



Difference in safety and humoral response to mRNA SARS-CoV-2 vaccines in patients with autoimmune neurological disorders: the ANCOVAX study

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Abstract

Background Assessing the safety of SARS-CoV-2 mRNA vaccines and the effect of immunotherapies on the seroconversion rate in patients with autoimmune neurological conditions (ANC) is relevant to clinical practice. Our aim was to assess the antibody response to and safety of SARS-CoV-2 mRNA vaccines in ANC.

Methods This longitudinal study included ANC patients vaccinated with two doses of BNT162b2 or mRNA-1273 between March and August 2021. Side effects were assessed 2–10 days after each dose. Neurological status and anti-spike receptor binding domain antibody levels were evaluated before vaccination and 4 weeks after the second dose. Healthcare-workers served as controls for antibody levels.

Results We included 300 ANC patients (median age 52, IQR 40–65), and 347 healthcare-workers (median age 45, IQR 34–54). mRNA-1273 vaccine was associated with an increased risk of both local (OR 2.52 95% CI 1.45–4.39, $p=0.001$) and systemic reactions (OR 2.51% CI 1.49–4.23, $p=0.001$). The incidence of relapse was not different before and after vaccine (Incidence rate ratio 0.72, 95% CI 0.29–1.83). Anti-SARS-CoV-2 IgG were detected in 268 (89.9%) patients and in all controls ($p<0.0001$). BNT162b2 vaccine (OR 8.84 95% CI 2.32–33.65, $p=0.001$), anti-CD20 mAb (OR 0.004 95% CI 0.0007–0.026, $p<0.0001$) and fingolimod (OR 0.036 95% CI 0.002–0.628, $p=0.023$) were associated with an increased risk of not developing anti-SARS-CoV-2 IgG.

Conclusion SARS-CoV-2 mRNA vaccines were safe in a large group of ANC patients. Anti-CD20 and fingolimod treatment, as well as vaccination with the BNT162b2 vaccine, led to a reduced humoral response. These findings could inform vaccine policies in ANC patients undergoing immunotherapy.

Keywords Sars-Cov2-mRNA vaccine · Autoimmune neurological disorders · Humoral response · Immune therapy · Multiple sclerosis

Tiziana Lazzarotto and Rocco Liguori contributed equally to this work.

The members of the ANCOVAX study group was processed under acknowledgements section.

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Introduction

The BNT162b2/Pfizer and the mRNA-1273/Moderna SARS-CoV-2 vaccines have shown high efficacy in preventing symptomatic SARS-CoV2 infection in the general population and a good safety profile [1, 2]. However, concerns have been raised regarding theoretical risks of vaccination in patients with autoimmune conditions which might be exacerbated by immunization, and conversely possible lack of vaccine efficacy in patients receiving immunotherapies. Nevertheless, data on the safety and immunogenicity of mRNA vaccines in patients with autoimmune neurological conditions (ANC) undergoing immunosuppressive treatment are

scarce, mainly focused on multiple sclerosis (MS) patients vaccinated with BNT162b2 [3–11].

In this interim study, we prospectively evaluated the safety of both the SARS-CoV-2 BNT162b2 and mRNA-1273 vaccines and the serologic status one month after the second dose in a large cohort of patients with different ANC compared with a group of healthcare-workers (HCW) who served as vaccinated controls.

Methods

Study design and participants

The ANCOVAX is a longitudinal observational study evaluating the safety and efficacy of the SARS-CoV2 vaccines in patients with a range of ANC over 12 months from the second vaccine dose through serial blood sampling and clinical evaluations (Supplementary Fig. 1). Only the results of T1 will be presented here.

We included patients with a range of ANCs (i.e. myasthenia gravis [MG], MS, chronic inflammatory neuropathy [CIPD], autoimmune encephalitis and other antibody-mediated CNS disorders, i.e. Neuromyelitis Optica Spectrum disorder [NMOSD], stiff-person syndrome [SPS]). Patients fulfilling the inclusion criteria (age ≥ 18 years, ascertained ANC, ability to sign the consent form) were recruited 1–10 days before the first vaccine dose by the treating neurologist at the IRCCS Istituto delle Scienze Neurologiche di Bologna. At enrollment, both the investigator and the patient were blind to the vaccine administered. The allocation of patients to the BNT162b2 or mRNA-1273 group was independent from the study protocol. Exclusion criteria were concomitant medical conditions interfering with the immune response or the adherence to the study protocol and a previous COVID-19 infection ascertained through history or baseline serology (see below).

The study protocol was approved by the Istituto Superiore di Sanità Ethical committee. All patients signed the informed consent to participate in the study.

Once the patient's recruitment was completed, a cohort of HCW, without autoimmune pathologies or immunodeficiency, was selected from a larger control cohort to match the time of patients' sampling. HCW were only used for comparison with the patients' T1 antibody levels. The HCW's data were collected in the occupational risks surveillance environment as requested by Italian laws and have been processed in an aggregate and pseudo-anonymous procedure.

Blood sampling and testing

Serum was collected at baseline (T0, 1–10 days before the first dose) and 1 month (T1) after the second vaccine dose.

Collected samples were stored at $-80\text{ }^{\circ}\text{C}$ if not immediately used. Researchers performing the antibody assays were blind to the neurological disorder, immunosuppression status and administered vaccine.

The Elecsys[®] anti-SARS-CoV-2 ECLIA assay (Roche Diagnostics AG, Rotkreuz, Switzerland) performed on the cobas e801 analyzer (Roche Diagnostics) was used to assess antibodies against the nucleocapsid (N) and spike (S) receptor binding domain (RBD) proteins in the baseline samples. The assay is CE marked and FDA's EUA. The cut-off value for reactivity (positivity) anti-N was equal to 1.0 cut-off index (COI). To establish more accurate criteria for interpretation of the positivity and the levels of IgG anti-S(RBD), the evaluation of the results was launched after an initial validation study, including two well-defined groups of serum samples obtained from 50 HCW before and a month after receiving a second dose of vaccine. Based on the results from the fifty true-positive and the fifty true-negative SARS-CoV-2 samples, antibody responses were stratified into the following groups:

Anti-S(RBD): negative (0.8 BAU/mL); inconclusive (≥ 0.8 to < 5 BAU/ml); positive (≥ 5 BAU/ml). Patients with values below 5 BAU/ml were classified as non-responders. This choice is motivated by the fact that low levels of IgG antibodies in serum samples present a challenge for interpretation; indeed, low IgG levels may be associated with a true positive or a false positive. Furthermore, among patients with ANC undergoing immunosuppressive treatment, a stable humoral immune response with good IgG levels may not be quickly achieved after vaccination because antibody development is highly dynamic.

Patients with evidence of previous infection, that is anti-N and anti-S(RBD) IgG antibodies at T0, were excluded from the study. At T1, only the levels of IgG anti-S(RBD) antibodies were analyzed.

Side effects and neurological status assessment

Adverse events were assessed 2–10 days after each dose by a structured phone interview.

Concomitant disorders, medications, and neurological status were assessed at each study visit using a structured survey and anonymized data were stored on an electronic CRF. For patients on anti-CD20 mAb therapy, total cumulative treatment duration and treatment interval between last infusion and each vaccine dose were recorded. Absolute CD19+ and lymphocyte count and CD19% within one month prior to vaccine were collected from medical records. Disability was assessed using a specific assessment scale for each ANC, i.e. MG-Activities of Daily Living (MG-ADL) for MG, Expanded Disability Status

Scale (EDSS) for MS and NMOSD, Overall Neuropathy Limitations Scale (ONLS) for CIDP patients. The modified Rankin scale (mRS) was used in all patients. To evaluate the impact of vaccination on neurological status, the number of relapses or neurological symptoms worsening requiring hospitalization in the previous two years and 6 months was investigated at T0.

Statistical analysis

Comparisons were performed using the χ^2 test or Fisher exact test for categorical variables and the Mann–Whitney test or the Kruskal Wallis test for continuous variables, correcting for multiple comparisons when necessary. Adjusted odds ratios (OR) and 95% Confidence Interval (95% CI) were calculated using multivariable logistic regression for adverse events, including the covariates of sex, age, vaccine, neurological disease, and comorbidities and for serological status (non-responders vs responders), including the covariates of sex, age, vaccine, neurological disease, and immune therapy. The Incidence rate ratio (IRR) was used to compare the frequency of relapse in the two months before and after the first dose of vaccine. A multivariable quantile regression model was used to evaluate the median difference of the antibody levels between the vaccine groups adjusted for age, sex, neurological disease and immune therapy. A multivariable quantile regression model adjusted for sex and age was

used to evaluate if the presence of moderate/severe local or systemic reaction could predict the antibody levels. The antibody levels were transformed on a Log10 scale only for graphical purposes. Statistical analysis was conducted using STATA, SPSS version 25 and GraphPad Prism version 7. The significance threshold was set at $p < 0.05$.

Results

Participants and vaccine

Between the 15th of March and the 4th of August 2021, 361 patients with ANCs were screened and 308 (186 F and 122 M) enrolled in the study (Fig. 1). After T0 serological testing, seven patients were excluded due to positive serological results, although none reported symptoms or known exposure, and one dropped out. The final cohort included 300 patients (median age 52, IQR 40–65), who underwent the safety assessment. Of them one dropped out before T1 and one skipped sampling at T1 (Fig. 1). Patients' characteristics are reported in Table 1.

Overall, 144 (48%) received the BNT162b2 and 156 (52%) the mRNA-1273 vaccine.

The HCW group included 347 individuals (median age 45, IQR 34–54), 190 (54.8%) F and 157 (45.2%) M. HCW were significantly younger than patients ($p < 0.001$). Of

Fig. 1 Study patient flow chart

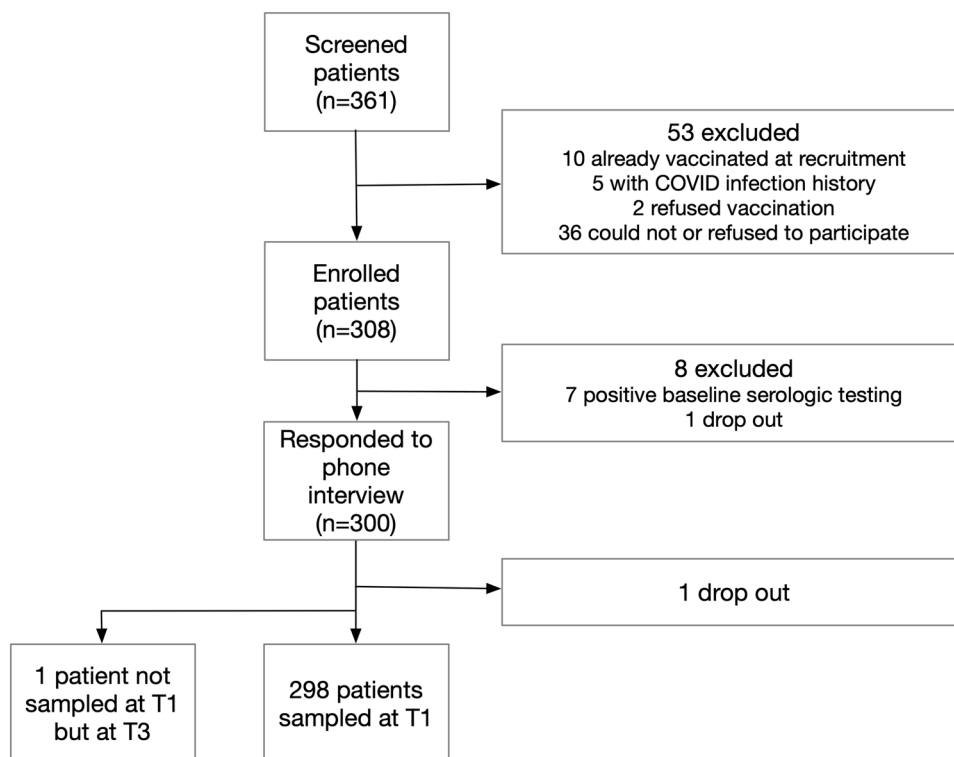


Table 1 Demographic and clinical features of enrolled patients

	Whole population (n = 300)	MG (n = 88)	MS (n = 169)	CIDP (n = 34)	Other (n = 9)	p value
Gender						
Male, number (%)	119 (39.7)	43 (48.9)	49 (29)	21 (61.8)	6 (66.7)	< 0.0001
Female, number (%)	181 (60.3)	45 (51.1)	120 (71)	13 (38.2)	3 (33.3)	
Age, median (IQR)	52 (40–65)	64 (53.2–72)	45 (36–52)***°°°	66 (61.7–76.5)	68 (26–73)	< 0.0001
Disease duration, months, median(IQR)	109.5 (51–180)	78 (42–144)	130 (65.7–216)***	88.5 (61–156)	13.5 (12–75) §§§°	< 0.0001
Immunotherapy, number (%)						
None	74 (24.7)	32 (36.4)	35 (20.7)	5 (14.7)	2 (22.2)	0.021
Steroids (± IVIG or PLEX)	31 (10.3)	28 (31.8)	0	2 (5.9)	1 (11.1)	
IVIG/PLEX	25 (8.3)	0	1 (0.6)	23 (67.6)	1 (11.1)	
AZA (± steroids, IVIG or PLEX)	31 (10.3)	23 (26.1)	4 (2.4)	3 (8.8)	1 (11.1)	
Anti-CD20 mAb (± steroids)						
<i>Ocrelizumab</i>	21 (7)	0	21 (12.4)	0	0	
<i>Rituximab</i>	25 (8.3)	5 (5.7)	15 (8.9)	1 (2.9)	4 (44.4)	
DMF	43 (14.3)	0	43 (25.4)	0	0	
Cladribine	3 (1)	0	3 (1.8)	0	0	
Natalizumab	24 (8)	0	24 (14.2)	0	0	
Fingolimod	9 (3)	0	9 (5.3)	0	0	
Glatiramer	7 (2)	0	7 (4.1)	0	0	
Interferon	2 (0.7)	0	2 (1.2)	0	0	
Teriflunamide	5 (1.7)	0	5 (3)	0	0	
Immunotherapy duration, months, median (IQR)	29.18 (14.2–58)	16.3 (5.6–59.8)	31 (17.7–54.3)	62.5 (21.7–88.2)**	1.6 (0.8–14.7)°°°§§	< 0.0001
mRS at baseline, number (%)						
No disability (0–1)	213 (71)	79 (89.8)	113 (66.9)	18 (52.9)	3 (33.3)	< 0.0001
Mild-moderate disability (2–3)	53 (17.7)	7 (8)	26 (15.4)	14 (41.2)	6 (66.7)	
Severe disability (4–5)	34 (11.3)	2 (2.3)	30 (17.8)	2 (5.9)	0	
Comorbidity, number (%)						
Other AI disorders	43 (14.4)	22 (25.3)	13 (30.2)	5 (15.2)	7 (33.3)	0.001
Diabetes type 2	21 (7)	9 (10.2)	5 (3)	5 (14.3)	2 (22.2)	0.008
Hypertension	72 (24)	30 (34.1)	18 (10.7)	19 (55.9)	5 (55.6)	< 0.0001
Dislipidemia	48 (16)	27 (30.7)	11 (6.5)	8 (23.5)	2 (22.2)	< 0.0001
Vaccine						
mRNA-1273, number (%)	156 (52.3)	50 (56.8)	96 (56.8)	8 (24.2)	2 (25)	0.002
BNT162b2, number (%)	142 (47.7)	38 (43.2)	73 (43.2)	25 (75.8)	6 (75)	

***vs MG, $p < 0.0001$; **vs MG $p < 0.01$; °°°vs CIDP, $p < 0.0001$; ° vs CIDP, $p < 0.05$

§§§vs MS, $p < 0.0001$; §§vs MS $p < 0.01$

them, 341 (98.2%) received the BNT162b2 and 6 (7.2%), the mRNA-1273 vaccine; this difference was due to the availability of vaccines at the beginning of the vaccination campaign when HCW were prioritized.

Side effects

Overall, 244 (81.3%) participants reported local and 220 (73.3%) systemic side effects after either vaccine dose. Local and systemic side effects were more common after the

second dose for both vaccines. Solicited reports of injection-site pain, fatigue, headache, and myalgia were the most frequent reactions for both vaccines (Supplementary Table 1). No patient reported side effects requiring hospitalization with either vaccine.

Since side effects are more common in patients < 55 years for the BNT162b2 vaccine [1], we compared the frequency of local and systemic side effects between the two vaccines stratifying the patients according to this age cut-off (Fig. 2). Independently from the recipient age, local reactions after either dose and fever after the second dose were more common in patients receiving the mRNA-1273 vaccine (Fig. 2). Headache, muscle and joint pain were more common in patients > 55 years receiving mRNA-1273 (Fig. 2). Side effects frequency after each vaccine dose according to age cut-off for each pathology subgroup are shown in Supplementary Fig. 2. Independently from the recipient age and neurological diagnosis, the most common side effects were local pain after the either vaccine dose and systemic symptoms including headache, fever, fatigue and joint/muscle pain after the second dose; side effects were overall more frequent in patients receiving the mRNA-1273 vaccine (Fig. 3).

Logistic regression analysis to investigate factors predicting moderate-severe local or systemic side effects showed that male sex and older age were associated with a lower risk of both local (OR 0.28, 95% CI 0.15–0.52, $p=0.003$ and 0.96 95% CI 0.94–0.99, $p=0.010$, respectively) and systemic reactions (OR 0.42, 95% CI 0.25–0.74, $p<0.0001$ and OR 0.97, 95% CI 0.95–0.99, $p=0.002$, respectively) (Supplementary table 2), whereas mRNA-1273 vaccine was associated with an increased risk of both local (OR 2.52 95% CI 1.45–4.39, $p=0.001$) and systemic reactions (OR 2.51 95% CI 1.49–4.23, $p=0.001$). Neurological diagnosis, comorbidities, and presence of immunotherapy had no influence (Supplementary table 2). However, an association analysis showed that patients treated with IVIG/PLEX had moderate/severe local ($p=0.008$) and systemic ($p=0.002$) side effects less frequently than untreated patients (Supplementary table 3).

Neurological events after vaccination

In the two months prior to the first vaccine dose, 8/300 (2.7%) patients had a clinical relapse, whereas at T1 evaluation, 11 (3.7%) patients, reported symptoms worsening (Supplementary table 4). Overall, the incidence of relapse was not different in the two months before and after vaccination (Incidence rate ratio (IRR) 0.72, 95% CI 0.29–1.83). The results remained unchanged also when considering each ANC separately (data not shown).

Of these 11 patients, 7 (63.6%) had MG, 3 (27.3%) MS, and one (9.3%) CIDP. Five patients (45.4%) were not receiving immunotherapy at vaccination. Most patients were female (8, 72.2%) and received mRNA-1273 (7, 63.6%). In most cases, symptom worsening was transitory and resolved spontaneously after a few days without specific interventions before the T1 evaluation. Two patients, affected by MS and CIDP respectively, required treatment (i.v. steroids). The one affected by MS presented with two independent neurological events after each dose, even though the second dose was administered 62 days after the first. Importantly, no patients had neurological manifestations unrelated to the known neurological diagnosis.

Serostatus at T1

One month after the second dose, anti-S(RBD) specific IgG were detected in 268 (89.9%) patients and in all controls ($p<0.0001$), with no difference in the antibody median levels between the two groups (Fig. 4A). Overall, only 30 (10.1%) patients were classified as non-responders.

In patients, moderate/severe systemic reactions after either vaccine dose were associated with higher antibody levels, with a median increment of 709 BAU/ml (146–1271) ($p=0.014$), after adjustment for age and sex. A similar trend was observed for moderate/severe local reactions ($p=0.084$).

To evaluate the impact of the immunotherapy on antibody levels, patients were divided into categories accordingly to the immune treatment in use at vaccination: no immunotherapy, steroids, intravenous immunoglobulin (IVIG) or plasmapheresis (PLEX), azathioprine (AZA), anti-CD20 mAb, including rituximab and ocrelizumab, first line MS therapies (dimethyl fumarate (DMF), glatiramer acetate (GLA), interferon (IFN) and teriflunomide (TFM)), natalizumab (NTZ), and fingolimod. Three patients treated with cladribine were excluded from this analysis due to the small sample.

Antibody levels were significantly different among these groups ($p<0.0001$). After adjustments for multiple comparisons, significantly lower antibody levels were found in patients undergoing anti-CD20 mAb ($p<0.0001$), fingolimod ($p<0.0001$), AZA ($p=0.011$) and steroids ($p=0.035$) compared to patients without immunotherapy (Fig. 4B). Both untreated and immunosuppressed patients ($p<0.0001$) vaccinated with mRNA-1273 had significantly higher levels of anti-S(RBD) antibodies compared to those vaccinated with BNT162b2 (Fig. 4C,D). The crude median difference of antibody levels between the vaccine groups was 2135 BAU/ml (95% CI 1893–2377, $p<0.001$).

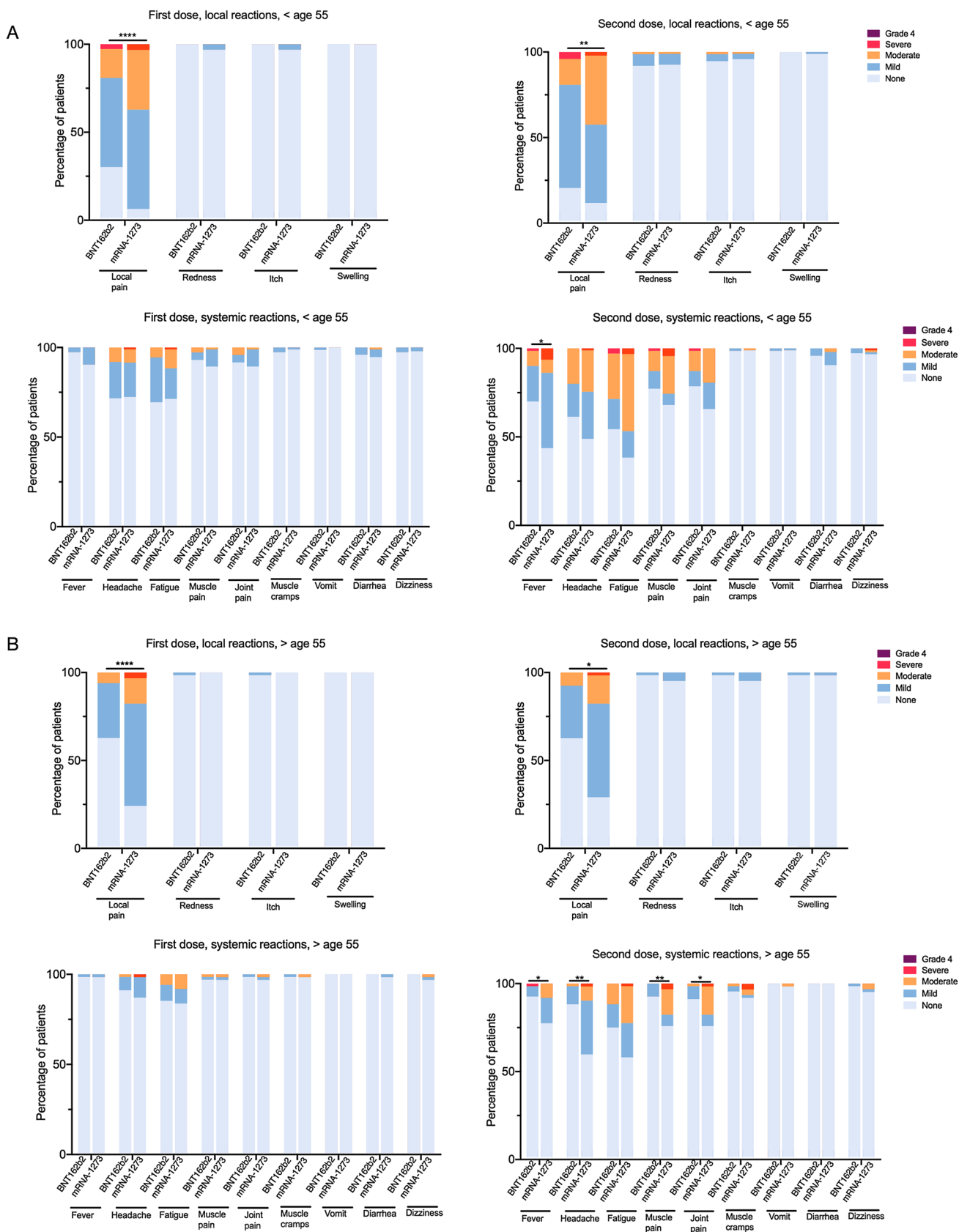


Fig. 2 Solicited side effects reported after injection of BNT162b2 or the mRNA-1273 in patients younger (A) or older (B) than 55 years. Symptoms' severity was assessed according to the following scale: mild, does not interfere with activity; moderate, interferes with activity; severe, prevents daily activity; and grade 4, life-threatening. Fever was graded as follows: mild, temperature >37 to 38.4 °C; moderate, temperature >38.4 to 38.9 °C; severe, temperature >38.9 to 40 °C; grade 4, >40 °C. *Indicates significant different values.

and after adjusting for age, sex, neurological disease and immune therapy, the difference was 916 BAU/ml (95% CI 707–1125, $p < 0.001$).

Non-responders were mainly in the anti-CD20 mAb group (25, 83.3%) (Table 2). No differences in demographic and clinical features were observed between responders and non-responders. However, the latter more often had received the BNT162b2 vaccine ($p < 0.0001$) (Table 2). A sub-analysis comparing responders and non-responders in the anti-CD20 mAb group showed that non-responders were more frequently treated with rituximab than ocrelizumab (18% vs 7%, $p = 0.013$). No difference was observed in the time gap within vaccination and last dose between responders (median 3.7 months, IQR 3.2–4.9) and non-responders (3.5 months, IQR 2.6–5.2, $p = 0.92$), nor in the length of treatment before vaccination (21, IQR 11–26.9, vs 25.3 months, IQR 13.2–39.3, $p = 0.33$), nor in the lymphocyte typing or IgG/IgM levels (data not shown). However, a mild correlation between antibody titers and interval between last anti-CD20 mAb infusion and second dose was observed (Sperman's $\rho = 0.38$, $p = 0.016$).

Logistic regression analysis to investigate predictors of non-responder status showed that BNT162b2 vaccine (OR 8.84 95% CI 2.32–33.65, $p = 0.001$), anti-CD20 mAb (OR 0.004, 95% CI 0.0007–0.026, $p < 0.0001$) and fingolimod (OR 0.036, 95% CI 0.002–0.628, $p = 0.023$) therapies were associated with an increased risk of not developing anti-SARS-CoV-2 IgG. Age, sex and neurological disorder had no influence (Supplementary table 5).

Discussion

Since the beginning of the vaccination campaign, clinicians have been confronted with the question of the safety and effectiveness of novel mRNA vaccines in immunosuppressed patients, who were excluded from the phase III vaccine trials [1, 2]. A few data have now been published, but they are still sparse, and data on patients with ANC other than MS, are very limited. This study provides some answers about the safety and efficacy of new BNT162b2 or mRNA-1273 mRNA vaccines in a

heterogenous large group of patients with ANC undergoing immunotherapy.

Overall, 264 (88%) patients reported adverse events after either vaccine dose, mostly graded as moderate (136, 45.3%). Only 31 (10.3%) patients reported severe reactions and no life-threatening adverse events were observed. Solicited side effects occurred more frequently in the mRNA-1273 group and were not related to the underlying neurological disorder. Moreover, side effects were more common in patients younger than 55 years, as already observed [12]. The frequency and features of solicited adverse events were similar to those reported in the phase III vaccine trials [1, 2]. Interestingly, IVIG/PLEX immunotherapy was associated with a lower risk of moderate/severe side effects. Finally, moderate/severe systemic reactions were associated with higher antibody levels at T1.

Neurological symptom worsening was observed, mostly after the second dose, in 11 (3.6%) cases, requiring medical intervention in two; 1.77% (3/169) MS, 8% (7/88) MG and 3% (1/33) of CIDP patients, overall supporting the safety of SARS-Cov2 mRNA vaccines in ANCs. Two previous studies evaluated the relapse rate in MS patients who had received the BNT162b2 vaccine [10, 13] and found a non-significant increase of relapse rate after vaccination. Notably, in our cohort, among 4 MS events, 3 occurred in the absence of any disease-modifying therapy and the 4th occurred on AZA. Although our study suffers from some of the limitations of the previous ones, including a limited observation time and the lack of MRI studies, this is the first study also involving patients who underwent the mRNA-1273 vaccine and since is still ongoing, it will provide further information on any long-term changes in these patients. Only one study investigated the vaccination effect in a small MG patients' cohort in the 4 weeks after the first dose [14]. Although only 60% of patients had completed the vaccine cycle, 2/22 (9.1%) patients reported mild and self-limiting symptom worsening, similarly to our cases. Despite the small sample, in our cohort only one patient with CIDP reported symptom worsening after vaccination.

Overall, 89.9% of ANC patients had antibody responses after two doses of mRNA vaccines. Despite this high seroconversion rate, however, antibody levels in patients receiving steroids, AZA, anti-CD20 mAb and fingolimod were significantly lower than in untreated patients. Only 44.4% of patients on anti-CD20 mAb therapy, showed seroconversion, according to previous findings [3, 5, 7, 10, 11, 15]. The importance of this observation is related to the expanding indication and use of these mAbs in a plethora of neurological conditions besides MS, due to the high efficacy and relatively safe profile. Different

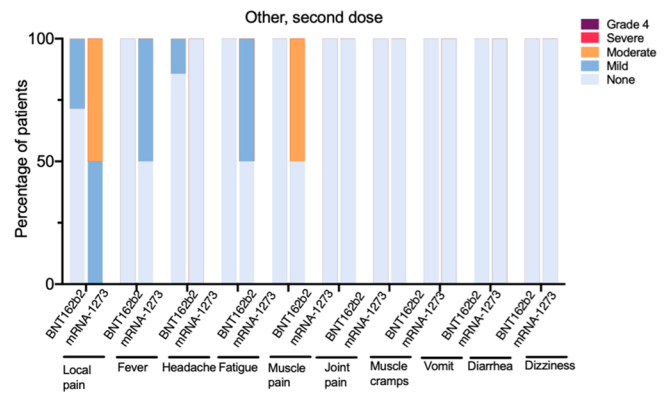
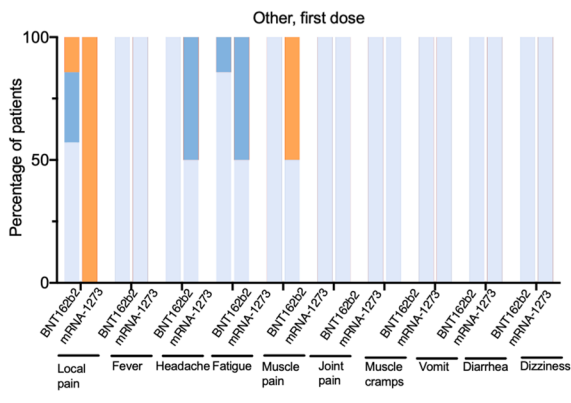
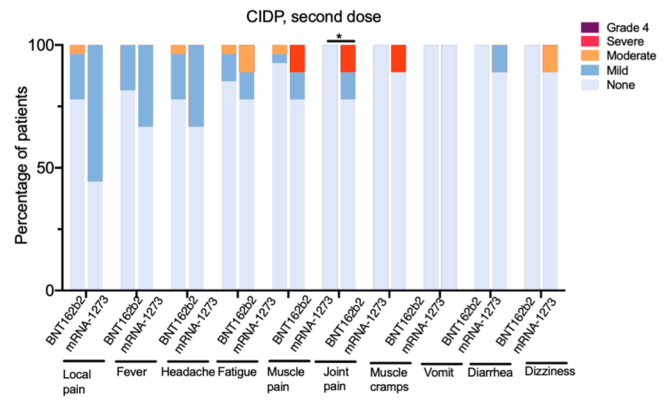
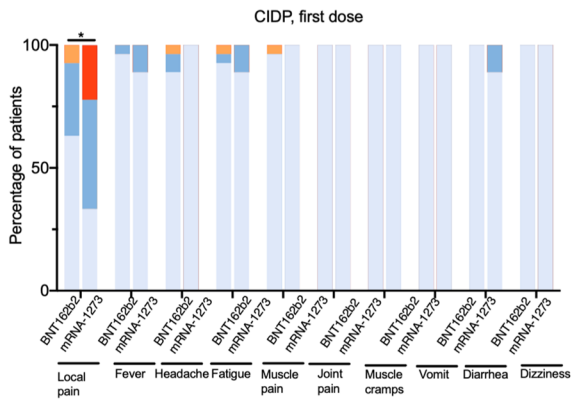
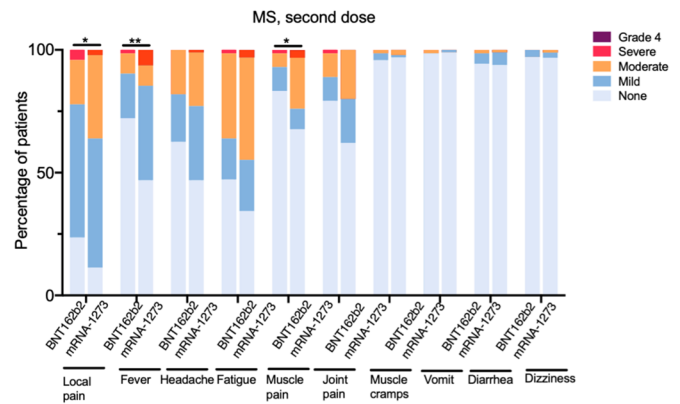
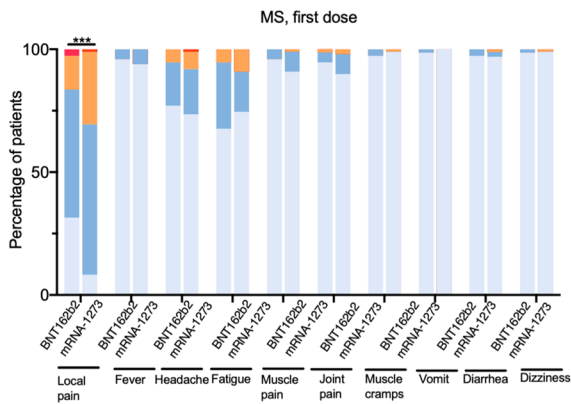
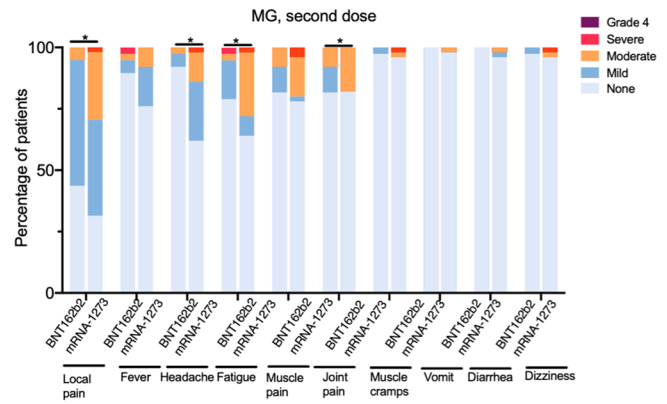
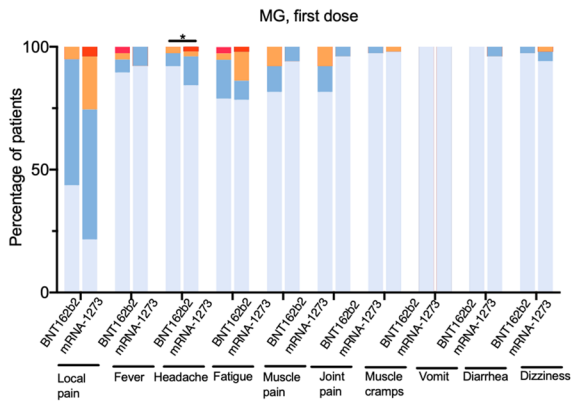


Fig. 3 Side effects in patients according to neurological diagnosis. Solicited side effects reported after injection of BNT162b2 or the mRNA-1273. Symptoms' severity was assessed according to the following scale: mild, does not interfere with activity; moderate, interferes with activity; severe, prevents daily activity; and grade 4, life-threatening. Fever was graded as follows: mild, temperature > 37 to 38.4 °C; moderate, temperature > 38.4 to 38.9 °C; severe, temperature > 38.9 to 40 °C; grade 4, > 40 °C. *Indicates significant different results.

from some previous studies however, we could not find a relation between seropositivity and interval between last anti-CD20 mAb infusion and vaccination, nor with the length of treatment before vaccination [3, 7]. However, we found a positive correlation between antibody levels and the interval between the last infusion and the second vaccine dose, as observed by others [5, 11]. Reasons for these discrepancies could include differences in the methods used to assess anti-S(RBD) antibodies or to the overall features of selected patients, including not only MS but also patients with other ANCs, such as MG, SPS and NMOSD. Similarly, we did not find a correlation between CD19⁺ cells levels prior vaccination and antibody titers [3].

Antibody responses were also reduced in patients treated with fingolimod as already found [3, 7, 10, 11, 15], as well as in patients treated with steroids and AZA, although only a minority of them remained seronegative. Given the diffusion of steroids and AZA treatment beyond neurological disorders this is a relevant observation. Previous studies showed either normal [16] or reduced [17, 18] antibody levels in patients treated with steroids. Another study showed reduced antibody levels in patients undergoing antimetabolite therapy after solid organ transplant [19] whereas others found no significant differences [18]. This result variability could be due to differences in the antibody detection methods or in the treatment schemes and need further assessment.

As already shown [3, 11], we also found that untreated patients and those treated with first-line MS therapies, NTZ, IVIG/PLEX developed anti-S(RBD) antibody similarly to controls, indicating that not all patients on immunotherapy have blunted immune response to vaccine. This notion could be useful to inform future policies on prioritizing further vaccine boost in patients receiving treatments that are more likely to inhibit the immune response.

Two previous studies, comparing the BNT162b2 and the mRNA-1273 vaccine, showed an influence of the vaccine type on antibody titers in either healthy [20] or

immunosuppressed subjects [11]. Our study confirms and expand to other ANCs the finding of a higher seroconversion rate and higher antibody levels in immunosuppressed patients vaccinated with the mRNA-1273 vaccine compared to those vaccinated with the BNT162b2 [11]. It is likely that the higher mRNA content (100 µg in the mRNA-1273 vs 30 µg in the BNT162b2 vaccine), together with the longer interval between the two doses for mRNA-1273 (4 weeks vs 3 weeks for the BNT162b2 vaccine) could account for the higher seroconversion rate and RBD antibody levels as well as for the higher reactogenicity associated with the mRNA-1273 vaccine [1, 2, 11, 20]. Although this finding needs replication in larger cohorts, it could be useful in guiding the vaccine choice in specific patient groups. The use of a heterologous vaccine scheme, which has been shown to induce a stronger immune response [21], might represent a possible further option.

Our study has limitations. Firstly, we assessed solely the vaccine-induced humoral response. However, compared to previous studies using the same approach, our study has the strength of using a commercially available test which has shown high sensitivity [22] and which should ensure results reproducibility. Although a correlation between antibody levels and protection against SARS-CoV2 infection has not been established yet, a sustained humoral response has been associated with a faster recovery [23] and decreased risk of symptomatic infection [24]. Secondly, we did not evaluate the neutralizing antibody responses. However, SARS-Cov-2-RBD IgG levels have been shown to correlate with virus neutralization antibody titers [25–27]. Moreover, although neutralizing antibodies are considered more reliable predictors of protection [28], their titers might not correlate with clinical effectiveness against SARS-CoV2 symptoms [29].

A further limitation of this study is that we did not investigate cellular immunity, which could still confer significant protection against the infection, despite reduced or absent antibody levels. Indeed, previous studies in MS patients showed T cells reactivity also in serological non-responders [3, 7], highlighting possible disparities between cellular and humoral responses. Therefore, caution is needed in interpreting the serology results in non-responders, since they could still harbor significant level of protection against infection through cellular responses. However, since a correlation has not yet been established between cellular and humoral response and risk of symptomatic infection, epidemiological studies on infection after vaccination in immunosuppressed

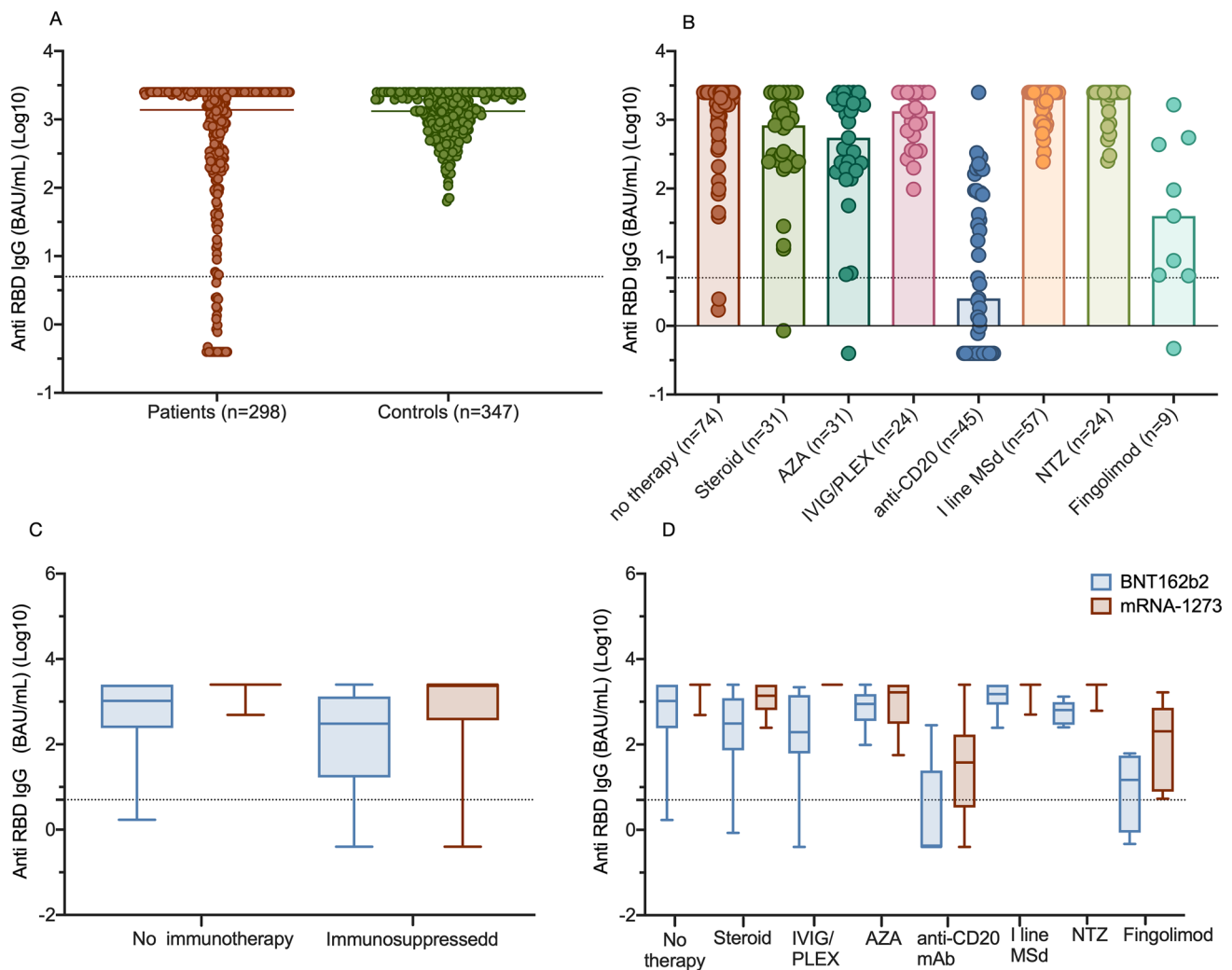


Fig. 4 SARS-CoV2 RBD IgG titers in patients and controls. **A** Antibody levels were not significantly different in patients and health-care-workers. **B** Antibody levels were significantly lower in patients treated with steroids, AZA, anti-CD20 and fingolimod compared to untreated patients and to those treated with IVIG/PLEX, first line MS modifying therapies and NTZ. **C** Antibody levels were lower in

patients who received BNT162b2 compared to those vaccinated with mRNA-1273, independently from immunosuppression. **D** Antibody levels were significantly different between patients receiving the BNT162b2 and the mRNA-1273 vaccine, in the steroid ($p < 0.0001$), AZA ($p < 0.0001$), anti-CD20 and fingolimod ($p < 0.0001$) groups

individuals are needed to confirm the real-world impact of in vitro studies.

Despite these limitations, our data demonstrated for the first time in a large group of patients with various ANC that most patients with a large selection of immunosuppressive therapies can mount an effective immune response after SARS-CoV-2 mRNA vaccination without significant side effects or short-term worsening of their neurological status.

Moreover, one month after the second dose, no patient reported SARS-Cov2 infection, supporting the clinical effectiveness of the vaccination. Therefore, our study supports the recommendation to vaccinate immunosuppressed patients with ANC. The continuous evaluation of antibody titers as well as the rate of infection will provide more information about the efficacy of anti-SARS-CoV-2 vaccination in this vulnerable population.

Table 2 Comparison of demographic and clinical features between responders and non-responders

	Responders	Non-responders	<i>p</i>
Number	268 (89.9)	30 (10.1)	
Sex, F, number (%)	161 (60.1)	18 (60)	0.994
Age, median (IQR), years	52 (39.2–65)	54.5 (45–65)	0.500
Disease duration, months, median (IQR)	96 (48–178)	144 (58.7–243)	0.108
Immunosuppression duration, months, median (IQR)	29.3 (14.1– 59.8)	26.5 (14.4–41.7)	0.339
Other autoimmune disorders, number (%)	37 (13.9)	6 (20.7)	0.321
Type II diabetes, number (%)	18 (6.7)	3 (10)	0.505
Immunosuppressive therapy, number (%)			
No immunotherapy	72 (26.9)	2 (6.7)	< 0.0001
Steroid	30 (11.2)	1 (3.3)	
IVIG/PLEX	24 (9)	0	
AZA	30 (11.2)	1 (3.3)	
Anti-CD20	20 (7.5)	25 (83.3)	
First line MS therapies	57 (21.3)	0	
Natalizumab	24 (9)	0	
Fingolimod	11 (4.1)	1 (3.3)	
Vaccine			
mRNA-1273, number (%)	150 (56)	6 (20)	< 0.0001
BNT162b2, number (%)	118 (44)	24 (80)	

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Data sharing After publication, anonymised data will be made available upon reasonable request.

Declarations

Conflict of interest MPG, VV, ML, FC, CZ, IP, AB, MT, AB, FS, RR, GDF, VL, TZ, declare no competing interest; AL, has served as a Biogen, Bristol Myers Squibb, Merck Serono, Novartis, Roche, Sanofi/Genzyme and Teva Advisory Board Member. She received congress and travel/accommodation expense compensations or speaker honoraria from Biogen, Merck, Mylan, Novartis, Roche, Sanofi/Genzyme, Teva and Fondazione Italiana Sclerosi Multipla (FISM). Her institutions received research grants from Novartis and Sanofi Genzyme. RL has received: Lecture fees from LT3 s.r.l., NICO s.r.l., SUMMEET s.r.l. and GALEN SYMPOSIUM s.r.o.; fees for consultancy (Advisory Board) from LT3 s.r.l. and PREX s.r.l.; fees for scientific meeting organization from I&C s.r.l.; Chair meeting fees from PLANNING CONGRESSI s.r.l.

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