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Brief Article

B Cell Aplasia Is the Most Powerful Predictive Marker for Poor Humoral Response after BNT162b2 mRNA SARS-CoV-2 Vaccination in Recipients of Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Little is known about the immune response to SARS-CoV-2 vaccination in recipients of allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, several studies have reported that adequate protection could be provided to this population. The purpose of this study was to evaluate which factors can predict the efficacy of SARS-CoV-2 vaccination in these specifically immunosuppressed patients. Specific anti-Spike (S) antibody responses were assessed in a cohort of 117 allo-HSCT recipients after 2 injections of BNT162b2 mRNA SARS-CoV-2 vaccine (V1 and V2). Factors considered liable to influence the antibody response and analyzed in this series were the interval between allo-HSCT and V1, donor source, recipient and donor age, current immunosuppressive/ chemotherapy (I/C) treatment, and levels of CD4$^+$and CD8$^+$ T cells, B cells, and natural killer cells at the time of V1. Overall, the S-antibody response rate, evaluated at a median of 35 days after V2, was 82.9% for the entire cohort, with 71 patients (61%) reaching the highest titer. In univariate analysis, a lower pre-V1 median total lymphocyte count, lower CD4$^+$ T cell and B cell counts, ongoing I/C treatment, and a haploidentical donor were characteristic of nonhumoral responders. However, multiparameter analysis showed that B cell aplasia was the sole factor predicting the absence of a specific immune response (odds ratio, 0.01; 95% confidence interval, 0.00 to 0.10; $P < 10^{-3}$). Indeed, the rate of humoral response was 9.1% in patients with B cell aplasia versus 95.9% in patients with a B cell count >0 ($P < 10^{-9}$). These results advocate for the administration of anti-SARS-CoV-2 vaccination in allo-HSCT recipients as early as peripheral B cell levels can be detected, and also suggest the need for close monitoring of B-cell reconstitution after Allo-HSCT.

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INTRODUCTION

Coronavirus 19 (COVID-19) due to infection by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been responsible for more than 4 million deaths worldwide. Immunocompromised individuals, such as patients treated for hematologic malignancies [1−4], including recipients of allogeneic hematopoietic stem cell transplantation (allo-HSCT)

[5], represent a particularly high-risk population, with a reported mortality rate of 25% to 40%. The results of anti-SARS-CoV-2 vaccination are now being progressively reported in such patients and show a surprisingly high efficacy of 70% to 80%, a rate lower than that observed in the general population, however [6−8].

Recently, Ram et al [9]. published a study examining immune responses after anti-SARS-CoV-2 vaccination in a cohort of patients who underwent allo-HSCT or anti-CD19 chimeric antigen receptor T cell therapy that found a better post-vaccination humoral response in patients with higher levels of peripheral B cells. Notwithstanding that study, data remain scarce regarding factors predicting the humoral response after

Table 1
Patient Characteristics

Characteristics	All (N = 117)	Responders (N = 97)	Nonresponders (N = 20)	P Value
Recipient age, yr, median (IQR)	57.1 (44.2-65.9)	56.4 (44.1-65.9)	60.8 (45.3-65.1)	.55
Sex, n (%)				
Male	70 (59.8)	56 (57.7)	14 (70)	.44
Female	47 (40.2)	41 (42.3)	6 (30)	
Underlying disease, n (%)				
Myeloid	77 (65.8)	63 (64.9)	14 (70)	1
Lymphoid	36 (30.8)	30 (30.9)	6 (30)	
Recipient blood type, n (%)				
O	55 (47.0)	46 (47.4)	9 (45.0)	.39
A	43 (36.8)	37 (38.1)	6 (30.0)	
B	12 (10.3)	9 (9.3)	3 (15.0)	
AB	2 (1.7)	1 (1.0)	1 (5.0)	
Donor blood type, n (%)				
O	56 (47.9)	47 (48.5)	9 (45.0)	.55
A	45 (38.5)	37 (38.1)	8 (40.0)	
B	11 (9.4)	10 (10.3)	1 (5.0)	
AB	2 (1.7)	1 (1.0)	1 (5.0)	
Donor type, n (%)*				
Matched	79 (67.5)	70 (72.2)	9 (45.0)	**.02**
Haploidentical	36 (30.8)	25 (25.8)	11 (55.0)	
Donor age, yr, median (IQR)	38.6 (28.2-48.7)	37.8 (28.1-46.4)	42.4 (30.7-52.5)	.39
Conditioning regimen, n (%)				
Reduced intensity	87 (74.4)	70 (72.2)	17 (85.0)	.11
Myeloablative	23 (19.7)	22 (22.7)	1 (5.0)	
Sequential	7 (6.0)	5 (5.2)	2 (10.0)	
D0-V1 interval, d, median (IQR)	654 (372-1367)	914 (454-1455)	271 (198-395)	$<10^{-5}$
GVHD history, n (%)				
Yes	62 (53.0)	51 (52.6)	11 (55.0)	1
No	55 (47.0)	46 (47.4)	9 (45.0)	
Current I/C treatment, n (%)				
Yes	32 (27.4)	20 (20.6)	12 (60.0)	$<10^{-3}$
No	85 (72.6)	77 (79.4)	8 (40.0)	
Pre-V1 lymphocyte count, $\times 10^9$/L, median (IQR)	1.40 (0.71-2.27)	1.61 (1.01-2.33)	0.62 (0.47-1.24)	$<10^{-4}$
T lymphocytes	0.82 (0.42-1.32)	0.97 (0.49-1.39)	0.39 (0.15-0.85)	**.01**
TCD4	0.31 (0.16-0.49)	0.35 (0.22-0.52)	0.13 (0.08-0.23)	$<10^{-3}$
TCD8	0.38 (0.19-0.86)	0.45 (0.21-0.87)	0.23 (0.07-0.52)	.06
B lymphocytes	0.24 (0.08-0.46)	0.28 (0.16-0.51)	0.00 (0.00-0.00)	$<10^{-6}$
NK	0.20 (0.14-0.30)	0.21 (0.15-0.30)	0.14 (0.10-0.23)	.14

* Two patients received a graft from a 9/10 mismatched unrelated donor and were not considered for univariate analysis.

such vaccinations in immunocompromised hosts. Consequently, in the present study we retrospectively investigated factors, including immune status at the time of vaccination, that might influence the postvaccination antibody response after allo-HSCT.

METHODS

The main objective of the study was to decipher which factors can predict the humoral response after 2 injections (V1 and V2) of the BNT162b2 (Pfizer-BioNTech) vaccine in a cohort of 117 allo-HSCT recipients. The characteristics and outcomes of these patients have been reported previously [7,8].

Antibody response to the SARS-CoV-2 spike (S) protein receptor-binding domain was tested (Elecsys; Roche, Rotkreuz, Switzerland) at a median of 35 days (range, 18 to 77 days) post-V2. As recommended by the manufacturer, titers ≥0.8 U/mL were considered positive; the highest value recorded was >250 U/mL.

Factors considered for analyses were sex, underlying disease (myeloid versus lymphoid), recipient/donor ABO blood type, donor type (matched versus haploidentical), conditioning regimen (myeloablative versus reduced-intensity versus sequential), graft-versus-host-disease (GVHD) history, current immunosuppressive/chemotherapy (I/C) treatment, delay between transplantation and V1, and pre-V1 CD3 T cell, CD4 T cell, CD8+ T cell, B cell,

and natural killer (NK) cell counts. Total lymphocyte counts and quantitative lymphocyte subsets were evaluated by flow cytometry before V1.

Statistical analyses were performed with R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Patient characteristics were compared using the chi-square test for discrete variables and the Wilcoxon test for continuous variables, and generalized linear models were used to conduct multivariate analyses. All participants provided informed consent, and the study was approved by the Ethics Review Board of Nantes University Hospital.

RESULTS

The 117 allo-HSCT recipients enrolled were vaccinated between January 20 and April 17, 2021 (Table 1). The cohort had a median age of 57 years, with a predominance of males (60%) and patients being treated for a myeloid disease (66%). Donor source was matched in 67.5% of cases and haploidentical in 30.8%. Two patients who received a graft from a 9/10 mismatched unrelated donor were not considered for univariate analysis. At the time of V1, 62 of 117 patients (53%) had a previous history of GVHD, and 32 of 117 (27.4%) were

receiving ongoing I/C therapy. The average interval from allo-HSCT (day 0) to V1 (D0-V1) was 654 days (interquartile range [IQR], 372 to 1367 days). As reported previously [8], the S-antibody response rate post-V2 was 82.9% for the entire cohort, with 71 patients (61%) reaching the highest titer for this assay. Nonhumoral responders (NHRs) post-V2 (n = 20) had a lower D0-V1 interval (median, 271 days versus 914 days; $P < 10^{-5}$) and lower pre-V1 median total lymphocyte counts (0.62×10^9/L versus 1.61×10^9/L; $P < 10^{-4}$). Regarding lymphocyte subsets, NHRs displayed lower median CD3 (0.39×10^9/L versus 0.97×10^9/L; $P = .01$), CD4 (0.13×10^9/L versus 0.35×10^9/L; $P \leq 10^{-3}$), and B cell (0.00 versus 0.28×10^9/L; $P < 10^{-6}$) counts. NK and T CD8 counts were not statistically lower in NHRs (0.14×10^9/L versus 0.21×10^9/L [$P = .14$] and 0.23×10^9/L versus 0.45×10^9/L [$P = .06$], respectively). In addition, no influence was observed when considering the age of donors ($P = .39$) or recipients ($P = .55$), underlying disease ($P = 1$), allo-HSCT conditioning ($P = .11$), blood groups (donor, $P = .55$; recipient, $P = .39$) or a previous history of GVHD (83.1% versus 83.6%; $P = 1$). Conversely, ongoing I/C treatment and a haploidentical graft were associated with lower responses to vaccination (62.5% versus 90.5% [$P < 10^{-3}$] and 69.4% versus 88.6% for patients with matched donors, respectively [$P = .02$]). These data are shown in Table 1.

In multivariate analysis also including D0-V1 interval, donor source, current I/C treatment, and TCD4 lymphocyte count, only B cell aplasia remained statistically associated with lack of antibody response after 2 vaccinations (odds ratio, 0.01; 95% confidence interval, 0.00 to 0.10; $P < 10^{-3}$) (Figure 1). The rate of humoral response was 9.1% in patients with B cell aplasia versus 95.9% in patients with a B cell count >0 ($P < 10^{-9}$).

The characteristics of patients with B cell aplasia (n = 11) were compared with those of patients with a documented B cell count >0 (n = 73). B cell aplasia was related mainly to rituximab administration post-allo-HSCT and not to BTK inhibitor or CD19-directed treatments, which were given to only 4 patients.

Indeed, more patients with B cell aplasia had received rituximab post-allo-HSCT (63% versus 24.7%; $P = .01$). The indication for rituximab was EBV reactivation in all but 2 patients, in whom it was as part of chemotherapy for relapse. The median number of rituximab infusions was not statistically different between patients with B cell aplasia and those without B cell aplasia (6 [IQR, 3.5 to 6.5] versus 3 [IQR, 2 to 4]; $P = .06$), but, as expected, the time from the last rituximab infusion was shorter in patients with B cell aplasia (6 months [IQR, 5.2 to 8.8 months] versus 32.3 months [IQR, 17.0 to 43.8 months]; $P < .001$). No between-group difference was observed in the number of patients who had received rituximab before undergoing allo-HSCT (18% versus 8.2%; $P = .28$).

DISCUSSION

This study attempted to identify factors impairing a protective immune response after anti-SARS-CoV-2 vaccination in allo-HSCT recipients. The S-antibody response rate post-V2 was high, reaching 82.9% for the entire cohort, with 61% reaching the highest titer for this assay and thus likely much higher protective levels [10]. B cell aplasia clearly appeared as the major predictor of the absence of antibody response after 2 doses of anti-SARS-CoV-2 mRNA vaccine in this population. The overall response rate (83%) in our cohort is similar to that reported by Ram et al [9]. when taking into account the real population of 47 responders among 57 patients actually tested for humoral response in the Israeli cohort of allo-HSCT recipients. Of note, although B cell levels were correlated with humoral response, the cohort of Ram et al [9]. included only 1 allo-HSCT recipient with total B cell aplasia. Similarly, in a recent study by Malard et al [11]. including 41 allo-HSCT recipients, those with a B cell level $<120/\mu$L had significantly lower anti-S IgG levels at day 42 after the V2. One explanation is, of course, that low B cell numbers, as a reflection of immunodepression and/or previous anti-B cell therapy, may prevent antibody production after transplantation. Given that in healthy populations, SARS-CoV-2 mRNA vaccination induces persistent human germinal center responses [12], it also can be hypothesized that these specific responses are abolished in immunocompromised hosts. Finally, the possibility that these patients have developed a cellular response, which has not been studied here but was demonstrated by Ram et al [9]. in 7 of 37 patients, should be considered. In any event, these results advocate for close immune monitoring after allo-HSCT to administrate the vaccine immediately on B cell detection without waiting for a defined period as is currently the case. For patients with B cell aplasia or about to receive post-allo-HSCT rituximab therapy, other strategies, such as neutralizing antibodies for the prevention of COVID-19 infection, should be explored as well [13].

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Variable		N	Odds ratio		p
D0V1 intervall		82		1.00 (1.00, 1.01)	0.5
Pre-V1 T CD4 count		82		1.00 (1.00, 1.01)	0.8
Pre-V1 B count	Ctrl	71		Reference	
	B-cell aplasia	11		0.01 (0.00, 0.10)	<0.001
Current IS drug	No	60		Reference	
	Yes	22		0.46 (0.05, 4.42)	0.5
Donor	haploidentical	27		Reference	
	matched	55		2.48 (0.27, 22.84)	0.4

Figure 1. Multivariate analysis.

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