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Chronic graft *vs.* host disease and hypogammaglobulinemia predict a lower immunological response to the BNT162b2 mRNA COVID-19 vaccine after allogeneic hematopoietic stem cell transplantation

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Abstract. – OBJECTIVE: Due to the high mortality rate of COVID-19, the assessment of BNT162b2 SARS-CoV-2 mRNA vaccine (Pfizer-BioNTech) efficacy in allogeneic hematopoietic stem cell transplant (HSCT) recipients is mandatory.

PATIENTS AND METHODS: We conducted a single-center pilot study with the main objective of evaluating the immunogenicity of the BNT162b2 mRNA vaccine in 31 hematological patients who underwent hematopoietic stem cell transplantation within the previous 12 months and/or were affected by chronic graft-vs.-host-disease (cGVHD), by the assessment of antibody levels at 30-45 days after the second dose of vaccine.

RESULTS: After the second dose of vaccine, 23 out of 31 patients (74%) showed a positive immune response. The presence of severe cGVHD or Ig deficiency identified 7 out of 8 (85%) of non-responders. The median absolute cluster of differentiation 19 (CD19) count was significantly lower in non-responders vs. responders (109/μl *vs.* 351/μl). Underlying pathology, comorbidities, type of donor, time intervals from transplant and cluster of differentiation 3/cluster of differentiation 4/cluster of differentiation 8 (CD3/CD4/CD8) subsets were not significantly associated with an effective immune response to vaccination.

CONCLUSIONS: Despite the limited sample of patients enrolled, our findings suggest that hypogammaglobulinemia and cGVHD could be associated with poor humoral response to the BNT162b2.

Key Words:
Transplantation, COVID-19, Hypogammaglobulinemia, Chronic graft-versus-host-disease, cGVHD, BNT162b2, Vaccine.

Introduction

Patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) and other immune cell therapies are well-known to exhibit a lower response rate to vaccines than the general population[1,2]. In December 2020, the BNT162b2 mRNA vaccine (Pfizer-BioNTech) received conditional marketing authorizations in Europe for active immunization to prevent COVID-19 caused by SARS-CoV-2 in individuals 16 years of age and older[3].

Onco-hematological and other immunocompromised patients were excluded from initial studies investigating SARS-CoV-2 mRNA vaccine efficacy. However, cancer patients have at least a two-fold higher risk of experiencing COVID-19-associated intensive care unit admission, invasive ventilation, and death than the general population[4,5]. In addition, a recent meta-analysis of more than 3,000 patients with underlying hematologic malignancies and COVID-19 showed a mortality rate of 34%, which is three times higher than the general population[6].

It has been reported that HSCT recipients who contract COVID-19 have poor outcomes, with an overall survival (OS) rate of 68% in the 30 days following diagnosis[7]. Hence, HSCT recipients can be defined as an especially fragile group with high susceptibility to severe COVID-19 infection, with a strong clinical need to undergo effective immunization with the BNT162b2 mRNA vaccine. However, clinicians must consider that, in

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the early period after HSCT, immunological alterations, such as graft-*vs*.-host-disease (GVHD), can delay immune reconstitution, resulting in a poor response to the SARS-CoV-2 vaccine[8].

Higher mortality due to the COVID-19 pandemic among allogeneic HSCT recipients has encouraged clinicians to suggest vaccination, despite the low probability of response. According to the guidelines of the Italian Society of Hematology (SIE, Società Italiana di Ematologia) and the Italian Group of Bone Marrow Transplantation (GITMO, Gruppo Italiano Trapianto Midollo Osseo), published in April 2021, all consecutive patients who underwent allogeneic HSCT at our center within the previous 12 months and all patients suffering from grade III-IV chronic graft-*vs*.-host-disease or who previously underwent cluster of differentiation antigen 20 (anti-CD20) monoclonal antibodies (MoAb) based therapies (i.e. rituximab) regardless of the time since transplantation, were proposed for vaccination at least three months after HSCT[9].

Until now, data on outcomes of HSCT recipients with COVID-19 have been limited to small case series and single-center experiences[8-11]. Furthermore, data concerning the immunogenicity and efficacy of mRNA SARS-CoV-2 vaccines in HSCT patients remain limited to single-center reports or small studies reporting impaired humoral and/or cellular immune responses[12]. The primary aim of the present study was therefore to evaluate the antibody response to the BNT162b2 mRNA vaccine and its safety in a cohort of HSCT recipients. The secondary aim was to explore the immunological status of patients and other clinical and laboratory parameters which may potentially predict vaccine response. Finally, we reported the occurrence of possible symptomatic COVID-19 even after vaccination.

Patients and Methods

We conducted a single-center pilot study with the main aim of evaluating the immunogenicity of the BNT162b2 mRNA vaccine in 31 consecutive patients who underwent HSCT for hematologic malignancies within the previous 12 months and/or were affected by cGVHD. Patients with a previous history of SARS-CoV-2 infection were excluded.

Patient baseline demographic and disease characteristics are summarized in Table I.

All patients received two vaccine doses (30 μg per dose, on days 0 and 21 according to the manufacturer's instruction) between March and June 2021. Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki. Anti-spike antibody titers were assessed 30-45 days after the second dose of vaccination in accordance with the manufacturer's instructions using the Liaison® TrimericS IgG Diasorin, Rome, Italy (sensitivity 98.7%, specificity 99.5%)[13]. Patients with IgG SARS-CoV-2 < 15.0 arbitrary units/ml (AU/ml) were considered non-responders and those with IgG SARS-CoV-2 > 15.0 AU/ml were defined as responders, in accordance with the manufacturer's instructions[13].

We further performed immunophenotyping (peripheral blood, BD Bioscences FACSLyric System, Franklin Lakes, New Jersey, USA), focusing on the analysis of T, B, and NK lymphocytes before the first vaccine dose, in order to identify any possible correlation between single patient immune response and quantitative lymphocyte subsets.

Chronic GVHD was graded according to National Institutes of Health (NIH) Consensus Criteria and medical impairment and comorbidities were assessed using the Cumulative Illness Rating Scale (CIRS) at the time of vaccination. Hypogammaglobulinemia was defined as IgG titer < 700 mg/dl, IgM < 40 mg/dl, and IgA < 70 mg/dl. A deficit of more than one Ig class was defined as Combined Antibody Deficiency (CAD).

Statistical Analysis

Categorical variables were compared using the chi-square test and Fisher's exact test, whereas continuous variables were compared using the Mann-Whitney test. The binary logistic regression models were fitted to assess odds ratios (OR). All analyses were performed using the statistical software Prism 5.04 (GraphPad Software, La Jolla, CA, USA); statistical significance was set at $p < 0.05$. Univariate analysis was performed to correlate the antibody response to cGVHD, donor type (matched-sibling, haploidentical, matched-unrelated), immunosuppressive therapy, hypogammaglobulinemia, cluster of differentiation 19/ cluster of differentiation 4/cluster of differentiation 8/cluster of differentiation 3/natural killer (CD19/CD4/CD8/CD3/NK) counts, comorbidities, and infections within 12 months prior to the vaccine. The status of all enrolled patients was updated on November 15, 2021.

Table I. Patient baseline demographic and disease characteristics.

	Total	Negative <15.0 AU/ml	Positive >15.0 AU/ml	*p*-value
Sex				
Male	21	6	15	0.69
Female	10	2	8	
Infections in the past year				
Yes	21	5	16	0.68
No	10	3	7	
Age				
>50 yrs	15	4	11	1
<50 yrs	16	4	12	
CIRS				
<6	22	5	17	0.659
>6	9	3	6	
Time from HSCT				
>12 M	16	5	11	0.47
<12 M	15	3	12	
Underlying disease				
Myeloid neoplasm/leukemia	21	6	15	0.69
Lymphoid or plasma-cell neoplasm	10	2	8	
N° of line of treatment				
<1	18	4	14	0.68
>1	13	4	9	
Donor				
Haploidentical	8	2	6	0.94
Related HLA-identical	14	4	10	
MUD	9	2	7	
cGVHD				
Absent to moderate	24	4	20	**0.03**
Severe	7	4	3	
N° of IST drugs at the time of vaccination				
0-1	18	3	15	0.17
>1	13	5	8	
ALC				
>900/mcl	24	6	18	0.84
<900/mcl	7	2	5	
CD3				
>690/mcl	20	6	14	0.47
<690/mcl	11	2	9	
CD4				
>410/mcl	11	4	7	0.31
<410/mcl	20	4	16	
CD8				
>190/mcl	25	7	18	0.56
<190/mcl	6	1	5	
CD19				
>50/mcl	28	6	22	0.08
<50/mcl	3	2	1	
N° of Ig class deficit				
0-1	26	4	22	**0.008**
2-3	5	4	1	
Severe cGVHD and/or >1 Ig class defict				
Absent	18	1	17	**0.002**
Present	13	7	6	

CIRS, Cumulative Illness Rating Scale; HSCT, Hematopoietic Stem Cell Transplantation; MUD, Matched Unrelated Donor; cGVHD, Chronic Graft-versus-Host-Disease; IST, Immunosuppressive Therapy; ALC, absolute lymphocyte count.

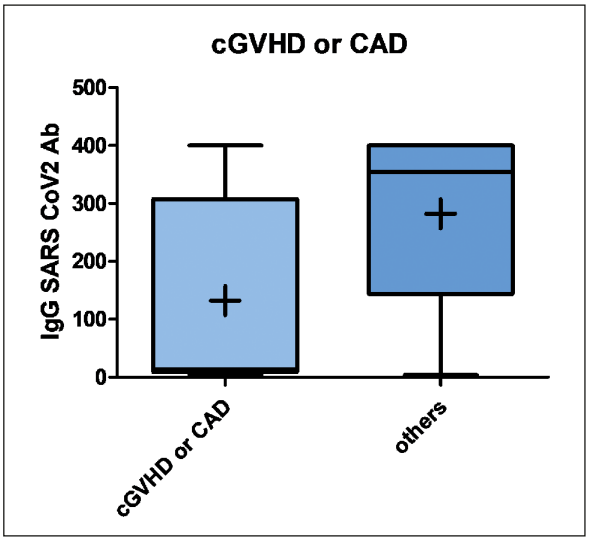

Figure 1. IgG SARS-Cov2 titer in non-responders patients with severe cGVHD or Ig deficiency.

Results

The median age at transplantation was 44 years (range 19-66). The median time between HSCT and the first vaccine dose was 16 months (range 4-300 months). Overall, the most common hematological disease was acute myeloid leukemia (n = 21/31, 68%), followed by lymphoid and plasma cell neoplasms (n = 10/31, 32%). Twenty-two patients out of 31 (71%) were on active immunosuppressive therapy at the time of vaccination for either cGVHD treatment or prophylaxis and seven (22%) were taking a low dose (< 0.5 mg/Kg/die) of oral steroids for respiratory and skin complications not related to cGVHD. Nineteen out of 31 patients (61%) had cGVHD (16 mucocutaneous, one liver, one lung, one joint and fascia), graded severe in seven out of 19 (37%) cases (Table I).

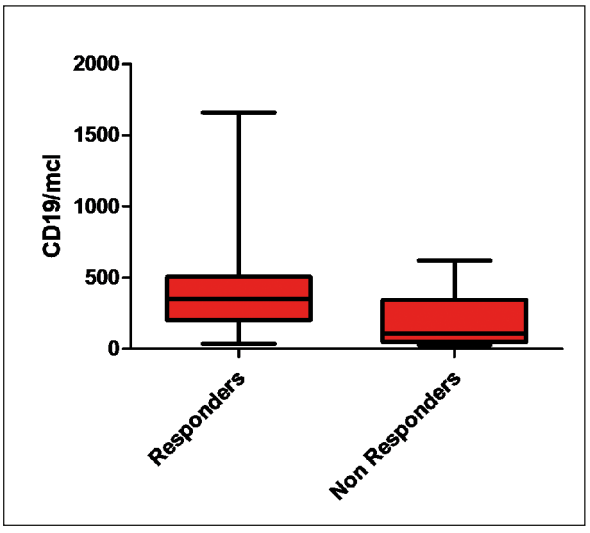

Figure 2. Median absolute CD19 count in Responders (351/µl; 95% CI 263-553) vs. non-responders (109/µl; 95% CI 20-370).

After the second dose of the vaccine, 23/31 patients (74%) showed a positive immune response with a median titer of 303 AU/mL (36-400).

Only one of the 31 vaccinated patients, with a previous effective vaccine response (IgG > 400 AU/ml) developed completely asymptomatic SARS-CoV-2 infection 4 months after the second dose of the vaccine. Non-COVID-19 related infection occurred in 68% of the patients (n = 21/31) during the 12 months prior to vaccination.

Factors associated with vaccine response failure were represented by the presence of severe cGVHD (odds ratio [OR] 0.09, 95% confidence interval [CI] 0.013-0.588 p = 0.008) and CAD (OR, 0.045; 95% CI 0.004–0.51 p = 0.0045). The presence of severe cGVHD or hypogammaglobulinemia identified 85% of non-responders (OR 0.05 95% CI 0.005–0.499 p = 0.001) (Figure 1). Baseline IgG level < 700 mg/dl was observed in six patients (four non-responders and two responders, OR 0.09; 95% CI 0.01-0.70 p = 0.016), IgA < 70 mg/dl in six patients (four non-responders, two responders, OR 0.09; 95% CI 0.01-0.70 p = 0.016), and IgM < 40 mg/dl in four patients (two non-responders, two responders, p = 0.26). None of the 31 patients had received immunoglobulin replacement therapy.

The median absolute CD19 count was 351/µl in responders (95% CI 263-553) *vs.* 109/µl (95%-CI 20-370) in non-responders (p = 0.018) (Figure 2). No association was observed between underlying disease (myeloid *vs.* lymphoid neoplasm) and CD19 lymphocyte count < 50/µl. Underlying pathology (p = 0.94), CIRS (median score of 3, range 0-9) (p = 0.6), type of donor (p = 0.94), age at time of vaccination, timing from transplant (before or after 12 months, p = 0.47), and CD3/CD4/CD8 subsets (p = 0.47; p = 0.31; p = 0.56, respectively) were not significantly associated with the immune response to vaccination. In addition, the dose and number of immunosuppressive drugs administered at the time of vaccination did not affect the antibody response (p = 0.17). Mild adverse events after vaccination were reported in 6 out of 31 patients (19%) and were mainly constituted by injection site pain, while systemic reactions such as asthenia and worsening cGVHD were found in less than 10% (n = 3/31) of patients.

Discussion

The identification of predictive factors of immune SARS-CoV-2 vaccine response appears to be essential in HSCT recipients and clinical and biological parameters with a predictive value for serum conversion still need to be identified[7,14]. However, the lack of a correlation between post-vaccine antibody titer and clinical protection from severe infection in humoral responder patients, and the possibility of cell-mediated immune response in humoral non-responders need to be considered. In agreement with previously reported data[5], we observed in our cohort a rate of seroconversion similar to that of the general population and we confirmed that hypogammaglobulinemia and cGVHD are associated with poor humoral response to the BNT162b2 vaccine. The impact of cGVHD on vaccine failure appears to be greater than the dose and number of immunosuppressants.

It has been reported that a CD19 lymphocytes count < 20/μl is a sign of delayed recovery of the cellular B compartment and is consequently a predictive factor for poor or ineffective vaccine response[6]. Furthermore, in our cohort the median absolute CD19 count was significantly higher in responders than in non-responders and no cases of death or severe COVID-19 were reported. This evidence could suggest a possible predictive role of CD19 count in preventing severe SARS-CoV-2 infection.

Our study confirmed the immunogenicity of the BNT162b2 mRNA vaccine in HSCT patients and underlined the impact of hypogammaglobulinemia and cGVHD which predict a poor humoral response to the vaccination. Optimizing humoral response with specific vaccination strategies, especially in patients with lack of serologic response, is a topic of current and future investigations, and will be important for guiding subsequent strategies of immunization such as boosting dose, pre-interventional vaccination, and eventually, pausing of concomitant immunosuppression.

Our results, although statistically significant, are limited by the small size of the cohort. Further longitudinal data are required to verify whether the generated anti-spike antibody titer is adequate to provide antiviral protection over time.

Conclusions

We observed that allogeneic-HSCT recipients with chronic GVHD and deficit of more than one Ig class presented lower antibody levels if compared to patients without severe cGVHD and normal Ig titers. Similarly, using flow cytometry analyses at the time of vaccination we noticed that median absolute CD19 count was significantly lower in non-responders. Of note, rates of adverse events to vaccination were low and we didn't observe cases of severe SARS-CoV-2 infection.

In conclusion, we confirm the safety and the immunogenicity of vaccination and the importance of immunization in allogeneic HSCT recipients.

Conflict of Interests

All the authors declare no conflict of interest.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee of the ARNAS Brotzu Hospital, Cagliari, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Availability of Data and Material

Data supporting the reported results can be obtained by contacting the corresponding author.

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Authors' Contributions

LB, AG, RM designed and performed the study, analyzed the data and wrote the manuscript. MG, GA contributed essential reagents or tools. OM, CT, EP, AV, VF, AC collected the data. G C, GLN project administration.

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