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

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Abstract

Objectives. To analyse humoral and cellular immune response to messenger RNA (mRNA) COVID-19 vaccines in patients with giant cell arteritis (GCA).

Methods. Consecutive patients with a diagnosis of GCA receiving two doses of BNT162b2 vaccine were assessed at baseline and three weeks from the second vaccine dose. Healthy subjects (n=51) were included as controls (HC). Humoral response was assessed with Spike-specific IgG antibody response (S-IgG) and neutralising antibodies (NtAb). Specific T-cell response was assessed by Enzyme linked immunospot (ELISpot).

Results. Of 56 included patients with GCA, 44 were eligible after exclusion of previous evidence of COVID-19 and incomplete follow-up. A significant proportion of patients with GCA (91%) demonstrated antibody (S-IgG) response, however this was significantly lower than HC (100%); p<0.0001. Neutralising activity was not detected in 16% of patients with GCA. Antibody titres (S-IgG and NtAb) were significantly lower compared to HC. Humoral response (S-IgG and NtAb) was significantly hampered by treatment with methotrexate (MTX). Cellular response was lacking in 30% of patients with GCA (vs 0% in HC); p<0.0001. Cellular response was significantly influenced by the levels of baseline peripheral T-lymphocytes and by glucocorticoid treatment. Treatment with tocilizumab did not affect any level of the immune response elicited by vaccination.

Conclusions. Although patients with GCA apparently achieve a robust antibody seroconversion, there is a significant impairment of the neutralising activity. MTX significantly reduced all levels of the humoral response. Up to one third of patients do not develop a cellular immune protection in response to COVID-19 vaccination.

Key Words: giant cell arteritis, vasculitis, COVID-19, vaccination, immune response, cellular response

Key Messages

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

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 undoubtfully 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.

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

Material and Methods

Consecutive patients with a clinical diagnosis of GCA followed at the Department of Rheumatology of Policlinico S. Matteo IRCCS Fondazione who received two doses of SARS-CoV-2 BNT162b2 vaccine (Pfizer/BioNtech) between April and June 2021 were enrolled. We had previously described the seroconversion rate following the first and second vaccine dose in a different cohort of patients with GCA(9); in the current study, patients were included to assess neutralising and cellular response to vaccination. Humoral response was reported to find correlations with the other two types of immunological response and was assessed with a different methodology (new generation recombinant trimeric spike glycoprotein) to optimise sensitivity and specificity. Only patients with complete data and follow-up were considered. Patients with GCA continued their regular treatment for the rheumatological disease around the time of vaccination. A group of HC ≥ 50 years of age according to the epidemiological distribution of GCA was collected. To exclude a role of age or disease-specific effect on the degree of immune response, two adjunctive age-matched pathological groups were considered as controls: patients from an historic cohort with RA or spondyloarthritis treated with biologic DMARDs with or without MTX, and patients with psoriasis treated with biologic DMARDs. All patients provided written informed consent (Ethical approval reference P-20210000232).

Blood samples were collected prior to vaccination, and three weeks after the second vaccine dose. Serum was stored at -80°C until analysed. Serum samples were collected for evaluation of SARS-CoV-2 total and neutralising antibodies (NtAb) while heparinised whole blood samples were used for peripheral mononuclear cells (PBMC) isolation and quantification of Spike-specific T-cell response.

Assessment of humoral response elicited by SARS-CoV-2 vaccine

Immunoglobulins (IgG) directed to SARS-CoV-2-specific Spike protein in their trimeric form (SIgG) were determined by chemiluminescent assay (Liaison SARS-CoV-2 trimeric, Diasorin, Saluggia, Italy), according to manufacturer's instructions. A result above the cutoff of 13 AU/ml (33.8 BAU/ml) was considered positive.

SARS-CoV-2 specific NtAb titre was determined as previously reported (Percivalle et al., 2020)(15). Results were considered positive when the titre was ≥ 1:10.

Assessment of T cell response elicited by SARS-CoV-2 vaccine and T cell count

SARS-CoV2 specific-T cells were determined by Enzyme linked immunospot (ELISpot) assay as previously reported (Cassaniti et al., 2021)(16). Briefly, PBMC were plated at a concentration of $2x10^5$/100µl culture medium per well and were stimulated in duplicate for 24 h in 96-well plates (coated with anti-IFN-γ monoclonal capture antibody) with peptide pools (15mers, overlapping by 10 aminoacids, Pepscan, LelystadThe Netherlands) representative of the Spike protein (S) at the final concentration of 0.25 µg/ml. Phytoheamagglutinin (PHA; 5 µg/mL) was used as positive control, and medium alone as negative control. Results were expressed as IFN-γ spots forming units (SFU)/10^6 PBMC. A result ≥10 net spots/million PBMC was considered positive. This cutoff was based of the mean value plus two x standard deviations of PBMC stimulation with negative control.

The methods for detection of total and naïve T and B cells are reported in the Supplementary Material, available at *Rheumatology* online.

Statistical analysis

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

Results

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

SARS-CoV-2 specific anti-Spike trimeric antibodies response

After two vaccine doses, 40 (91%) patients developed SARS-CoV-2 specific S-IgG, while 4 (9%) patients had no detectable humoral response. Among HC 51 (100%) developed S-IgG. The median titre of S-IgG in patients with GCA was significantly lower compared to HC (median 595 AU/ml, IQR 251-850 vs 791, IQR 570-850, p=0.02) (Figure 1, Panel A). Treatment with GC did not affect humoral response (Figure 1, Panel B).

The four patients without seroconversion were 3 females/1 male, median age 77 years old, range 70-82, all receiving MTX. Patients treated with MTX showed significantly lower levels of SARS-CoV-2 specific S-IgG compared to patients not receiving MTX treatment (p<0.0001) (Figure 1, Panel C). Patients treated with a combination of GC and MTX (n=12) showed a decreased S-IgG response compared to either agent alone: median S-IgG titre for combination treatment 209 (IQR 12.7-265) vs 850 (IQR 611-850) for GC (n=18); p<0.0001; vs 850 (773-850) for MTX monotherapy (n=6); p=0.008.

SARS-CoV-2 specific neutralising antibodies response

Among patients with GCA, 37 (84%) were NtAbs responders, while 7 (16%) did not develop NtAbs. All HC developed NtAbs. The titre of NtAbs was significantly lower for patients with GCA compared to HC (median 80, IQR 20-160 vs 320, IQR 320-1280 p<0.0001) (Figure 1, Panel D).

The analysis of the factors associated with NtAbs response revealed no association with sex or disease phenotype (cranial vs large-vessel GCA). Moreover, GC therapy, regardless of the dose, did not influence the development of NtAbs (Table 2; Figure 1, Panel E). Similarly, treatment with tocilizumab (TCZ) did not affect the neutralising response. On the other hand, patients treated with MTX did not develop NtAbs in 39% of cases. All NtAbs non-responders were receiving MTX (Table 2). Furthermore, patients treated with MTX showed significantly lower levels of SARS-CoV-2 specific NtAbs (Figure 1, Panel F).

Patients treated with a combination of GC and MTX showed a decreased NtAb response compared to patients treated with GC only. Median NtAb for combination treatment: 10, IQR 5-40 vs 80, IQR 40-640 for GC; p<0.0001.

SARS-CoV-2 specific cellular response

Thirteen (30%) patients with GCA did not develop SARS-CoV-2 specific T-cell response. On the other hand, in the control group of HC, all 51 (100%) subjects developed a cellular response.

The level of T-cell response was significantly lower for GCA patients compared to HC (median 28 IFN-γ SFU/10^6 PBMC, IQR 5-69 vs HC, median 90, IQR 45-155; p<0.0001) (Figure 2, Panel A).

The analysis of the factors associated with T-cell response did not show any association with sex, age, or disease phenotype.

Treatment with GC negatively influenced cellular response to the vaccine. Twelve (92%) patients in the T-cell immunity non-responders received GC, compared to 21 (67%) of responders. The level of T-cell response was significantly influenced by treatment with GC (Figure 2, Panel B). Treatment with MTX did not influence the degree of T-cell response (Figure 2, Panel C). Similarly, TCZ did not affect cellular response (Table 2). The combination therapy of MTX and GC (median 10, IQR 0-55) did not influence cellular response compared to either agent alone (median 27.5, IQR 5-68.5 for GC, and median 45, IQR 20-1515 for MTX).

Cellular response was significantly influenced by the levels of peripheral T-lymphocytes. Patients who developed a T-cell response showed higher levels of $CD4^+$ T-cells (1002 cells/μl blood, range 389-1586 vs non-responders, p=0.03). $CD8^+$ T-cells did not influence the rate of response (Figure 3).

No significant difference was found between the percentage of circulating $CD45RA^+CCR7^+$ CD4 naïve or CD8 naïve T cells and the detection of a T-cell response (Supplementary Figure S1, available at *Rheumatology* online).

Moreover, in a multivariate model, treatment with GC remained an independent risk factor for cellular response, regardless of baseline $CD4^+$ or $CD8^+$ T-cells (Supplementary Table S1, available at *Rheumatology* online).

Humoral response and B cells

There was a significant correlation between the number of circulating B-cells (Supplementary Figure S2, Panel A and B, available at *Rheumatology* online) and IgD^+CD27^- naïve B-cells (Supplementary Figure S2, Panel C and D, available at *Rheumatology* online) with both S-IgG and NtAb responses.

Multiple linear regression to assess the factors influencing the different types of immune response

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

Correlation between different types of immune response

There was a significant correlation between levels of SARS-CoV-2 specific S-IgG and NtAbs (r^2=0.573; p<0.0001). (Figure 4, Panel A).

Moreover, there was a significant correlation between levels of SARS-CoV-2 specific S-IgG and T-cell response (r^2=0.0932; p=0.04) (Figure 4, Panel B). There was also a correlation between NtAb response and cellular response (r^2=0.102; p=0.0347).

At a single patient level, B and T-cell non-responders differed. Absence of both B (humoral) and T-cell responses was observed in two patients. Ten patients developed S-IgG and NtAbs responses without cellular response, while one patient developed S-IgG only. T-cell response was possible even in the absence of any type of humoral response (one patient), or only combined with S-IgG response but without neutralising activity (two patients).

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

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

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

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

Recent evidence has reported a potential safety warning with increased diagnoses of GCA and polymyalgia rheumatica following COVID-19 vaccination (32). Nevertheless, none of our patients experienced relapses following two doses of BNT162b2 vaccine, offering reassuring safety results. Moreover, patients enrolled in

the study were all in remission at the time of vaccination, limiting the possibility that the underlying disease activity might have influenced the immunological response.

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

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

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References

1. WHO Coronavirus (COVID-19) Dashboard [Internet]. [cited 2021 Nov 30]. Available from: https://covid19.who.int
2. Dong Y, Dai T, Wang B, Zhang L, Zeng L hui, Huang J, et al. The way of SARS-CoV-2 vaccine development: success and challenges. Signal Transduct Target Ther 2021 Dec [cited 2021 Nov 30];6:387.
3. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med 2021 [cited 2021 Nov 30];384(5):403–16.
4. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med 2020 Dec 31 [cited 2021 Jul 27];383:2603–15.
5. Hungerford D, Cunliffe NA. Real world effectiveness of covid-19 vaccines. BMJ 2021 Aug 20 [cited 2021 Nov 30];374:n2034.
6. Ribeiro ACM, Guedes LKN, Moraes JCB, Saad CGS, Aikawa NE, Calich AL, et al. Reduced seroprotection after pandemic H1N1 influenza adjuvant-free vaccination in patients with rheumatoid arthritis: implications for clinical practice. Ann Rheum Dis 2011 Dec 1 [cited 2022 May 5];70:2144–7.
7. Alten R, Bingham CO, Cohen SB, Curtis JR, Kelly S, Wong D, et al. Antibody response to pneumococcal and influenza vaccination in patients with rheumatoid arthritis receiving abatacept. BMC Musculoskelet Disord 2016 May [cited 2022 May 5];17:231.
8. Kapetanovic MC, Saxne T, Sjöholm A, Truedsson L, Jönsson G, Geborek P. Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis. Rheumatology 2006 [cited 2022 May 5];45(1):106–11.
9. Delvino P, Bartoletti A, Cassaniti I, Bergami F, Lilleri D, Baldanti F, et al. Impact of immunosuppressive treatment on the immunogenicity of mRNA Covid-19 vaccine in vulnerable patients with giant cell arteritis. Rheumatol Oxf Engl 202;keab776.
10. Jena A, Mishra S, Deepak P, Kumar-M P, Sharma A, Patel YI, et al. Response to SARS-CoV-2 vaccination in immune mediated inflammatory diseases: Systematic review and meta-analysis. Autoimmun Rev. 2021;102927.
11. Ny P, As P, Vt C, Dy W. Understanding neutralising antibodies against SARS-CoV-2 and their implications in clinical practice. Mil Med Res [Internet]. 2021 [cited 2022 May 5];8(1).
12. Cell-mediated immune responses to influenza vaccination in healthy volunteers and allogeneic stem cell transplant recipients. Bone Marrow Transpl 2005;36:411-5. [cited 2022 May 5].
13. McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, Kleppinger A, et al. T cell responses are better correlates of vaccine protection in the elderly. J Immunol Baltim Md 1950 2006;176:6333–9.
14. Mahil SK, Bechman K, Raharja A, Domingo-Vila C, Baudry D, Brown MA, et al. The effect of methotrexate and targeted immunosuppression on humoral and cellular immune responses to the COVID-19 vaccine BNT162b2: a cohort study. Lancet Rheumatol 2021 [cited 2022 May 5];3(9):e627–37.
15. Percivalle E, Cambiè G, Cassaniti I, Nepita EV, Maserati R, Ferrari A, et al. Prevalence of SARS-CoV-2 specific neutralising antibodies in blood donors from the Lodi Red Zone in Lombardy, Italy, as at 06 April 2020. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull. 2020;25.
16. Cassaniti I, Percivalle E, Bergami F, Piralla A, Comolli G, Bruno R, et al. SARS-CoV-2 specific T-cell immunity in COVID-19 convalescent patients and unexposed controls measured by ex vivo ELISpot assay. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 2021;27:1029–34.
17. Feng C, Shi J, Fan Q, Wang Y, Huang H, Chen F, et al. Protective humoral and cellular immune responses to SARS-CoV-2 persist up to 1 year after recovery. Nat Commun 2021 [cited 2022 Jan 13];12:4984.
18. Haberman RH, Herati R, Simon D, Samanovic M, Blank RB, Tuen M, et al. Methotrexate hampers immunogenicity to BNT162b2 mRNA COVID-19 vaccine in immune-mediated inflammatory disease. Ann Rheum Dis 2021;annrheumdis-2021-220597.
19. Deepak P, Kim W, Paley MA, Yang M, Carvidi AB, El-Qunni AA, et al. Glucocorticoids and B Cell Depleting Agents Substantially Impair Immunogenicity of mRNA Vaccines to SARS-CoV-2. MedRxiv Prepr Serv Health Sci 2021:21254656.
20. Naaber P, Tserel L, Kangro K, Sepp E, Jürjenson V, Adamson A, et al. Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. Lancet Reg Health 2021.
21. Chmielewska AM, Czarnota A, Bieńkowska-Szewczyk K, Grzyb K. Immune response against SARS-CoV-2 variants: the role of neutralization assays. Npj Vaccines 2021 [cited 2022 Jan 13];6:1–8.
22. Bertoletti A, Le Bert N, Qui M, Tan AT. SARS-CoV-2-specific T cells in infection and vaccination. Cell Mol Immunol 2021;18:2307–12.
23. Bange EM, Han NA, Wileyto P, Kim JY, Gouma S, Robinson J, et al. CD8+ T cells contribute to survival in patients with COVID-19 and hematologic cancer. Nat Med 2021;27:1280–9.

24. Schulz E, Hodl I, Forstner P, Hatzl S, Sareban N, Moritz M, et al. CD19+IgD+CD27- Naïve B Cells as Predictors of Humoral Response to COVID 19 mRNA Vaccination in Immunocompromised Patients. Front Immunol 2021 [cited 2022 May 5];12:803742.
25. Weisblum Y, Schmidt F, Zhang F, DaSilva J, Poston D, Lorenzi JC, et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. eLife 2020;9:e61312.
26. Van Laethem F, Baus E, Smyth LA, Andris F, Bex F, Urbain J, et al. Glucocorticoids attenuate T cell receptor signaling. J Exp Med 2001;193:803–14.
27. Jani M, Barton A, Warren RB, Griffiths CEM, Chinoy H. The role of DMARDs in reducing the immunogenicity of TNF inhibitors in chronic inflammatory diseases. Rheumatol Oxf Engl 2014 [cited 2015 Feb 27];53:213–22.
28. Nived P, Pettersson Å, Jönsson G, Bengtsson AA, Settergren B, Skattum L, et al. Methotrexate reduces circulating Th17 cells and impairs plasmablast and memory B cell expansions following pneumococcal conjugate immunization in RA patients. Sci Rep 2021 [cited 2022 May 6];11:9199.
29. Arad U, Tzadok S, Amir S, Mandelboim M, Mendelson E, Wigler I, et al. The cellular immune response to influenza vaccination is preserved in rheumatoid arthritis patients treated with rituximab. Vaccine 2011;29:1643–8.
30. Bulatović Ćalasan M, Vastert SJ, Scholman RC, Verweij F, Klein M, Wulffraat NM, et al. Methotrexate treatment affects effector but not regulatory T cells in juvenile idiopathic arthritis. Rheumatol Oxf Engl 2015;54:1724–34.
31. Kremer JM, Lawrence DA, Hamilton R, McInnes IB. Long-term study of the impact of methotrexate on serum cytokines and lymphocyte subsets in patients with active rheumatoid arthritis: correlation with pharmacokinetic measures. RMD Open 2016;2(:e000287.
32. Mettler C, Jonville-Bera AP, Grandvuillemin A, Treluyer JM, Terrier B, Chouchana L. Risk of giant cell arteritis and polymyalgia rheumatica following COVID-19 vaccination: a global pharmacovigilance study. Rheumatol Oxf Engl 2022;61:865–7.

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)	**0.008**	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)	**0.02**	11 (30)	7 (100)	**0.0008**	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

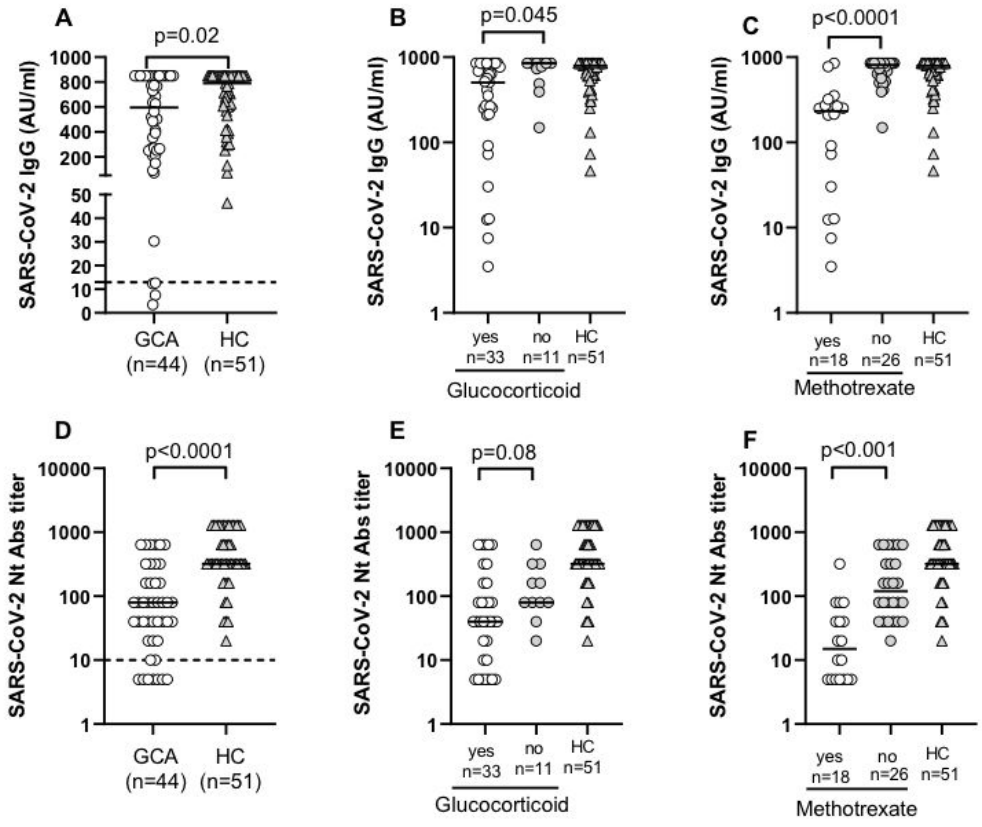

Figure 1. Humoral response in patients with giant cell arteritis according to treatment and compared to healthy controls. SARS-CoV2 specific IgG and neutralising immune response in patients with giant cell arteritis compared to healthy controls. Panel A. SARS-CoV-2 specific anti-Spike IgG antibodies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

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

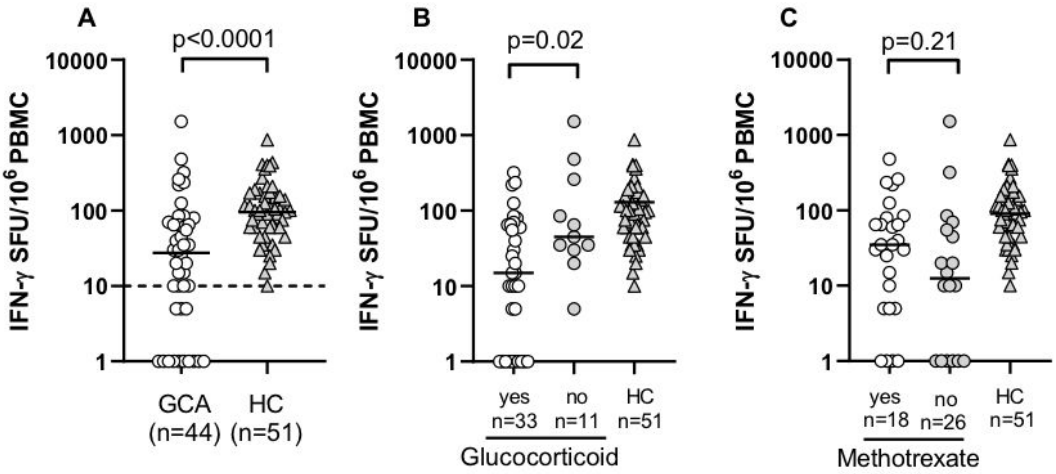

Figure 2. **Cellular response according to type of treatment in patients with giant cell arteritis and healthy controls.** Panel A. T-cell response measured as IFN-γ SFU (spot forming units) in patients with GCA compared to HC; Panel B. T-cell response measured as IFN-γ SFU (spot forming units) in patients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

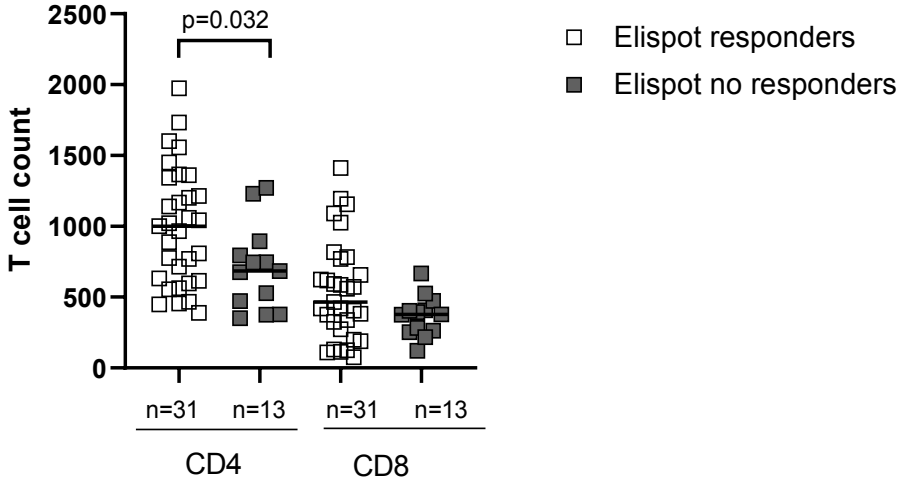

Figure 3. Levels of peripheral T-cell count and SARS-CoV-2 cellular cellular response. Levels of T cells (CD4+ and CD8+ T-cell count) in patients with giant cell arteritis displaying a SARS-CoV-2 specific T-cell response (n=31) compared to those who did not respond (n=13).

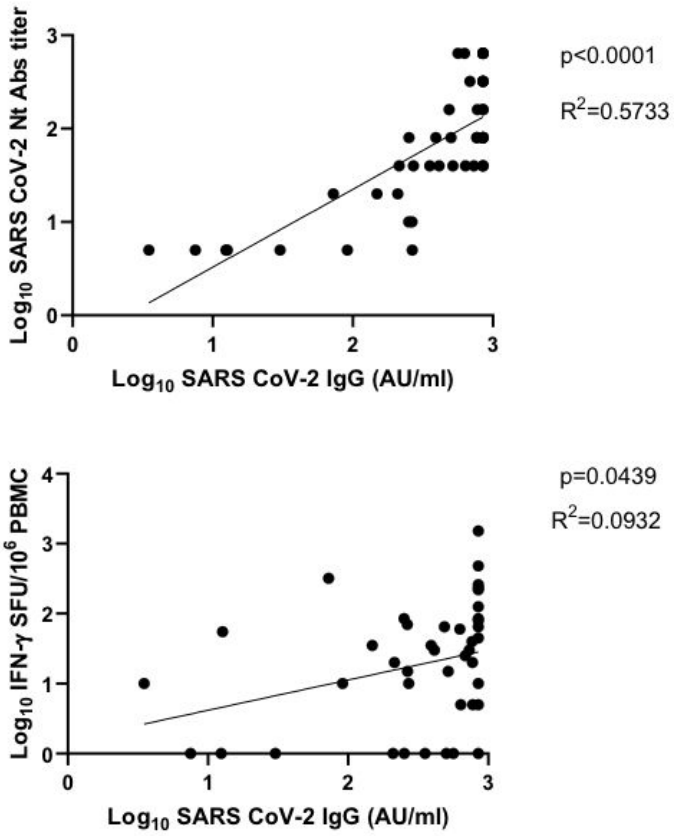

Figure 4. Linear regression of the anti-Spike humoral response with the neutralising activity and the cellular response. Panel A. Correlation between levels of SARS-CoV-2 specific anti-Spike IgG and SARS-CoV-2 specific neutralising antibodies. Panel B. Correlation between levels of SARS-CoV-2 specific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