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## Immunosuppressive treatments selectively affect the humoral and cellular response to SARS-CoV-2 in vaccinated patients with vasculitis

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7 Abstract

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9 Objectives. To analyse humoral and cellular immune response to messenger RNA (mRNA) COVID-19  
10 vaccines in patients with giant cell arteritis (GCA).  
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12 Methods. Consecutive patients with a diagnosis of GCA receiving two doses of BNT162b2 vaccine were  
13 assessed at baseline and three weeks from the second vaccine dose. Healthy subjects (n=51) were included  
14 as controls (HC). Humoral response was assessed with Spike-specific IgG antibody response (S-IgG) and  
15 neutralising antibodies (NtAb). Specific T-cell response was assessed by Enzyme linked immunospot  
16 (ELISpot).  
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18 Results. Of 56 included patients with GCA, 44 were eligible after exclusion of previous evidence of COVID-19  
19 and incomplete follow-up. A significant proportion of patients with GCA (91%) demonstrated antibody (S-  
20 IgG) response, however this was significantly lower than HC (100%);  $p < 0.0001$ . Neutralising activity was not  
21 detected in 16% of patients with GCA. Antibody titres (S-IgG and NtAb) were significantly lower compared to  
22 HC. Humoral response (S-IgG and NtAb) was significantly hampered by treatment with methotrexate (MTX).  
23 Cellular response was lacking in 30% of patients with GCA (vs 0% in HC);  $p < 0.0001$ . Cellular response was  
24 significantly influenced by the levels of baseline peripheral T-lymphocytes and by glucocorticoid treatment.  
25 Treatment with tocilizumab did not affect any level of the immune response elicited by vaccination.  
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28 Conclusions. Although patients with GCA apparently achieve a robust antibody seroconversion, there is a  
29 significant impairment of the neutralising activity. MTX significantly reduced all levels of the humoral  
30 response. Up to one third of patients do not develop a cellular immune protection in response to COVID-19  
31 vaccination.  
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33 Key Words: giant cell arteritis, vasculitis, COVID-19, vaccination, immune response, cellular response  
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35 Key Messages

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- 38 • Immune response to COVID-19 vaccine is reduced in patients with GCA versus healthy controls.
  - 39 • Despite good seroconversion rate for anti-Spike antibodies, neutralising and T-cellular response are  
40 significantly hampered.
  - 41 • Different treatments (methotrexate and glucocorticoids) selectively affect the response to  
42 vaccination (humoral and cellular, respectively).  
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## Introduction

The pandemic caused by severe acute respiratory coronavirus 2 (SARS-CoV2) has spread since 2019 leading to the death of 5.200.267 people worldwide as of November 2021(1). While efforts are ongoing to find efficacious treatment strategies for COVID-19, the scientific community has focused on the development of preventive immunisation since early 2020. The speed of advance in vaccine production and testing in large trials has shown the unprecedented successful results achievable by modern scientific research in case of a global emergency. The mRNA vaccines developed against COVID-19 (BNT162b2 and mRNA-1273) have been at the forefront of vaccine development (2). Efficacy of COVID-19 vaccines has been reported to be as high as 95% against infection and even higher in preventing severe disease in the first trials(3,4). The effectiveness in real-life has been shown to be reduced in containing the disease, particularly after the selection of new variants of the virus, while still preventing severe outcomes in a significant proportion of the general population(5). While vaccines against SARS-CoV-2 have undoubtedly reduced the mortality and morbidity of COVID-19, a number of challenges remain. One of the main concerns around the protective role of COVID-19 vaccines is their effectiveness in selected populations, especially those receiving immunosuppressive treatments. Experience from previous H1N1 influenza pandemic, and data evaluating the efficacy of seasonal flu and pneumococcal vaccines suggest reduced sero-protection for patients with rheumatoid arthritis (RA) treated with methotrexate (MTX), but not with other disease modifying anti-rheumatic drugs (DMARDs), including biologic treatments (6,7, 8). Whether DMARDs impact mRNA vaccines against SARS-CoV-2 in a similar way is still largely to be elucidated.

Preliminary reports on the efficacy of COVID-19 vaccines in patients with rheumatological conditions have focused on seroconversion, suggesting lower rates of antibody response to mRNA vaccines compared to healthy controls (HC), especially after a single vaccine dose(9), and a significant association of a poorer response with certain classes of drugs (eg. rituximab)(10). Nevertheless, the value of dosing circulating antibodies against SARS-CoV-2 Spike protein as an assurance of effective protection is still a matter of debate. Indeed, seroconversion, when considered alone, may fail to be representative of the complex immune response to COVID-19 vaccines. Humoral response itself can be stratified to include the neutralising activity of circulating antibodies, known to be crucial in the clearance of the virus (11). Moreover, cell-mediated immunity has been shown to be a more reliable indicator for protection than humoral response against respiratory viruses, including influenza, especially in immunocompromised or elderly patients (12,13). Different immunosuppressive regimens and specific disease characteristics may influence the response to vaccination in patients with rheumatic diseases.(14). Therefore, further studies on several levels of immunity elicited by COVID-19 vaccination are required, especially in patients who are clinically at-risk but with a low, undetectable, or waning humoral response.

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3 In this prospective study we assessed the rates, levels, and correlations of specific trimeric anti-Spike  
4 antibodies, neutralising antibodies, and T-cellular response in a group of patients with giant cell arteritis  
5 (GCA) following two BNT162b2 vaccine doses.  
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## 10 11 Material and Methods

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14 Consecutive patients with a clinical diagnosis of GCA followed at the Department of Rheumatology of  
15 Policlinico S. Matteo IRCCS Fondazione who received two doses of SARS-CoV-2 BNT162b2 vaccine  
16 (Pfizer/BioNtech) between April and June 2021 were enrolled. We had previously described the  
17 seroconversion rate following the first and second vaccine dose in a different cohort of patients with GCA(9);  
18 in the current study, patients were included to assess neutralising and cellular response to vaccination.  
19 Humoral response was reported to find correlations with the other two types of immunological response and  
20 was assessed with a different methodology (new generation recombinant trimeric spike glycoprotein) to  
21 optimise sensitivity and specificity. Only patients with complete data and follow-up were considered.  
22 Patients with GCA continued their regular treatment for the rheumatological disease around the time of  
23 vaccination. A group of HC  $\geq 50$  years of age according to the epidemiological distribution of GCA was  
24 collected. To exclude a role of age or disease-specific effect on the degree of immune response, two  
25 adjunctive age-matched pathological groups were considered as controls: patients from an historic cohort  
26 with RA or spondyloarthritis treated with biologic DMARDs with or without MTX, and patients with psoriasis  
27 treated with biologic DMARDs. All patients provided written informed consent (Ethical approval reference P-  
28 20210000232).  
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32 Blood samples were collected prior to vaccination, and three weeks after the second vaccine dose. Serum  
33 was stored at  $-80^{\circ}\text{C}$  until analysed. Serum samples were collected for evaluation of SARS-CoV-2 total and  
34 neutralising antibodies (NtAb) while heparinised whole blood samples were used for peripheral mononuclear  
35 cells (PBMC) isolation and quantification of Spike-specific T-cell response.  
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### 39 40 41 42 43 44 45 46 47 48 *Assessment of humoral response elicited by SARS-CoV-2 vaccine*

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51 Immunoglobulins (IgG) directed to SARS-CoV-2-specific Spike protein in their trimeric form (SIgG) were  
52 determined by chemiluminescent assay (Liaison SARS-CoV-2 trimeric, Diasorin, Saluggia, Italy), according to  
53 manufacturer's instructions. A result above the cutoff of 13 AU/ml (33.8 BAU/ml) was considered positive.  
54 SARS-CoV-2 specific NtAb titre was determined as previously reported (Percivalle et al., 2020)(15). Results  
55 were considered positive when the titre was  $\geq 1:10$ .  
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### *Assessment of T cell response elicited by SARS-CoV-2 vaccine and T cell count*

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5 SARS-CoV2 specific-T cells were determined by Enzyme linked immunospot (ELISpot) assay as previously  
6 reported (Cassaniti et al., 2021)(16). Briefly, PBMC were plated at a concentration of  $2 \times 10^5/100 \mu\text{l}$  culture  
7 medium per well and were stimulated in duplicate for 24 h in 96-well plates (coated with anti-IFN- $\gamma$   
8 monoclonal capture antibody) with peptide pools (15mers, overlapping by 10 aminoacids, Pepscan,  
9 LelystadThe Netherlands) representative of the Spike protein (S) at the final concentration of 0.25  $\mu\text{g/ml}$ .  
10 Phytohemagglutinin (PHA; 5  $\mu\text{g/mL}$ ) was used as positive control, and medium alone as negative control.  
11 Results were expressed as IFN- $\gamma$  spots forming units (SFU)/ $10^6$  PBMC. A result  $\geq 10$  net spots/million PBMC  
12 was considered positive. This cutoff was based of the mean value plus two x standard deviations of PBMC  
13 stimulation with negative control.  
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15 The methods for detection of total and naïve T and B cells are reported in the Supplementary Material,  
16 available at *Rheumatology* online.  
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### 20 *Statistical analysis*

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28 The Mann-Whitney U-test was used to compare quantitative variables between two groups and the Kruskal-  
29 Wallis test for more than two groups. Differences in frequencies were analyzed by Fisher's test. Linear  
30 regression analysis was used to show the relationship between two quantitative variables. A multiple linear  
31 regression analysis was used to identify independent predictors of immune response to the vaccine. Immune  
32 parameters were log-transformed for the analysis. P values  $< 0.05$  were considered significant. GraphPad  
33 Prism 8.3.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for the analyses.  
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## 40 Results

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42 The cohort consisted of 56 patients with GCA, of which four were excluded due to evidence of previous SARS-  
43 CoV-2 infection, five were lost to follow-up, and three were excluded due to lack of availability of PBMC. Of  
44 the 44 included patients, 31 (70%) were female, median age 72 (IQR 68-77). A group of 51 HC was included,  
45 female 40 (80%), median age 56 (IQR 53-61). The general characteristics and type of treatment of patients  
46 with GCA are presented in Table 1. Median glucocorticoid (GC) dose was 5 mg/day (IQR 3.1-7.5), with 20.5%  
47 of patients receiving  $\geq 7.5$  mg of prednisone-equivalent/day.  
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### 54 ***SARS-CoV-2 specific anti-Spike trimeric antibodies response***

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56 After two vaccine doses, 40 (91%) patients developed SARS-CoV-2 specific S-IgG, while 4 (9%) patients had  
57 no detectable humoral response. Among HC 51 (100%) developed S-IgG. The median titre of S-IgG in patients  
58 with GCA was significantly lower compared to HC (median 595 AU/ml, IQR 251-850 vs 791, IQR 570-850,  
59  $p=0.02$ ) (Figure 1, Panel A). Treatment with GC did not affect humoral response (Figure 1, Panel B).  
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3 The four patients without seroconversion were 3 females/1 male, median age 77 years old, range 70-82, all  
4 receiving MTX. Patients treated with MTX showed significantly lower levels of SARS-CoV-2 specific S-IgG  
5 compared to patients not receiving MTX treatment ( $p<0.0001$ ) (Figure 1, Panel C). Patients treated with a  
6 combination of GC and MTX ( $n=12$ ) showed a decreased S-IgG response compared to either agent alone:  
7 median S-IgG titre for combination treatment 209 (IQR 12.7-265) vs 850 (IQR 611-850) for GC ( $n=18$ );  
8  $p<0.0001$ ; vs 850 (773-850) for MTX monotherapy ( $n=6$ );  $p=0.008$ .  
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### 15 ***SARS-CoV-2 specific neutralising antibodies response***

16 Among patients with GCA, 37 (84%) were NtAbs responders, while 7 (16%) did not develop NtAbs. All HC  
17 developed NtAbs. The titre of NtAbs was significantly lower for patients with GCA compared to HC (median  
18 80, IQR 20-160 vs 320, IQR 320-1280  $p<0.0001$ ) (Figure 1, Panel D).  
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20 The analysis of the factors associated with NtAbs response revealed no association with sex or disease  
21 phenotype (cranial vs large-vessel GCA). Moreover, GC therapy, regardless of the dose, did not influence the  
22 development of NtAbs (Table 2; Figure 1, Panel E). Similarly, treatment with tocilizumab (TCZ) did not affect  
23 the neutralising response. On the other hand, patients treated with MTX did not develop NtAbs in 39% of  
24 cases. All NtAbs non-responders were receiving MTX (Table 2). Furthermore, patients treated with MTX  
25 showed significantly lower levels of SARS-CoV-2 specific NtAbs (Figure 1, Panel F).  
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30 Patients treated with a combination of GC and MTX showed a decreased NtAb response compared to patients  
31 treated with GC only. Median NtAb for combination treatment: 10, IQR 5-40 vs 80, IQR 40-640 for GC;  
32  $p<0.0001$ .  
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### 38 ***SARS-CoV-2 specific cellular response***

39 Thirteen (30%) patients with GCA did not develop SARS-CoV-2 specific T-cell response. On the other hand, in  
40 the control group of HC, all 51 (100%) subjects developed a cellular response.  
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42 The level of T-cell response was significantly lower for GCA patients compared to HC (median 28 IFN- $\gamma$   
43 SFU/ $10^6$  PBMC, IQR 5-69 vs HC, median 90, IQR 45-155;  $p<0.0001$ ) (Figure 2, Panel A).  
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45 The analysis of the factors associated with T-cell response did not show any association with sex, age, or  
46 disease phenotype.  
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50 Treatment with GC negatively influenced cellular response to the vaccine. Twelve (92%) patients in the T-cell  
51 immunity non-responders received GC, compared to 21 (67%) of responders. The level of T-cell response was  
52 significantly influenced by treatment with GC (Figure 2, Panel B). Treatment with MTX did not influence the  
53 degree of T-cell response (Figure 2, Panel C). Similarly, TCZ did not affect cellular response (Table 2). The  
54 combination therapy of MTX and GC (median 10, IQR 0-55) did not influence cellular response compared to  
55 either agent alone (median 27.5, IQR 5-68.5 for GC, and median 45, IQR 20-1515 for MTX).  
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3 Cellular response was significantly influenced by the levels of peripheral T-lymphocytes. Patients who  
4 developed a T-cell response showed higher levels of CD4<sup>+</sup> T-cells (1002 cells/ $\mu$ l blood, range 389-1586 vs non-  
5 responders,  $p=0.03$ ). CD8<sup>+</sup> T-cells did not influence the rate of response (Figure 3).  
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8 No significant difference was found between the percentage of circulating CD45RA<sup>+</sup>CCR7<sup>+</sup> CD4 naïve or CD8  
9 naïve T cells and the detection of a T-cell response (Supplementary Figure S1, available at *Rheumatology*  
10 online).  
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13 Moreover, in a multivariate model, treatment with GC remained an independent risk factor for cellular  
14 response, regardless of baseline CD4<sup>+</sup> or CD8<sup>+</sup> T-cells (Supplementary Table S1, available at *Rheumatology*  
15 online).  
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### 20 ***Humoral response and B cells***

21 There was a significant correlation between the number of circulating B-cells (Supplementary Figure S2, Panel  
22 A and B, available at *Rheumatology* online) and IgD<sup>+</sup>CD27<sup>-</sup> naïve B-cells (Supplementary Figure S2, Panel C  
23 and D, available at *Rheumatology* online) with both S-IgG and NtAb responses.  
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### 28 ***Multiple linear regression to assess the factors influencing the different types of immune response***

29 In a multiple linear regression model assessing the factors associated with the different types of immune  
30 response, accounting for potential confounding factors, including age, having the disease, and type of  
31 treatment, we confirmed the independent negative correlation between MTX and S-IgG and NtAbs  
32 responses, and the effect of GC on T-cell response (Supplementary Table S2, available at *Rheumatology*  
33 online). The correlations for all types of immune response were confirmed to be independent of age. Further  
34 data on the influence of age are reported in the Supplementary Material, available at *Rheumatology* online.  
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### 41 ***Correlation between different types of immune response***

42 There was a significant correlation between levels of SARS-CoV-2 specific S-IgG and NtAbs ( $r^2=0.573$ ;  
43  $p<0.0001$ ). (Figure 4, Panel A).  
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46 Moreover, there was a significant correlation between levels of SARS-CoV-2 specific S-IgG and T-cell response  
47 ( $r^2=0.0932$ ;  $p=0.04$ ) (Figure 4, Panel B). There was also a correlation between NtAb response and cellular  
48 response ( $r^2=0.102$ ;  $p=0.0347$ ).  
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51 At a single patient level, B and T-cell non-responders differed. Absence of both B (humoral) and T-cell  
52 responses was observed in two patients. Ten patients developed S-IgG and NtAbs responses without cellular  
53 response, while one patient developed S-IgG only. T-cell response was possible even in the absence of any  
54 type of humoral response (one patient), or only combined with S-IgG response but without neutralising  
55 activity (two patients).  
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## Discussion

A better understanding of the immunological response elicited by mRNA COVID-19 vaccines is pivotal to ensure protection against the infection, especially in the most at-risk categories of the population. This is, to the best of our knowledge, the first study to thoroughly assess and correlate the humoral, neutralising and cellular response to BNT162b2 COVID-19 vaccine in a homogenous cohort of patients with vasculitis. Our study demonstrated that, albeit a significant proportion of patients with GCA (91%) develop detectable anti-Spike antibodies, a deeper analysis of the immunological levels of response reveals that 16% do not display neutralising antibody activity, and up to 30% of patients do not develop an efficacious T-cell response against the virus as a result of the vaccination. Moreover, the study shows that both the qualitative (e.g. type of response) and quantitative immunisation (e.g. titres of antibody production and degree of T-cell reactivity) is significantly reduced compared to HC, and according to the type of treatment.

Available vaccines selected the Spike glycoprotein as an immunogen given its crucial role in the viral entry process. Neutralising activity targets the receptor-binding domain (RBD) that specifically recognises the host-cell receptor ACE2. Serum NtAbs reflect the functionality of serum protection directed against SARS-CoV-2. Moreover, the levels of NtAbs significantly correlate with COVID-19 protection being able to effectively prevent the disease (17). Nevertheless, while NtAbs activity is regarded as the most effective, other mechanisms of the humoral response may still contribute to infection control, such as antibody-dependent complement deposition and antibody-dependent cellular cytotoxicity. Even though specific thresholds for immune protection are difficult to identify, in our cohort of patients with GCA, both levels of S-IgG and NtAbs were significantly lower compared to HC. This finding was significantly associated with specific treatments, particularly MTX, as reported for other rheumatic diseases (18). Moreover, 100% of patients lacking a humoral response (both S-IgG and NtAb) were treated with MTX. Since MTX is the anchor drug for a number of immune mediated inflammatory diseases, this finding is particularly important for the rheumatological community. Further research on temporary drug withdrawal strategies, to be balanced with the risk of disease relapse, or on the frequency of re-vaccination strategies in these patients is needed. In our study we did not identify a significant impact of GC therapy on the neutralising activity, however it cannot be excluded that higher doses of GC might have an influence on the humoral response (19).

In the setting of suboptimal NtAb titres, rapid waning of the humoral response over the first few months following vaccination (20), and emerging variants of concern, T-cell responses may become particularly important in the protection from COVID-19 (21). T-cells act by reducing viral replication and containing the pathogenicity of the infection (22). It has been shown that in patients with impaired humoral responses due to deficient B cells during anti-CD20 treatments highly functional SARS-CoV-2 specific T-cells are crucial to improve the severity of the disease and survival (23). Indeed, circulating naïve B cells have been found to be associated with antibody response in immunocompromised patients (24). In our study, we confirmed that a higher number of B cells and B naïve cells are good predictors of both types of the humoral response. T-cell

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3 immunity is believed to wane less rapidly than detectable circulating antibodies, possibly leading to some  
4 degree of protection in time (17). T-cell response might therefore prove particularly important in securing  
5 vaccine efficacy against viral variants. Indeed, despite reported immune elusion and cases of re-infection  
6 (25), viral variants are often responsible for mild or asymptomatic disease in those who have received a two-  
7 dose vaccination regimen, likely as a result of an effective cellular immunity. Indeed, the novel mutations  
8 mainly occur in the RBD domain and could therefore escape the neutralising humoral protection. Vaccine-  
9 induced T cells are multispecific and recognise different regions of the Spike protein interfering with the virus  
10 ability to fully escape cellular immunity (22). In this scenario, it is a worrisome finding of our study that 30%  
11 of patients with GCA lack T-cell specific immune response despite having fully completed the vaccine  
12 schedule. Moreover, in patients developing cellular immune response, this was significantly lower compared  
13 to the control group. Possible contributors to this finding were the type of treatment, with (92%) of non-  
14 responders receiving GC compared to 67% of responders, and the dose of GC. We identified a negative  
15 correlation of GC treatment and the level of T-cell response. While high-dose GC are usually required to  
16 significantly impact the action of B cells, GC, even at lower doses, have been demonstrated to regulate the  
17 peripheral immune responses by inhibiting T-cell immunity and attenuating T-cell receptor signaling (26).

18 On the other hand, MTX or TCZ did not seem to play a significantly influence on the rate and quality of cellular  
19 response. It was interesting to observe how MTX seems to influence B-cell but not T-cell response. Our  
20 findings are supported by existing data showing how MTX attenuates humoral immunity. MTX decreases  
21 serological response to influenza or pneumococcus vaccines (6,8), and has a role in preventing anti-drug  
22 antibody development in combination with biologic drugs (27). MTX may act on B cells by inhibiting  
23 activation, and blocking the expansion of switched memory cells and plasmablasts after antigen  
24 stimulation(28). Despite the known therapeutic effect of MTX on T-cell regulation and cytokine production,  
25 previous evidence did not report a significant impairment of the cellular response to influenza vaccination in  
26 patients with RA receiving DMARDs (29). Similar findings have been found following COVID-19 vaccine in  
27 other disease types (14). Although the exact mechanisms responsible for a preserved cellular response to  
28 mRNA vaccines under MTX treatment still has to be demonstrated, evidence suggests that MTX leads to an  
29 enhancement, rather than diminishing CD4+ and CD8+ T cell count, resulting in a preserved T-cell effector  
30 function and improved T-cell regulatory activity (30,31).

31 We did not find any association between cellular response and older age or other disease characteristics.  
32 Whether such a significant rate of inadequate cellular response elicited by vaccination will expose our  
33 patients with GCA to a more severe disease in case of re-infection is still unknown, but certainly warrants  
34 consideration.

35 Recent evidence has reported a potential safety warning with increased diagnoses of GCA and polymyalgia  
36 rheumatica following COVID-19 vaccination (32). Nevertheless, none of our patients experienced relapses  
37 following two doses of BNT162b2 vaccine, offering reassuring safety results. Moreover, patients enrolled in  
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3 the study were all in remission at the time of vaccination, limiting the possibility that the underlying disease  
4 activity might have influenced the immunological response.

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6 This study has some limitations. The HC group, although recruiting only subjects  $\geq 50$  years of age, similarly  
7 to the epidemiology of GCA, still included subjects that were slightly younger than the study population, this  
8 should be regarded as a major limitation of the study. Nonetheless, age did not influence the seroconversion  
9 rate, nor the cellular response, even after adjusting for a number of confounders. Moreover, further analyses,  
10 including the comparison with different age-matched pathological control groups confirmed that, despite  
11 comparable age, the different types of immunological response were mainly influenced by the type of  
12 treatment rather than by the diagnostic group. Although the numerosity of patients with GCA was significant  
13 given the rarity of the disease and the extension of the immunological analyses performed, the number of  
14 observations for some subgroup analyses stratified by type of treatment might have been too low to ensure  
15 statistical significance in some cases. The potential confounding effect of disease resistance requiring MTX  
16 treatment on the impairment of the humoral response observed with MTX could not be completely ruled  
17 out, however, the similar finding reported for other rheumatological conditions such as RA, where MTX is  
18 used as first-line agent, supports a drug-specific effect. Finally, we assessed T-cell response, without  
19 distinguishing between CD4 and CD8 responses. Nonetheless, the study offers important insights on the  
20 different mechanisms of vaccine-induced protective immune response and their correlation with specific  
21 immunosuppressive treatments and patients' characteristics.

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23 In conclusion, despite a good response rate in terms of seroconversion to COVID-19 vaccination, neutralising  
24 and especially cellular response are significantly reduced in patients with GCA. Drugs used to treat GCA, but  
25 also relevant to a number of different rheumatic conditions, affected the immune response to COVID-19  
26 vaccination at different levels, with MTX significantly impairing humoral and especially neutralising activity,  
27 and GC hampering the cellular response, possibly adding further risk factors in case of infection in this  
28 population of elderly patients.

29  
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33 the recruitment of the pathological control groups affected by rheumatoid arthritis/spondyloarthritis and  
34 psoriasis.

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40 design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision  
41 to submit the paper for publication.

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43 Data availability: Data will be made available upon request

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**Table 1. General characteristics of the study population**

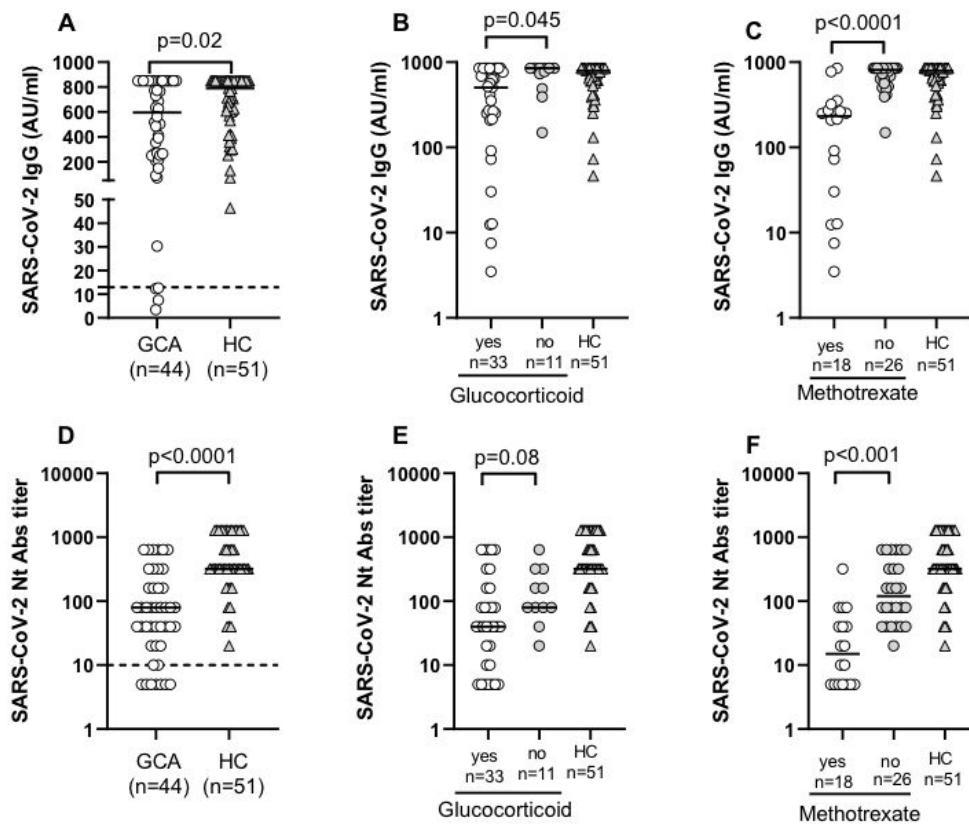
	GCA patients (n=44)
Age, median (IQR)	72 (68-77)
Female, n (%)	31 (70)
Disease duration (months), median (IQR)	42 (26; 80)
Cranial-GCA, n (%)	24 (55)
Large-vessel GCA, n (%)	9 (20)
Cranial and large-vessel GCA, n (%)	11 (25)
Number of previous relapses, median (IQR)	1 (0; 2)
Erythrocyte sedimentation rate at enrollment (mm/hour), median (IQR)	14 (7.5; 26.5)
C-reactive protein at enrollment (mg/dl), median (IQR)	0.3 (0.1; 0.6)
GC therapy, n (%)	33 (75)
GC dose, median (IQR), mg/dl	5 (3.1-7.5)
GC dose ≤ 5 mg/day, n (%)	22 (50)
GC dose ≥ 7.5 mg/day, n (%)	9 (20)
GC dose ≥ 10 mg/day, n (%)	6 (14)
MTX, n (%)	18 (41)
GC + MTX, n (%)	12 (27)
TCZ, n (%)	5 (11)

**Table 2. Frequency of anti-Spike trimeric antibodies, neutralising antibodies, cellular immunity according to clinical characteristics and treatment**

	Total (n=44)	S-IgG Responders (n=40)	S-IgG Non- responders (n=4)	P value	NtAbs Responders (n=37)	NtAbs Non- responders (n=7)	P value	ELISpot Responders (n=31)	ELISpot Non- responders (N=13)	P value
Age, median (IQR)	72 (68-77)	72 (68-76)	77 (72-81)	0.13	71 (68-75)	77 (77-78)	<b>0.008</b>	73 (70-77)	71 (68-77)	0.71
Female, n (%)	31 (70)	27 (67)	4 (100)	0.30	25 (68)	6 (86)	0.65	21 (68)	10 (77)	0.72
Cranial-GCA, n (%)	24 (55)	22 (55)	2 (50)	0.99	19 (51)	5 (71)	0.43	14 (45)	10 (76)	0.09
Large-vessel GCA, n (%)	9 (20)	9 (23)	0 (0)	0.57	9 (24)	0 (0)	0.31	8 (26)	1 (8)	0.24
Cranial and large-vessel GCA, n (%)	11 (25)	9 (23)	2 (50)	0.26	9 (24)	2 (29)	0.99	9 (29)	2 (15)	0.46

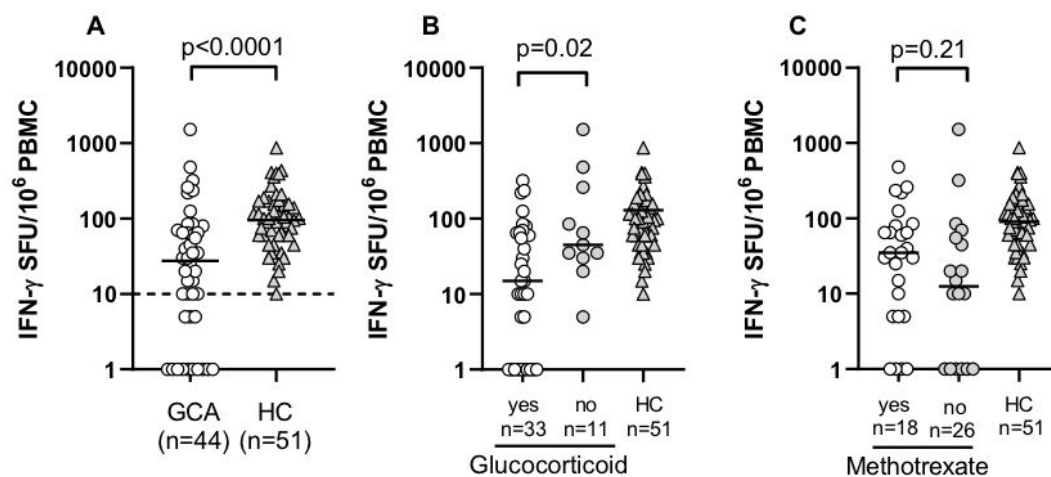
GC therapy, n (%)	33 (75)	29 (72)	4 (100)	0.56	26 (70)	7 (100)	0.17	21 (68)	12 (92)	0.13
GC dose, median (IQR), mg/dl	5 (3.1-7.5)	5 (2.5; 8.12)	5.63 (5; 6.25)	0.52	5 (2.5; 8.12)	5 (3.75; 6.25)	0.91	5 (3.75; 8.12)	5 (2.50; 7.19)	0.66
GC dose ≤ 5 mg/day, n (%)	22 (50)	20 (50)	2 (50)	0.59	18 (49)	4 (57)	0.99	14 (45)	8 (62)	0.99
GC dose ≥ 7.5 mg/day, n (%)	9 (20)	9 (23)	0 (0)	0.56	8 (22)	1 (14)	0.65	6 (19)	3 (23)	0.99
GC dose ≥ 10 mg/day, n (%)	6 (14)	6 (15)	0 (0)	0.99	6 (16)	0 (0)	0.30	4 (13)	2(15)	0.99
MTX, n (%)	18 (41)	14 (34)	4 (100)	<b>0.02</b>	11 (30)	7 (100)	<b>0.0008</b>	12 (39)	6 (46)	0.74
TCZ, n (%)	5 (11)	5 (12)	0 (0)	0.99	5 (14)	0 (0)	0.57	4 (13)	1 (8)	0.99

S-IgG: anti-Spike specific immunoglobulins; NtAbs: neutralising antibodies; ELISpot: anti-Spike specific T-cell response; GCA: giant cell arteritis; GC: glucocorticoid; IQR: interquartile range; MTX: methotrexate; TCZ: tocilizumab

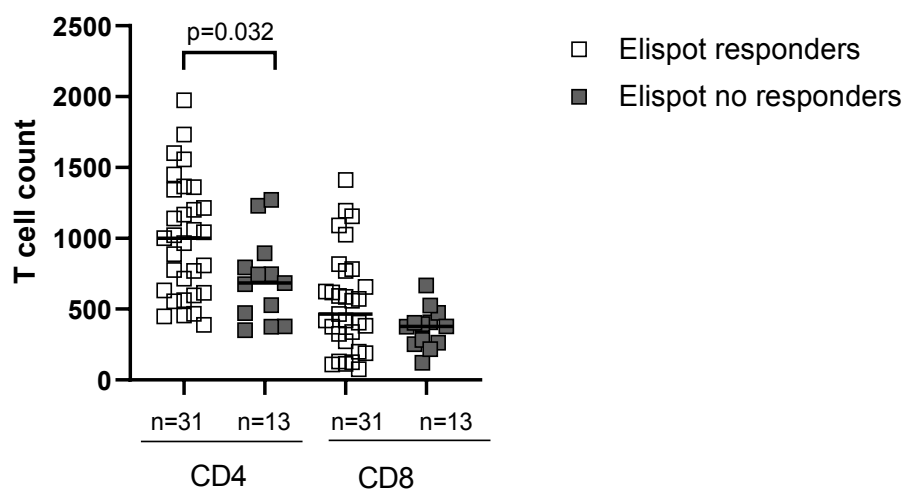


**Figure 1. Humoral response in patients with giant cell arteritis according to treatment and compared to healthy controls.** SARS-CoV2 specific IgG and neutralising immune response in patients with giant cell arteritis compared to healthy controls. Panel A. SARS-CoV-2 specific anti-Spike IgG antibodies in patients with GCA compared to HC; Panel B. SARS-CoV-2 specific anti-Spike IgG antibodies in patients with GCA

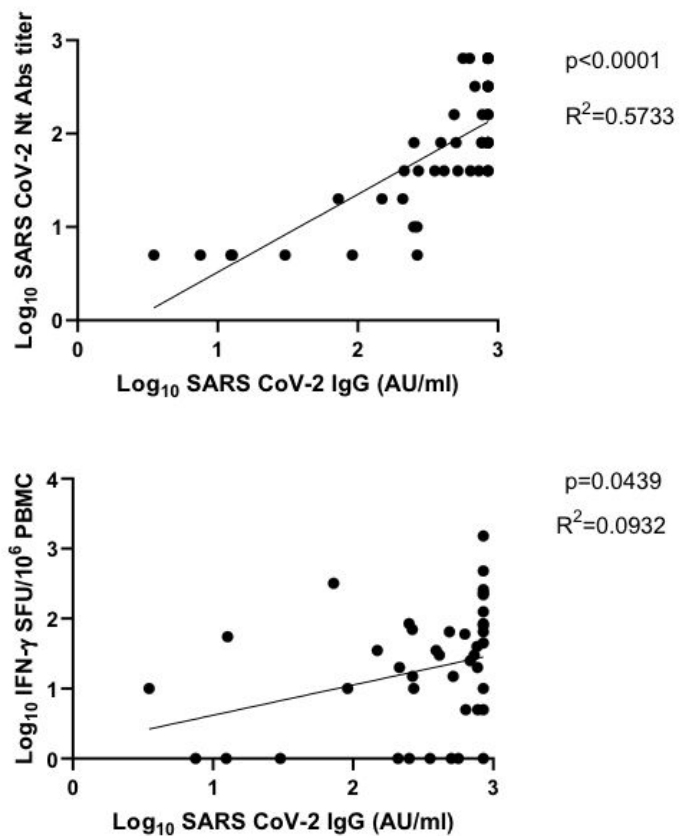
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3 treated with glucocorticoid compared to those not receiving glucocorticoids, and HC; Panel C. SARS-CoV-2  
4 specific anti-Spike IgG antibodies in patients with GCA treated with methotrexate compared to those not  
5 receiving methotrexate, and HC; Panel D. SARS-CoV-2 specific neutralising antibodies in patients with GCA  
6 compared to HC; Panel E. SARS-CoV-2 specific neutralising antibodies in patients treated with  
7 glucocorticoids compared to those not receiving glucocorticoids, and HC; Panel F. SARS-CoV-2 specific  
8 neutralising antibodies in patients treated with methotrexate compared to those not receiving  
9 methotrexate, and HC. Dotted line: cut-off for test positivity. GCA: giant cell arteritis; HC: healthy controls.



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33 **Figure 2. Cellular response according to type of treatment in patients with giant cell arteritis and healthy**  
34 **controls.** Panel A. T-cell response measured as IFN- $\gamma$  SFU (spot forming units) in patients with GCA  
35 compared to HC; Panel B. T-cell response measured as IFN- $\gamma$  SFU (spot forming units) in patients with GCA  
36 receiving glucocorticoids compared to those not receiving glucocorticoids, and HC; Panel C. T-cell response  
37 measured as IFN- $\gamma$  SFU (spot forming units) in patients with GCA receiving methotrexate compared to those  
38 not receiving methotrexate, and HC. Dotted line: cut-off for test positivity. GCA: giant cell arteritis; HC:  
39 healthy controls.



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59 **Figure 3. Levels of peripheral T-cell count and SARS-CoV-2 cellular response.** Levels of T cells  
60 (CD4+ and CD8+ T-cell count) in patients with giant cell arteritis displaying a SARS-CoV-2 specific T-cell  
response (n=31) compared to those who did not respond (n=13).



**Figure 4. Linear regression of the anti-Spike humoral response with the neutralising activity and the cellular response.** Panel A. Correlation between levels of SARS-CoV-2 specific anti-Spike IgG and SARS-CoV-2 specific neutralising antibodies. Panel B. Correlation between levels of SARS-CoV-2 specific anti-Spike IgG and SARS-CoV-2 specific T-cell response.