Monitoring immune response to BNT162b2 mRNA COVID-19 vaccine in a woman with multiple myeloma and breast cancer anamnesis

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ABSTRACT

Vaccination against SARS-CoV-2 is an important preventive strategy against COVID-19 particularly in frail patients. Recent literature reports about suboptimal antibody response to COVID-19 vaccination in people with weakened immune system, including those with hematological malignancies and who are receiving or have received treatment for cancer. Here we report the case of a 63-year-old woman who was diagnosed a breast cancer and a stage 3 multiple myeloma with multiple bone lesions both in 2016. Following specific radio and chemotherapy and autologous stem cell transplantation, the diseases completely remitted. Currently, she presents serum hypogammaglobulinemia, a condition that usually prevents the immune system from producing circulating antibodies in adequate amounts. The immune response of the patient to the mRNA BNT162b2 vaccine against SARS CoV-2 is presented and compared with that of a cohort of healthcare professionals. It was possible to conclude that the patient exhibits a good SARS-CoV-2 immune response comparable to that of the control population.

Keywords: *BNT162b2, breast carcer, multiple myeloma*

CASE PRESENTATION

The overall risk of immunocompromised cancer patients becoming infected and developing critical symptoms related to COVID-19 disease is very high. Immunocompromised patients with hematological malignancies or solid cancer are more susceptible to infection from SARS-CoV-2 and at higher risk of severe complications and worse outcomes compared with the general population (1). Unfortunately, the same mechanisms that avoid immunocompromised patients controlling COVID-19 infection also reduce their capability to generate an immune response from vaccination (2).
Despite this, the era of mRNA vaccine might be a game-changer. In Italy, the "Vaccine Day" on December 27, 2020, gave the official start to the vaccination campaign, which primarily involved frail individuals and healthcare workers. ASL Roma 2 promoted a seroprevalence campaign for monitoring anti-SARS-CoV-2 antibodies concentration following vaccination among its employees. The present report describes the case of a 63-year-old woman who, in 2016 received a diagnosis of breast cancer (pT1c N0 G2) and multiple myeloma (MM) (IgG lambda IIIA at stage 3 according to Durie Salmon staging system and stage 1 according to international staging system) with multiple bone lesions; the MM progressed from a monoclonal gammopathy of uncertain significance (MGUS) diagnosed in 2003. The patient underwent a quadrantectomy of the left breast with subsequent radiotherapy and contextual partial resection of the seventh rib. The hormonal therapy with aromatase inhibitor Letrozole, which continued for

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five years was also administered. At the same time, the patient was treated with 6 MV x-rays at the left breast (40.05 Gy fractioned at 267cGy/die for four weeks). Then, the patient started four cycles of chemotherapy to treat MM with Bortezomib, Thalidomide, and Dexamethasone and one cycle with Bortezomib and Dexamethasone for the onset of a severe peripheral neuropathy affecting arms and legs as side effect of Thalidomide. In January 2017 she was subjected to high-dose Cyclophosphamide for peripheral blood mobilization of hematopoietic stem cells before hematopoietic progenitor cells apheresis collection. She underwent two subsequent autologous transplantations (ASCT) after high-dose of Melphalan in March and November 2017. Currently, the patient is reasonably well and in complete remission from diseases; however low levels of serum immunoglobulins are observed, a condition associated with lower antibody responses to COVID-19 among patients with hematological malignancies (1). Because of the anamnesis, the patient was considered to be more likely to develop severe COVID-19 complications and was elected to receive early vaccination. The vaccination with the Pfizer/BioNTech BNT162b2 (Comirnaty, Pfizer, New York, USA) mRNA-based vaccine anti-SARS-CoV-2, was then administered (two doses of 30 mg, on January 4 and January 25, 2021). This paper describes the patient post-COVID-19 vaccination humoral immunity by measuring the antibodies anti-S1 subunit of spike protein (anti-S1) (Ortho Clinical Diagnostics, Raritan, USA), anti-receptor binding domain (anti-RBD) (Abbott, Wiesbaden, Germany) and anti-trimeric form of S protein (anti-trimericS) (DiaSorin Saluggia, Italy). The patient antibody titres were compared with those measured in a control population of vaccinated healthcare employees. Immunoassays used to study the time course of antibody response to the COVID-19 vaccine were the following: VITROS® Anti-SARS-CoV-2 IgG Quantitative (Ortho Clinical Diagnostics, Raritan, USA), performed on the VITROS 3600 (sample positive if ≥17.8 BAU/mL); LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin, Saluggia, Italy), performed on LIAISON XL analytical system (sample positive if ≥33.8 BAU/mL); ARCHITECT® SARS-CoV-2 IgG II Quant assay (Abbott Laboratories, Wiesbaden, Germany) performed on the ARCHITECT i-2000 using chemiluminescent microparticle immunoassay technology, (sample positive if ≥7.1 BAU/mL). Antibodies against the N recombinant protein were also measured in order to exclude natural SARS-CoV-2 infection (3) by using Elecsys® Anti-SARS-CoV-2 antibodies (Roche, Basel, Switzerland) performed on the Cobas e411 analytical system (qualitative results are reported as signal sample/cutoff (COI); sample positive if ≥1.0).

Ethical approval

This study was approved by the Institutional Ethics Committee of ASL Roma 2, Roma, Italy (Prot. N. 0223816/2021 of 17/11/2021) in accordance with the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice. All subjects provided written informed consent prior to enrollment in the study.

DISCUSSION

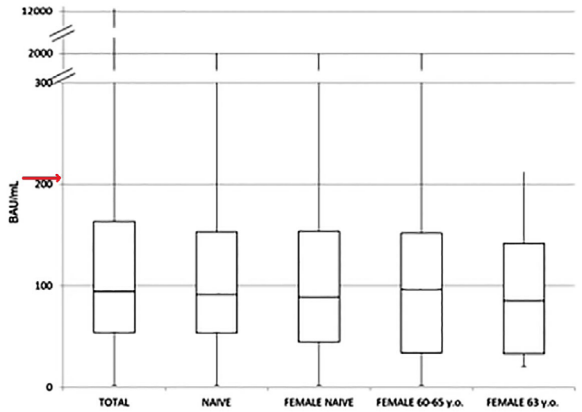
To evaluate the effects of Cyclophosphamide treatment on patient immunological memory we analyzed the data available in the laboratory database about antibodies anti-HBsAg (HBsAb) deriving from HBV vaccine inoculated at the age of 35 years correlating with HBcAb negative and measles IgG that was referred to an infection happened during the pediatric age. Both HBsAb and measles IgG become negative, after the first and the second ASCTs respectively. In particular, HbsAb was 108 mUI/mL at PreASCT and resulted of 0.2 mUI/mL after the first ASCT, measles IgG was 50.4 mUI/mL after the first ASCT and resulted 9.9 AU/mL after the second one (reference values: HBsAb, negative <8 mUI/mL, grey zone 8-12 mUI/mL, positive >12 mUI/mL; Measles IgG, negative <13.5 AU/mL, grey zone 13.5-16.5 AU/mL, positive >16.5 AU/mL). Following the BNT162b2 mRNA vaccine inoculation, the patient in active surveillance underwent serological tests measuring antibodies against the anti-S protein at 8-12-21-28 days from the first dose, as suggested by Mahase et al. (4) and at 49, 256, and

Table 1
Antibodies measured after BNT162b2 mRNA COVID-19 vaccination in the patient (second dose was administered at day 21)

Days post first dose	anti-RBD IgG (BAU/mL)	anti-S1 IgG (BAU/mL)	anti-TrimericSIgG (BAU/mL)
8	1.4	<2	<4.81
12	50.5	93.6	136
21	217.1	392	488
28	1634.2	2800	2590
49	2947.3	3910	4930
256	208	219	635
273	199	202	495

273 days following the first dose (Table 1). Blood was sampled immediately before vaccine injection on the 21st day, corresponding with the second dose of vaccination date. Levels of IgG anti-S1 are shown in Table 1. For all assays, IgG anti-S are expressed in BAU/mL according to the World Health Organization (WHO, NIBSC code 20/136) first International Standard (IS) in order to standardize and harmonize humoral immune response to SARS-CoV-2 (5). To exclude previous SARS-CoV-2 infection or exposure, antibodies anti-NC protein were also measured at the fourth and sixth sampling, resulting negative (COI, 0.089 and 0.025, respectively; r.v. <1.0). Timing was initially chosen based on indications provided by literature reporting that immunity starts about 12 days after the first dose (4), then in accordance with the seroprevalence campaign promoted by the ASL Roma 2. As shown in Table 1, IgG to S protein tested eight days from the first dose of vaccine resulted negative, but turned positive reaching a peak at the fifth sampling, when anti-TrimericS IgG was higher than anti-RBD and anti-S1 IgG values. This is not surprising as only 50% of neutralizing antibodies binds the RBD region, the other 50% binds the N-terminal part of the S protein; the trimeric assay can capture all the antibodies produced. In conclusion, anti-TrimericS antibodies can explore a wider humoral response which includes both anti-RBD and antibodies against other epitopes of spike protein with neutralizing capacity (6). Unexpectedly, at the 4th sampling anti-S1 IgG was higher than anti-TrimericS IgG (this value was confirmed by testing the sample at successive dilutions to exclude the hook effect). This is probably due to the type of anti-S antibodies produced at that time by the patient. In line with literature, at the 6th and 7th sampling a general reduction of detectable IgG to S was observed (7). This trend was maintained across the assays. As it has been reported that values from different test systems are not interchangeable, even when converted to BAU/mL (8), individual immune monitoring should be performed with the same method (5). Furthermore, anti-RBD and anti-TrimericS IgG values at the 7th sampling correspond to a good anti SARS-CoV-2 antibody titer based on the manufacturer declarations in concordance with neutralizing antibody titers values of 1050 AU/mL (149 BAU/mL) for anti-RBD IgG or 520 BAU/mL for anti-TrimericS IgG correspondent to 1:80 titer of neutralizing antibodies in patients with previous SARS-CoV-2 infection (9). At 273 days from the first dose, the amount of IgG anti-S1 detected in the serum of the patient was compared with those detected at 270 ± 2 days following the first dose in a cohort of 483 health workers included in the ASL Roma 2 seroprevalence campaign. Of these, 460 were SARS-CoV-2 infection-naïve, 300 were female; 39 of these ranged 60-65 years old and 10 were exactly 63 years old.

Efficacy of conventional vaccination is affected by proliferative disease-related factors in MGUS, MM and cancer patients. Antitumoral treatments aggravate the immunosuppression affecting T-cell, antibody function and production. This raised questions about the efficacy and safety of mRNA vaccines in immunocompromised cancer patients, which seems to be generally well tolerated but showed a reduced performance and lower rates of seroconversion. Patients receiving treatment for MM had significantly lower SARS-CoV-2 S-binding IgG antibody levels after two vaccine doses compared to patients not receiving therapy (2). Dexamethasone has been strongly associated with lower antibody anti-S levels, while high doses of melphalan did not show any association with changes in antibody anti-S levels (10); no data are available about the effects of Letrozole. The patient of the present case shows a good response of anti-S1 antibodies to the BNT162b2 vaccine (202 BAU/mL, Figure 1, arrow) in line with the mean of anti-S1 antibodies detected in the control population. This is probably due to the complete remission of the disease and/or the long treatment-free period. When we excluded from the controls, the subjects who had been infected by SARS-CoV-2 before or during the vaccination phases,



	Total	Naïve	Female Naïve	Female 60-65 years	Female 63 years
Number	483	460	300	39	10
Median	94,2	91,2	101	115	100,8
IQR-I	53,95	53,675	56,875	52,75	49
IQR-III	163,5	153	165,5	170,5	157,5

Figure1
Comparison of levels of IgG anti-S1 at 270 ± 2 days following the first dose of vaccine in five subgroups: total control population; health workers naïve; female naïve; female between 60 and 65 years and female of 63 years old. Arrow indicates the value of IgG anti-S1 obtained from the patient. Data of median and Interquartile ranges (IQR) are reported in the table in BAU/mL.
Naïve indicates infection-naïve subjects

the median of of the IgG anti-S1 values obtained in the control groups matched for age and sex was even lower than those observed in the patient (Figure 1). These results correlate with literature reporting that patients who completed their treatment and remained on remission at the time of vaccination, are more likely to produce antibodies, probably due to a reconstitution of humoral immunity (1). Data from the literature suggest that the risk of symptomatic COVID-19 decreases with increasing levels of anti-S and anti-RBD IgGs, pseudovirus and live-virus neutralization titers (10). Moreover, comparative studies conducted among different populations with cancer provide reasonable evidence that the value of 260 BAU/mL represents the minimum threshold in cancer patients to guarantee protection from symptomatic infection (7). Overall, serological tests prove to be an excellent tool for assessing post-vaccination antibody status, but it is important to understand the strengths and limitations of such tests. In particular, standardization of SARS-CoV-2 antibody binding tests still needs to be improved. SARS-CoV-2 immunity does not arise only from antibodies, but is multidimensional including humoral and cellular response as well as memory B and T cells (1, 6) so, although the methods used are strongly related to each other, individual measurements differ between assays, even after conversion to standardized units (BAU/mL). Nonetheless, serum antibody measurements remain the most accessible mean to evaluate humoral immunity.

CONFLICT OF INTEREST

None.

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