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dose of SARS-CoV-2 vaccination, highlighting the benefit of a three-dose vaccination schedule for these patients. We also identified a subset of patients, namely those on regimens containing either mycophenolate or CD20-depleting therapy, in which antibody responses remained suboptimal after a third dose. These patients are ideal candidates for prophylactic therapies or might require additional vaccine doses to confer increased protection against COVID-19 infection. The rapidly evolving SARS-CoV-2 requires continued development and refinement of medical countermeasures such as antibody testing, vaccine schedule, and prophylactic therapies to enhance the protection of these vulnerable patients.

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Methotrexate and TNF inhibitors affect long-term immunogenicity to COVID-19 vaccination in patients with immune-mediated inflammatory disease

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Studies have revealed that patients with immune-mediated inflammatory diseases, especially those on immunomodulatory medication, have attenuated immunogenicity to COVID-19 vaccination.^{1,2} These findings have informed American College of Rheumatology (ACR) and European Alliance of Associations for Rheumatology (EULAR) recommendations regarding use of immunomodulatory therapies peri-vaccination. Recent longitudinal studies in immunocompetent adults have found waning

humoral immunity by 6-months post-vaccination.^{3,4} However, despite an already diminished initial response to immunisation in patients with immune-mediated inflammatory diseases, there are scarce data regarding their longer-term humoral response.

We hypothesised that patients with immune-mediated inflammatory diseases who are treated chronically with certain disease-modifying rheumatic drugs (ie, methotrexate) or anti-cytokine therapies (ie, TNF inhibitors), would have lower rates of

adequate humoral response over time compared with patients without these diseases or those receiving other immunomodulatory medications. Using the New York University SAGA cohort, we obtained post-vaccination blood samples from participants with immune-mediated inflammatory diseases (n=245) and healthy controls (n=27) and analysed SARS-CoV-2-spike-specific antibody titres and neutralisation capacity at 4-week, 3-month, and 6-month timepoints after vaccination. This study was approved by the NYU institutional review board (20-01078) and all patients provided informed consent for participation. Full methods can be found in the appendix (p 2).

Healthy individuals and those with immune-mediated inflammatory diseases were similar in age, sex, and history of previous SARS-CoV-2 infection (appendix pp 3-4). Diagnoses included predominantly psoriatic disease, rheumatoid arthritis, and inflammatory bowel disease. The proportion of participants who achieved an adequate humoral response (ie, anti-spike IgG titre ≥ 5000 RU/mL) among healthy individuals and participants with immune-mediated inflammatory diseases remained stable between 4 weeks and 3 months after vaccination: 26 (96%) of 27 healthy controls were seropositive at 4 weeks versus 25 (100%) of 25 at 3 months, as were 216 (88%) of 245 participants with immune-mediated inflammatory diseases versus 193 (89%) of 217 (appendix p 5). Adequate humoral response rates decreased significantly in both groups by the 6-month timepoint (17 [65%] of 26 controls [$p=0.0017$] and 96 [56%] of 170 participants with IMIDs [$p<0.0001$]), as did IgG titres, percentage of antibodies with neutralising capacity, and rates of adequate neutralising response (ie, $\geq 35\%$) (figure). Neutralising capacity correlated strongly with IgG antibody response ($r=0.812$, appendix p 14). No singular diagnosis demonstrated significant differences in adequate humoral response compared with healthy controls (appendix p 5). Previous COVID-19 infection was associated with increased antibody titres and neutralisation capacity at all timepoints and an increased adequate humoral response rate at 6 months (appendix p 6).

At week 4, when compared with healthy controls, patients with immune-mediated inflammatory diseases who were not receiving methotrexate achieved a similar rate of adequate humoral response (150 [93%] of 161; appendix p 7), whereas those on methotrexate had a

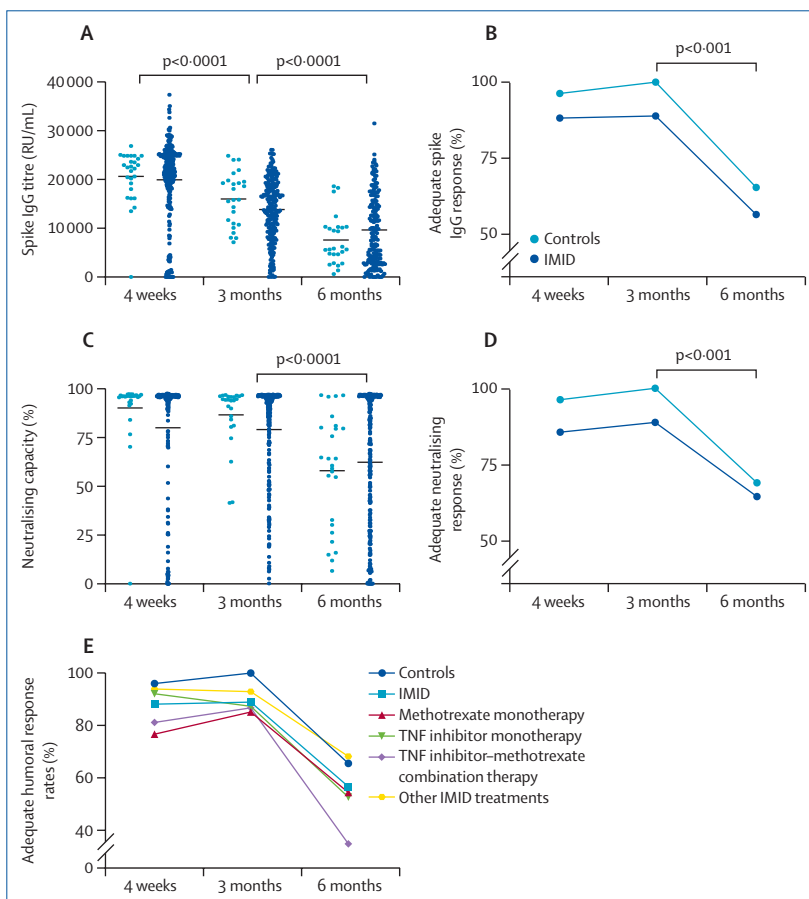


Figure: Longitudinal humoral response to COVID-19 vaccination in healthy controls and patients with IMID Humoral response represented by the spike IgG titres (A), the proportion of patients achieving an adequate spike IgG response as defined by greater than or equal to 5000 RU/mL (B), the proportion of patients achieving an adequate neutralising capacity (C), and proportion of patients achieving an adequate neutralising capacity as defined by greater than or equal to 35% (D). (E) Proportion of patients achieving an adequate spike IgG response, by immunomodulatory use, as defined by spike IgG titres greater than or equal to 5000 RU/mL. The 4-week timepoint is defined at 4 weeks after the first dose of Janssen Ad26.COV2.S (Johnson & Johnson) and BNT162b2 (Pfizer-BioNTech) and 5 weeks after the first dose of mRNA-1273 (Moderna). IMID=immune-mediated inflammatory disease. RU=relative units.

lower rate of adequate humoral response (66 [79%] of 84; $p=0.002$). Patients on methotrexate tended to be older, female, and have rheumatoid arthritis, and had a mean weekly dose of 14.5 mg. Mean IgG titres and neutralising antibodies were similarly lower in patients on methotrexate than in healthy controls and those not on methotrexate. At 3 months and 6 months after vaccination, patients on methotrexate had numerically lower rates of adequate response and titres than did healthy controls and patients not on methotrexate (66 [86%] of 77 vs 25 [100%] of 25 and 127 [91%] of 140, respectively, $p=0.10$ at 3 months; and 28 [46%] of 61 vs 17 [65%] of 26 and 68 [62%] of 109, respectively, $p=0.08$ at 6 months) with similar trends seen in antibody titre and neutralising antibodies. The overall adequate

For more on New York University SAGA see <https://clinicaltrials.med.nyu.edu/clinicaltrial/1360/serologic-testing-genomic-analysis/>
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humoral response rate of patients receiving methotrexate differed significantly from healthy controls at 4 weeks ($p=0.039$), and was numerically lower, but did not differ significantly at 3 and 6 months (figure; appendix p 8).

The unadjusted odds ratio (OR) of achieving an adequate response to COVID-19 vaccination at week 4 for all methotrexate use (ie, alone or in combination with other medications) was 0.27 (95% CI 0.12–0.60, $p=0.001$), when compared with those not on methotrexate (appendix p 9). This effect remained significant when adjusting for age and sex and when restricting analysis to methotrexate monotherapy. The unadjusted OR for methotrexate use at 3 months was 0.61 (95% CI 0.26–1.45, $p=0.26$) and at 6 months was 0.51 (95% CI 0.27–0.97, $p=0.039$; appendix pp 10–11). The results at 3 and 6 months remain similar after adjusting for age and sex (appendix pp 10–11).

At 4 weeks, 37 (44%) of 84 patients on methotrexate held at least one dose immediately before or after vaccination (appendix p 7). Among patients receiving methotrexate, those who held their medication during the peri-vaccination period had an unadjusted OR of 3.50 (95% CI 1.04–11.75, $p=0.043$) achieving an adequate response compared with those who did not hold any doses (appendix p 9). This difference remained true at 3 months (unadjusted OR 8.33, 1.01–68.87, $p=0.049$) and 6 months (5.60, 1.68–18.70, $p=0.005$; appendix pp 10–11). There was no difference in adequate humoral response rates between the use of high-dose methotrexate (≥ 15 mg) and low-dose methotrexate (≤ 12.5 mg) in any combination of use (appendix p 12).

Patients with immune-mediated inflammatory diseases on TNF inhibitors had similar rates of adequate humoral response compared with those not on TNF inhibitors at 4 weeks (88 [88%] of 100 vs 128 [88%] of 145) and 3 months (74 [87%] of 85 vs 119 [90%] of 132), and compared with healthy controls. However, by 6 months the proportion of adequate antibody response was lower, although not statistically significant, in patients on TNF inhibitors compared with patients receiving other medications (30 [45%] of 66 vs 66 [63%] of 104, $p=0.053$; appendix p 13). Although adequate neutralising response was only significantly lower in the TNF group at 6 months, neutralising antibody concentration was significantly decreased at all timepoints, as were antibody titres at 3 and 6 months. Compared with those not receiving TNF inhibitors, the unadjusted OR of having an adequate humoral response following

vaccination while receiving a TNF inhibitor was 0.48 (95% CI 0.26–0.90, $p=0.022$; appendix p 11) at 6 months post-vaccination, no such difference was seen at the 4-week and 3-month timepoints (appendix pp 9–10). The overall adequate humoral response rate for patients on TNF inhibitors declined significantly from 88 (88%) of 100 at 4 weeks and 74 (87%) of 85 at 3 months, to 30 (45%) of 66 at 6 months ($p<0.0001$ for both 4 weeks vs 6 months and 3 months vs 6 months; appendix pp 8–9).

Concomitant TNF inhibitor use attenuated the early suppressive effect of methotrexate (appendix p 8). However, at 6 months, combined TNF inhibitor–methotrexate use had the lowest adequate humoral response rate of any medication (9 [35%] of 26), even when compared with all methotrexate use (figure, appendix p 8). This combination had an unadjusted OR of 0.35 (95% CI 0.14–0.83, $p=0.018$; appendix p 11) of achieving adequate humoral response at 6 months compared with those not on this combination. This effect remained significant when adjusting for age and sex.

In the New York University SAGA cohort, participants with immune-mediated inflammatory diseases had a decline to 56% in adequate humoral response at 6 months. Importantly, healthy controls had a lower humoral response rate at 6 months (65%) than reported in previous studies (81–84%).^{4,5} We continue to observe that methotrexate hampers the humoral immune response to COVID-19 vaccination. This effect is somewhat attenuated at the 3-month and 6-month timepoints, which might reflect the fact that methotrexate slows, rather than prevents, antibody production. In particular, the 3-month timepoint might reflect the peak immunogenicity of the vaccination captured in our study, thereby overcoming any observable effect on humoral response. Although dose of methotrexate did not affect humoral response; importantly, participants who held at least one dose of methotrexate during the peri-vaccination period had much higher odds of achieving an adequate humoral response than those who did not. These findings support the notion that this drug can substantially affect the biological response to vaccination and support the rationale behind current guidelines from ACR and EULAR for methotrexate use during this time.

Crucially, by 6 months, TNF inhibitors (especially in combination with methotrexate) led to further decreased rates of immunogenicity compared with earlier timepoints. Most initial studies did not demonstrate

any effect of TNF inhibitors on adequate humoral response.^{1,2,6} However, Chen and colleagues⁷ observed reduced antibody activity against the SARS-CoV-2 delta (B.1.617.2) variant in patients receiving TNF inhibitors, especially at 3-month and 5 or 6-month timepoints. Like methotrexate,⁸ TNF inhibitors have previously been shown to impair the immune response to vaccines against other viral infections.^{9,10} Mechanistic studies are needed to evaluate the seemingly synergistic effect of these drugs on weakening the antibody response of COVID-19 and other vaccines.

Although the relevance of our findings are constrained by the small sample size and scarcity of established correlates of levels of immunogenicity to efficacy, they show that both methotrexate and TNF inhibitors might lead to a dampened humoral response to COVID-19 vaccinations. Although TNF inhibitors do not demonstrate an initial effect on immunogenicity, persistence of adequate humoral response is significantly decreased by month 6 (appendix p 11). Taken together, these findings support the use of supplemental booster dosing in patients with immune-mediated inflammatory diseases, and specifically for those being treated with TNF inhibitors or TNF inhibitor-methotrexate combination therapy. Larger studies are needed to validate these results and to assess the effects of other immunomodulatory therapies, which will help to identify optimal timing and strategy of COVID-19 (and potentially other) vaccines.

RHH and JUS designed the study, designed the data collection tools, analysed and curated the data, and drafted and revised the paper. SU and SA designed the data collection tools, analysed and curated the data, and revised the paper. JEA and DPH designed the study, acquired data, and revised the draft. All authors approved the final version to be published and agree to be accountable for all aspects of the work. RHH and JUS verified the underlying data and had final responsibility for the decision to submit for publication. RBB, ZU, SCA, ALN, MJM, RSH, SJH, SCH, AM, GG, PMI, AS, GS, NA, JS, BDG, and PR acquired data and revised the draft.

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