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## Evaluation of humoral and cellular response to third dose of BNT162b2 mRNA COVID-19 vaccine in patients treated with B-cell depleting therapy<sup>☆</sup>

Davide Firinu<sup>a,\*,1</sup>, Giuseppe Fenu<sup>b,1</sup>, Giuseppina Sanna<sup>c</sup>, Giulia A. Costanzo<sup>a</sup>, Andrea Perra<sup>d</sup>, Marcello Campagna<sup>a</sup>, Roberto Littera<sup>e</sup>, Carlotta Locci<sup>a</sup>, Alessandra Marongiu<sup>c</sup>, Riccardo Cappai<sup>f</sup>, Maurizio Melis<sup>b</sup>, Germano Orrù<sup>a</sup>, Stefano Del Giacco<sup>a</sup>, Ferdinando Coghe<sup>e</sup>, Aldo Manzin<sup>c</sup>, Luchino Chessa<sup>a</sup>

<sup>a</sup> Department of Medical Sciences and Public Health, University of Cagliari. and Unit of Internal Medicine, Policlinico Universitario – AOU di Cagliari, Azienda Ospedaliero Universitaria, SS 554-Bivio Sestu, 09042, Monserrato, CA, Italy

<sup>b</sup> Department of Neuroscience, ARNAS Brotzu, 09100, Cagliari, Italy

<sup>c</sup> Microbiology and Virology Unit, Department of Biomedical Sciences, University of Cagliari, 09042, Monserrato, Italy

<sup>d</sup> Oncology and Molecular Pathology Unit, Department of Biomedical Sciences, University of Cagliari, 09100, Cagliari, Italy

<sup>e</sup> Medical Genetics, Department of Medical Sciences and Public Health, University of Cagliari, 09100, Cagliari, Italy

<sup>f</sup> Laboratory Clinical Chemical Analysis and Microbiology, University Hospital of Cagliari, 09042, Monserrato, Italy

### ARTICLE INFO

#### Keywords:

COVID-19

Vaccination

CD-20

Autoimmune disease

IMID

BNT162b2

### ABSTRACT

**Objective:** to investigate the responses to mRNA COVID-19 vaccines in a cohort of immunosuppressed patients affected by immune-mediated inflammatory diseases (IMID).

**Methods:** we have measured humoral and cellular immunity using quantitative IgG anti-SARS-CoV-2 Spike antibody (anti-S-IgG), neutralization assays and specific interferon-gamma (IFN-g) release assay (IGRA) before and after the third dose of BNT162b2. The response of those on anti-CD20 (n = 18) was then compared with healthy controls (HC, n = 18) and IMID naïve to anti-CD20 drugs (n = 13).

**Results:** a third BNT162b2 dose is highly immunogenic in IMID patients naïve to anti-CD20, as 100% of the subjects seroconverted compared to the 55% in anti-CD20. The rate of IGRA response was of 79% in anti-CD20, 50% in IMID naïve to anti-CD20, 100% in HC. Among those who have seroconverted, IMID patients had significantly reduced anti-S-IgG and neutralization titers compared to HC, whereas no significant difference was observed when comparing anti-CD20 and HC. Furthermore, 13% of anti-CD20 and 7.7% of IMID were simultaneously negative for both neutralizing antibodies and IGRA after three doses. Conclusion: these data draw attention to the immunogenicity of COVID-19 vaccination in treated IMID, taking specific groups into consideration for vaccination program.

### 1. Introduction

The COVID-19 pandemic has been the main focus of the entire scientific community in the last two years due to the high rate of morbidity and mortality directly related to Sars-CoV-2 infection and the virus' wide impact on global health and society. Vaccination is currently the

only truly effective means of containing the spread of the virus.

The Pfizer-BioNTech COVID-19 (BNT162b2) vaccine consists of a lipid nanoparticle-formulated mRNA vaccine which encodes the SARS-CoV-2 spike protein (S-protein), a large class I trimeric fusion protein. Its use was authorized in Europe and the U.S. in late 2020, and data of immune response to BNT162b2 against the original strain have been

**Abbreviations:** CLIA, chemiluminescent analytical system; COVID-19, coronavirus disease 2019; csDMARDs, conventional synthetic and targeted synthetic DMARDs; IMID, immune-mediated inflammatory diseases; HCW, healthy healthcare workers; PRNT, plaque reduction neutralization test; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

<sup>☆</sup> We also wish to thank Bianca Maria Manzin for the revision of the English language.

\* Corresponding author.

E-mail address: [davide.firinu@unica.it](mailto:davide.firinu@unica.it) (D. Firinu).

<sup>1</sup> Contributed equally to this work.

<https://doi.org/10.1016/j.jaut.2022.102848>

Received 26 April 2022; Received in revised form 3 June 2022; Accepted 5 June 2022

Available online 13 June 2022

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reported [1]; said vaccine induces durable SARS-CoV-2 specific spike-protein (and/or its RBD) B-cells and neutralizing antibodies and generates polyspecific CD8<sup>+</sup> and CD4<sup>+</sup> T-cell clones [2].

The profile of immune response of the BNT162b2 vaccine remains to be investigated in this context, especially in patients affected by diseases such as immune-mediated inflammatory diseases (IMID), which rendered them vulnerable during the pandemic period [3]. Therefore, since a considerable fraction of patients with IMID has displayed insufficient vaccine responses, alternative SARS-CoV-2 vaccination strategies, i.e. rapid re-exposure of patients who have not responded to double vaccination, should be considered to achieve adequate protection for this susceptible group.

It is well known that infections represent an important cause of morbidity and mortality in patients suffering from IMID [4], due to both an altered regulation of the immune system inherent to these diseases, and drug-induced immunosuppression. Immunosuppressive agents - whether traditional, synthetic or biological - modulate or inhibit the expression of key target molecules for the immune response, and can therefore reduce the immunogenicity and efficacy of vaccines [5]. Anti-CD20 represent a class of drugs targeting B-cells used to treat IMID, including subjects who do not respond to other treatments [6] and patients treated with B-cell depleting agents are at higher risk of hospitalization and a more severe course of COVID-19 [7,8]. Several real-world reports show proof of a reduced humoral response in patients exposed to immunotherapies after vaccination against COVID-19 [9-13].

The proportion and magnitude of response in treated IMID is considerable, even though it is lower than that of immunocompetent subjects with a progressive measured decline influenced by age and immunomodulatory treatments [14,15], and it is more marked for anti-CD20 drugs [16,17]. The emergence of variants of concern (VOC) has highlighted the importance of a booster dose to restore declining effectiveness a few months after completing the primary vaccine course [18]. The response elicited by the booster dose guarantees a renewed protection in line with the role of neutralizing antibody titers in protection [19], but also increases the rate of vaccine response by inducing humoral and/or cellular immunity [20].

Therefore, in this prospective observational study focused on COVID-19 infection and vaccination (CORIMUN study), we have tested the

outcomes and determinants of immune response induced by the third dose of mRNA-based vaccine BNT162b2 in subjects affected by IMID who had previously already completed the regular vaccine schedule.

## 2. Methods

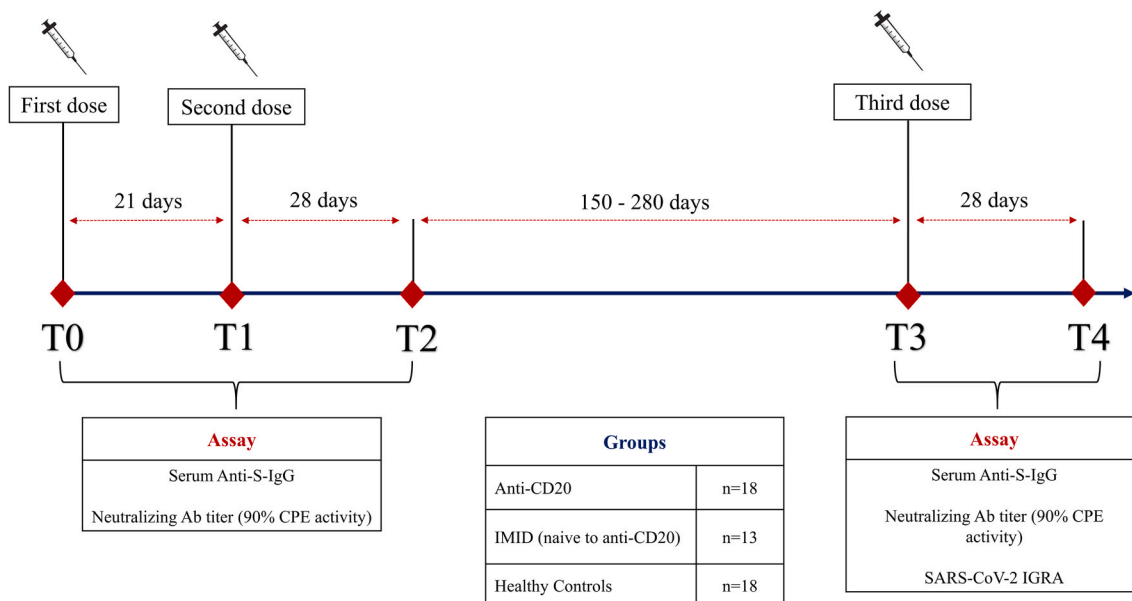
### 2.1. Study design

We have measured humoral and cellular immunity using quantitative IgG anti-SARS-CoV-2 Spike antibody (anti-S-IgG) and neutralization assay, and specific interferon gamma (IFN-g) release assay (IGRA) before and after the third dose of BNT162b2, in order to investigate the responses in a prospective cohort of IMID patients treated with anti-CD20. The responses were compared then with healthy controls and patients treated with drugs different from anti-CD20 (Fig. 1). Patients' diagnosis is detailed in Supplementary Table 1.

### 2.2. Patient selection

We enrolled prospectively consecutive subjects aged >18 years, who had received COVID-19 vaccine Pfizer/BioNTech BNT162b2 (two doses 21-days apart of 30 µg mRNA vaccine Comirnaty by Pfizer Inc, NY, USA) and deliberately given their Informed Consent to participate in the study. Pregnancy, transplantation, known primary immunodeficiency or lymphoproliferative disorders were considered exclusion criteria. Among enrolled subjects, we have then defined three groups:

- vaccinated subjects at our hospital with concurrent IMID, treated with anti-CD20 drugs (rituximab or ocrelizumab) for multiple sclerosis or other diseases.
- vaccinated subjects at our hospital with concurrent IMID, never treated with anti-CD20 drugs and with a stable dose of DMARDs (mophetil mycophenolate, methotrexate and/or adalimumab or etanercept) and/or low-dose oral glucocorticoids.
- a control group of subjects enrolled among healthy healthcare workers (HC) recruited at our hospital, not affected by any of the immune-mediated diseases listed above, no evidence of immunodeficiency or relevant medications intake.



**Fig. 1.** Study design. Anti-CD20: patients treated with anti-CD20 drugs; IMID: immune-mediated inflammatory diseases, naïve to anti-CD20 drugs; anti-S-IgG levels: serum SARS-CoV-2 trimeric anti-spike assay; Neutralizing ab: serum neutralizing antibody titer assessed by SARS-CoV-2 Microneutralization assay (90% Protective activity of neutralizing Ab against the CPE induced by the virus); IGRA: Interferon gamma (IFN-g) release assay to SARS-CoV-2 Spike-1 tube (Wuhan/Hu-1/2019 and 20I/501Y.V1 “alpha” variant).

To exclude prior SARS-CoV-2 infection, subjects were asked if they had tested positive on PCR in the past and were cross-matched with the database of positive rt-PCR tests at the laboratory and hospital records. We have also checked data of serological testing for health surveillance in HCW of both IgM and IgG antibodies with the 2019-nCov (Snibe, Shenzhen, China) chemiluminescent analytical system (CLIA) assay on MAGLUMI platform, which detects antibodies of natural infection to SARS-CoV-2 Spike-(S) protein and N-protein with high sensitivity and specificity.

Each group included only naïve subjects who had not been previously infected by SARS-CoV-2 (repeatedly tested negative rt-PCR; with consistently undetectable IgG or IgM antibodies and negative anti-N response in specific IGRA assay).

### 2.3. Sample collection and storing

10 ml of peripheral blood was obtained by venepuncture immediately before each vaccine dose and defined as T0, before the first dose; T1, at the second vaccine dose (+21 days from T0); T2 at day 51 (T1+28 days); T3, before the additional dose (3rd) and T4 (at T3+21–28 days). The serum was separated by centrifugation (2000×g for 15 min) within 3 h of collection and aliquots were stored at –80 °C until use.

### 2.4. Serological studies

The primary outcome was the rate of seroconversion and anti-S-IgG and 90% protective response of neutralizing Abs at week 3 after the third vaccine dose.

Secondary outcomes included the assessment of residual response in a whole blood SARS-CoV-2 IGRA before the third dose and the kinetics after.

The anti-S-IgG was assessed with the LIAISON SARS-CoV-2 Trimeric IgG assay (DiaSorin, Saluggia Italy), a chemiluminescence immunoassay which measures the anti-trimeric spike glycoprotein of SARS-CoV-2 in serum samples on the LIAISON XL (DiaSorin, Saluggia, Italy). The measuring range is 4.81–2080 BAU/mL, and, per the manufacturer's instructions, values more than 2080.00 BAU/mL were diluted 1:20; values > 33.8 BAU/mL were considered positive.

**SARS-CoV-2 Microneutralization assay.** MNA was performed in a Biosafety Level 3 (BSL-3) laboratory (Section of microbiology and virology, Cittadella Universitaria di Monserrato). Serum samples were diluted (1:2; 1:5; 1:10; 1:40; 1:160; 1:640) in triplicates and mixed with 100 TCID<sub>50</sub> of SARS-CoV-2 virus (clinical isolate, strain VR PV10734, kindly donated by the Lazzaro Spallanzani Hospital of Rome, Italy) at 37 °C, serum/virus mixes were transferred to 96-wells containing 5 × 10<sup>5</sup>/ml adherent Vero E6 (ATCC, Manassas, Virginia, United States) cells seeded the day before in. Monolayers were incubated at 37 °C for 72 h prior evaluation of CPE via microscope and then fixed and stained with Gram's crystal violet solution. The neutralization percentage of individual dilutions was calculated by setting the mean OD<sub>595</sub> of the serum control equal to 100%. Virus dilution used for infection was titrated in each experiment. Cell growth and serum controls were run in each experiment. Neutralization titers of serum samples were determined by the highest serum dilution protecting 90% of the infected wells [21].

### 2.5. SARS-CoV-2 specific cellular immunity

We investigated cell-mediated immunity by measuring IFN- $\gamma$  secreted by T cells in response to SARS-CoV-2 antigens, using a specific IGRA kit with enzyme-linked immunosorbent assay (ELISA) (Covi-FERON ELISA, SD Biosensor, Suwon, Republic of Korea). Whole blood specimens from the participants were collected, and 1 ml was injected into each Covi-FERON tube (Nil tube, SARS-CoV-2 spike protein antigen (Sp)1 tube, Sp2 tube, and Mitogen tube). The Sp1 tube contained spike protein antigens derived from the original SARS-CoV-2 (Wuhan/Hu-1/

2019) and 20I/501Y.V1 variant, while the Sp2 tube contained those derived from the B.1.351 (20H/501.V2) and P.1 (20J/501Y.V3) variants. After incubating at 37 °C for 16–24 h, plasma was collected by centrifuging the tubes at 2200–2300 g for 15 min. IFN- $\gamma$  was detected by ELISA and the measured optical density was converted to IFN- $\gamma$  concentration (IU/mL) using ELISA Report Software (SD Biosensor). The positive cut-off for S and N tubes minus that of the Nil tube was >0.25 IU/ml, according to manufacturer specification.

Blood samples were processed for flow cytometric lymphocyte phenotyping: ethylenediaminetetraacetic acid whole blood was stained for CD3, CD4, CD8, CD45, CD16, CD56 and CD19.

### 2.6. Statistical analysis

Patient characteristics were summarized using appropriate means, medians, standard deviations, ranges, and percentages. Chi squared tests of independence and Fischer's exact tests were used for categorical data. Mann-Whitney U and Kruskal-Wallis tests were used for unpaired continuous data and nonparametric Spearman's rank for correlation test. Linear regression was used to evaluate the relationship between the dependent variable (e.g. antibody titer) and the clinical and demographic characteristics of patients as independent variables. All reported p-values represent 2-tailed tests, with  $p \leq 0.05$  considered statistically significant. All variables were analysed using SPSS.

### 2.7. Ethical aspects

Patients were recruited and enrolled in the study protocol at the University Hospital of Cagliari. Written informed consent was obtained from all patients and controls in accordance with the ethical standards (institutional and national) of the local human research committee. The study protocol, including informed consent procedures, conforms to the ethical guidelines of the Declaration of Helsinki and was approved by the responsible ethics committee (Ethics Committee of the Cagliari University Hospital approval May 27th, 2020; protocol number GT/2020/10,894 and extension approved Jan 27th, 2021). Records of written informed consent are kept on file and are included in the clinical record of each patient.

## 3. Results

We have collected complete data and results of immunity assays throughout all the established time intervals from 18 anti-CD20 patients, 13 IMID naïve to anti-CD20, and 18 HC. Baseline characteristics of enrolled subjects, both vaccinated with 3 doses of BNT162b2 and naïve to SARS-CoV-2 infection, are shown in Table 1.

The cohort included only Caucasian participants, and patients continued their treatment schedule for the vaccine, without stopping or tapering their drug(s), only avoiding the administration of injective drugs  $\pm$  3 days from the vaccine date.

There was no difference in age, gender and analysis time after the vaccine booster when comparing these groups (Kruskal-Wallis  $\chi^2$ ,  $p > 0.05$ ).

There was a significant difference in the time elapsed between the 2nd and 3rd vaccine dose, which was shorter for the anti-CD20 group than for IMID and HC (respectively  $p = 0.004$  and  $p < 0.0001$ , Mann-Whitney). Patients of the anti-CD20 group previously had a median of 3 (IQR 2.1) courses of rituximab or ocrelizumab, and the median time between the last drug infusion and the first vaccine dose was 98 days (IQR 43.3), while the interval between the last anti-CD20 administration and the 3rd dose was of 158 days (IQR 65.7).

The overall results and rates of response as evaluated by humoral and IGRA assays are shown in Table 2.

At T2, after the second dose, the rate of vaccine response (defined as seropositive anti-S-IgG) was of 50% among the anti-CD20 group, 76% among IMID, and 100% among HC (chi-square  $p$  value = 0.002). Among

**Table 1**  
Characteristics of enrolled subjects.

Subjects characteristics	Anti-CD20 n = 18	IMID n = 13	Healthy Controls n = 18
Age (years), median (IQR)	46 (17.2)	59 (26.3)	51.5 (24.2)
Female, %	9 (50%)	8 (61.5%)	9 (50%)
Disease	MS, SLE, RA, CTD	SLE, RA, CTD	–
Treatment	RTX, OCRE	ADA, ETN, MTX, MMF	–
csDMARDs (MTX, MMF)	–	77%	–
Combination of MTX plus anti-TNF	–	23%	–
Treatment, current low-dose prednisone	16.6%	18.75%	–
Days between 2nd and 3rd vaccine dose, median (IQR)	152 (117.5)	200 (70.3)	280.5 (16.5)
Analysis time after vaccine booster (days), median (IQR)	24 (3.5)	22.5 (7)	19 (10)

Anti-CD20: patients treated with anti-CD20 drugs; IMID: immune-mediated inflammatory diseases, naïve to anti-CD20 drugs; MS: multiple sclerosis; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; CTD: connective tissue disease; RTX: rituximab; OCRE: ocrelizumab; ADA: adalimumab; ETN: etanercept; MTX: methotrexate; MMF: mophetil mycophenolate; csDMARDs: conventional synthetic DMARDs.

the responders, the rate of subjects still seropositive for anti-S-IgG before third dose was 66% in anti-CD20, 72% in IMID and 100% in HC (chi-square p value = 0.03).

At T3, before the third dose, there was no significant difference between anti-S-IgG (p = 0.07) or neutralizing titers (p = 0.27) among responders after second vaccine dose (Fig. 2), but the longer time elapsed after 2nd dose for HC might need to be taken into consideration.

The rate of subjects with a response still detectable in IGRA before third dose was of 64.3% in anti-CD20, 43% in IMID and 71.4% in HC (chi-square p value = 0.43); moreover, 43% of anti-CD20, 14% of IMID and 70% of HC tested positive on both (chi-square p value = 0.05).

At T4, after the third vaccine inoculation, the rate of responders (defined as those seropositive for anti-S-IgG or with an increase of antibody titer) was 55% among the anti-CD20 group, 100% in IMID and HC, respectively (chi-square p value = 0.0003).

Therefore, the additional dose had fully rescued the humoral immunogenicity rate in IMID, while the rate of response remained below 60% among the anti-CD20 group.

While there was no difference between groups regarding quantitative IGRA response before (p = 0.19) or after the third dose (p = 0.44) (Fig. 3), a statistically significant difference was present in anti-S-IgG antibody after third dose (KW p = 0.0033), which were significantly lower in IMID than in HC (MW p = 0.0016). Accordingly, there was a significant difference in neutralization titers after third dose (KW p = 0.05), as they were significantly lower in IMID than in HC (MW p = 0.029).

The rate of subjects with a response in IGRA after third dose was of 79% in anti-CD20, 50% in IMID and 100% in HC (chi-square p value = 0.01), and there was a positivity for both tests in 60% of anti-CD20, 50% of IMID and 100% of HC (chi-square p value = 0.01). Notably, there was an overall scant fraction of subjects negative for both anti-S-IgG antibody (or neutralizing antibody) and IGRA, respectively 13% in the anti-CD20 and 7.7% in the IMID group (chi-square p value = 0.31).

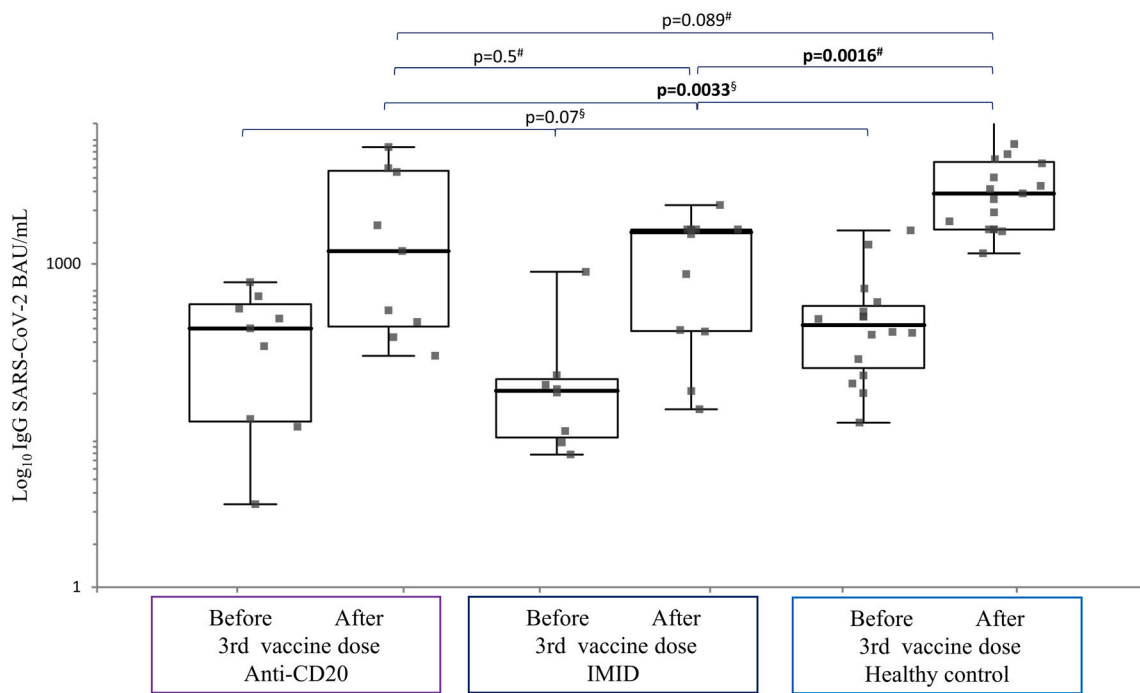
**3.1. Correlation with subjects' characteristics**

In a linear regression model, the anti-spike antibody titer after the third dose was significantly related to CD19<sup>+</sup> absolute count (p < 0.001) after controlling for age and gender. A similar result was found for neutralizing titers after the third dose (p = 0.02), while the IGRA spike response was not related to CD19<sup>+</sup> cell count (p = 0.19).

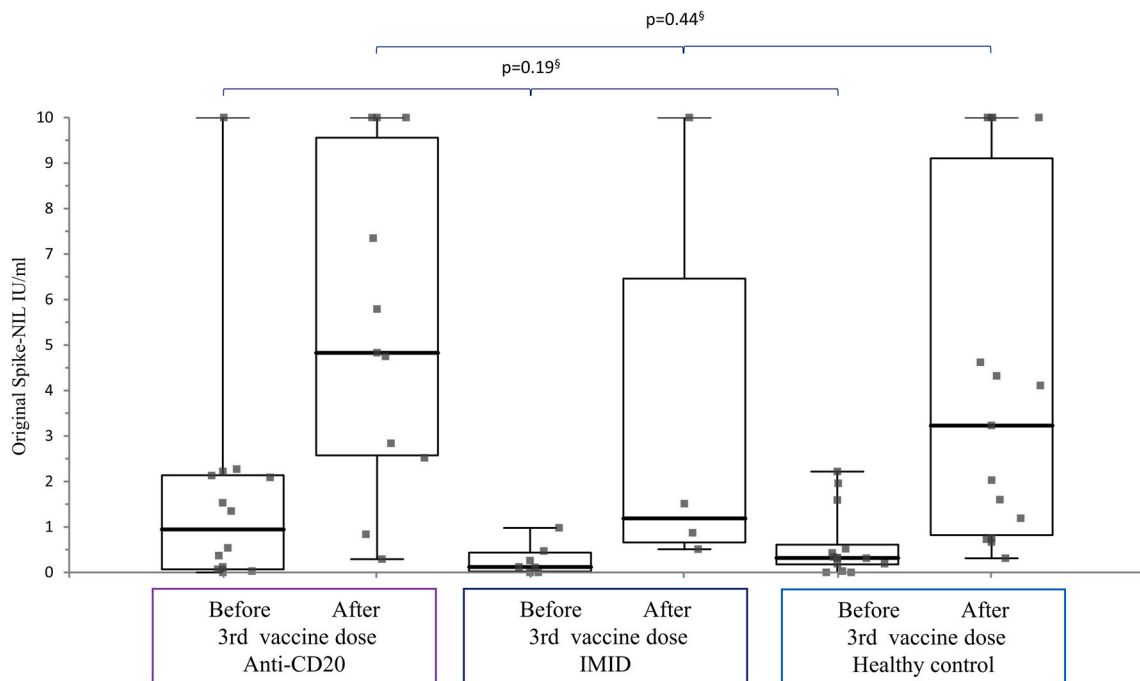
**Table 2**  
The main study variables of enrolled subjects assessed 4 weeks after BNT162b2 vaccine booster (T2), and before (T3) and after (T4) the additional vaccine dose.

	T2			T3			T4			
	After 2nd vaccine dose			Before additional (3rd) vaccine dose			After additional (3rd) vaccine dose			
	Rate of response (ab)	Anti-S-IgG BAU/mL	Neutralizing Ab titer [1:X]	Rate of residual responders to IGRA	IGRA IU/mL	Anti-S-IgG BAU/mL	Rate of response (ab)	Neutralizing Ab titer [1:X]	IGRA IU/mL	Rate of double negative for anti-S-IgG and IGRA
Anti-CD20 n = 18	50%	251 (34.36–421.6)	10 (5–10)	64%	0.945 (0.069–2.138)	1310 (261.33–7300)	55%	10 (10–140)	4.83 (2.57–9.55)	13%
IMID n = 13	76%	66.25 (24.5–85.19)	5 (5–10)	43%	0.12 (0.018–0.435)	1965 (237.92–2080)	100%	40 (10–40)	1.19 (0.66–6.46)	7.7%
Healthy Control n = 18	100%	270 (107.77–407.83)	10 (9.6–10)	71%	0.320 (0.177–0.609)	4490 (2080–8790)	100%	40 (40–160)	3.23 (0.82–9.1)	0%

Anti-CD20: patients treated with anti-CD20 drugs; IMID: immune-mediated inflammatory diseases, naïve to anti-CD20 drugs; rate of response (ab): rate of subjects having serum antibody IgG to trimeric SARS-CoV-2 spike protein above the cut-off of 33.8 BAU/mL or presence of neutralizing ab; anti-S-IgG levels: serum SARS-CoV-2 trimeric anti-spike assay, positive >33.8 BAU/mL; Neutralizing ab: serum neutralizing antibody titer assessed by SARS-CoV-2 Microneutralization assay (90% Protective activity of neutralizing Abs against the CPE induced by the virus); IGRA: Interferon gamma (IFN-γ) release assay to Spike-1 tube (Wuhan/Hu-1/2019 and 2019Y.V1 “alpha” variant); Rate of response to IGRA: rate of subjects testing IGRA to Spike-1 tube (minus the nil) above >0.25 IU/ml.



**Fig. 2.** SARS-CoV-2 trimeric anti-S-IgG serum immune responses in patients and control subjects. Antibody anti-S-IgG (BAU/mL) elicited after two BNT162b2 doses at T3 (before 3rd dose), and at T4 (28 days after 3rd dose) among anti-CD20 (IMID treated with anti-CD20 agents), IMID (naïve to anti-CD20 treatments) and Healthy controls (HC). All subjects were vaccinated with 2 doses, 21 days apart. #: Mann-Whitney test; § for Kruskal-Wallis test; bold indicates statistical significance.



**Fig. 3.** Response of a SARS-CoV-2 specific interferon gamma (IFN-g) release assay of whole blood in patients and control subjects. Specific interferon gamma release assay IU/mL to original spike protein of SARS-CoV-2, elicited after two BNT162b2 doses at T3 (before 3rd dose), and at T4 (28 days after 3rd dose) among anti-CD20 (IMID treated with anti-CD20 agents), IMID (naïve to anti-CD20 treatments) and Healthy controls (HC). All subjects were vaccinated with 2 doses, 21 days apart. #: Mann-Whitney test; § for Kruskal-Wallis test; bold indicates statistical significance.

**3.2. COVID-19 course in subjects vaccinated with 3 doses**

During the study period a total of 12/49 subjects of the study cohort tested positive for SARS-CoV-2 after receiving of the third vaccine dose. According to viral sequencing or date of infection, a single infection occurred in the B.1.617.2 (Delta) wave and the remaining cases during

the circulation of Omicron variant (BA.1 since early January, replaced by BA.2 since March 2022). Three cases occurred in the anti-CD20 group, five cases in the IMID group and four cases in the HC and median symptoms duration was 14 (IQR 9.2) days, 8.5 (IQR 3.9) and 4 days (IQR 2), respectively (KW  $p > 0.05$ ). Symptoms were present in all patients and were classified as mild according to WHO clinical progression

scale of COVID-19: most of them reported upper airways symptoms or influenza-like illness, requiring paracetamol or NSAIDs. None required oxygen supplementation or hospitalization or died for COVID-19.

The median duration of rt-PCR positivity was respectively of 7 days (IQR 4.17 days), 16 days (IQR 4.33 days) and 11 days (IQR 3.17 days), with a statistically significant difference among groups (KW  $p = 0.044$ ), as it was longer in the IMID group. One patient affected by SLE and interstitial lung disease in the anti-CD20 group had absent humoral response to vaccination and was treated with monoclonal antibodies in an outpatient service.

#### 4. Discussion

The ongoing COVID-19 pandemic is currently being limited by an unprecedented vaccine program and an innovative use of vaccine technologies, which includes using mRNA [22]. Several real-world data have reported a reduction of humoral response in patients exposed to immunotherapies after vaccination against COVID-19. Patients undergoing this category of therapy were not included in the clinical trials that have led to the approval of the vaccines. This issue has raised public health concerns and has resulted in main changes in vaccination strategies, also in the context of emerging of SARS-CoV-2 VOCs. In this scenario, providing data about immunogenicity in this population to guarantee informed health policies is of the utmost importance.

Our study aimed to compare the humoral and cellular response after the third dose of vaccine against COVID-19 in patients exposed to anti-CD20 with healthy controls, and patients with immune-mediated diseases not exposed to anti-CD20, as these groups show various degrees of impaired response after 2 or 3 doses and different kinetics of waning immunity [23–25].

Several studies have shown that the humoral response after vaccination is dampened in patients exposed to immunotherapies, especially anti-CD20 [16,26].

Our findings largely agree with those recently published about seroconversion after the third dose in anti-CD20 treated patients affected by MS, RA, or other diseases against which these drugs are increasingly used; seroconversion has been reported to vary between 21 and 70% after 2 doses and 33–57% after 3 doses [16,24,26–28]. The rate of responders among the anti-CD20 group in our cohort after the second dose was 50% and after the third it became 55%, thus still markedly reduced.

This study demonstrates that a third BNT162b2 dose is highly immunogenic in IMID patients naïve to anti-CD20, as 100% of those tested have seroconverted. Among responders, IMID patients have shown a significant difference in the levels of anti-S-IgG and neutralization titers compared with HC, whereas no difference was observed comparing anti-CD20 responders with HC. Thus, considering the potential role of IgG and neutralizing titers in the response to SARS-CoV-2, some degree of concern remains about the outcomes of infection in the current scenario [29]. The absence of significant differences in IgG and neutralization among the responders in anti-CD20 compared to HC may be in agreement with previous studies, which have shown different patterns of perturbation in  $T_{FH}$ , CD4 and CD8 T-cell response according to anti-RBD IgG positivity or peripheral B-cell depletion after two vaccine doses [30].

Peripheral lymphocytes subpopulations counts (especially  $CD19^+ > 1\%$  or counts  $> 27$  cells/ $\mu$ L) are predictive of vaccine response; in fact, studies have shown that a decrease in peripheral lymphocytes is accompanied by a risk of breakthrough infection shortly after vaccination [31–33], which indicates the importance of detecting primary vaccine failure. In this context, the potential of impaired specific IgG anti-RBD as a proxy of low circulating  $T_{FH}$ , a robust CD8 response, and the immunological restoration following booster doses should be further investigated. Initial immunogenicity data available for at-risk subgroups showed a variable proportion of non-responders or low antibody responses to the SARS-CoV-2 spike protein and reduced clinical

effectiveness in patients with IMID [14,34], in those with solid-organ transplantation [35] or lymphoid malignancies. However, this may be partially overcome by an additional (third) dose [36,37]. To date, available studies show that a complete mRNA vaccination course (three-doses) in patients with IMID may elicit an increase in the response rate and humoral response [14,38–41], albeit with reduced serum anti-S-IgG titers and neutralizing activity if compared to HC [39,41]. The third dose also adds breadth to SARS-CoV-2 VOC immune response [18,42].

Previous longitudinal studies have revealed a gradual decline in antibody and neutralization levels parallel to a constant increase in the frequency of Spike+ and Spike + RBD + memory B-cells 3–6 months post-vaccination (two doses) in SARS-2 naïve individuals [2].

Cellular response to SARS-CoV-2 is probably one of the key determinants of severe disease protection, especially months after viral and/or vaccine exposure in a context of declining or absent humoral immunity. In addition to antibodies and memory B cells, memory T cells can contribute to protection upon exposure to the virus, and the latter has also shown to be less affected by VOCs ability to overcome the protective effect of neutralizing antibodies produced as a result of natural infection and/or vaccination [43,44].

In agreement with other observations [2,45,46], our data show a detectable residual cellular response in 43–64% of included patients that underwent the first two doses of BNT162b2 5–6 months prior. Most importantly there is an increase in the rate of responders and in the magnitude of IFN-gamma release after the additional dose, even though response is remarkably lower in the IMID group than in HC. A cellular response comparable to or even stronger than controls after two doses has been recently reported in anti-CD20-treated patients [16,40]. The robust anamnestic B-cell response found in the IMID group seems uncoupled to cellular response. The dampened cellular response in the said group may be linked to the effect of treatments used (MTX and anti-TNF agents or MMF) which have a strong effect on T-cell response.

Lastly, our data show that 13% of anti-CD20 and about 7% of IMID are simultaneously negative for both neutralizing antibodies and detectable IFN-gamma response, which highlights the potential role of a fourth vaccine dose in these at-risk groups of patients.

The number of vaccine administrations necessary to obtain adequate protection from SARS-CoV-2 as well as the best timing concerning treatment is still a matter of research.

The data reported here about the course of COVID-19 in a small number of subjects that had previously completed a three-dose BNT162b2 schedule and later had COVID-19 during the Delta and Omicron waves indicate a mild disease course, with a low risk of hospitalization or death. However, these observations should be confirmed by larger studies.

The magnitude of response impairment to mRNA COVID-19 vaccine in patients with ongoing B-cells depleting therapies suggests that precise vaccination strategies should be implemented in the near future [10,13,47]. This may imply delaying treatment, repeating the vaccine course or administering a fourth dose [48,49], which is ongoing in some countries but has yet to be investigated in specific studies. Our findings preliminary suggest that patients treated with anti-CD20 may deserve attention on B-cell reconstitution and/or antibody testing and strict adherence to personal protective measures, and they might be prioritized for antivirals or monoclonal ab therapy. Passive immunoprophylaxis such as Tixagevimab-Cilgavimab may also be an option for immunocompromised subjects [50].

The total number of recruited subjects as well as the absence of adenoviral-vector based vaccinated subjects (or heterologous schedules) and B-cell antigen specific studies and also data about SARS-CoV-2 infection and its clinical outcomes on a longer period have limited our research. The accurate assessment of the humoral immune response, including neutralization with a validated assay and specific anti-S-IgG against the total spike protein, together with the T-cell response in terms of specific IGRA strengthen the validity of our study. Moreover,

said methods to detect SARS-CoV-2 specific responses are highly reproducible.

This data highlight the immunogenicity and importance of COVID-19 vaccination in patients affected by IMID as a measure to contrast pandemics, and the relevance of taking special care of specific groups such as those treated with B-cell depleting agents. They could also highlight the potential importance of analysing SARS-CoV-2 immunity status in specific categories such as IMID vaccinated subjects to recognize those with inadequate responses who need revaccination [51]. Epidemiological and efficacy studies focused on special groups are needed to understand whether there is a different immune kinetics response after a mRNA booster, to differentiate the risk for specific subgroups, to determine if further vaccine doses are warranted, and to establish the best timing for said doses, in relation to immunomodulatory drugs.

### Statement of authors contribution

Study conceptualization and design: DF, GF, GS, MC, LC. Investigation GS, GC, CL, AM, GC, GO, RC. Resources: FC, AP, LC, DF, AM, GO. Data curation: AM, RL, MM. Formal analysis: RL, GF, DF, SDG. Drafting original manuscript draft: DF, GF, CL, GC. Critical revision: SDG, GC, DF, GF, MC, AM, GS. Funding: AP, LC, DF, AM.

### Declaration of competing interest

The authors state no relevant conflict of interest.

### Data availability

Data will be made available on request.

### Acknowledgements

Funding: This research was supported by an unrestricted grant of C.S. L. Behring Italy for research in primary and secondary immunodeficiency, by Fondazione di Sardegna (#2020.2197); Fondo Coesione e Sviluppo - Regione Autonoma della Sardegna (# 9417); PRIN 2017 "Natural and pharmacological inhibition of the early phase of viral replication (VirSudNet)" (# 2017M8R7N9); Associazione per l'Avanzamento della Ricerca per i Trapianti O.D.V. The funding sources had no role in study design, collection, data interpretation and decision to submit.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaut.2022.102848>.

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