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Short communication

Humoral and cellular responses to SARS-CoV-2 BNT162b2 vaccination in allogeneic hematopoietic stem cell transplantation recipients



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ABSTRACT

Previous studies reporting the response to SARS-CoV-2 mRNA vaccination in alloHSCT recipients used serological and/or cellular assays, but no study has evaluated vaccine-induced neutralizing antibodies.

We prospectively studied 28 alloHSCT recipients who received two BNT162b2 doses. Two patients groups were defined according to time from alloHSCT and immunosuppressive treatment, and had different baseline immunologic status. Study end-point was the evaluation of humoral and cellular responses one month after the second vaccine.

All patients seroconverted. Anti-S IgG levels and neutralizing antibodies percentages were not significantly different between both groups. Using IFN γ ELISpot assay, five patients showed a strong increase, without correlation with the humoral response. Using flow cytometry lymphocyte proliferation assay, 14 patients exhibited responding T cells, without difference between both groups or correlation with anti-S IgG levels. A few low serological responders had a detectable CD4 + T cell proliferative response. This finding should be confirmed in a larger cohort.

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1. Introduction

SARS-CoV-2 induced coronavirus disease 2019 (COVID-19) is associated with a mortality rate around 25% among allogeneic hematopoietic stem cell transplantation (alloHSCT) recipients [1,2]. In the general population, BNT162b2 mRNA vaccine confers a 90–95% protection against symptomatic COVID-19 [3,4]. However, the recent emergence of the B.1.1.529 (omicron) variant challenged the efficacy of vaccination, especially before the injection of a booster dose [5–8]. Previous studies of influenza and pneumococcus vaccines showed a weaker antibody response in alloHSCT recipients compared with the general population [9]. Previous studies [10–14] reporting responses to SARS-CoV-2 mRNA vaccination in alloHSCT recipients used serological and/or cellular assays,

but no study has evaluated vaccine-induced neutralizing antibodies (nAb) in this population.

2. Material and methods

Twenty-eight adult alloHSCT recipients transplanted for a hematological malignancy, who received two doses of the BNT162b2 vaccine from six months post-transplantation, were prospectively included between January and March 2021. All patients had baseline serological and immunological evaluation before vaccination (T0). The study end-point was the evaluation of humoral and cellular responses to the SARS-CoV-2 vaccine at a median of 30 days (range: 7–56) after the second dose (T2). All patients gave their consent for data collection before transplantation.

Testing for SARS-CoV-2 IgG was performed on the Abbott Alinity system, with qualitative detection of IgG antibodies against nucleocapsid protein (N) and quantitative detection of IgG against the RBD of the S1 subunit of the spike protein. Test

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results ≥ 50 arbitrary units (AU)/ml were reported as reactive and interpreted as positive for anti-S IgG. This assay has an analytical measuring interval of 21–40 000 AU/ml (up to 80 000 AU/ml with on-board 1:2 dilution). Non-responders were defined as anti-S IgG levels < 50 AU/mL, and low-responders as anti-S IgG > 50 but $< 4 160$ AU/mL, as this threshold corresponds to a 0.95 probability of virus neutralization in *in vitro* neutralization tests [15,16]. A SARS-CoV-2 surrogate neutralization assay, based on antibody-mediated blockade of ACE-2-spike protein interaction, was used (ichromax Covid-19 nAB, Boditech, South Korea). A fluorescence inhibition above 30% (meaning 30% interference with the SARS-CoV-2 spike RBD protein and ACE-2 receptor by neutralizing antibodies) is considered positive. This semi-quantitative assay correlates with a neutralizing SARS-CoV-2 Ab ELISA assay (Boditech Package insert).

Cellular responses were evaluated using a flow cytometry-based lymphocyte proliferation assay: Cell Trace Violet (CTV)-stained PBMCs (2×10^6 /mL) were stimulated for seven days at 37 °C in the absence (medium alone) or presence of the SARS-CoV-2 spike protein (2.5 μ g/mL, R&D systems), as reported [12]. Proliferation was quantified by flow cytometry according to CTV dilution in T cells, following surface staining with anti-CD3, -CD4 and -CD8 antibodies (BD Biosciences) and live/dead exclusion. Acquisitions were performed on a BD FACSCanto IITM flow cytometer and analyses performed using BD FACSDiva™ software version 7. Results were considered positive if $> 0.5\%$ of cells proliferated in presence of the spike protein (after subtracting proliferation in medium alone). Cellular responses were also evaluated using IFN γ enzyme-linked immunoassay (ELISpot) following overnight stimulation with 15-mer peptides spanning the SARS-Cov-2 spike protein (2 mg/mL, Miltenyi Biotec). Spots were counted using an automated ELISpot Reader System (Autoimmun Diagnostika

GmbH). In the absence of a control cohort, ELISpot results were evaluated as a fold increase (FI), normalizing the difference between T2 and T0 on the T0 value ($FI = (T2-T0)/T0$).

Categorical variables were compared using the two-sided chi-square test. Comparisons between groups of patients were performed using the Mann-Whitney test, and a comparison between timepoints using the Wilcoxon matched-pairs test. Correlations were performed using the Spearman test. $P < 0.05$ was determined significant. All statistical tests were performed with GraphPad Prism software version 8.4.0.

3. Results

Patient characteristics, immunological baseline evaluation and post-vaccination humoral response are detailed in Table 1. Two groups of patients were defined according to the time from alloHSCT and immunosuppressive treatment (IST) at vaccination. Group 1 comprised 14 patients within two years from alloHSCT or still receiving IST at vaccination ($n = 10, 61\%$). Group 2 included 14 patients beyond two years from alloHSCT, without IST. No patient had a clinical history of previous COVID-19 and the T0 serological assay was anti-N and anti-S IgG negative for all patients. Before vaccination, group 1 patients had significantly lower total peripheral blood lymphocytes, total and naive CD4 + T cells, and gamma globulins.

No patient had a clinical history of COVID-19 after vaccination and anti-N IgG were still negative at T2. No patient was a non-responder to the vaccine, but nine were low responders (seven patients from group 1 and two from group 2, $p = 0.043$) (Table 1). Median anti-S IgG level in the total cohort was 11,498 UA/mL (range: 236–80 000), with a trend towards lower levels in group 1 ($p = 0.08$) (Fig. 1A). The median anti-S IgG level was not signifi-

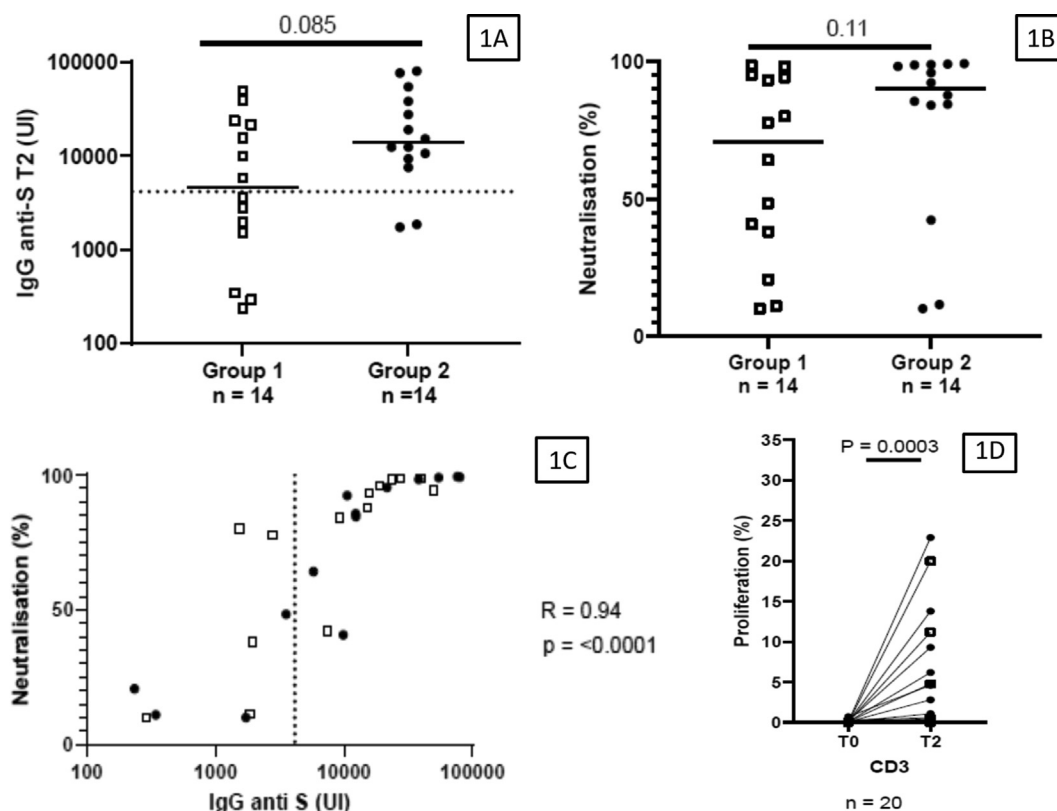


Fig. 1. A. Median titers of anti-S IgG in both groups. B. Comparison of neutralizing antibodies in both groups. C. Correlation between anti-S IgG level and neutralization. D. Flow cytometry CD3 + T cells proliferation assay.

cantly different between both groups. The median percentage of nAb was 86.8% for the entire population. Five patients had nAb titers below 30% (three in group 1 and two in group 2). In patients above 30%, median percentage of nAb was 92.4% (range: 40.7–99.4%), without a significant difference between groups (Fig. 1B). Anti-S IgG levels and nAb proportions were strongly correlated (R = 0.94, p < 0.0001) (Fig. 1C).

IFN γ T cell responses to spike peptide pools were available in 21 patients (10 in group 1 and 11 in group 2). Ten patients (48%) showed no increase in cellular response at T2 (FI < 1) and only five (24%) showed a strong increase (FI > 10). There was no correlation between the intensity of the cellular response and the anti-S IgG level or percentage of nAb.

T cell proliferative response to the spike protein was evaluated by flow cytometry in 21 patients (nine in group 1 and 12 in group 2). Fourteen patients (50%) exhibited spike-specific responding T cells at T2 (median 7.75%, range: 0.6–22.9), without difference between both groups (Fig. 1D). The proportion of proliferating cells was higher among CD4 + T cells than CD8 + T cells. Correlation between the CD4 + T cell proliferative response and anti-S IgG levels was barely significant (p = 0.06).

We did not expect a 100% seroconversion rate after SARS-CoV-2 vaccination in our cohort, as previous reports in alloHSCT recipients showed post-vaccine serological responses of 69% to 83%. [10–13] This high seroconversion rate could be related to the rather long interval from alloHSCT to vaccination in our cohort (median 5.6 years). In previous publications, Redjoul et al. report a 78% rate of seroconversion among 88 alloHSCT recipients vaccinated a median of six months after alloHSCT, while Le Bourgeois et al. report an 83% seroconversion rate among 97 alloHSCT recipients vaccinated a median of 645 days after alloHSCT. In these two publications, although the median time from alloHSCT to vaccination was quite different (six months, versus 1.7 years), the seroconversion rates were around 80%. This suggests that time from alloHSCT to vaccination by itself is not the most important factor for post-vaccination seroconversion, but has to be taken into account, alongside other factors, such as immunological recovery and immunosuppressive treatments, among others. Our results also highlight the fact that patients with acute and/or chronic GVHD requiring immunosuppressive treatment should still be offered anti-SARS-CoV-2 vaccination, as it may lead to a significant antibody and/or cellular response.

Table 1
Patient characteristics.

	Total	Group 1	Group 2	P value
Patients (n =)	28	14	14	
Median age at vaccination (range) (years)	51.5 (35–70)	56 (38–68)	58 (35–70)	0.83
Male sex	17 (61)	10 (71)	7 (50)	
Hematological disease				0.47
Acute myeloblastic leukemia	16 (56)	6 (43)	10 (71)	
Non-Hodgkin lymphoma	5 (18)	3 (21)	2 (14)	
Myelodysplastic syndrome	4 (14)	3 (21)	1 (7)	
Myeloproliferative neoplasm	3 (11)	2 (14)	1 (7)	
Other*	0	0	0	
Median age at transplantation (range) (years)	51.5 (29–65)	51 (35–65)	51.5 (29–65)	0.33
Myeloablative conditioning	9 (32)	5 (36)	5 (36)	0.87
Conditioning, including anti-thymocyte globulin	13 (46)	6 (43)	7 (50)	
Donor type				0.24
Matched related	12 (43)	7 (50)	5 (36)	
Matched unrelated	9 (32)	4 (28)	5 (36)	
Mismatched unrelated	5 (18)	1 (7)	4 (28)	
Haplo-identical	2 (7)	2 (14)	0	
Stem cell source				0.34
Peripheral blood	26 (93)	14 (100)	12 (86)	
Bone marrow	1 (3.5)	0	1 (7)	
Cord blood	1 (3.5)	0	1 (7)	
Acute or Chronic Graft-versus-Host-Disease		11 (79)	0	<0.0001
Immunosuppressive treatment		10 (61)	0	<0.0001
Immunological status before first vaccine dose				
Peripheral blood lymphocytes (G/L)	1.78	1.585 (0.7–3.65)	2.16 (1.46–3.6)	0.05
CD4+ (/mm3)	548	333 (79–1,212)	667 (334–1,459)	0.01
Naive CD4+ (/mm3)	93 (20Pt)	28 (8–187)	179 (25–375)	0.04
CD8+ (/mm3)	673	629 (45–2645)	673 (270–1,915)	0.6
CD19+ (/mm3)	353	270 (40–975)	428 (205–1,656)	0.09
CD56+ (/mm3)	243	234 (42–892)	313 (116–730)	0.35
Gamma globulins (g/L)	8.2 (27Pt)	6.4 (2.4–15.5)	10.7 (7.1–13.3)	0.008
(13Pt)				
Median time from alloHSCT to first vaccine injection (years) (range)	5.6 (0.6–16.8)	2 (0.6–14.1)	8.6 (3.1–16.8)	0.004
Serological response after two vaccine injections				
Anti-S IgG > 50 AU/mL	28 (100%)	14 (100%)	14 (100%)	
Anti-S IgG > 4 160 AU/mL	19 (68%)	7 (50%)	12 (86%)	0.043
Median levels of anti-S IgG levels (UA/mL)	11 498	4 680	13 774	0.08
(236–80 000)		(236–49 721)	(1 857–80 000)	
Median levels of anti-S IgG levels (if > 4,160 AU/mL)	18 985	21 671	17 067	0.9
(5 805–80 000)		(5 805–49 721)	(7 514–80 000)	
Neutralizing antibodies > 30%	23 (82%)	11 (78.6%)	12 (85.7%)	>0.99
Median titer of neutralizing antibodies	86.8% (<10–99.4)	77.8% (<10–98.7)	92.4% (<10–99.4)	0.11
Median titer of neutralizing antibodies (if > 30%)	92.4% (38.1–99.4)	80.2% (38.1–98.7)	94.2% (42.3–99.4)	0.08

* : Acute lymphoblastic leukemia, chronic lymphocytic leukemia, multiple myeloma, n = 1 each.

Anti-S IgG levels and nAb percentages were not significantly different between both groups, although their baseline immunological status was clearly different. The strong correlation between anti-S IgG levels and neutralization is particularly interesting with the emergence of SARS-CoV-2 variants, which question the threshold for positivity of antibody response. Although cellular responses were less frequently detected than serologic ones, we observed that a few patients considered as low serological responders had a detectable CD4 + T cell proliferative response. In the context of the emergence of SARS-CoV-2 variants associated with a lower efficacy of vaccine-induced nAb, the evidence of a CD4 + T cell proliferative response to the spike protein is important to maintain an immune response against SARS-CoV-2.

In view of these results, we studied six additional non-responder patients, who received a third vaccine dose a median of 56 days (range: 33–78) after the second vaccine injection. Median time from alloHSCT to first dose was 3.3 years (range: 0.4–10.9), and three patients were less than two years from alloHSCT or still receiving IST. A median of 38 days (range: 27–51) after the third dose, anti-S IgG were < 50 AU/mL in one patient and > 4 160 AU/mL in two others, leaving three low responders. The nAb were < 10% in non-responders and low responders, while a significant nAb response was observed in the two responders. The flow cytometry lymphocyte proliferation assay showed a specific CD4 + T cell response in three patients (two responders and one non-responder). Thus, a third dose of SARS-CoV-2 vaccine may induce a protective antibody response in a subset of alloHSCT patients, as previously reported [12].

4. Conclusions

Although based on a small cohort of alloHSCT recipients, our data show a high rate of serological response, including a high proportion of neutralizing antibodies, after BNT162b2 SARS-CoV-2 vaccination. We show that a cellular response can be detected in the absence of serological response. A similar observation has already been described in CAR-T cell recipients with complete B cell aplasia [10]. That a cellular response can be detected in the absence of a humoral response, particularly in the context of the lack of B cell recovery, should be further documented in a larger cohort, comprising patients receiving three vaccine doses.

CRedit authorship contribution statement

Alienor Xhaard: designed the study, recruited patients, collected data, analyzed the data, wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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