CLINICAL SCIENCE

Pausing methotrexate improves immunogenicity of COVID-19 vaccination in elderly patients with rheumatic diseases

Amanthi Nadira Arumahandi de Silva,1 Leonie Maria Frommert,1 Fredrik N Albach,1 Jens Klotsche,2 Veronika Scholz,1 Lara Maria Jeworowski,3 Tatjana Schwarz,3 Alexander ten Hagen,1 Jan Zernicke,1 Victor Max Corman,3,4 Christian Drosten,3 Gerd-Rüdiger Burmester,1,4 Robert Biesen1,5

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-221876).

For numbered affiliations see end of article.

Correspondence to Dr Robert Biesen, Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, Berlin, Berlin, Germany; robert.biesen@charite.de

ANAdS, LMF, G-R, and RB contributed equally.

Abstact

Objective To study the effect of methotrexate (MTX) and its discontinuation on the humoral immune response after COVID-19 vaccination in patients with autoimmune rheumatic diseases (AIRD).

Methods In this retrospective study, neutralising SARS-CoV-2 antibodies were measured after second vaccination in 64 patients with AIRD on MTX therapy, 31 of whom temporarily paused medication without a fixed regimen. The control group consisted of 21 patients with AIRD without immunosuppressive medication.

Results Patients on MTX showed a significantly lower mean antibody response compared with patients with AIRD without immunosuppressive therapy (71.8% vs 92.4%, p<0.001). For patients taking MTX, age correlated negatively with immune response (r=−0.49; p<0.001). All nine patients with antibody levels below the cut-off were older than 60 years. Patients who held MTX during at least one vaccination showed significantly higher mean neutralising antibodies levels after second vaccination, compared with patients who continued MTX therapy during both vaccinations (83.1% vs 61.2%, p=0.001). This effect was particularly pronounced in patients older than 60 years (80.8% vs 51.9%, p=0.001). The impact of the time period after vaccination was greater than of the time before vaccination with the critical cut-off being 10 days.

Conclusion MTX reduces the immunogenicity of SARS-CoV-2 vaccination in an age-dependent manner. Our data further suggest that holding MTX for at least 10 days after vaccination significantly improves the antibody response in patients over 60 years of age.

INTRODUCTION

Until November 2021, SARS-CoV-2 had infected at least 250 million people worldwide and caused about 5 million deaths in a 23-month period.1 At the same time, enormous knowledge about SARS-CoV-2 and the related disease COVID-19 have been generated and the possibilities for prevention, diagnostics and treatments have improved remarkably.

Methotrexate (MTX) has been used for decades to treat a wide variety of immune-mediated diseases in oncology, rheumatology, dermatology, gastroenterology and neurology. Following prednisolone, MTX is the most prescribed anti-inflammatory drug worldwide with 1 million patients on MTX in the USA alone.2 Various immunosuppressants reduce the immune response after COVID-19 vaccination.3 Although several research groups have recently described a reduced vaccination response under MTX,4 5 in some cohorts MTX had no negative influence.6 7 Most of these studies did not collect data on whether or not patients had paused MTX during vaccinations, although more than one-third of patients had modified their vaccination strategies.8

What is already known about this subject?

⇒ Patients receiving methotrexate (MTX) have a reduced immune response after COVID-19 vaccination and holding MTX has shown to increase the immunogenicity after influenza vaccination.

⇒ Yet, no previous studies have analysed the effect of MTX-hold for COVID-19 vaccination.

What does this study add?

⇒ This study identified old age (≥60 years), short vaccine interval and MTX continuation as critical factors for an inadequate antibody response.

⇒ We found a minimum of 10 days between vaccination and re-intake of MTX as the critical threshold to increase immunogenicity for patients ≥60 years of age.

How might this impact on clinical practise or future developments?

⇒ Regarding ongoing booster vaccinations, our data suggest that especially older patients on MTX should hold MTX for at least 10 days after receiving a COVID-19 vaccination.
medication on their own or on the advice of their rheumatologist, according to a recent survey. The discontinuation of immunosuppressive medication can improve the vaccination response as recently shown for mycophenolate. A reduced vaccination response under MTX was first described in 2016 for influenza vaccination. Follow-up data showed the increase in humoral immune response when pausing MTX 2 weeks before and after vaccination or only 2 weeks after vaccination. The time after and not before vaccination was decisive. However, data regarding MTX hold during COVID-19 vaccination are still lacking, which is why current guidelines are based on experience with influenza vaccines, not considering mRNA-based technology used for COVID-19 vaccinations. Although current guidelines by the American College of Rheumatology as well as the German Society for Rheumatology recommend holding MTX 1–2 weeks after COVID-19 vaccination, the European League Against Rheumatism does not recommend pausing MTX.

Therefore, our main objective was to study the effect of MTX and its discontinuation on the humoral immune response after COVID-19 vaccination in patients with autoimmune rheumatic diseases (AIRD). Secondary objective

### Table 1 Characteristics of patients on MTX who held and continued MTX

|                          | MTX continued (n=33) | MTX hold (n=31) | MTX all (n=64) | P value* |
|--------------------------|----------------------|-----------------|----------------|---------|
| Age, mean (SD)           | 62.4 (14.2)          | 59.6 (11.1)     | 61.1 (12.8)    | 0.391   |
| Female, n (%)            | 21 (63.6)            | 24 (77.4)       | 45 (70.3)      | 0.251   |
| BMI, mean (SD)           | 26.4 (4.52)          | 24.7 (3.30)     | 25.6 (4.03)    | 0.102   |
| Rheumatic diagnosis      |                      |                 |                | 0.759   |
| Rheumatoid arthritis, n (%) | 21 (63.6)         | 23 (74.2)       | 44 (68.8)      |         |
| Psoriatic arthritis, n (%) | 5 (15.2)            | 2 (6.5)         | 7 (10.9)       |         |
| Others, n (%)†           | 7 (21.2)             | 6 (19.4)        | 13 (20.3)      |         |
| Medication               |                      |                 |                | 0.553   |
| MTX-mono, n (%)          | 14 (42.4)            | 12 (38.7)       | 26 (40.6)      |         |
| MTX+prednisolone, n (%)  | 7 (21.2)             | 5 (16.1)        | 12 (18.8)      |         |
| MTX+anti-TNF-α, n (%)‡   | 4 (12.1)             | 7 (22.6)        | 11 (17.2)      |         |
| MTX+anti-TNF-α+prednisolone, n (%)‡ | 5 (15.2)       | 2 (6.5)         | 7 (10.9)       |         |
| MTX+others, n (%)§       | 3 (9.1)              | 5 (16.1)        | 8 (12.5)       |         |
| Additional prednisolone, n (%) | 12 (36.4)          | 8 (25.8)        | 20 (31.3)      | 0.377   |
| Prednisolone dose (mg/day), mean (SD) | 3.0 (1.8)   | 2.6 (1.1)       | 2.9 (1.6)      | 0.572   |
| MTX dose (mg/week), mean (SD) | 13.2 (4.5)      | 13.1 (4.1)      | 13.2 (4.3)     | 0.973   |
| MTX oral application, n (%) | 16 (48.5)         | 10 (32.3)       | 26 (40.6)      | 0.205   |
| Vaccination              |                      |                 |                | 0.896   |
| BNT162b2, n (%)          | 24 (72.7)            | 23 (74.2)       | 47 (73.4)      |         |
| mRNA-1273, n (%)         | 5 (15.2)             | 3 (9.7)         | 8 (12.5)       |         |
| AZD1222, n (%)           | 3 (9.1)              | 4 (12.9)        | 7 (10.9)       |         |
| AZD1222+AZD162b2, n (%)  | 1 (3.0)              | 1 (3.2)         | 2 (3.1)        |         |
| Vaccine interval in days, mean (SD) | 39.0 (14.8)     | 41.9 (15.3)     | 40.4 (15.0)    | 0.444   |
| Immune response          |                      |                 |                |         |
| Days from second vaccination, mean (SD) | 35 (23)           | 28 (22)         | 32 (22)        | 0.237   |
| Anti-RBD-IgG, S/CO, mean (SD) | 3.7 (3.4)         | 6.3 (2.6)       | 5.0 (3.3)      | 0.001   |
| Neutralising capacity, mean (SD) | 61.2 (30.2)      | 83.1 (21.2)     | 71.8 (28.3)    | 0.001   |
| Responders, neutralisation capacity, n (%)*| 25 (75.8)        | 30 (96.8)       | 55 (85.9)      | 0.017   |
| Responders, anti-RBD-IgG response, n (%)** | 21 (63.6)        | 30 (96.8)       | 51 (79.7)      | 0.002   |
| MTX hold                 |                      |                 |                |         |
| For both vaccinations, n (%) | NA                 | 24 (77.4)       |         |         |
| For only the first vaccination, n (%) | NA              | 2 (6.5)         |         |         |
| For only the second vaccination, n (%) | NA              | 5 (16.1)        |         |         |
| Duration of MTX hold for first vaccination (days), mean (SD) | NA                | 15.1 (6.6)      |         |         |
| Duration of MTX hold for second vaccination (days), mean (SD) | NA               | 16.9 (6.6)      |         |         |

Significant results are in bold.

* P values compare MTX continued and MTX hold and were calculated using the exact unconditional z-pooled test for binary variables (female, additional prednisolone, MTX oral application, responders neutralisation capacity, responders anti-RBD-IgG response); χ² test for categorical variables (rheumatic diagnosis, medication, vaccination) and unpaired t-test with Welch’s correction for continuous variables.

†For MTX continued: ANCA-associated vasculitis (n=1), axial spondyloarthritis (n=1), polymyalgia rheumatica (n=2), systemic sclerosis (n=1), myositis (n=1), systemic lupus erythematosus (n=1), for MTX hold: axial spondyloarthritis (n=1), polymyalgia rheumatica (n=1), primary Sjögren’s syndrome (n=1), systemic sclerosis (n=2), myositis (n=1).‡Adalimumab, certolizumab, etanercept, golimumab, infliximab.*§For MTX continued: hydroxychloroquine (n=1), secukinumab (IL-17 inhibitor, n=1), ustekinumab (IL-12/IL-23 inhibitor, n=1). For MTX hold: hydroxychloroquine (n=1), leflunomide (n=2), leflunomide+prednisolone (n=1), secukinumab (IL-17 inhibitor, n=1).¶Defined as neutralising capacity against SARS-CoV-2 >30%.

** Defined as anti-RBD-IgG levels >1.0 S/CO.

ANCA, antineutrophil cytoplasmic antibody; BMI, body mass index; IL, interleukin; MTX, methotrexate; NA, not available; S/CO, signal cut-off; TNF, tumour necrosis factor.
was to determine additional influencing factors on antibody response in these patients.

METHODS

Study design and participants

This is a retrospective subanalysis of the VACCIMMUN study, which is an observational cohort study among patients with autoimmune rheumatic diseases (AIRD) without immunosuppression and with methotrexate (MTX) therapy. Neutralising capacity measured using surrogate virus neutralisation test after second vaccination in patients on MTX (n=64) represented by red dots versus patients with AIRD who were under no immunosuppressive therapy during both vaccinations (n=21) represented by green dots. P values were calculated using the parametric unpaired t-test with Welch’s correction.

Figure 1 Comparison of neutralising capacity in patients with autoimmune rheumatic diseases (AIRD) without immunosuppression and with methotrexate (MTX) therapy. Neutralising capacity measured using surrogate virus neutralisation test after second vaccination in patients on MTX (n=64) represented by red dots versus patients with AIRD who were under no immunosuppressive therapy during both vaccinations (n=21) represented by green dots. P values were calculated using the parametric unpaired t-test with Welch’s correction.

Neutralising capacity measured using surrogate virus neutralisation test after first vaccination in patients with AIRD under MTX therapy were considered, receiving either only MTX or MTX combined with low-dose prednisolone (defined as ≤5 mg/day), tumour necrosis factor-α inhibitors, hydroxychloroquine, leflunomide, interleukin (IL)-17 or IL-12/IL-23 inhibitors, since these immunosuppressive comediations are not known to have a remarkable impact on the immune response after vaccination. Additionally, patients with AIRD who were vaccinated under no immunosuppressive therapy served as controls. Information regarding medical history including COVID-19 vaccination status and immunosuppressive therapy were provided directly by patients and additionally validated with medical records. At the time of blood drawing, patients were asked about their MTX intake schedule around vaccinations. The decision on continuing or holding MTX was made by the patient or the attending physician and was only observed in the study. Patients who reported to have changed their MTX-intake schedule resulting in an MTX interval longer than 7 days around first or second vaccination were compared with patients who continued MTX therapy throughout both vaccinations.

Laboratory analyses

Antibody response was measured predominantly about 2 weeks after the second dose of vaccination with maximum range from 11 to 112 days. Neutralising antibody levels were assessed using a surrogate virus neutralisation test (ePass Neutralisation, Medac, Wedel, Germany). Following the manufacturer’s protocol, patients who reached inhibition rates ≥30% were considered to have demonstrated a SARS-CoV-2-specific humoral response and are further defined as responders, while patients with inhibition rates <30% are defined as non-responders. Additionally, IgG antibodies against nucleocapsid, receptor binding domain (RBD), full spike and the S1 domain of the spike protein were tested using SeraSpot anti-SARS-CoV-2 IgG microarray-based immunoassay (Seramun Diagnostica, Heidesee, Germany) and served here for further validation purposes. Hence, all calculations were additionally performed using anti-RBD-IgG levels and can be found in the supplements. The threshold for reactivity for anti-SARS-CoV-2 IgG levels was set at >1.00 signal/cut-off in accordance with manufacturer’s protocol.

Statistical analysis

Descriptive statistics included mean with SD and absolute and relative frequencies. The exact unconditional z-pooled test and χ² test were applied for binary and categorical data and the unpaired t-test with Welch’s correction for continuously distributed variables to perform hypotheses tests for group differences, as appropriate. The likelihood of response to vaccination was modelled by a Poisson generalised linear model with robust error variances and log link function including the covariates age, gender, MTX monotherapy, MTX in combination with prednisolone, MTX in combination with other disease-modifying antirheumatic drugs (DMARDs)=prednisolone, MTX-hold and vaccine interval as suggested by Zou. These covariates were selected based on the theoretical assumption that they could affect vaccination success and on the results of the univariate analysis. The association between antibody results (dependent variables anti-RBD-IgG concentrations or neutralising capacity) and the covariates age, gender, MTX monotherapy, MTX in combination with prednisolone and MTX in combination with other DMARDs=prednisolone, MTX-hold, vaccine interval and timing and duration of MTX-hold was estimated by a linear regression model. The unstandardised and standardised beta-coefficients were calculated for linear regression analysis in order to compare the strengths of association between parameters. The area under the curve (AUC) was calculated after fitting a logistic regression model to provide a measure of strengths of association for dichotomous outcomes. The Youden index was used to estimate thresholds for age and time of MTX break before and after vaccination from receiver operating characteristics (ROC). Statistical analyses were performed using GraphPad Prism V9.2.0, R V4.1.2 and STATA V12.1.
the no-\terisation is given in online supplemental table 1. Patients in group (mean age 61, 90.5% women). Detailed clinical charac-

receiving any kind of immunosuppressive therapy as a control

consisted of 64 patients with AIRD taking MTX (mean age 61

years, 70.3% women) and 21 patients with AIRD who did not

had continued their MTX therapy without any interruption

(MTX continued, table 1). Blood sampling occurred slightly earlier in the MTX-hold group than in the MTX continued
group. There were no significant differences between these
two groups regarding age, BMI, distribution of sex, vaccination
regimes, diagnoses and immunosuppressive comedications
(table 1).

Table 2  Comparison of vaccination responders and non-responders among patients with AIRD taking MTX

|                          | Responders* (n=55) | Non-responders (n=9) | P value† |
|--------------------------|--------------------|---------------------|----------|
| Age, mean (SD)           | 59.5 (12.9)        | 70.3 (6.67)         | 0.001    |
| Female, n (%)            | 42 (76.4)          | 3 (33.3)            | 0.010    |
| BMI, mean (SD)           | 25.4 (4.09)        | 26.6 (3.70)         | 0.389    |
| Medication               |                    |                     | 0.616    |
| MTX-mono, n (%)          | 23 (41.8)          | 3 (33.3)            |          |
| MTX+prednisolone, n (%)  | 8 (14.5)           | 4 (44.4)            |          |
| MTX+anti-TNF-α, n (%)‡   | 10 (18.2)          | 1 (11.1)            |          |
| MTX+anti-TNF-α+prednisolone, n (%)‡ | 6 (10.9) | 1 (11.1) |          |
| MTX+HCQ, n (%)           | 2 (3.6)            | 0                   |          |
| MTX+leflunomide, n (%)§  | 3 (5.5)            | 0                   |          |
| MTX+anti-IL-17, n (%)¶   | 2 (3.6)            | 0                   |          |
| MTX+anti-IL-12/IL-23, n (%)** | 1 (1.8) | 0                  |          |
| MTX dose (mg/week), mean (SD) | 13.0 (4.29)     | 14.2 (4.33)         | 0.469    |
| MTX oral application, n (%) | 25 (45.5)       | 1 (11.1)            | 0.057    |
| Additional prednisolone, n (%) | 15 (27.3)     | 5 (55.6)            | 0.103    |
| Prednisolone dose (mg/day), mean (SD) | 2.5 (1.4)       | 3.8 (1.6)            | 0.174    |

Vaccination

|                      | Responders* (n=55) | Non-responders (n=9) | P value† |
|----------------------|--------------------|---------------------|----------|
| BNT162b2, n (%)      | 39 (70.9)          | 8 (88.9)            |          |
| mRNA-1273, n (%)     | 7 (12.7)           | 1 (11.1)            |          |
| AZD1222, n (%)       | 7 (12.7)           | 0                   |          |
| AZD1222+BNT162b2, n (%) | 2 (3.6)         | 0                   |          |
| Vaccine interval in days, mean (SD) | 42 (15)         | 31 (9)              | 0.011    |
| Days from second vaccination, mean (SD) | 30 (22)       | 40 (22)             | 0.259    |
| MTX-hold, n (%)      | 30 (54.5)          | 1 (11.1)            | 0.017    |
| For both vaccinations, n | 23 (41.8)       | 1 (11.1)            |          |
| For only the first vaccination, n | 2 (3.6)        | 0                   |          |
| For only the second vaccination, n | 5 (9.0)         | 0                   |          |

*Defined by neutralising capacity against SARS-CoV-2 ≥30%.
†P values were calculated using the exact unconditional z-pooled test for binary variables (female, MTX oral application, additional prednisolone, MTX-hold), χ² test for categorical variables (medication, vaccination) and unpaired t-test with Welch’s correction for continuous variables.
‡Adalimumab, certolizumab, etanercept, golimumab, infliximab.
§Additional low-dose prednisolone for n=1.
¶Secukinumab.
**Ustekinumab.
AIRD, autoimmune rheumatic diseases; BMI, body mass index; HCQ, hydroxychloroquine; IL, interleukin; MTX, methotrexate; TNF, tumour necrosis factor.

Patient and public involvement
This study aimed to provide evidence for future recommendations due to questions asked regarding MTX intake by patients and physicians. However, patients and the public were not directly involved in process of designing.

RESULTS

Patient characteristics
Of 73 eligible patients receiving MTX, 9 were excluded due to unacceptable immunosuppressive comedication, irregular medication regimens and unclassifiable MTX-hold. The final cohort consisted of 64 patients with AIRD taking MTX (mean age 61 years, 70.3% women) and 21 patients with AIRD who did not receive any kind of immunosuppressive therapy as a control group (mean age 61, 90.5% women). Detailed clinical characterisation is given in online supplemental table 1. Patients in the no-therapy group were of similar age and body mass index (BMI), but more often female. They were less often diagnosed with rheumatoid arthritis and more often with systemic sclerosis.

Of 64 patients on MTX, 31 patients reported to have held MTX for at least one vaccination (MTX-hold) while 33 patients had continued their MTX therapy without any interruption (MTX continued, table 1).
Factors influencing antibody response in patients on MTX

To identify factors influencing the antibody response under MTX, we compared COVID-19 vaccination responders (n=55, 85.9%) and non-responders (n=9, 14.1%) defined by neutralisation activity. Both groups were comparable in BMI, vaccine type, MTX application form, additional prednisolone intake, time of blood draw and immunosuppressive comedication (table 2). Dosage of MTX was not significantly associated with vaccination success (Spearman’s rank correlation, r=−0.02, p=0.867). However, a higher neutralisation capacity was significantly associated with young age, MTX-hold and female gender in univariable analysis (table 2) and multivariable analysis (table 3). If classification into responders and non-responders was based on anti-RBD-IgG results, 13 patients would fall into the non-responder group. While the effects of age and MTX-hold were still significant using anti-RBD-IgG levels, this was not the case for gender (online supplemental table 2, table 3). A longer vaccine interval was associated with an adequate humoral response to vaccination in our cohort (significant in t-test for neutralisation capacity and anti-RBD-IgG levels; only significant in multivariable analysis for anti-RBD-IgG levels). In the following, we will analyse the effect of age and MTX-hold in more detail.

Effect of MTX-hold and age

Patients who had changed their MTX intake schedule for at least one vaccination showed a significantly higher antibody response than patients who continued their MTX intake (p=0.001, figure 2A, online supplemental figure 2A for anti-RBD-IgG).

Mean neutralisation was 61.2% for patients who continued their therapy and 83.1% for patients who held MTX (table 1). There was only one non-responder (3.2%) in the MTX-hold group, while there were eight non-responders (24.2%) in the MTX continued group. The effect of pause persisted in patients with MTX monotherapy, indicating that this effect cannot be explained by the existing comedication (online supplemental figure 3).

Vaccination response correlated significantly with age (Spearman’s rank correlation, −0.49, p<0.001, figure 3, online supplemental figure 4 for anti-RBD-IgG). No patient younger than 60 years was classified a non-responder which is why we further distinguished the MTX-hold and continued groups into patients older and younger than 60 years of age (figure 2B, online supplemental figure 2B for anti-RBD-IgG). Considering only patients who continued their MTX intake, patients ≥60 years of age (mean 51.9%) had a 30.7 percentage points lower mean inhibition rate than patients <60 years (mean 82.6%). Vice versa, neutralisation levels were 28.9 percentage points higher in patients older than 60 years who held MTX (mean 80.8%) compared with those who continued MTX (mean 51.9%). In contrast, when regarding patients under 60 years there were no significant differences in neutralisation rates between patients who held or continued MTX therapy.

Effect of timing and duration of MTX-hold

In the following, we considered all 64 patients and analysed the MTX interval at the time of vaccination, which was defined by the time between last MTX intake and vaccination (time before vaccination=TBV) and the time between vaccination and re-intake of MTX (time after vaccination=TAV, figure 4). One patient could not recall on which day MTX was taken and was therefore not considered for calculations of T_AV and T_BV. We found that the duration of the MTX interval (T_BV+T_AV) significantly correlates with
Epidemiology

neutralising capacity (Spearman’s rank correlation, \( r = 0.47, p < 0.001 \)). We further analysed which of these time periods is most likely to determine antibody response. By using linear regression analysis, we found time after vaccination (TAV) to be highly significant for adequate neutralisation rate and anti-RBD-IgG concentration in the elderly, but not for younger patients (table 4). Here, 10 days between vaccination and MTX re-intake (TAV) were determined as the critical cut-off based on the Youden index from ROC curve.

DISCUSSION

Our study found a reduced COVID-19 vaccination response in patients on MTX, demonstrates the effect of age and provides first data on the effect of MTX-hold around COVID-19 vaccinations.

Using neutralising capacity and the manufacturer’s cut-off, we found a slightly higher rate of vaccination responders among patients taking MTX (85.9%) than previously reported (47%–72%). Using ROC analysis and an untreated control group, we determined an adapted cut-off value and found adequate immune response in only 40.6% of patients on MTX. Hence,
we confirmed the observations from previous studies that the antibody response is reduced under MTX therapy.\(^4,5\) In contrast, others described no effect of MTX on vaccination response.\(^6,7\)

These varying results may be due to a lower effect size of MTX on vaccination response compared with other immunosuppressive therapies such as rituximab or mycophenolate, different test systems and statistical analyses used and other influencing factors such as age and pausing of MTX therapy.

We determined young age, MTX-hold and longer vaccine interval as the main factors improving antibody response after vaccination. The negative influence of age on vaccination response was already known.\(^20,21\) However, the consideration of age was not yet differentiated in previous studies investigating immune response under MTX therapy. Therefore, our data allow the assumption that continuous MTX intake and old age are potentiating negative factors. The positive effect of a longer vaccine interval on humoral immune response is in line with previously published works.\(^22,23\) These results were statistically significant in the t-test for both antibody testing systems, but in the generalised linear model only for anti-RBD-IgG levels. This discrepancy is likely due to the higher statistical power of the t-test.

Patients who held MTX for at least one vaccination had a significantly higher immune response than those who continued MTX, which has not yet been described for COVID-19 vaccination. Nevertheless, our findings are in line with studies by Park et al investigating the effect of MTX-hold on the immune response to influenza vaccination.\(^11\) More detailed analysis showed that time after vaccination is crucial, which was also described by Park et al who recommended an MTX discontinuation of 2 weeks after influenza vaccination.\(^12,13\) In our study, we found a minimum time of 10 days after vaccination to be critical for immune response in patients ≥60 years. Additionally, the positive effect of MTX-hold was only statistically significant for patients 60 years or older. An effect also in younger patients might be observed in a larger cohort.

A strength of our study was that we validated all our neutralisation test results with an additional test system measuring anti-RBD-IgG levels. The latter defined four more patients as non-responders compared with the neutralisation test. This small number of conflicting test results is to be expected when using different test systems. The uneven distribution of gender among patients who had conflicting test results caused our analyses to suggest a significant influence of gender on the neutralisation result. This may be due to a statistical artefact and the effect of gender should be interpreted with caution.

This study has limitations. Since data regarding the MTX intake schedule during vaccination were assessed retrospectively, recall bias cannot be excluded. Due to our small sample size, we had to limit factors in the multivariable logistic regression modelling, which may lead to bias and residual confounding. For instance, confounding due to duration from vaccination to blood sampling, disease activity or AIRD diagnosis cannot with certainty be excluded in our analyses. We did not assess disease activity and safety of pausing MTX in our cohort, but current data do not indicate a significantly higher flare occurrence or disease activity in association with MTX discontinuation of 2 weeks.\(^24\) Also, T-cell response was not part of our study design. However, according to current studies, it can be assumed that measuring humoral vaccination response is an adequate mean to determine vaccine immunogenicity\(^25\) and that higher antibody levels correlate with a better clinical outcome.\(^26,27\)

To address these limitations, a randomised controlled clinical trial to generate evidence for optimal management of MTX in COVID-19 vaccinations should be performed.

In conclusion, we present real-world data of clinical relevance regarding ongoing booster vaccinations. We determined age and MTX-hold as the main factors influencing antibody response during SARS-CoV-2 vaccinations and both aspects should be considered when discussing MTX regimens. Our data suggest that, if possible, patients older than 60 years of age should hold MTX for at least 10 days after receiving a COVID-19 vaccination.

**Author affiliations**

1 Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität, Berlin, Germany

---

**Table 4** Association of neutralising capacity and anti-RBD-IgG concentration with MTX intake timing using linear regression analysis (n=64)*

|                          | All patients† | Patients <60 years‡ | Patients ≥60 years‡ |
|--------------------------|--------------|---------------------|---------------------|
|                          | β            | P value        | 95% CI             | β            | P value        | 95% CI             | β            | P value        | 95% CI             |
| Neutrailisation capacity |              |                |                   |              |                |                   |              |                |                   |
| **TBV**                  |              |                |                   |              |                |                   |              |                |                   |
| T<sub>1</sub>            | 0.00         | 0.976          | −0.20 to 0.19     | 0.00         | 0.749          | −0.37 to 0.27     | −0.06         | 0.807          | −0.26 to 0.33     | 0.03         |
| T<sub>2</sub>            | 0.19         | 0.005          | 0.06 to 0.32      | 0.30         | 0.301          | −0.09 to 0.28     | 0.20         | 0.012          | 0.06 to 0.43      | 0.32         |
| T<sub>≥10</sub> days     | −0.85        | 0.367          | −2.73 to 1.02     | −0.08        | −0.36          | 0.789            | −3.12 to 2.40   | −0.05        | −1.98          | 0.094            | −4.31 to 0.35   |
| T<sub>≥10</sub> days     | 2.00         | 0.008          | 0.53 to 3.47      | 0.27         | −0.22          | 0.833            | −2.34 to 1.91   | −0.04        | 2.91           | 0.001            | 1.29 to 4.53    |
| T<sub>≥10</sub> + T<sub>off</sub> | 0.13 | 0.035 | 0.01 to 0.25 | 0.25 | 0.04 | 0.626 | −0.13 to 0.22 | 0.11 | 0.039 | 0.01 to 0.35 | 0.29 |
| Anti-RBD-IgG             |              |                |                   |              |                |                   |              |                |                   |
| **TBV**                  |              |                |                   |              |                |                   |              |                |                   |
| T<sub>1</sub>            | 0.80         | 0.244          | −0.56 to 2.15     | 0.09         | 0.47           | 0.575            | −1.24 to 2.17   | 0.10         | 1.26           | 0.236            | −0.86 to 3.39   |
| T<sub>2</sub>            | 1.02         | 0.016          | 0.19 to 1.84      | 0.19         | 0.11           | 0.739            | −0.56 to 0.78   | 0.04         | 1.63           | 0.036            | 0.12 to 0.34    |
| T<sub>≥10</sub> days     | −1.86        | 0.777          | −14.96 to 11.24   | −0.02        | 0.49           | 0.950            | −15.49 to 16.47 | 0.01         | −6.43          | 0.323            | −19.43 to 6.57  |
| T<sub>≥10</sub> days     | 13.70        | 0.004          | 4.60 to 22.79     | 0.21         | −2.53          | 0.583            | −11.96 to 6.90  | −0.07        | 20.03          | 0.005            | 6.57 to 33.50   |
| T<sub>≥10</sub> + T<sub>off</sub> | 0.95 | 0.003 | 0.33 to 1.58 | 0.22 | 0.21 | 0.487 | −0.41 to 0.84 | 0.09 | 1.51 | 0.019 | 0.26 to 2.77 |

Significant results are in bold.

*One patient who did not hold MTX could not recall on which exact day MTX was taken and was therefore only considered for calculations of TBV+TAV (=7 days).
†Adjusted for female, age, MTX monotherapy, MTX+prednisolone, MTX combination+prednisolone, vaccine interval.
‡Adjusted for female, MTX monotherapy, MTX+prednisolone, MTX combination+prednisolone, vaccine interval. β (unstandardised beta-coefficient); β† (standardised beta-coefficient); TBV (time before vaccination) time between last MTX intake and vaccination; T<sub>off</sub> (time after vaccination), time between vaccination and re-intake of MTX; T<sub>≥10</sub> + T<sub>off</sub> MTX interval at the time of vaccination.

MTX, methotrexate.
Epidemiology

2 Epidemiology Unit, German Rheumatism Research Center Berlin – a Leibniz Institute (DRFZ), Berlin, Germany
3 Institute of Virology, Charité Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, and German Centre for Infection Research (DZIF), Associated Partner Site, Berlin, Germany
4 Labor Berlin, Charité - Vivantes GmbH, Berlin, Germany

Acknowledgements
We would like to thank Tanja Braun and Vera Höhne-Zimmer for their support in obtaining the ethics vote and for their organisational support.

Contributors
All authors contributed to the acquisition, analysis or interpretation of data and critical revision of the manuscript for important intellectual content. RB had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the analysis. RB is responsible for the overall content as the guarantor: FNA, JZ, G-RB, RB were involved in the study design. Sample collection was done by ANAdS, LF, FNA, VS, AHT, JZ. Experiments and data analysis were performed by ANAdS, LF, FNA, JK, LMI, TS, JZ, VMC, CD, G-RB and RB. ANAdS, LF, FNA and RB were responsible for tables and figures. Data interpretation was done by all authors. Statistical analyses were done by ANAdS, LF, FNA, JK, and RB. Writing of the manuscript were performed by ANAdS, LF, FNA, JK, G-RB and RB. All authors were involved in critical proof reading of the manuscript.

Funding
The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests
VCM is named together with Euroimmun GmbH on a patent application filed recently regarding the diagnostic of SARS-CoV-2 by antibody testing.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication
Not applicable.

Ethics approval
This study was ethically approved by the Regional Office for Health and Social Affairs Berlin, Germany (21/0098-IV E 13). All patients provided written informed consent.

Provenance and peer review
Not commissioned; externally peer reviewed.

Data availability statement
All data relevant to the study are included in the article. Data are available on reasonable request.

Supplemental material
This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Please see the additional information supplied for details on the content.

Open access
This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) licence, which permits others to copy, redistribute, remix, transform and build upon this work for any purpose, provided the original work is properly cited, a link to the licence is given, and indication of whether changes were made. See: https://creativecommons.org/licenses/by/4.0/.

ORCID iDs
Aamirni Nadira Arumahandi de Silva http://orcid.org/0000-0002-5977-9747
Leonie Maria Frommert http://orcid.org/0000-0003-0110-9190
Fredrik N Albach http://orcid.org/0000-0002-2967-9080
Jens Klotzsche http://orcid.org/0000-0002-2954-5755
Veronika Scholz http://orcid.org/0000-0001-9259-8406
Lara Maria Leworowski http://orcid.org/0000-0002-4159-4189
Fatjana Schwarz http://orcid.org/0000-0002-6054-4750
Alexander ten Hagen http://orcid.org/0000-0002-8657-8019
Jan Zernicke http://orcid.org/0000-0002-5163-2177
Victor Max Corman http://orcid.org/0000-0002-3605-0136
Christian Drosten http://orcid.org/0000-0001-7923-0519
Gerhard-Rüdiger Burmester http://orcid.org/0000-0001-7518-1131
Robert Blohm http://orcid.org/0000-0002-0434-7832

REFERENCES
1 Ritchie H, Mathieu E, Rodés-Guirao L, et al. Coronavirus pandemic (COVID-19): our world in data, 2020. Available: https://ourworldindata.org/coronavirus-data [Accessed 14 Nov 2021].
2 Kane S. Methotrexate 2021. Available: https://clinicaltrial.gov/DrugStats/Drugs/Methotrexate [Accessed 14 Nov 2021].
3 Friedman MA, Curtis JR, Winthrop KL. Impact of disease-modifying antihumneic drugs on vaccine immunogenicity in patients with inflammatory rheumatic and musculoskeletal diseases. Ann Rheum Dis 2021;80:1255–65.
4 Mahil SK, Behkan K, Raharja A, 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;3:e627–37.
5 Haberman RH, Herati R, Simon D, et al. Methotrexate hampers immunogenicity to BNT162b2 mRNA COVID-19 vaccine in immune-mediated inflammatory disease. Ann Rheum Dis 2021;80:1339–44.
6 Braun-Mossovici Y, Kaplan MA, Kraun M, et al. Disease activity and humoral response in patients with inflammatory rheumatic diseases after two doses of the Pfizer mRNA vaccine against SARS-CoV-2. Ann Rheum Dis 2021;80:1317–21.
7 Ruddy JA, Connolly CM, Boyansky BI, et al. High antibody response to two-dose SARS-CoV-2 messenger RNA vaccination in patients with rheumatic and musculoskeletal diseases. Ann Rheum Dis 2021;80:1351–2.
8 Barthschaer M, Levine JM, Bykerk VP, et al. Immunomodulatory and immunosuppressive medication modification among patients with rheumatic diseases at the time of COVID-19 vaccination. Lancet Rheumatol 2022;4:e485–7.
9 Connolly CM, Chiang TP-Y, Boyansky BJ, et al. Temporary hold of mycophenolate augments humoral response to SARS-CoV-2 vaccination in patients with rheumatic and musculoskeletal diseases: a case series. Ann Rheum Dis 2022;81:293–5.
10 Winthrop KL, Silverfield J, Racevicz A, et al. The effect of tofacitinib on pneumococcal and influenza vaccine responses in rheumatoid arthritis. Ann Rheum Dis 2016;75:687–95.
11 Park JK, Lee YJ, Shin K. Impact of temporary methotrexate discontinuation for 2 weeks on immunogenicity of seasonal influenza vaccination in patients with rheumatoid arthritis: a randomised clinical trial. Ann Rheum Dis 2018;77:898–904.
12 Park JK, Lee MA, Lee EY, et al. Effect of methotrexate discontinuation on efficacy of seasonal influenza vaccination in patients with rheumatoid arthritis: a randomised clinical trial. Ann Rheum Dis 2017;76:1559–65.
13 Park JK, Choi Y, Winthrop KL, et al. Optimal time between the last methotrexate administration and seasonal influenza vaccination in rheumatoid arthritis: post hoc analysis of a randomised clinical trial. Ann Rheum Dis 2019;78:1283–4.
14 Curtis JR, Johnson SR, Anthony DD. American College of rheumatology: COVID-19 in: Vaccine clinical guidance summary for patients with rheumatic and musculoskeletal diseases. 4, 2021.
15 Speker C, Aries F, Braun J, et al. Aktualierte Handlungsempfehlungen der Deutschen Gesellschaft für Rheumatologie für die Betreuung von Patienten mit entzündlich-rheumatischen Erkrankungen im Rahmen der SARS-CoV-2/COVID-19-Pandemie. Zeitschrift für Rheumatologie 2021;80:570–87.
16 EULAR. EULAR View-points on SARS-CoV-2 vaccination in patients with RDMDs 2021, 2021. Available: https://www.eular.org/eular_sars_cov_2_vaccination_rmd_patients.cfm
17 Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockade of ACE2–spike–protein–protein interaction. Nat Biotechnol 2020;38:1073–8.
18 Lydersen S, Langaa M, Øv Lundv, et al. The exact unconditional z-pooled test for equality of two binomial probabilities; optimal choice of the Berger and Boos confidence coefficient. J Stat Comput Simul 2012;82:1131–6.
19 Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702–6.
20 Schwarz T, Tober-Lau P, Hillus D, et al. Delayed antibody and T-cell response to BNT162b2 vaccination in the elderly. Emerg Infect Dis 2021;27:2174–8.
21 Muller L, Andreé M, Moszkow W. Age-dependent immune response to the Biotech/Pfizer BNT162b2 COVID-19 vaccination. Clin Infect Dis. 2021.
22 Payne RP, Longet S, Austin JA, et al. Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. Cell 2021;184:5699–714.
23 Grunau B, Goldfarb DM, Asamoah-Boaheng M, et al. Immunogenicity of extended mRNA SARS-CoV-2 vaccine dosing intervals. JAMA 2022;327:279–81.
24 Park JK, Kim MJ, Choi Y, et al. Effect of short-term methotrexate discontinuation on rheumatoid arthritis disease activity; post-hoc analysis of two randomized trials. Clin Rheumatol 2020;39:375–9.
25 Khoury DS, Cromer D, Reynolds A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021;27:1205–11.
26 Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nat Med 2021;27:2032–40.
27 Gilbert PB, Montefiori DC, McDermott A. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy trial. medRxiv 2021;20210201202108.09.21621920.