimmune disease.[1 19] Since factors such as coimmunosuppression and circulating lymphocyte subpopulations could assist in predicting immune responses to vaccination,[3] prospective studies should focus on these factors to enable individualised vaccination strategies and to determine optimal timing and number of additional vaccine doses in the immunocompromised.

The present study has some limitations. First, while we observed a decline of antibodies and CMI in the peripheral circulation, this is a natural phenomenon because SARS-CoV-2-specific T and B memory cells persist within lymph nodes and cannot directly be assessed in the peripheral blood.[32] Next, the present analysis did not include enough longitudinal measurements to allow for an in-depth modelling of the antibody decay dynamics that has been demonstrated in SARS-CoV-2 infection studies[33 34] or in selected reports of vaccinated healthy volunteers.[31] Furthermore, the magnitude of immune responses required for protection from severe disease in immunocompromised patients is unclear. Once the concentrations of circulating or neutralising antibodies required for protection of severe disease in immunocompromised patients are understood relative to those in the general population,[35] prediction models based on neutralising or total antispike antibody clearance rates and circulating peripheral immune cells could guide the administration of future vaccine doses. Also, immunogenicity in relation to different degrees of autoimmune disease activity is poorly understood. Finally, our study was underpowered to gain conclusive evidence from patient subgroup analyses, for example, according to type of disease. More insight into these phenomena is essential, because the heterogeneity of patients with anti-CD20 therapies and their immune response, the differences in their comorbidities, coimmunosuppression and in their environmental exposure to COVID-19 hinder the establishment of a simple generic algorithm for SARS-CoV-2 vaccination in this population. We, therefore, recommend to closely observe this population and invest in targeted public health strategies for different subsets of immunocompromised patient groups.

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Contributors MBM and DS conceived the study. MBM, MPH, CH, BM and DS designed the study. MBM, DA, AB, BM, LN, CM, AA-S, LB, SR-H, AC, RH, UB, L-YM, BM and DS recruited participants. MBM, SS, AB and DS performed computational chart review. MBM, FS-R, MPH, JMI, MN, CH, BM and DS performed analyses. MBM, BM and DS wrote the manuscript with input from all authors. MBM acts as the guarantor responsible for the overall content of the present work. All authors approved the final version of the manuscript.

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