

LETTER TO THE EDITOR



The booster dose of BNT162b2 mRNA vaccine in patients after hematopoietic stem cell transplantation: humoral and clinical response and serological conversion predictors

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Despite the approval and introduction of new drugs into clinical practice for the treatment of patients with COVID-19 (molnupiravir, Nirmatrelvir) [1,2], vaccination remains the most effective weapon in the fight against the pandemic. The administration of two doses of the BNT162b2 vaccine has already been well-described as a safe and effective form of COVID-19 prophylaxis in hematopoietic stem cell transplantation (HSCT) patients [3]. Furthermore, risk factors that predispose HSCT recipients to a poor humoral response, such as the use of immunosuppressive drugs, a low peripheral blood B-lymphocyte count, and a low absolute lymphocyte count, are becoming better identified and understood [4–6].

In January 2022, we published data on the efficacy and safety of two doses of the BNT162b2 vaccine in patients after autologous and allogeneic HSCT [4]. Out of the 93 patients vaccinated with two doses of the BNT162b2 vaccine in March and April 2021, only four developed symptomatic COVID-19 as confirmed by PCR test during the observation period, with data censored at the end of September 2021. Among them, one patient died due to COVID-19. It is worth noting that this patient received multiagent aggressive immunosuppressive treatment for GvHD and did not develop antibodies after two doses of the BNT162b2 vaccine.

Herein, we report long-term follow-up in the same study group, in which we continued clinical observation for the occurrence and severity of COVID-19, late vaccine complications, the course or occurrence of GvHD, and other post-transplant complications until August 2022. Moreover, in October 2021, we also offered these patients vaccination with a third dose of the vaccine in accordance with the European Hematology Association (EHA) recommendations. In patients who remained under observation and agreed to be vaccinated, we assessed the concentration of antibodies before and after the administration of the third dose. Additionally, peripheral blood leukocyte analysis by flow

cytometry was performed to confirm earlier observations of lymphocyte subpopulations associated with vaccine efficacy. We also collected data on the incidence and course of COVID-19 after the booster dose of the vaccine during a 6-month follow-up period. Patients who opted not to receive a third dose of the BNT162b2 vaccine were evaluated solely for SARS-CoV-2 infection and COVID-19 symptoms, allowing us to compare clinical data between patients who had received either two or three doses of the vaccine. The protocol was conducted according to the Declaration of Helsinki, approved by the local ethics committee, and informed consent was obtained from all patients.

For the current analysis, a full evaluation of the clinical course was possible in 83 out of the 93 subjects described in our previous study, with data censored at the end of August 2022 [4]. Ten patients were lost to follow-up due to distinct reasons, including non-response to questions about their COVID-19 status, death, or progression of underlying disease before a final evaluation. Among the 83 evaluable patients, 68 (21 after autologous and 47 after allogeneic HCT) were vaccinated with a booster dose and evaluated for antibodies before and after the third dose, while 15 patients refused the booster dose. The median time between the 2nd and 3rd doses of the vaccine was 168 days (range 144–244). The median antibody concentration assessed within 48 h before vaccination with the 3rd dose was significantly lower ($p < 0.0001$) than the concentration determined 2–4 weeks after the 2nd dose: 2694.22 BAU/mL (range from 0 to ≥ 5680.00) vs 343.72 BAU/mL (range 0 to ≥ 5680.00), $p < 0.001$. The median ratio of anti-SARS-CoV-2 antibody concentration before the 3rd vaccine dose to the concentration after 2nd vaccine dose was as low as 0.15 (range 0.03–1.0). Interestingly, this ratio was positively correlated with the number of circulating NKT CD3+ CD16+ CD56+ cells ($R = 0.47$, $p = 0.002$) and CD3+ CD4- CD8- cells ($R = 0.35$, $p = 0.03$). The

booster dose resulted in a significant increase in antibody concentration, with a median anti-SARS-CoV-2 antibody concentration after the 3rd dose of 4486.65 BAU/mL (range from 0.62 to ≥ 5680.00) ($p < 0.001$). Of note, the percentage of B lymphocytes among all lymphocytes correlated positively with concentration of antibodies before and after administration of third dose ($R\ 0.56$; $p = 0.0001$ and $R\ 0.50$; $p = 0.0032$, respectively).

In August 2022, we conducted a re-evaluation of the clinical effectiveness of the vaccine. Among the 83 evaluated patients, 12 tested positive for SARS-CoV-2 infection based on PCR or antigen tests. Out of these 12 patients, 5 developed COVID-19 symptoms. Positive results were obtained from asymptomatic patients who were tested due to contact with a COVID-19 patient or during routine testing prior to hospital admission. Notably, 2 of the patients who developed a severe course of the disease did not develop antibodies after vaccination. Among the 68 patients who received a booster dose, 65 responded to the questionnaire in August 2022. Out of these respondents, 8 (12%) reported testing positive for SARS-CoV-2, and 3 of them experienced mild symptoms. Among the 15 patients who did not receive the booster dose, only 8 responded to the COVID-19 questionnaire. Out of these respondents, 4 (50%) tested positive for SARS-CoV-2 through antigen or molecular testing, and 2 developed symptomatic infection. This infection rate is significantly higher compared to the rate observed in patients who received 3 vaccine doses ($p = 0.021$). However, these results should be interpreted with caution due to the limited number of patients and the reliance on self-reported questionnaire responses.

In terms of toxicities, based on our experience, vaccination with the third dose was as safe as the two previous doses. There was no increase in cytopenia, exacerbation of graft-versus-host disease (GvHD) symptoms, or other grade III-IV adverse events, which is consistent with observations from other study groups. Most of the literature also supports the safety and immunogenicity of the BNT162b2 vaccine in HSCT patients [7–9].

Additionally, other authors have confirmed a decrease in antibody levels over time after the second vaccine dose [10]. It has also been demonstrated that the use of a booster dose results in an increase in antibody concentration, although this growth is highly dependent on patient-related factors such as immunosuppressive treatment, chemotherapy, or immunotherapy at the time of vaccination. It has also been shown that low absolute B lymphocyte count, CD4⁺ lymphocyte count, and total absolute count are associated with a low post-vaccination antibody concentration [3,4,11,12].

In this paper, we confirmed the positive correlation between the percentage of B lymphocytes and the concentration of antibodies after approximately 6 months after the second dose. Moreover, in our present analysis, we found a positive correlation between the relative decrease in the anti-SARS-CoV-2 antibodies (measured by the ratio of antibody concentration before the 3rd vaccine dose to concentration after the 2nd vaccine) with the number of CD3⁺ CD16⁺ CD56⁺ NKT cells and double negative T lymphocytes (CD3⁺ CD4[–] CD8[–]) count, which has not been previously reported. While this might seem like a

coincidental correlation, there are a number of publications indicating the effect of NKT cells on the response to vaccination [13,14]. As for the percentage of CD3⁺ CD4[–] CD8[–] cells, it has been observed in patients with moderate and severe course of COVID-19 [15]. It is worth noting that the above-mentioned populations of lymphocytes are an object of interest in modern immunology. It is difficult to clearly determine their role in the immune response, but changes in the percentages of these populations are described in autoimmune diseases and distinct neoplasms [16,17].

The use of drugs directed against the CD20 antigen is one of the main risk factors of the poor response to SARS-CoV-2 vaccination [18]. It is worth noting that the only patients who did not develop antibodies after the booster dose were those who received rituximab at the time of vaccination. The use of anti-SARS-CoV-2 vaccination in patients treated with rituximab remains controversial. It is well known that these patients have an impaired humoral response to vaccination, although there is mounting evidence that vaccination induces a cellular response [19]. Considering the safety of vaccination and the fact that later doses of the vaccine induce a stronger response, it seems reasonable to continue vaccination during anti-CD20 treatment [20,21].

A weakness and, at the same time, a strength of our publication is the significant group of patients who did not receive the third dose. This allowed us to evaluate the clinical efficacy of the vaccine in patients who received three doses and those who were vaccinated with only two doses, under similar epidemic conditions. Importantly, patients who received three doses only developed asymptomatic SARS-CoV-2 infections or mild COVID-19 symptoms. This observation strongly suggests the high efficacy of the booster dose in preventing severe COVID-19 in patients after HSCT. It is noteworthy that the period of observation between January 2022 and August 2022 coincided with a sudden increase in SARS-CoV-2 infections in general. Additionally, this was the time when the omicron variant became dominant in the Polish population [22].

We believe that sharing experiences and collecting new data on the use of SARS-CoV-2 vaccination in post-HSCT patients, especially those on immunosuppressive treatment, will help determine the optimal timing and protocol for vaccination. This is particularly important in the context of the emergence of a new prophylactic option based on monoclonal antibodies for patients on immunosuppressive treatment.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [M.M.], upon reasonable request.

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