Impaired T-cell response to mRNA vaccination heralds risk of COVID-19 in long-term allogeneic hematopoietic stem cell transplantation survivors

Initial reports suggested a COVID-19-associated mortality rate approaching 30% among allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients.[1] However, patient outcomes have improved with the introduction of effective vaccines and advancements in patient management, such as rapid diagnostic testing and antiviral therapies. Although new Omicron virus variants show increased transmissibility, they have also been associated with reduced risk of severe disease among vaccinated individuals.[2] Recent data suggests that mortality in fully vaccinated allo-HSCT recipients has decreased to as low as 1%.[3] Our previous findings show that a weak T-cell response following the first two doses of vaccination leads to reduced humoral immunity[4] and that allo-HSCT recipients who experience vaccine-related adverse events demonstrate augmented T-cell responsiveness.[5] While various studies have evaluated COVID-19 vaccination response in this patient cohort,[6,7] to our knowledge, this is the first report of allo-HSCT recipients with poor T-cell response following two doses of mRNA vaccine being at increased risk of acquiring subsequent COVID-19.

This study was conducted between March 2021 and November 2022 at the Sahlgrenska University Hospital in Gothenburg, Sweden (CONSORT diagram in Figure 1). Allo-HSCT recipients without prior history of COVID-19 (n=50), identified in local transplant registries in the Region Västra Götaland (population of approximately 1.7 million), were recruited to participate in this sub-study within the DurIRVac study. All participants gave written informed consent before enrollment. Enrolled allo-HSCT recipients fulfilled the following predetermined criteria: (i) to be at least 3 months post allo-HSCT, (ii) to not have received rituximab during the last 6 months, and (iii) to be without uncontrolled acute graft-*versus*-host disease (GvHD) (grade 3-4), all according to EBMT guidelines for COVID-19 vaccination (version 7, 2021). Patients were sampled before

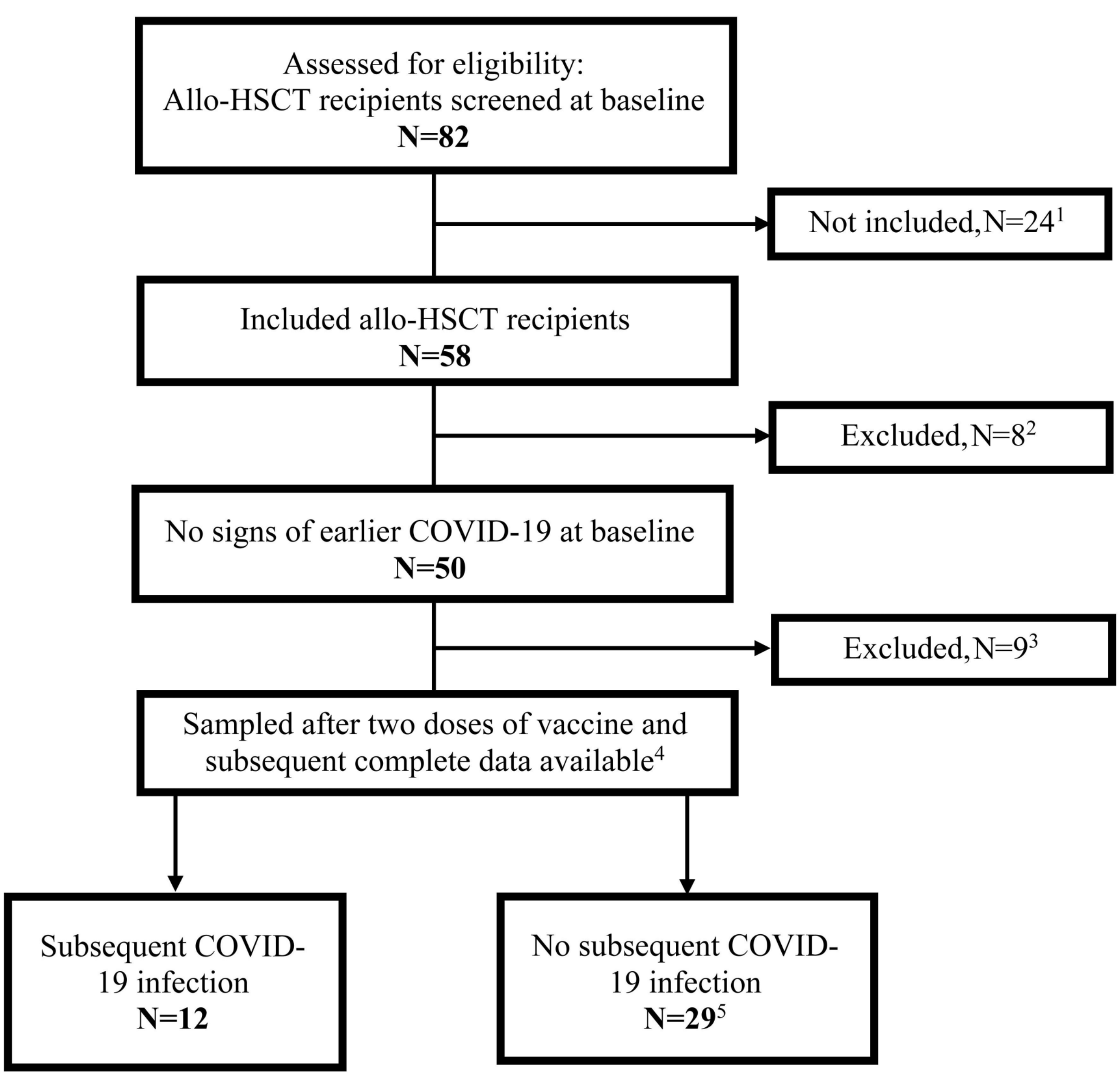

Figure 1. CONSORT-diagram for allogeneic stem cell transplantation recipients in the study. [1]Previously COVID-19 vaccinated (N=13); recent treatment with rituximab (N=4); hesitant to COVID-19 vaccine (N=2), current infection (N=2), declined participation (N=3). [2]Previously polymerase chain reaction (PCR)-confirmed COVID-19 infection (N=5) or seropositive at baseline (N=3).[3]PCR-positive between dose 1 and 2 (N=1); dead due to complications of severe graft-*versus*-host disease (N=1); relapse of underlying disease (N=3); lost to follow-up (N=4). [4]At least 2 blood samples available at the first 2 vaccine doses and data available on occurrence of COVID-19. [5]Complete data available on interleukin-2 (N=27) and anti-nucleocapsid immunoglobulin G (N=28).

and after the first two vaccine doses and then every 6 months. The cohort mainly consisted of long-term survivors with a median time following transplant of 90 months (range, 10-330), with no participant receiving any COVID vaccine dose prior to transplantation. The DurIRVac study was approved by the Swedish Ethical Review Authority (Etikprövningsmyndigheten; permit no. 2020-03276, 2021-00374 and 2021-00539) and by the Swedish Medical Products Agency (Dnr: 5.1-2021-11118) and has been registered at the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT no. 2021-000349-42). The researchers in this study had neither an influence on which mRNA COVID-19 vaccine (mRNA-1273 Moderna Spikevax® or BNT162b2 Pfizer-BioBTech Comirnaty®) participants received nor on the number or time point of vaccinations, as doses were administered in accordance with regional prioritization.

Chemiluminescent microparticle immunoassays (CMIA) were performed on serum using the automated Alinity system for quantitative measurement of immunoglobulin (Ig)G antibodies against the receptor-binding-domain (RBD) of the spike protein of SARS-CoV-2 (SARS-CoV-2 IgG II Quant, Abbott, Abbott Park, Illinois, USA) with levels reported in the World Health Organization international standard binding antibody units (BAU)/mL (quantitative detection range: 14-5,680 BAU/mL or equivalent to 1.14-3.75 log10 BAU/mL; samples reaching 5,680 BAU/mL were diluted with sero-negative serum and reanalyzed). Anti-nucleocapsid antibodies were analyzed at baseline and during follow-up sampling (November 2022).

Peripheral blood, collected in vacutainer lithium-heparin tubes (BD, Plymouth, UK), was stimulated with 15-mer peptides with 11-amino acid overlap spanning the S1 domain of the SARS-CoV-2 surface glycoprotein as described previously.[8,9] Plasma from whole blood cultures was recovered and stored at -80°C until analysis of IL-2. Levels of interleukin (IL)-2 in whole blood supernatants were determined by the FirePlex®-96 Key Cytokines Immunoassay (antibody [ab] 243549 and ab 285173, Abcam) according to the manufacturer's instructions. Samples were acquired on a BD LSR Fortessa (BD) and analyzed with FirePlex Analysis Workbench (Abcam). S1-specific responses were calculated by subtracting levels of IL-2 in unstimulated control samples from those in S1-stimulated samples. The S1 peptide-induced IL-2 has been shown to be contributed foremost by T cells, in particular $CD4^+$ T cells.[10]

All 41 patients were COVID-19-naïve 5 months after the second vaccine dose as per polymerase chain reaction (PCR), COVID-19 antigen test, analysis of antibodies against the nucleocapsid protein of SARS-CoV-2 (SARS-CoV-2 IgG, Abbott, Abbott Park, Illinois, USA), review of medical records, or evaluation of participant self-reporting questionnaire.

During the follow-up period after receiving the initial two vaccine doses, the patients were divided into two groups based on whether they had subsequent breakthrough COVID-19 infection or not (baseline characteristics detailed in Table 1). All 12 documented COVID-19 infections had detectable SARS-CoV-2 RNA (n=8), reactive COVID antigen test (n=3), and/or presence of antibodies against the nucleocapsid (n=2) in a sample obtained after the second vaccine dose. No severe breakthrough infections, i.e., requiring hospitalization or leading to death, were observed during the study. The Omicron variants (B.1.1.529) BA.1 and BA.2 dominated the local circulation during this study.

At 5 months after the second vaccine dose, the group of patients who experienced subsequent breakthrough COVID-19 infection (n=12) had a significantly lower median level of S1-induced IL-2 (52 pg/mL; range 7-141) compared to the group without later breakthrough infection (n=27) (174 pg/mL; range, 1-2,344; *P*=0.016, Mann-Whitney) (Figure 2). As baseline characteristics stratified according to COVID-19 breakthrough infection demonstrated a significant difference in the mRNA vaccine used (i.e., Moderna *vs.* Pfizer-BioNTech) for primary vaccination, this association between T-cell response 5 months after the second vaccination and risk of infection was confirmed in a subgroup of the cohort consisting of those <65 years of age, where all patients received BNT162b2 (Pfizer-BioBTech Comirnaty®) and were 11 of 12 infections occurred (*Online Supplementary Figure S1*). A receiver operating characteristics (ROC) analysis determined 145 pg/mL of S1-induced IL-2 as a cut-off level that best discriminated patients at risk for subsequent breakthrough infections. No patients with S1-induced IL-2 levels above this value after two doses of vaccination experienced breakthrough infection, compared to 52% of those with levels below (*P*<0.001, Fischer's exact test).

The antibody levels against RBD within the spike protein after two vaccine doses did not differ significantly between patients who experienced breakthrough infection and those who did not. The median antibody levels were 207 BAU/mL; range, 0-1,778 (2.32 log10 BAU/mL; range <1-3.25) in the group with subsequent COVID-19 *versus* 479 BAU/mL; range, 0-1413 (2.68 log10 BAU/mL; range <1-3.15) in the group without subsequent infection (*P*=0.89, Mann-Whitney; Figure 2). However, there was a significant correlation between antibody levels and IL-2 response following vaccination (r=0.51; *P*=0.001, Spearman's Ρ).

Of the 41 patients, 15 were above or equal to the age of 65 years. This elderly portion of the cohort differed in two ways from younger allo-HSCT recipients; only one of 15 patients had a breakthrough infection, and all were vaccinated with mRNA-1273 (Moderna Spikevax®). In contrast, all study participants below 65 years of age were vaccinated with BNT162b2 (Pfizer-BioBTech Comirnaty®), and 11 of 26 experienced breakthrough infection. A subgroup analysis showed that, also in the latter younger cohort, high levels

Table 1. Baseline characteristics of allogeneic stem cell transplantation recipients stratified by COVID-19 breakthrough infection status after receiving two mRNA vaccine doses and analyzing the complete sample data.

Characteristics	All patients N=41	COVID-19† following 2nd vaccination N=12	No COVID-19† following 2nd vaccination N=29	*P*
Age in years, median (range)	54 (29-78)	46.5 (29-78)	62 (40-75)	0.16*
Sex, N (%)				
Male	21 (51)	5 (42)	16 (55)	0.51**
Female	20 (49)	7 (58)	13 (45)	
Vaccine Moderna/Pfizer-BioNTech, N (%)	15/26 (37/63)	1/11 (8/92)	14/15 (48/52)	0.03**
Time in months from transplant to first vaccine dose, median (range)	90 (10-330)	108 (13-330)	89 (10-246)	0.58*
Conditioning intensity, N (%)				
RIC/MAC	20/21 (49/51)	5/7 (42/58)	15/14 (52/48)	0.73**
Donor source, N (%)				
RD /URD	10/31 (24/76)	4/8 (33/67)	6/23 (21/79)	0.44**
Diagnosis, N (%)				
AML	18 (44)	7 (44)	11 (44)	1.0**
CML	7 (17)	2 (13)	5 (20)	0.31**
MDS	4 (10)	1 (6)	3 (12)	1.0**
Other	12 (29)	6 (38)	6 (24)	0.13**
GvHD, N (%)				
Yes	15 (38)	4 (33)	11 (39)	1.0**
No	25 (63)	8 (67)	17 (61)	
Ongoing immunosuppression, N (%)				
Yes	7 (18)	2 (17)	5 (18)	1.0**
No	33 (83)	10 (83)	23 (82)	

†Definition: positive SARS-CoV-2 PCR, antigen test and or antibodies to nucleocapsid antigen. The presence of graft-*versus*-host disease (GvHD) was assessed at the visit prior to the first vaccine dose. Ongoing immunosuppression was registered if present at first vaccination. Statistics using Mann-Whitney U test (*) or Fischer´s exact test (**). Statistical analyses were performed using SPSS statistical software package (version 28). *P* values are designated as follows: *$P<0.05$ and **$P<0.01$. All indicated *P* values are two-sided. RIC: reduced intensity conditioning; MAC: maximal intensity conditioning; RD: related donor; URD: unrelated donor; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome.

of SARS-CoV-2 specific T cells, but not antibodies, following dual vaccination were protective against subsequent infection (*Online Supplemental Figure S1*). Notably, we did not observe significant differences in the immune responses elicited by two different vaccines (495 *vs.* 434 BAU/mL [2.69 *vs.* 2.64 log10 BAU/mL]; *P*=0.8; and 167 *vs.* 88 IL-2 pg/mL; *P*=0.3; following 2 doses of mRNA-1273 and BNT162b2, respectively). This finding suggests that the lower incidence of COVID-19 among individuals over the age of 65 might be attributed to their higher level of adherence to social distancing and other preventive measures, rather than differences regarding vaccine-induced immune responses.

The immunogenic mRNA COVID-19 vaccines elicit robust innate and adaptive immune responses.[11] Although T and B cells crosstalk, they have partially independent roles.[10] Higher neutralizing antibody levels against SARS-CoV-2 in healthy adults have been associated with protection against infection.[12] However, in the Omicron era, studies have reported a lack of association between antibody concentrations and the incidence of infection,[13] which was supported by our findings. The magnitude of the T-cell response has been shown to negatively correlate with disease severity.[14] However, an absolute protective effect against infection due to high T-cell activity in the absence of antibodies has not been established. A recent study of 572 vaccinated allo-HSCT recipients found a 1-year cumulative incidence of breakthrough infection of 15%, with antibody titers associated with both breakthrough infections and disease severity, though data on T-cell response were not reported.[15] As an increasing number of T-cell assays for SARS-CoV-2 (e.g., Qiagen QuantiFERON® SARS-CoV-2, Hyris' T-Cell Test, T-SPOT®.COVID, etc.) are becoming commercially available as complements to already accessible COVID-19 antibody tests, our findings might be easier to generalize to other healthcare settings.

Despite the small sample size, which may have resulted in some numerically very different results being statistically similar, and the inevitable risk of not detecting asymptomatic COVID-19 despite analyzing anti-nucleocapsid antibodies at baseline and follow-up, we conclude that a strong T-cell response might protect against acquiring

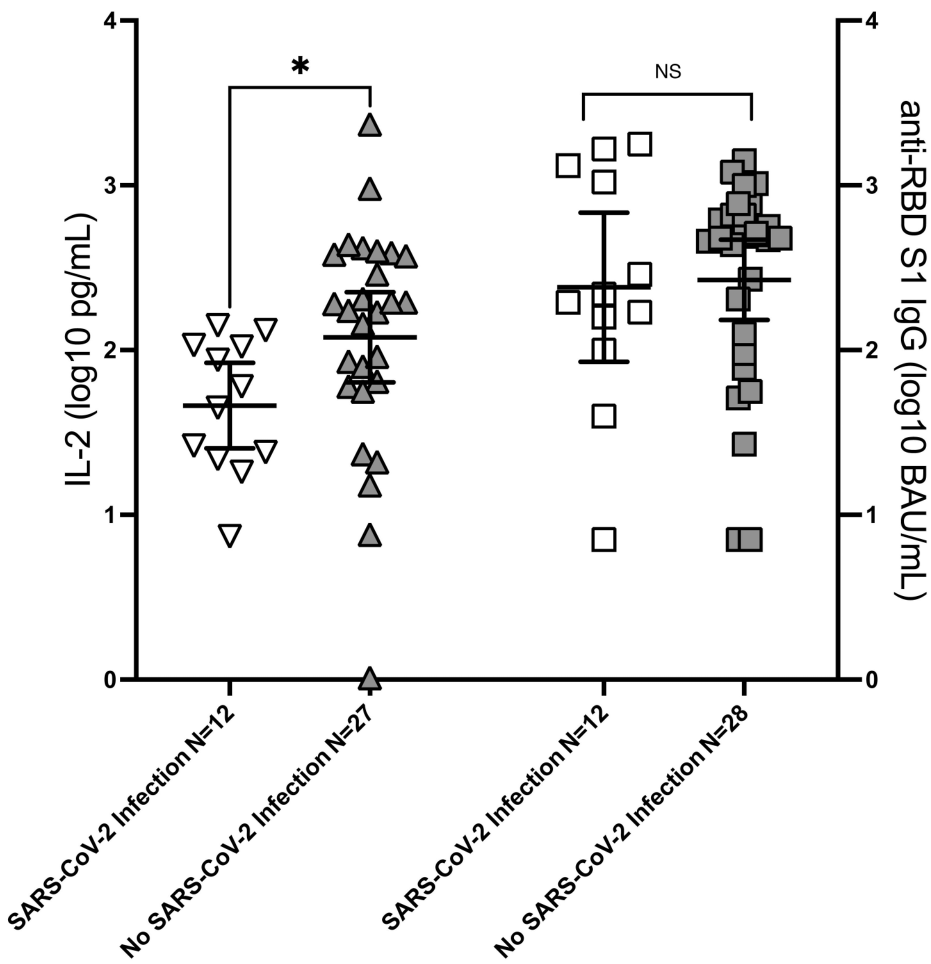

Figure 2. Humoral and T-cell-mediated immune responses 5 months after second mRNA COVID vaccination in allogeneic stem cell transplantation recipients stratified according to subsequent SARS-CoV-2 infection or not. Spike 1 (S1) specific immune responses in allogeneic stem cell transplantation recipients 3 months after the second vaccine dose demonstrated as T-cell production of interleukin (IL)-2 in supernatant plasma following stimulation of whole blood with S1 peptides and immunoglobulin (IgG) antibody levels in serum against the receptor-binding domain (RBD) in S1. Statistical comparisons were calculated by Mann-Whitney U test. Statistical analyses were performed using GraphPad Prism software (version 9). *P* value is two-sided and designated as $^*P<0.05$. BAU: standard binding antibody units.

breakthrough COVID-19. Additionally, antibody and T-cell responses following mRNA COVID vaccination were correlated in allo-HSCT recipients. This study suggests that antigen-specific T cells play a pivotal role in protection against COVID-19 and warrants future studies of their protective role against COVID-19 and infection with other respiratory viruses.

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https://doi.org/10.3324/haematol.2023.283551

Received: June 8, 2023.
Accepted: July 24, 2023.
Early view: August 3, 2023.

Disclosures

No conflicts of interest to disclose.

Contributions

ML, AM and KH were responsible for designing and writing the protocol, conducting the study, extracting and analyzing data, interpreting results, writing the manuscript, and updating the reference list. SE conducted the study, included all patients, participated in the extracting and analyzing of data, updated the reference list, and wrote the first draft of the manuscript. JW extracted and analyzed data, performed statistical analyses, created the figure and table, and participated in writing the manuscript. AM, AT, JR, JS and SM were responsible for the T-cell analyses, participated in the extraction and analysis of data and in writing of the manuscript. All authors have read and approved the last version of the manuscript.

Funding

This work was supported by the Swedish Medical Research Council (Vetenskapsrådet; grant no. 2021-04779), and the Gothenburg Society of Medicine (GLS-961772).

Data-sharing statement

For original data, please contact the corresponding author. As per Swedish law, individual participant data will not be shared.

References

1. Sharma A, Bhatt NS, St Martin A, et al. Clinical characteristics and outcomes of COVID-19 in haematopoietic stem-cell transplantation recipients: an observational cohort study. Lancet Haematol. 2021;8(3):e185-e193.
2. Ren SY, Wang WB, Gao RD, Zhou AM. Omicron variant (B.1.1.529) of SARS-CoV-2: mutation, infectivity, transmission, and vaccine resistance. World J Clin Cases. 2022;10(1):1-11.
3. Ljungman P, Tridello G, Piñana JL, et al. Improved outcomes over time and higher mortality in CMV seropositive allogeneic stem cell transplantation patients with COVID-19; an infectious disease working party study from the European Society for Blood and Marrow Transplantation registry. Front Immunol. 2023;14:1125824.
4. Einarsdottir S, Martner A, Waldenström J, et al. Deficiency of SARS-CoV-2 T-cell responses after vaccination in long-term allo-HSCT survivors translates into abated humoral immunity. Blood Adv. 2022;6(9):2723-2730.
5. Wiktorin HG, Einarsdottir S, Törnell A, et al. COVID-19 vaccine-induced adverse events predict immunogenicity among recipients of allogeneic haematopoietic stem cell transplantation. Haematologica. 2022;107(10):2492-2495.
6. Dhakal B, Abedin S, Fenske T, et al. Response to SARS-CoV-2 vaccination in patients after hematopoietic cell transplantation and CAR T-cell therapy. Blood. 2021;138(14):1278-1281.
7. Einarsdottir S, Martner A, Nicklasson M, et al. Reduced immunogenicity of a third COVID-19 vaccination among recipients of allogeneic hematopoietic stem cell transplantation. Haematologica. 2022;107(6):1479-1482.
8. Törnell A, Grauers Wiktorin H, Ringlander J, et al. Rapid cytokine release assays for analysis of SARS-CoV-2-specific T cells in whole blood. J Infect Dis. 2022;226(2):208-216.
9. Martner A, Grauers Wiktorin H, Törnell A, et al. Transient and durable T cell reactivity after COVID-19. Proc Natl Acad Sci U S A. 2022;119(30):e2203659119.
10. Törnell A, Grauers Wiktorin H, Ringlander J, et al. Induction and subsequent decline of S1-specific T cell reactivity after COVID-19 vaccination. Clin Immunol. 2023;248:109248.
11. Li C, Lee A, Grigoryan L, et al. Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine. Nat Immunol. 2022;23(4):543-555.
12. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med. 2021;27(7):1205-1211.
13. Smoot K, Yang J, Tacker DH, et al. Persistence and protective potential of SARS-CoV-2 antibody levels after COVID-19 vaccination in a West Virginia nursing home cohort. JAMA Netw Open. 2022;5(9):e2231334.
14. Rydyznski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell. 2020;183(4):996-1012.
15. Piñana JL, Martino R, Vazquez L, et al. SARS-CoV-2-reactive antibody waning, booster effect and breakthrough SARS-CoV-2 infection in hematopoietic stem cell transplant and cell therapy recipients at one year after vaccination. Bone Marrow Transplant. 2023;58(5):567-580.