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Synopsis

Temporary 2-week suspension of methotrexate treatment to enhance COVID-19 vaccine response in people with immune-mediated inflammatory diseases: the VROOM RCT

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Published April 2025
DOI: 10.3310/KYTK6537

Abstract

Objective: Methotrexate is first-line treatment for many immune-mediated inflammatory diseases. However, it inhibits vaccine-induced immunity – a major concern for this vulnerable group of patients. We evaluated if a 2-week interruption of methotrexate treatment immediately after COVID-19 booster improved antibody response against spike protein of the receptor binding domain and live virus neutralisation (ancestral Wuhan and Omicron BA.1) in patients with immune-mediated inflammatory diseases.

Design: Open-label, prospective, individually randomised, parallel-group, controlled superiority trial with 1 : 1 randomisation.

Setting: Multicentre, secondary-care rheumatology and dermatology outpatient clinics.

This synopsis should be referenced as follows:
Abhishek A, Peckham N, Pade C, Gibbons JM, Cureton L, Reynolds CJ, *et al.* Temporary 2-week suspension of methotrexate treatment to enhance COVID-19 vaccine response in people with immune-mediated inflammatory diseases: the VROOM RCT. [published online ahead of print April 30 2025]. *Efficacy Mech Eval* 2025. https://doi.org/10.3310/KYTK6537

Participants: Adults with immune-mediated inflammatory diseases attending rheumatology and dermatology clinics taking methotrexate (≤ 25 mg/week) for ≥ 3 months.
Intervention: Suspending methotrexate treatment for 2 weeks immediately after COVID-19 booster vaccination.
Main outcome(s) and measure(s): The primary outcome was spike protein of the receptor binding domain antibody level 4 weeks after COVID-19 booster vaccination. Secondary outcomes were spike protein of the receptor binding domain antibody levels 12 and 26 weeks after COVID-19 vaccine dose; live virus neutralisation (ancestral Wuhan Hu-1, Omicron BA.1) at weeks 4, 12 and 26; and self-reported inflammatory disease activity, flare-ups, quality of life, global assessment of inflammatory disease and adherence with trial allocation.
Results: A total of 383 participants (61% female, average age 59.0 years) were randomised to either suspend or continue methotrexate. The geometric mean (95% confidence interval) spike protein of the receptor binding domain antibody titre was 25,413 (22,227 to 29,056) and 12,326 (10,538 to 14,418) U/ml in those who suspended and continued methotrexate, respectively. The geometric mean ratio (95% confidence interval) was 2.08 (1.59 to 2.70), $p < 0.0001$. The intervention effect was present across prognostic subgroups, for example, age groups, methotrexate dose, methotrexate administration route, diseases and past severe acute respiratory syndrome coronavirus 2 infection. Enhanced antibody responses were sustained at 12 and 26 weeks with geometric mean ratio (95% confidence interval) 1.88 (1.44 to 2.46) and 1.50 (1.12 to 2.01), respectively. Interruption of treatment improved neutralisation of Wuhan and Omicron BA.1 at 4 weeks with geometric mean ratio (95% confidence interval) 2.56 (1.21 to 5.44) and 2.42 (1.45 to 4.05), respectively. Self-reported inflammatory disease activity initially deteriorated in the suspended methotrexate group, but the groups were comparable at week 12.
Conclusion: Two-week interruption of methotrexate treatment for immune-mediated inflammatory diseases enhanced antibody responses after COVID-19 vaccination that were sustained at 12 and 26 weeks.
Limitations: Lack of participant masking which could have affected self-reported outcomes. Condition-specific disease activity was not used as we recruited participants with a range of diseases, with many lacking validated outcome measures. We did not have data for memory B-cell and T-cell responses. Some hospitals declined to participate in the 26-week follow-up visit which was added to the study after interim analysis, due to lack of capacity, contributing to increased attrition at week 26.
Future work: Future research should evaluate whether interrupting other immune-suppressing treatments soon after vaccination against COVID-19 or other infectious diseases can improve immune responses. Further research should also evaluate whether a shorter hold in methotrexate would improve the immune response elicited by vaccination.
Funding: This synopsis presents independent research funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation programme as award number NIHR134607.
A plain language summary of this synopsis is available on the NIHR Journals Library Website https://doi.org/10.3310/KYTK6537.

Introduction

Rationale for research and background: Low-dose weekly methotrexate is the first-line glucocorticoid-sparing drug used in the treatment of immune-mediated inflammatory diseases (IMIDs) such as rheumatoid arthritis (RA), psoriatic arthritis and cutaneous psoriasis resistant to topical treatment and/or phototherapy.[1–5] It is used in the treatment of steroid-dependent inflammatory bowel disease (IBD), and is often combined with biologics to optimise their efficacy and to prevent antidrug antibody formation.[6–8] Unsurprisingly, methotrexate use has continued to increase in the biologic era with only a minority of patients (approximately 3%) with RA, and psoriasis ± arthritis prescribed biologics.[9–12] Its broad immune-suppressive effects attenuate immune response to COVID-19 vaccines.[13,14] Interrupting methotrexate treatment for 2 weeks immediately after vaccination against seasonal influenza enhanced the immunity from vaccination, with no effect of interrupting treatment for either 2 or 4 weeks before vaccination.[15,16]

In early 2021, soon after the launch of vaccination against COVID-19, there was no evidence as to whether patients taking immune-suppressive medicines should interrupt treatment peri-vaccination. This issue was debated within the British Society for Rheumatology and British Association of Dermatology COVID-19 working groups. In the absence of direct evidence, and after substantial discussion, the two professional societies advised patients to continue with their immune-suppressive therapies, peri-vaccination. However, many patients with inflammatory conditions did their own research and discontinued treatment before and/or after COVID-19 vaccinations '*just in case it may be helpful*' which was also reflected in the patient and public involvement (PPI) group's feedback. The PPI members said that potential consequences caused by a 2-week pause are likely to be low and such a pause in treatment is likely to be acceptable as interruption in treatment occurs when they, that is, patients on long-term methotrexate, become ill, for example, with an infection. The biggest consequence that may be seen is that participants may get a flare-up of their condition – if this

occurs, participants in the trial should be able to use all rescue therapies such as corticosteroids that they usually have available to them.

At about the same time, the American College of Rheumatology (ACR) advised patients with stable disease to discontinue methotrexate for 1 week post severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine while the American Academy of Dermatology advised continuation of treatment but monitoring of post-vaccination serology, which further confused the issue.[17,18]

Objectives: The Vaccine Response On/Off Methotrexate (VROOM) study had two components: a clinical study and a mechanistic study. In the clinical study, the primary objective was to assess the effect of a 2-week temporary suspension of methotrexate on an anti-spike-receptor binding domain (S1-RBD) antibody at 4 weeks post booster vaccination.

The secondary objectives were to assess the effect of a 2-week temporary suspension of methotrexate on:

- S1-RBD antibody at 12 and 26 weeks post booster vaccination
- inflammatory disease activity at weeks 2, 4, 12 and 26 post booster vaccination
- inflammatory disease flare-ups and actions taken to deal with the flare in the 26 weeks post booster vaccination
- drug treatments including rescue treatments for flare-ups in the 26 weeks post booster vaccination; change in disease activity in the 12 weeks post booster vaccination; and quality of life (QoL) at weeks 4, 12 and 26 post booster vaccination.

Inflammatory disease activity was self-reported at 2, 4, 12 and 26 weeks with a 1-week recall on an 11-point (0–10) numeric rating scale with higher scores reflecting better general health. Patients self-reported disease flare-up, actions taken to manage them, QoL using EuroQol-5 Dimensions, five-level version (EQ-5D-5L), 5-point ordinal patient global assessment of disease activity ranging from none/inactive to very severe activity with a 1-week recall at weeks 4, 12 and 26; and inflammatory disease control since vaccination using a 5-point ordinal scale ranging from much better to much worse at weeks 4 and 12. Participants self-reported adherence with trial allocation at week 2.

The objectives of the mechanistic study of 100 participants were to:

- assess the effect of a 2-week temporary suspension of methotrexate on neutralising antibody response against both ancestral Wuhan Hu-1, Omicron BA.1 (see *Appendix 1*) at weeks 4, 12 and 26 post booster vaccination
- explore the association between S1-RBD antibody and neutralisation titres pre booster, and at weeks 4, 12 and 26 post booster vaccination; and explore the validity of S1-RBD antibody and neutralisation titres in participants adherent to methotrexate at each time point, that is pre vaccination, weeks 4 and 12 post booster vaccination, based on validated biochemical assay (see *Appendix 2*).

Additionally, funded by the VROOM study, we collected peripheral blood mononuclear cells to assess the effect of a 2-week temporary suspension of methotrexate on T-cell and memory B-cell response at week 26 post booster vaccination (see *Appendix 3*). While the initial collection of samples, their separation at sites and transportation to Imperial College London were funded by the VROOM study grant, the assessment of T-cell and memory B-cell responses were not funded by the VROOM study and were undertaken using research funds of Professor RJ Boyton. These results therefore are only presented summarily in the funders' report but will be published in full separately under the leadership of Professor RJ Boyton with the wider VROOM trial team.

Methods

The study protocol is available at www.fundingawards.nihr.ac.uk/award/NIHR134607 and was published previously.[19] The methods are summarised below. Parts of this text have been reproduced from Abhishek *et al.*[19] This is an Open Access article distributed in accordance with the terms of the Creative Commons Attribution (CC BY 4.0) licence, which permits others to distribute, remix, adapt and build upon this work, for commercial use, provided the original work is properly cited. See: https://creativecommons.org/licenses/by/4.0/. The text below includes minor additions and formatting changes to the original text. Please see *Figure 1* for participant flow in the VROOM study.

The following table lists all external publications and what each publication covers, including links to these publications (*Table 1*). The findings are described in more detail in the Results summary.

This was an open-label, prospective, two-arm parallel-group, multicentre, superiority, randomised controlled

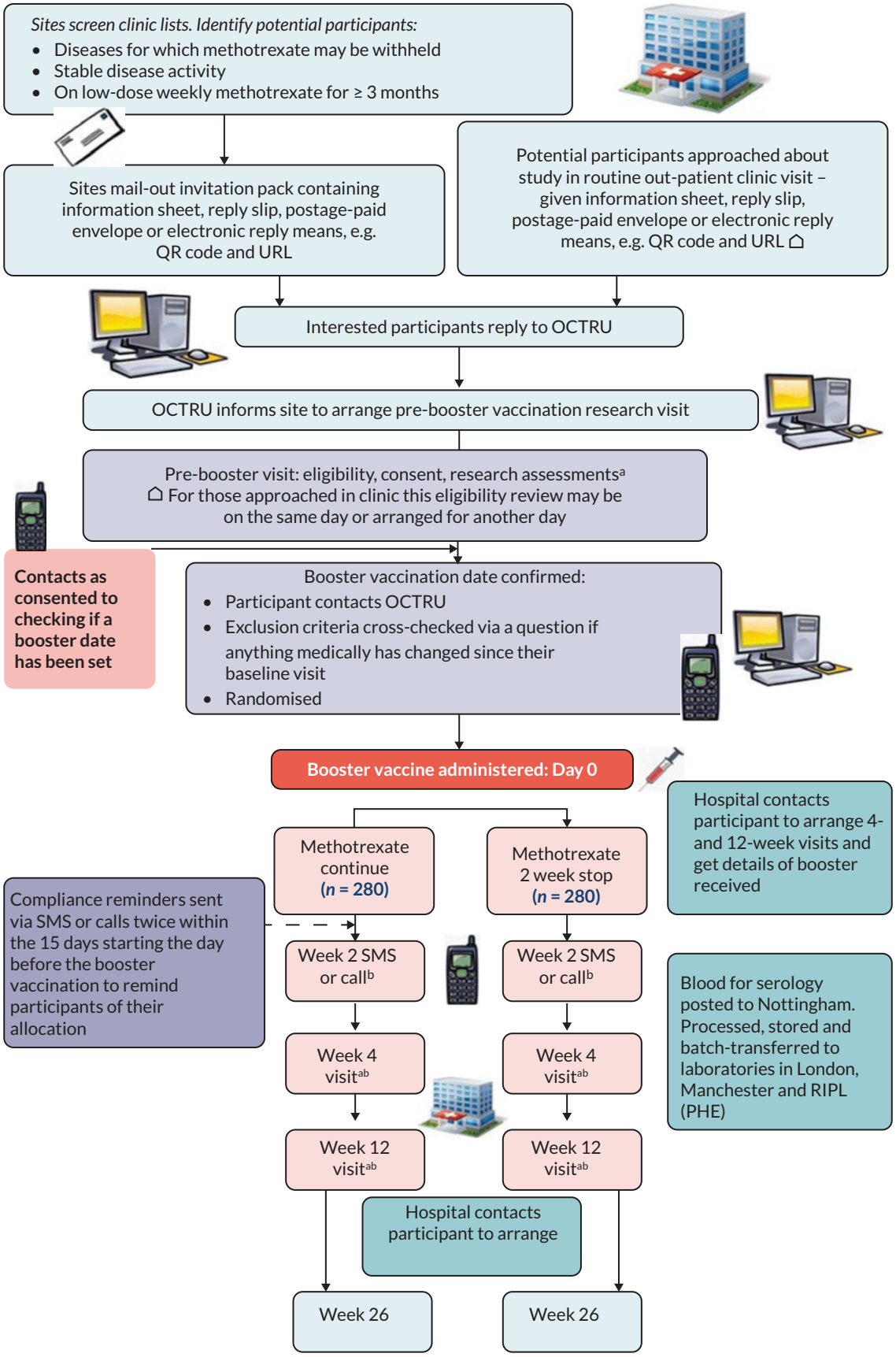

FIGURE 1 Participant flow in the VROOM study. a, Disease, demographic data; blood collection. b, Participant-reported outcome data collected. OCTRU, Oxford Clinical Trials Research Unit; PHE, formerly Public Health England, now UK Health Security Agency. Adapted from protocol paper.[19]

TABLE 1 List of external publications

External publication and weblink	What does it cover?
Effects of temporarily suspending low-dose methotrexate treatment for 2 weeks after SARS-CoV-2 vaccine booster on vaccine response in immunosuppressed adults with inflammatory conditions: protocol for a multicentre randomised controlled trial and nested mechanistic substudy VROOM study. Weblink: https://bmjopen.bmj.com/content/12/5/e062599.long	Protocol paper
Effect of a 2-week interruption in methotrexate treatment vs. continued treatment on COVID-19 booster vaccine immunity in adults with inflammatory conditions (VROOM study): a randomised, open-label, superiority trial. Weblink: www.thelancet.com/journals/lanres/article/PIIS2213-2600(22)00186-2/fulltext	Results of a pre-specified interim analysis
Effect of a 2-week interruption in methotrexate treatment on COVID-19 vaccine response in people with immune-mediated inflammatory diseases (VROOM study): a randomised, open-label, superiority trial. Weblink: www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(23)00298-9/fulltext	Results of the full analysis

trial with 1 : 1 randomisation. Briefly, participants were recruited from Rheumatology and Dermatology clinics in 26 UK hospitals. They were required to be ≥ 18 years old, diagnosed with an IMID such as RA, psoriasis etc., prescribed methotrexate (≤ 25 mg/week) for ≥ 3 months ±hydroxychloroquine, able to temporarily suspend methotrexate treatment for 2 weeks in the opinion of their clinical team, have received two vaccine doses from the UK COVID-19 Vaccination Programme and be eligible for an additional vaccine dose.

Key exclusion criteria were IMIDs for which treatment cannot be interrupted safely; recent or planned rituximab infusion; use of other steroid-sparing drugs in previous 2 months; use of prednisolone > 7.5 mg/day within previous 1 month; radiotherapy or chemotherapy for cancer in previous 6 months; and visceral cancer.

The VROOM study evaluated temporarily interrupting versus continuing methotrexate treatment immediately after the COVID-19 vaccine boosters (predominantly full-dose BNT162b2, half-dose or full-dose mRNA-1273; and full-dose AstraZeneca AZD1222) delivered through the UK COVID-19 Vaccination Programme. For the 'suspend group', methotrexate dosing was interrupted for 2 weeks immediately after receiving the COVID-19 vaccine. Participants vaccinated on the day on which they usually took methotrexate were asked to miss the methotrexate on the day of vaccination and another dose 1 week later. For others, the advice was to suspend the weekly methotrexate doses for 2 weeks immediately after vaccination. For the 'continue group', methotrexate was continued at the same dose on the same day. Any concomitant medicine including folic acid and hydroxychloroquine was continued and disease flares treated as per standard care. Participants could also stop or take methotrexate against trial allocation if clinically indicated, for example, if there was an intercurrent infection or flare, respectively.

Randomisation was done using a centralised validated computer randomisation programme through a secure (encrypted) web-based service provided by the Oxford Clinical Trials Research Unit (OCTRU). A minimisation algorithm including a random element ensured balanced allocation across treatment groups, and a 1 : 1 ratio to allocate to either suspend methotrexate use for 2 weeks or continue as usual. The trial used IMID type [rheumatic (±skin) disease or skin disease alone]; age (< 40 years, 40–64 years, ≥ 65 years); and primary vaccination technology (mRNA, vector or combination) as stratification factors in this minimisation algorithm. The stratification factors were chosen to balance IMIDs and key prognostic factors that impact COVID-19 vaccine response between trial arms. Self-reported prior SARS-CoV-2 infection was not controlled for despite it being a strong modifier of serological response to COVID-19 vaccination due to inconsistent access to diagnostic polymerase chain reaction (PCR) testing in the UK. Prior SARS-CoV-2 infection status was established by measuring *N*-serology at baseline and used in the statistical analysis.

The VROOM study was initially designed with visits at 4 and 12 weeks. There was a pre-specified plan to analyse the data once primary-outcome data for 250 participants were available in an interim analysis, and to present the findings to the independent data monitoring committee and trial steering committee. In view of the results of the interim analysis,[20] and supported by the independent data monitoring committee and trial steering committee and the funder, a 26-week visit was added in March 2022 to evaluate the durability of the improvement in immune response.

Statistical analyses were based on the as randomised ('intention to treat') population. The study was powered to detect ≥ 25% lower antibody response in the methotrexate continue group (Cohen's *d* effect of 0.29)

with 90% statistical power at two-sided 5% significance level. Using S1-RBD antibody response elicited by third dose of COVID-19 vaccine; this effect size translates to a target difference in S1-RBD antibody titre of ~5000 U/mL.

Antibody data were log-transformed (base10) to normalise distribution before analysis – model diagnostics can be found in *Appendix 4*, *Figures 7* and *8*. The difference in S1-RBD level at 4, 12 and 26 weeks between study groups was estimated using a multilevel mixed-effects regression model, allowing for repeated measures clustered within participants (treated as random effects). The model was adjusted for stratification factors, prior SARS-CoV-2 infection assessed using *N*-serology and COVID-19 vaccine technology received as booster dose as fixed effects. A treatment-by-time interaction was included, and the model used an unstructured covariance matrix. Adjusted geometric mean ratios (GMR) between the groups are presented, together with a 95% confidence interval (CI) and *p*-value for the primary-outcome measure.

Consistency of treatment effect for prognostic subgroups (age, rheumatic and skin disease, methotrexate dose and route, primary vaccination platform and prior SARS-CoV-2 infection) was explored at 4, 12 and 26 weeks using treatment by subgroup interactions. Other secondary outcomes were analysed using generalised linear models for binary and continuous data, as appropriate, with model adjustment as described above. The widths of the 95% CI have not been adjusted for multiplicity and these should not be interpreted as formal hypothesis tests. The number and details of serious adverse events (SAEs) were presented by treatment group.

Results summary

The results of a pre-specified interim analysis based on the recommendations of the independent data monitoring committee and trial steering committee were published previously.[20] Below, we describe the results of the full trial cohort.

By recruitment stop, 191 participants were randomly assigned to suspend methotrexate use for 2 weeks and 192 to continue methotrexate (see *Figure 2*). Seven and four participants in the suspend methotrexate and continue methotrexate groups, respectively withdrew consent before their 12-week visit (see *Appendix 4*, *Table 9*). The baseline characteristics of participants were well balanced between the groups (*Table 2*). However, there were numerically more people with RA 111 (57.8%) and numerically fewer non-smokers 95 (49.5%) in the continue methotrexate group than in the suspend methotrexate group, 97 (50.8%) and 104 (54.5%), respectively. The cohort's mean age and body mass index were 59.0 years and 29.2 kg/m^2, respectively; 61.4% (*n* = 235) were female, 54.3% (*n* = 208) had RA, 31.9% (*n* = 122) had psoriasis with/without arthritis and 17.8% (*n* = 68) had an inflammatory skin condition alone. The median methotrexate dose was 20 mg/week, and 94.5% (*n* = 362) received an mRNA vaccine booster, a mean of 178 days after the second dose of the primary vaccination.

Adherence to the intervention was high with 96.3% (*n* = 184) and 97.4% (*n* = 187) self-reported adherence with allocation in the suspend and continue methotrexate groups, respectively (see *Appendix 4*, *Table 10*). One participant in suspend arm and four participants in the continue arm were partially compliant with trial allocation taking one weekly dose. Compliance data were missing for seven participants. Participants were not excluded for non-compliance. Participants in both arms had high levels of adherence to methotrexate in a validated biochemical assay (see *Appendix 4*, *Tables 7* and *11*).

Primary outcome: The S1-RBD antibody response was significantly higher in the methotrexate suspend group compared to the continue treatment group at 4 weeks [geometric mean (GM) (95% CI) 25,413 (22,227 to 29,056) and 12,326 (10,538 to 14,418) U/mL, respectively]. In an adjusted mixed-effect model, the GMR (95% CI) of the S1-RBD antibody on suspending methotrexate for 2 weeks was 2.08 (1.59 to 2.70), $p < 0.001$ (*Table 3*). The results were unchanged on sensitivity analyses (see *Appendix 4*, *Table 12*). Planned exploratory subgroup analyses (*Figure 3*, *Appendix 4*, *Table 13*) suggested a greater treatment effect at higher methotrexate dose [interaction GMR effect (95% CI) 1.48 (1.04 to 2.12)]. The treatment effects were consistent across methotrexate administration route, rheumatic and skin disease, age, primary vaccination platform and prior SARS-CoV-2 infection status.

Secondary outcomes: The S1-RBD antibody level was higher in the methotrexate suspend group compared to the continue treatment group at 12 and 26 weeks (see *Table 3*). In a mixed-effect model, the GMR (95% CI) for S1-RBD antibody on suspending methotrexate for 2 weeks was 1.88 (1.44 to 2.46) at 12 weeks, and 1.50 (1.12 to 2.01) at 26 weeks. At 12 weeks, results were similar across subgroups except for methotrexate dose which indicated a greater treatment effect at higher doses [interaction GMR effect (95% CI) 1.56 (1.03 to 2.37)] (*Figure 4* and *Appendix 4*, *Table 13*). At 26 weeks, the subgroup results were similar across all prognostic factors (*Figure 5* and

Appendix 4, *Table 13*). A sensitivity analysis that excluded participants in receipt of an additional booster vaccination before their week-26 visit yielded similar results (see *Appendix 4*, *Table 12*).

The Wuhan Hu-1 inhibitory concentration half-maximal (IC50) neutralising antibody titre was higher in the suspend methotrexate group compared to the continue treatment group at four and 26 weeks (see *Table 3*). In a mixed-effect model, the GMR (95% CI) for Wuhan Hu-1 IC50 neutralising antibody titre on suspending methotrexate for 2 weeks was 2.56 (1.21 to 5.44) at 4 weeks, and 3.50 (1.34 to 9.18) at 26 weeks. The Omicron BA.1 IC50 cross-neutralising antibody titre was higher in the methotrexate suspend group compared to the continue treatment group at 4 weeks with GMR (95% CI) 2.42 (1.45 to 4.05). The Omicron BA.1 IC50 neutralising antibody titre was comparable between the two groups at other time points.

Clinical outcomes: Self-reported general health due to inflammatory disease and EQ-5D-5L utility values were comparable between the two groups at all time points (*Table 4*). Self-reported inflammatory disease activity was worse at 4 weeks in the suspend methotrexate group but was comparable in the two groups at 12 weeks and 26 weeks (see *Appendix 4*, *Tables 14* and *15*). Similarly, self-reported inflammatory disease control since vaccination was worse at 4 weeks but was comparable between the two arms by week 12. This was not assessed at week 26 to minimise any biased recall.

More participants self-reported ≥ 1 disease flare in the suspend methotrexate group than in the continue methotrexate group at week 4 [53.4% (*n* = 102) vs. 32.8% (*n* = 63), OR (95% CI) 2.28 (1.72 to 3.66)] and week 12 [64.9% (*n* = 124) vs. 46.4% (*n* = 89), OR (95% CI) 1.98 (1.33 to 2.90)]. However, disease flares were comparable at week 26 [69.1% (*n* = 132) vs. 60.9% (*n* = 117), OR (95% CI) 1.37 (0.72 to 2.17)]. Most flares were self-managed with a similar proportion of participants seeking medical or specialist-nurse help for flares in either arms, that is 12 (6.3%) and 8 (4.2%), in weeks 0–4, 25 (13.1%) and 25 (13.0%) in weeks 0–12, and 32 (16.8) and 39 (20.3%) in weeks 0–26 in the suspend and continue methotrexate arms, respectively (see *Appendix 4*, *Table 16*). More participants who suspended methotrexate self-reported using non-steroidal anti-inflammatory drugs (NSAIDs)/analgesics, corticosteroids and topical treatments for managing disease flare-ups up to week 12. SARS-CoV-2 infections were numerically higher for the period 13–26 weeks in the suspend methotrexate group [30/137 (21.9%)] than in the continue methotrexate group [24/151 (15.9%)] (see *Appendix 4*, *Table 17*). There were no hospitalisations or deaths reported due to COVID-19 in the study. There were no intervention-related SAEs. There were three SAEs (two in the suspend arm and one in the continue arm) unrelated to the intervention.

During the progression of the COVID-19 pandemic, the field rapidly progressed from reliance on neutralising antibody titres to the ancestral SARS-CoV-2 sequence to an appreciation that correlates of protection had necessarily become more complex, encompassing magnitude and breadth of T-cell and antibody responses. T-cell and memory B-cell studies were included in the initial submitted VROOM application to NIHR EME but were not funded. The VROOM study, therefore, did not collect any peripheral blood mononuclear cells (PBMCs) at baseline, 4- or 12-week time points from any participants. At the 26-week time point, NIHR EME and the VROOM study supported the additional collection of PBMC. However, by this time recruitment was already far advanced and the early pause of recruitment meant these samples were only collected from a limited number of participants. The number of patients recruited at 26 weeks where PBMC was collected (no interruption, *n* = 13- vs. 2-week interruption, *n* = 11) was unfortunately too low to allow a meaningful analysis of T-cell and memory B-cell responses between groups. The samples were prepared in Prof Boyton's laboratory as described in *Appendix 3* and have been analysed but the results were not written up. In this small sample size, there was no observed difference between the suspend and continue groups at week 26 in T-cell responses against ancestral or variant SARS-CoV-2 spike. A descriptive report of the findings will be published separately by Prof Boyton and her team in collaboration with the rest of the VROOM team.

Mechanistic outcomes

There was strong correlation between S1-RBD and neutralisation response at the baseline (*Table 5*). However, this became weaker at weeks 4, 12 and 26. The correlation coefficients were larger in magnitude in the suspend methotrexate arm than in the continue methotrexate arm (see *Table 5*, *Appendix 4*, *Figure 6*).

Discussion

Principal findings

A 2-week interruption of methotrexate treatment immediately after COVID-19 booster vaccination enhanced the S1-RBD antibody response that was maintained at 26 weeks. Subgroup analysis indicated that the effect was present across a range of prognostic factors including prior SARS-CoV-2 infection. There was a

TABLE 2 Baseline characteristics

Key baseline factors	Continue with methotrexate *N* = 192 (%)	Suspend methotrexate *N* = 191 (%)	Total *N* = 383 (%)
Sex, n (%)			
Male	75 (39.1)	73 (38.2)	148 (38.6)
Female	117 (60.9)	118 (61.8)	235 (61.4)
Age in years, mean (SD)	59.3 (11.9)	58.8 (12.5)	59.0 (12.2)
BMI (kg/m^2), mean (SD)	28.7 (6.0)	29.6 (5.7)	29.2 (5.9)
Type of inflammatory condition, n (%)			
Inflammatory rheumatic disease (±skin disease)	160 (83.3)	155 (81.2)	315 (82.2)
Skin disease only	32 (16.7)	36 (18.8)	68 (17.8)
Primary COVID-19 vaccine type, n (%)			
mRNA (Pfizer-BioNTech, Moderna)	73 (38)	70 (36.6)	143 (37.3)
Vector (AstraZeneca/University of Oxford)	118 (61.5)	119 (62.3)	237 (61.9)
Combination	1 (0.5)	2 (1.0)	3 (0.8)
Weekly dose of methotrexate (mg/week)			
n, Median (IQR)	20 (15–25)	20 (15–22.5)	20 (15–22.5)
Usual method of administration of methotrexate, n (%)			
Oral	106 (55.2)	106 (55.5)	212 (55.4)
Subcutaneous	86 (44.8)	85 (44.5)	171 (44.6)
Serum creatinine (µmol/l), mean (SD)	73.1 (14.0)	75.9 (14.5)	74.5 (14.3)
Serum albumin (g/l), mean (SD)	41.2 (3.5)	41.6 (4.0)	41.4 (3.7)
Randomisation to booster (days), mean (SD)	6.3 (7.1)	6.1 (7.2)	6.2 (7.1)
Baseline assessment to booster (days), mean (SD)	11.8 (12.0)	11.7 (11.4)	11.8 (11.6)
Previous vaccination to booster (days), mean (SD)	174.2 (43.8)	180.8 (42.2)	177.5 (43.1)
Ethnicity, n (%)			
White	182 (94.8)	177 (92.7)	359 (93.7)
Other ethnic group	10 (5.2)	12 (6.3)	22 (5.7)
Missing data	0 (0.0)	2 (1.0)	2 (0.5)
Smoking status, n (%)			
Never smoked	95 (49.5)	104 (54.5)	199 (52.0)
Ex-smoker	80 (41.7)	71 (37.2)	151 (39.4)
Current smoker	17 (8.9)	16 (8.4)	33 (8.6)
Residence, n (%)			
Own home	178 (92.7)	183 (95.8)	361 (94.3)
Residential care	1 (0.5)	1 (0.5)	2 (0.5)
Living with family/friends	12 (6.3)	7 (3.7)	19 (5.0)
Missing data	1 (0.5)	0 (0.0)	1 (0.3)

TABLE 2 Baseline characteristics (*continued*)

Key baseline factors	Continue with methotrexate *N* = 192 (%)	Suspend methotrexate *N* = 191 (%)	Total *N* = 383 (%)
Inflammatory condition (participants can have more than one condition), n (%)			
RA	111 (57.8)	97 (50.8)	208 (54.3)
Psoriasis with arthritis	37 (19.3)	38 (19.9)	75 (19.6)
Psoriasis without arthritis	22 (11.5)	25 (13.1)	47 (12.3)
Seronegative (axial) spondyloarthritis	3 (1.6)	2 (1.0)	5 (1.3)
Atopic eczema	9 (4.7)	9 (4.7)	18 (4.7)
Polymyalgia rheumatica	3 (1.6)	3 (1.6)	6 (1.6)
Systemic lupus erythematosus	3 (1.6)	2 (1.0)	5 (1.3)
Other rheumatic disease	8 (4.2)	14 (7.3)	22 (5.7)
Other skin disease	5 (2.6)	7 (3.7)	12 (3.1)
Comorbidities (participants can have more than one condition), n (%)			
Diabetes	23 (12)	20 (10.5)	43 (11.2)
Hypertension	49 (25.5)	44 (23)	93 (24.3)
Ischaemic heart disease	6 (3.1)	8 (4.2)	14 (3.7)
Congestive cardiac failure	0 (0.0)	1 (0.5)	1 (0.3)
Asthma	25 (13)	28 (14.7)	53 (13.8)
COPD	5 (2.6)	8 (4.2)	13 (3.4)
High cholesterol	25 (13)	25 (13.1)	50 (13.1)
Stroke (including transient ischaemic attack)	4 (2.1)	4 (2.1)	8 (2.1)
None of the above	105 (54.7)	105 (55.0)	210 (54.8)
Concomitant systemic medications (participants can take more than one medicine)			
Folic acid, *n* (%)	188 (97.9)	188 (98.4)	376 (98.2)
NSAIDs, *n* (%)	30 (15.6)	29 (15.2)	59 (15.4)
Hydroxychloroquine, *n* (%)	38 (19.8)	38 (19.9)	76 (19.8)
Hydroxychloroquine dose, mg/day median (IQR).	37, 200 (200–400)	37, 200 (200–200)	74, 200 (200–400)
Insulin, *n* (%)	4 (2.1)	1 (0.5)	5 (1.3)
Oral glucocorticoid, *n* (%)	3 (1.6)	7 (3.7)	10 (2.6)
None	3 (1.6)	2 (1.0)	5 (1.3)
Current use of topical glucocorticoid cream, n (%)			
Yes	28 (14.6)	29 (15.2)	57 (14.9)
No	164 (85.4)	162 (84.8)	326 (85.1)
Intra-articular or intramuscular, glucocorticoid in the past 3 months, n (%)			
Intra-articular corticosteroid	2 (1.0)	7 (3.7)	9 (2.3)
Intramuscular corticosteroid	3 (1.6)	5 (2.6)	8 (2.1)
Intravenous corticosteroids	0 (0.0)	0 (0.0)	0 (0.0)

continued

TABLE 2 Baseline characteristics (*continued*)

Key baseline factors	Continue with methotrexate *N* = 192 (%)	Suspend methotrexate *N* = 191 (%)	Total *N* = 383 (%)
COVID-19 disease history (participants could choose multiple options), n (%)			
COVID-19 hospitalisations	1 (0.5)	3 (1.6)	4 (1.0)
COVID-19 not requiring hospitalisations	22 (11.5)	27 (14.1)	49 (12.8)
COVID-19 PCR positive tests	15 (7.8)	24 (12.6)	39 (10.2)
No COVID-19 events	163 (84.9)	155 (81.2)	318 (83.0)
3rd/4th vaccination (booster), n (%)			
3rd	149 (77.6)	154 (80.6)	303 (79.1)
4th	43 (22.4)	37 (19.4)	80 (20.9)
COVID-19 booster, n (%)			
Pfizer-BioNTech	147 (76.6)	143 (74.9)	290 (75.7)
Oxford-AstraZeneca	8 (4.2)	4 (2.1)	12 (3.1)
Moderna	35 (18.2)	37 (19.4)	72 (18.8)
Unknown	0 (0.0)	2 (1.0)	2 (0.5)
Did not have booster	2 (1.0)	5 (2.6)	7 (1.8)

COPD, chronic obstructive pulmonary disease; IQR, inter-quartile range; SD, standard deviation.

Note
Data for serum creatinine, serum albumin, and hydroxychloroquine dose were missing for 15, 18 and 1 participants, respectively, in the continue methotrexate arm, and for 15, 19 and 1 participants, respectively, in the suspend methotrexate arm. Data for time between the latest previous vaccination before entering the trial to booster vaccination received in the VROOM study, baseline visit to booster vaccination received in the VROOM study, and randomisation to booster vaccination received in the VROOM study were missing for two participants in the continue methotrexate arm, and for three participants in the suspend methotrexate arm. Patient global assessment of disease activity was assessed on a 0–10 numeric rating scale with 0 being poor and 10 being excellent and a 1-week recall using the question: In all the ways that your condition affects you, over the last 7 days, how would you rate the way you felt?

TABLE 3 Serological outcomes at primary and secondary end points

	Continue methotrexate		Suspend methotrexate			
	N	GM (95% CIs)	*N*	GM (95% CIs)	Mixed-effects model: GMR (95% CI)[a]	*p*-value
S1-RBD antibody						
Baseline	191	948 (711 to 1263)	190	890 (677 to 1169)	–	–
4 weeks	187	12,326 (10,538 to 14,418)	180	25,413 (22,227 to 29,056)	2.08 (1.59 to 2.70)	< 0.001
12 weeks	184	8972 (7500 to 10,733)	179	17,131 (14,882 to 19,721)	1.88 (1.44 to 2.46)	< 0.001
26 weeks	151	9971 (8050 to 12,350)	137	15,318 (12,430 to 18,878)	1.50 (1.12 to 2.01)	0.006
Neutralisation of live SARS-CoV-2 virus						
Baseline						
Wuhan Hu-1 IC50	50	2229 (1096 to 4531)	50	1524 (736 to 3155)	–	–
Omicron BA.1 IC50	50	157 (103 to 239)	50	122 (80 to 185)	–	–

TABLE 3 Serological outcomes at primary and secondary end points (*continued*)

	Continue methotrexate		Suspend methotrexate			
	N	GM (95% CIs)	*N*	GM (95% CIs)	Mixed-effects model: GMR (95% CI)[a]	*p*-value
4 Weeks						
Wuhan Hu-1 IC50	50	18,342 (9059 to 37,139)	50	35,919 (17,628 to 73,191)	2.56 (1.21 to 5.44)	–
Omicron BA.1 IC50	50	339 (220 to 522)	50	724 (426 to 1230)	2.42 (1.45 to 4.05)	–
12 Weeks						
Wuhan Hu-1 IC50	50	21,879 (11,084 to 43,187)	50	22,150 (10,874 to 45,119)	1.32 (0.62 to 2.81)	–
Omicron BA.1 IC50	50	280 (172 to 454)	50	274 (170 to 443)	1.11 (0.67 to 1.86)	–
26 Weeks						
Wuhan Hu-1 IC50	29	11,161 (4517 to 27,578)	28	25,613 (9865 to 66,500)	3.50 (1.34 to 9.18)	–
Omicron BA.1 IC50[b]	29	881 (399 to 1946)	28	1001 (370 to 2703)	1.50 (0.69 to 3.29)	–

a Mixed-effects model, adjusted by baseline value, stratification factors (age, inflammatory condition, vaccine platform), prior infection, booster platform and included time by treatment interaction.
b Participants got vaccinated against COVID-19 in this period using a bivalent vaccine including Omicron and this explains a higher neutralisation titre at week 26 than at week 12.

TABLE 4 Self-reported clinical outcomes at primary and secondary end points

	Continue with methotrexate	Suspend methotrexate	Treatment effect (95% CI)[a]
EQ-5D Utility Scores, mean (SD)			
4 weeks	0.769 (0.181)	0.743 (0.213)	−0.024 (−0.063 to 0.015)
12 weeks	0.763 (0.191)	0.745 (0.220)	−0.014 (−0.052 to 0.025)
26 weeks	0.787 (0.183)	0.756 (0.201)	−0.033 (−0.104 to 0.037)
EQ VAS, mean (SD)			
4 weeks	77.0 (16.5)	73.6 (19.4)	−3.090 (−6.687 to 0.508)
12 weeks	75.3 (17.9)	72.0 (20.2)	−2.787 (−6.382 to 0.810)
26 weeks	77.9 (16.7)	75.1 (19.4)	−2.30157 (−6.075 to 1.562)
Patient assessment of inflammatory disease, mean (SD)			
2 weeks	7.3 (1.7)	6.8 (2.2)	−0.437 (−1.226 to 0.353)
4 weeks	7.4 (1.9)	6.9 (2.2)	−0.462 (−1.254 to 0.331)
12 weeks	7.2 (2.0)	7.0 (2.1)	−0.177 (−0.966 to 0.612)
26 weeks	7.5 (1.9)	7.0 (2.1)	−0.475 (−1.292 to 0.342)
Participants with at least one flare-up, n/N (%)[b]			
0–4 weeks	63/192 (32.8)	102/191 (53.4)	2.280 (1.723 to 3.655)
0–12 weeks	89/192 (46.4)	124/191 (64.9)	1.982 (1.334 to 2.901)
0–26 weeks	117/192 (60.9)	132/191 (69.1)	1.371 (0.721 to 2.165)

VAS, visual analogue scale.
a Mixed-effects model for EQ-5D, patient assessment of inflammatory disease outcomes, and flares adjusted by baseline value, stratification factors (age, inflammatory condition, vaccine platform), prior infection, booster platform, and included time by treatment interaction.
b Odds ratio for participants with at least one flare-up.

TABLE 5 Correlations between Wuhan and Omicron neutralisation titres and S1-RBD antibody titre

	Continue methotrexate		Suspend methotrexate		Total	
	Pearson correlation	95% CI	Pearson correlation	95% CI	Pearson correlation	95% CI
Baseline						
Wuhan Hu-1 IC50	0.824	(0.707 to 0.896)	0.856	(0.758 to 0.916)	0.841	(0.772 to 0.890)
Omicron BA.1 IC50	0.776	(0.634 to 0.867)	0.699	(0.522 to 0.818)	0.736	(0.631 to 0.815)
4 weeks						
Wuhan Hu-1 IC50	0.225	(-0.057 to 0.474)	0.495	(0.252 to 0.680)	0.379	(0.197 to 0.536)
Omicron BA.1 IC50	0.479	(0.231 to 0.668)	0.602	(0.389 to 0.754)	0.568	(0.418 to 0.688)
12 weeks						
Wuhan Hu-1 IC50	0.439	(0.183 to 0.639)	0.500	(0.257 to 0.683)	0.466	(0.296 to 0.607)
Omicron BA.1 IC50	0.550	(0.321 to 0.718)	0.800	(0.671 to 0.882)	0.668	(0.690 to 0.881)
26 weeks						
Wuhan Hu-1 IC50	0.447	(0.153 to 0.668)	0.572	(0.308 to 0.754)	0.513	(0.292 to 0.683)
Omicron BA.1 IC50	0.704	(0.499 to 0.834)	0.883	(0.786 to 0.938)	0.806	(0.690 to 0.881)

numerically greater effect on S1-RBD antibody response from interrupting treatment in those on higher doses of methotrexate and the S1-RBD antibody level in the suspend methotrexate group at 26 weeks was numerically greater than that in the continue methotrexate group at 4-weeks. The neutralising capacity was higher for the ancestral Wuhan Hu-1 strain at weeks 4 and 26, and for the Omicron BA.1 variant of concern (VOC) at week 4, an important finding as neutralising antibody IC_{50} titres are associated with protection against COVID-19 including severe disease.

High compliance with the intervention indicated patient acceptability. Interrupting methotrexate for 2 weeks did not impact on QoL, general health or patient assessment of inflammatory disease on a 10-point Numerical Rating Scale. A temporary deterioration of inflammatory disease control and an associated increased self-reported disease flare-up was apparent in the initial 4 weeks. However, there was no excess risk of self-reported flares, inflammatory disease activity and inflammatory disease control when longer follow-up periods were considered. The majority of flares were self-managed with no appreciable differences in seeking healthcare input across the two groups. Thus, interrupting treatment seemed to be associated with worsening self-reported inflammatory disease control in the following few weeks. Although the differences were absent when longer follow-up periods were considered, there will need to be a balancing of possible risk of a flare-up versus enhanced protection against COVID-19 to be considered together by the participants and physician.

Contribution to existing knowledge

Strategies to boost vaccine response will facilitate optimal benefits from vaccination in terms of longevity of protection and protection against VOC. A 2-week break in methotrexate treatment immediately after vaccination provided a simple, low-cost, easy-to-implement and effective intervention. It could potentially translate into greater vaccine efficacy and longer duration of protection for vulnerable groups.

Strengths and weaknesses

Strengths of our study included broad eligibility criteria with a range of IMIDs and recruitment of patients with prior SARS-CoV-2 infection, making the results generalisable, excellent adherence to intervention, and minimal attrition at the primary end point. Neutralisation assays used live viruses and included cross-neutralisation, derived from Wuhan Hu-1 spike exposure that was tested against an Omicron BA1 variant. Limitations included lack of participant blinding which could result in potential bias of self-reported inflammatory disease activity, QoL and flare outcomes. It was not possible to blind participants in this study without a matching placebo, which would have made this time-critical study unfeasible. Nevertheless, the pragmatic trial design used reflected real-world practice and patient experience making the results useful

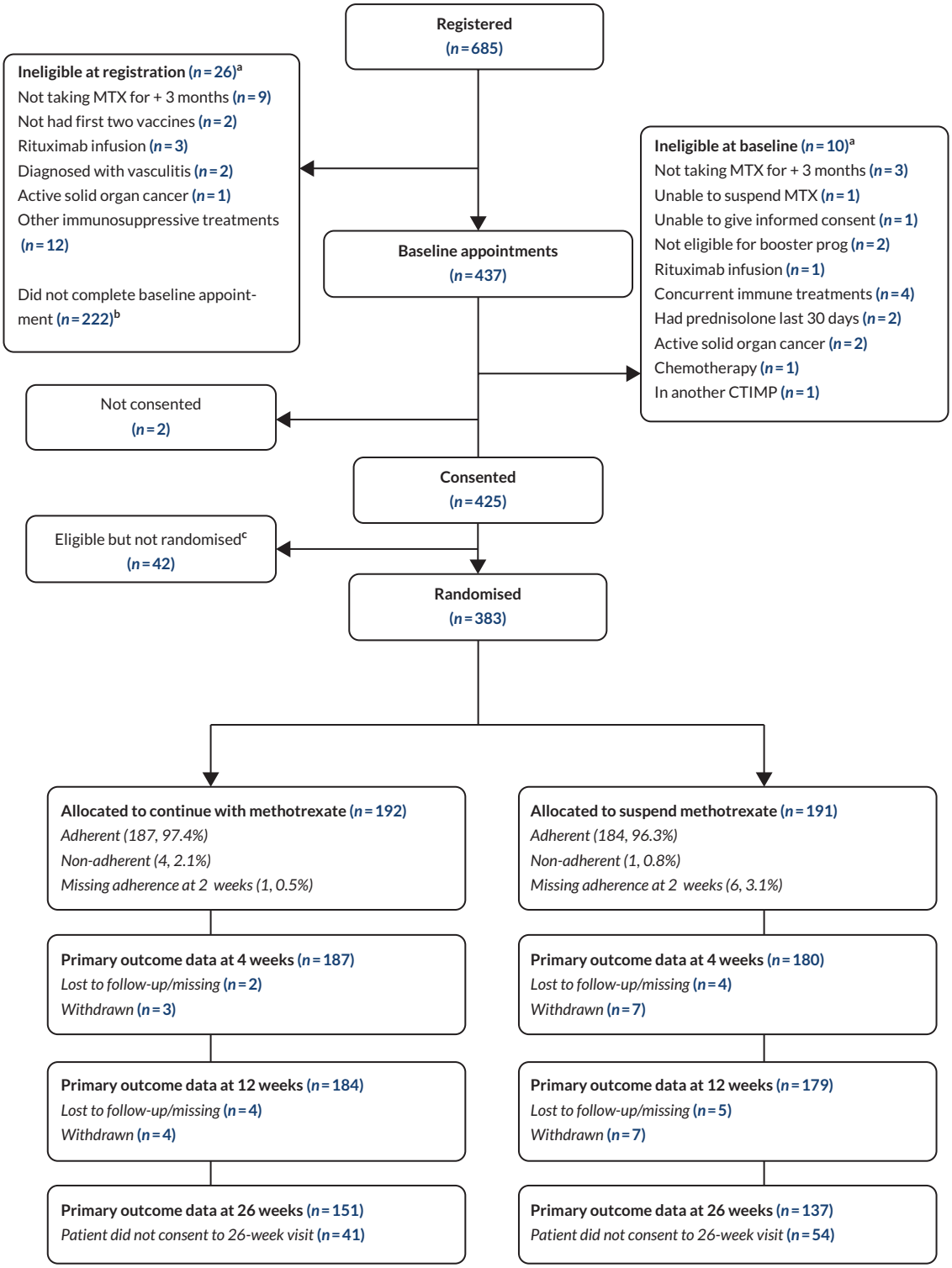

FIGURE 2 Consolidated Standards of Reporting Trials diagram. a, Each participant could have 1 or more than 1 reasons for being ineligible at registration. b, Due to recruitment being completed. These participants indicated willingness to enroll to the trial. c, Due to recruitment being completed. These participants completed their baseline visit but were not randomized.

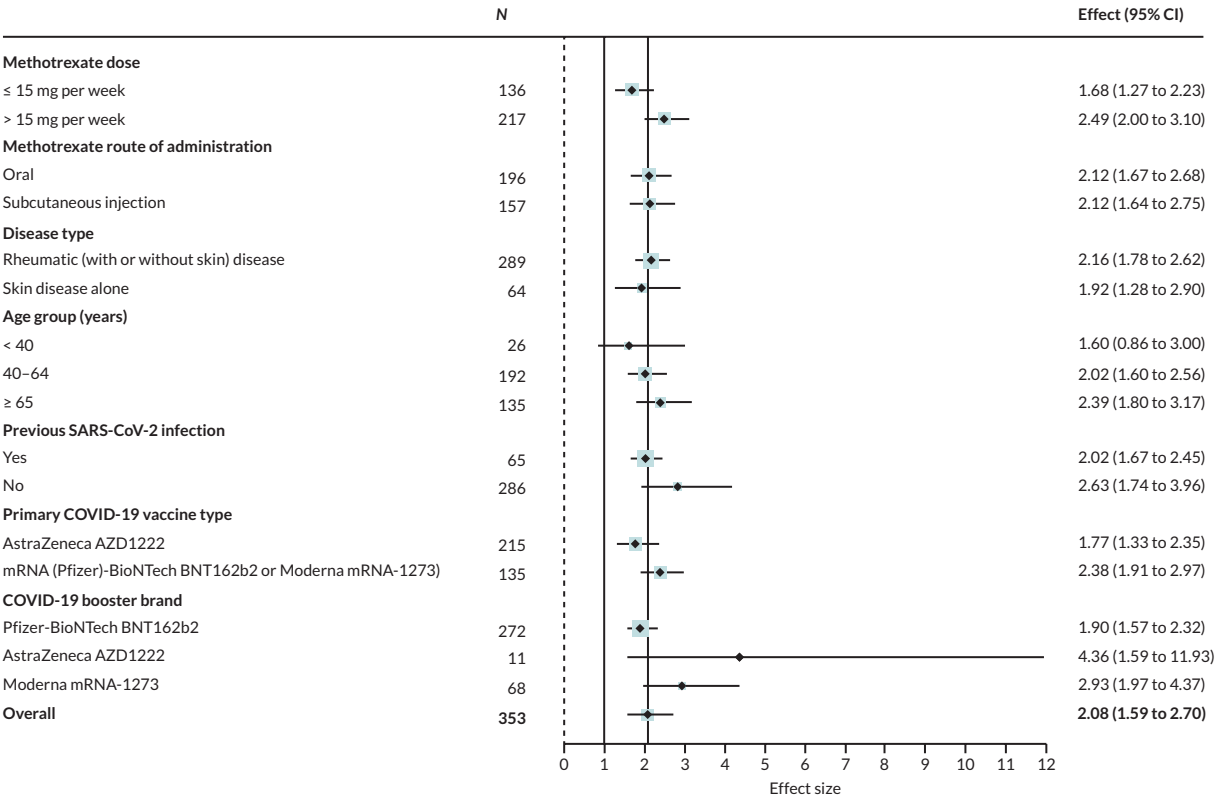

FIGURE 3 Subgroup analysis at week 4.

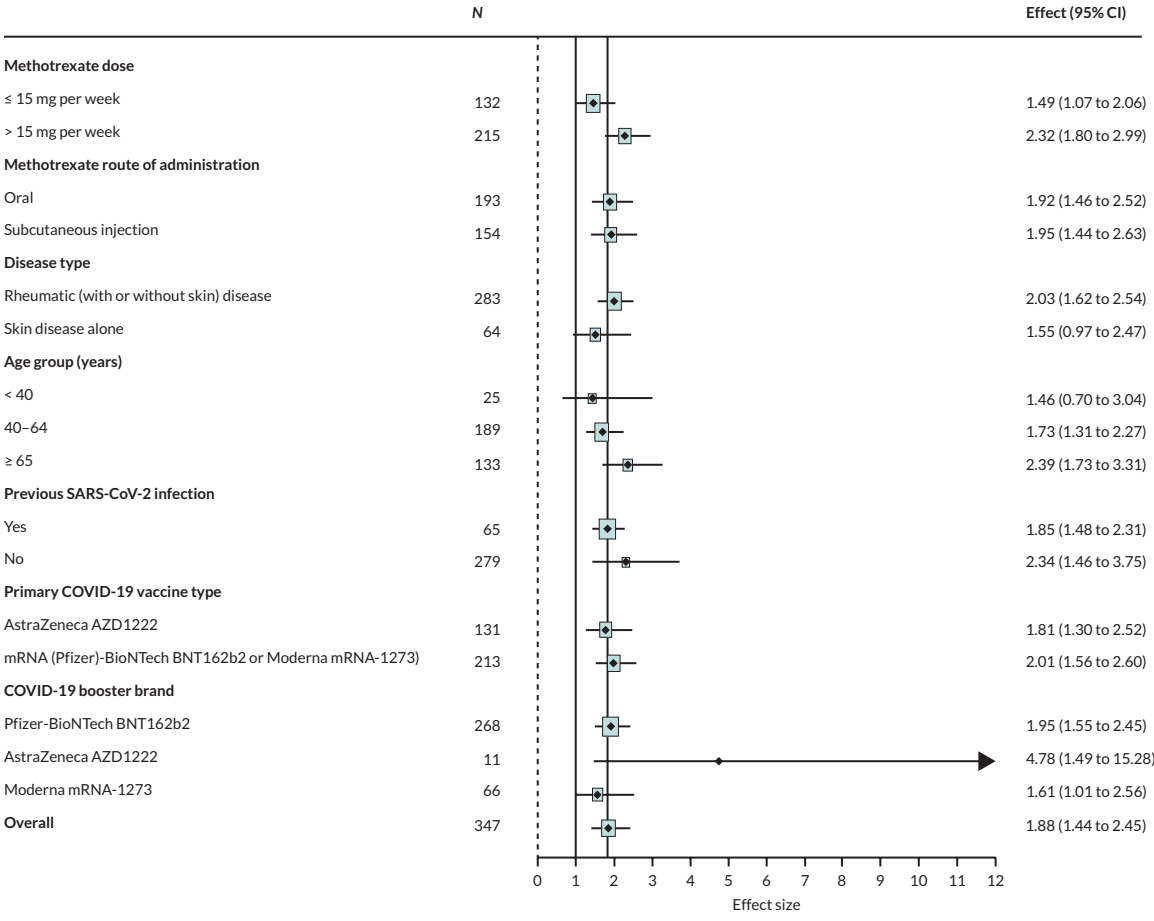

FIGURE 4 Subgroup analysis at week 12.

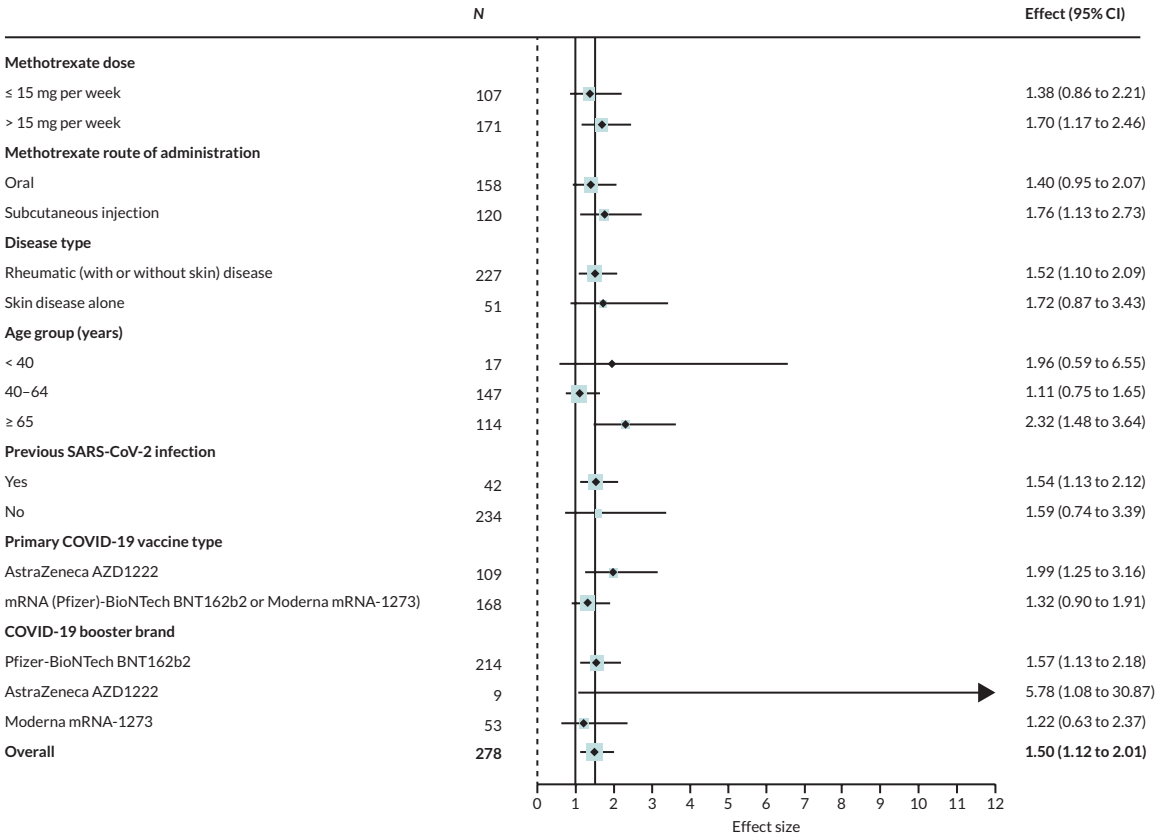

FIGURE 5 Subgroup analysis at week 26.

to clinicians and patients. Some hospitals declined to participate in the 26-week follow-up visit which was added into the study in March 2022 after the interim analysis, due to lack of capacity. This contributed to a substantially smaller number of participants at week 26 compared to weeks 4 and 12, and an increased attrition at week 26. Condition-specific inflammatory disease activity measures were not used as we recruited participants with a range of diseases, many without validated outcome measures to assess flare-ups. Another limitation was the absence of data for memory B-cell and T-cell responses at baseline, 4 and 12 weeks. A small number of PBMC samples were collected at 26 weeks. This small sample size was under-powered to detect differences between the suspend and the continue groups. In this small sample size, there was no observed difference between the suspend and continue groups at week 26 in T-cell responses against ancestral or variant SARS-CoV-2 spike. In the exploratory analysis (results not shown in this synopsis), there was no indication of a difference between the treatment groups at week 26 in B-cell memory frequency against wild type or BA1 variant spike. The exploratory analysis suggests that people with RA or psoriasis taking low-dose methotrexate made a T-cell and B-cell memory response against spike 26 weeks after their booster dose, but due to the extremely small sample size and lack of longitudinal sampling, no comment can be made about the impact of the intervention on the size, nature or longevity of the T-cell or memory B-cell responses. Additional laboratory experimental work and a sufficient sample size are required to address this.

We did not detect differences in the number of SARS-CoV-2 infections between the two groups and none of the participants experienced severe COVID-19 defined as either hospitalisation or death due to COVID-19. However, there was a numerically higher number of people with SARS-CoV-2 infection in the weeks between 13 and 26 in the suspend methotrexate group than in the continue methotrexate group. This study was not designed to detect a difference in clinical outcomes, and this finding should be interpreted with caution. We did not collect patient-reported data on COVID-19 symptom duration or severity and are unable to comment on whether patients with greater immunity also experienced milder symptoms.

Take-home message

In conclusion, we identified a sustained increase in binding S1-RBD antibody on interruption of methotrexate treatment for 2 weeks immediately after vaccination against COVID-19 with a short-term increase in risk of inflammatory disease flare-ups that were mostly self-managed. Patients and clinicians should consider

This synopsis should be referenced as follows:
Abhishek A, Peckham N, Pade C, Gibbons JM, Cureton L, Reynolds CJ, *et al.* Temporary 2-week suspension of methotrexate treatment to enhance COVID-19 vaccine response in people with immune-mediated inflammatory diseases: the VROOM RCT. [published online ahead of print April 30 2025]. *Efficacy Mech Eval* 2025. https://doi.org/10.3310/KYTK6537

suspending treatment with methotrexate for 2 weeks immediately after vaccination against COVID-19 to improve the immune response. The decision to suspend methotrexate should be individualised based on disease status and vulnerability to severe outcomes from COVID-19.

Reflections on the project

Due to recruitment being linked to the Autumn 2021 booster, it was important that the VROOM study was expedited in its set up. This involved out-of-hours working by the trials unit to get the trial open. Standard timeline trials also allow for new staff recruitment, this time was not available for VROOM, and so staff were seconded away from other trials to deliver VROOM.

Without the Health Research Authority (HRA) COVID-19 fast-track review and recruiting sites expediting their approvals – this would have negated the high recruitment to the study as we would have missed the peak of vaccination and recruitment. From funding confirmation, the first version of the protocol was circulated 3 days later and was through sponsor review and with the Research Ethics Committee (REC) 30 days later. REC and HRA approval came through within 18 days of submission.

Sites were opened to recruitment as quickly as possible following study approvals. Overall sites were very quick to open with 14/26 sites open within the first month and 23/26 sites open within 2 months. Sites were engaged with the study and recruitment each month was always on or above target. Participant retention was high with 95% (362/383) completing the week 12 follow-up (10 loss to follow-up and 11 withdrawn). However, completion of the additional follow-up visit at week 26 (added as an amendment during follow-up and implemented at participating sites between 8 May 2022 and 30 June 2022) was not as high at 75% (288/383). Retention at week 26 was a challenge because 2 of the 26 sites opted out of the additional visit and the visit was additional to what participants had originally consented to. It is likely we would have seen higher week 26 completion rates if the follow-up visit had been part of the study from the beginning.

The main way of recruiting – potential participants being able to register themselves online as eligible – needed new programming abilities to be created and added to our existing REDCap underlying systems. This meant expanding the functionality of our systems at pace. This was delivered by the programming team and allowed for recruitment in this way to be possible.

A substantial limitation of the study was that the T- and B-cell laboratory studies were not funded as requested in the original funding application. Had this been funded, a detailed and robust analysis of the impact of the drug pausing on the strength and nature of T-cell and memory B-cell responses against ancestral spike (contained in the COVID-19 vaccine) and subsequent VOCs would have been carried out. This would have included exploring immune responses against those VOCs currently dominating globally. Understanding how pausing methotrexate for 2 weeks impacted on T-cell and memory B-cell responses following vaccination would have allowed a much deeper and meaningful understanding of the impact of this intervention on these fundamentally important correlates of protection. Furthermore, it would have given an indication of the longevity of any impacts over time. It is well documented, for example, that T-cell and B-cell memory can be sustained for many years/decades. Now that the majority of the population has been vaccinated, a deeper understanding of and use of these important correlates of protection in future clinical studies looking at the impact of any intervention on COVID-19 vaccine responses in immunosuppressed population will be essential. During the study, funding was requested to be used for collection of PBMC at an additional time point. As this was received part-way though follow-up, many participants had already attended this study visit, some sites were not agreeable to asking participants to consider this additional time point and quite a few individuals did not want to contribute data or sample which led to only 28 participants donating an additional sample at week 26. PPI engagement at Trial Steering Committee (TSC) meetings was also a challenge. The study had two PPI representatives who were independent members of the TSC. However, due to the speed of the study and need to arrange TSC meetings at short notice, it was not possible for the PPI members to attend. PPI members were therefore sent minutes from the meetings. PPI input in other aspects of the study has occurred such as producing a patient education video and lay summary of results.

Individual training and capacity-strengthening activities

The research project allowed the Chief Investigator an opportunity to get funding from the NIHR EME programme and partner with OCTRU for the first time. OCTRU also built new database functionality to support rapid opening and speedy recruitment into the trial. The trial was open to the NIHR Associate Principal Investigator scheme. Five of the 24 recruiting sites nominated one Associate Principal Investigator each. They were trained on the trial procedures, obtained a GCP certificate and got the experience of working on the trial for at least 6 months

each. Therefore, this trial helped train the next generation of principal investigators (PIs).

Institutional capacity strengthening

The research project allowed new multidisciplinary collaborations to be developed between partnering institutions. It allowed triallists, statisticians, virologists, respiratory physicians, dermatologists and rheumatologists to come together to deliver an important study at speed with partnership from 24 NHS hospitals.

Engagement with partners and stakeholders

Stakeholders were identified early in the study and were contacted with the results of the interim analysis.[20] This allowed us to bring about health policy change with the Joint Committee on Vaccination and Immunisation (JCVI) recommending that patients on immune-suppressing treatments suspend their treatment for 2 weeks after vaccination against COVID-19. Similarly, the British Society of Rheumatology COVID-19 working group (www.rheumatology.org.uk/news/details/Boosting-COVID-19-vaccine-induced-immunity-in-methotrexate-patients, last accessed 15 August 2024) and the ACR COVID-19 working group changed their recommendations based on the results of the VROOM study.[21] We identified media as a strong mechanism for reaching the public and the study launch was publicised on BBC Radio-4, BBC Radio-2, BBC Radio Scotland and BBC Online, and the study interim results were publicised on the BBC (East Midlands TV), and several national and international newspapers including the *Independent*, *Financial Times*, *Express and Evening Standard* and *BMJ news*. Finally, the VROOM study allowed a multidisciplinary team of researchers from several institutions to work together, in that it allowed for the development of new collaborations across institutions and specialties.

Patient and public involvement

Patient and public involvement engagement

This study has been developed with PPI right from the start with patients based in Nottingham (lead applicant) and Oxford (PPI lead). Two PPI engagement meetings with eight people with lived experience of inflammatory conditions were held in March 2021.

Patients taking methotrexate for a variety of conditions (inflammatory arthritis, psoriasis, IBD) all felt that the study question was important to them and the study was 'definitely worth' conducting. Around half of the PPI volunteers temporarily held off their methotrexate before and/or after their primary COVID-19 vaccines after doing their own research – contrary to advice to continue with methotrexate treatment from British arthritis and skin disease experts.

All felt that most patients with inflammatory conditions would be keen to have the booster vaccine if offered and that hesitancy in getting vaccinated would not be a big risk for this study. PPI volunteers felt that they would stick to the study advice to continue or hold off methotrexate for 2 weeks if they were in the trial because the study question needed answering.

Results from the flu vaccine studies were presented and all PPI volunteers agreed that a 2-week pause in methotrexate offered the best balance of potential benefit without risking a disease flare that could happen with a longer 4-week treatment pause. Many had already experienced pausing methotrexate for 2 weeks from previous surgery or during an infection without their condition flaring up. There was mixed opinion on whether it would be easier to hold or to continue folic acid prescribed alongside methotrexate.

This study involved three extra visits to the hospital for blood tests around the booster vaccine dose. The PPI volunteers felt that this may be off-putting to some people but thought that most people would be happy to come for these visits with adequate travel expenses. One patient was concerned about the COVID-19 risk of attending the hospital but felt that the actual risk would be lower once most people in the country had been vaccinated and when transmission rates were low.

We discussed a finger prick blood collection kit (e.g. Mitra) that may be used by participants to collect a small amount of blood themselves. Although most were comfortable with the idea of using this kit, some were hesitant based on their own experience of diabetes monitoring and the high level of dexterity needed. The study team scientists also felt that samples from such devices may not give reliable information as a usual blood sample taken from veins, so we did not use the finger prick blood collection kit in the study.

Picking the best study outcomes was also discussed with our patient research partners. They supported the use of levels of proteins in the bloodstream produced in response to vaccination (antibodies) as the main outcome but also wanted us to include clinical outcomes to assess disease activity, flares and side effects of the vaccines. We had some good feedback on the questions to be used to assess these outcomes and the PPI volunteers supported the use

of a few questions covering all chronic illnesses, rather than using a different set of questions for each condition. Our PPI partners made some important decisions on the duration of the methotrexate pause, blood tests and choice of outcomes.

Dissemination

During the study, we continued working with our PPI co-applicant. We engaged with three new PPI members with personal lived experience of inflammatory conditions. The PPI volunteers reviewed the lay summary of the interim results, contributed to the lay summary of the final results, and to the content of video dissemination material that summarised the final results. One of our research participants took part in media engagement and featured on BBC Radio Nottingham and was quoted on BBC Online news when the results of the interim study were published. The plain language summary included in this report was also co-developed with input from PPI volunteers.

Once the trial was funded, it was publicised to the research and clinical communities to get sites onboard. Next, the trial launch was publicised to the patient communities and other stakeholders, such as self-help groups via popular media including print, radio and online. The interim results were presented by AA, RB and JC at a meeting set up by the Science Media Centre attracting much interest and coverage in the lay press. The results were published in *Lancet Respiratory Medicine* and again publicised to the clinical communities and patients through popular media and via lay summary made available on websites of patient charities such as the National Rheumatoid Arthritis Society. The manuscript reporting the interim results was published promptly in *Lancet Respiratory Medicine* and has, at the time of writing, an Altmetric attention score of 454 with 46 citations, putting it in the top 5% of all research outputs scored, with a high attention score compared to other outputs of the same age (99th percentile).

Equality, diversity and inclusion

The trial was set up to be inclusive (all patients who met the selection criteria at participating sites were candidates). Twenty-six sites from across England and Wales were open to recruitment, providing high geographic coverage of the UK ensuring a representative sample in the study across all demographic groups. Engagement from PPI was sought throughout all stages of the trial, and the study PPI advisory group was drawn from a mix of different ages, gender, ethnic and socioeconomic backgrounds, with a mix of inflammatory conditions. Oxford and Nottingham NIHR-BRC, and Versus Arthritis were used to draw PPI representatives from, with appropriate training offered to reduce barriers to entry. In addition to study participants, we included a representative sample of clinicians and trial personnel at recruiting sites.

The study was designed to have a low barrier to entry, with prospective participants able to express interest in the study both remotely (scanning QR codes, via web browser, phone call, post) and in-person via the study team. It was difficult to recruit patients who do not speak English at all into a trial due to the paucity of suitably translated outcome measure instruments. We considered this with our PPI team and chose to translate the patient information sheet (PIS) to the most-commonly spoken non-English languages in the UK, which were Polish, Urdu and Punjabi as per the Office for National Statistics 2011 census. During the total recruitment time period in the study (2021–March 2022), there were 696 PIS requested/downloaded in total. Of these, 67 (10%) were in Polish, 60 (9%) were in Urdu, 48 (8%) were in Punjabi and the rest 521 (75%) were in English. In addition, as a study carried out through the NHS, usual hospital and translator services were available.

Additionally, follow-up was designed to provide patients with as little disruption as possible with aid from PPI representatives, despite additional hospital visits required for the study.

Overall sample representativeness was reported for key demographic factors (sex, age, ethnicity, residential status, recruiting site, inflammatory conditions and key comorbidities). Outcome data were further disaggregated for key demographic factors (sex and age) in a subgroup analysis viewed as exploratory, using a treatment by subgroup interaction.

The recruited population was representative of the sex of patients with inflammatory conditions with more women recruited. There were fewer non-white participants than the UK population (6.3% vs. 18.3%) as per the Office for National Statistics population census 2021. This could potentially be related to greater vaccine hesitancy in the non-white population.

The trial population was less deprived than the general population in England, with a median [interquartile range (IQR)] Index of Multiple Deprivation of 4 (7–9) in the 352 trial participants recruited from England (*Table 6*). There were 31 patients recruited in Wales for whom these data

TABLE 6 Index of multiple deprivation scores for VROOM study participants

	Across study at baseline by decile
	N = 383, (%)
1 (Most deprived)	23 (6.0%)
2	34 (8.9%)
3	22 (5.7%)
4	26 (6.8%)
5	27 (7.0%)
6	26 (6.8%)
7	52 (13.6%)
8	50 (13.1%)
9	45 (11.7%)
10 (Least deprived)	47 (12.3%)
Welsh postcodes	31 (8.1%)
Median (IQR)	7 (4–9)
Mean (standard deviation)	6.2 (2.8)

Note
Only from patients in England by decile.

were not available. The diversity of participants is reflected in its multicentre nature (see *Appendix 4*, *Table 8*).

Impact and learning

The study has already had impact on health policy. We engaged with stakeholders and contacted them with the results of the interim analysis. This allowed us to change health policy with the JCVI recommending that patients on immune-suppressing treatments hold their treatment for 2 weeks after vaccination against COVID-19. Similarly, the British Society of Rheumatology COVID-19 working group (www.rheumatology.org.uk/news/details/Boosting-COVID-19-vaccine-induced-immunity-in-methotrexate-patients, last accessed 15 August 2024) changed their recommendations based on the results of the VROOM study.

Implications for decision-makers

The JCVI should consider the full study results and reiterate and/or firm up their initial recommendations that were based on interim analysis data. Health policy-makers should consider how the intervention will be implemented in the NHS.

Research recommendations

Future research should evaluate how interrupting other immune-suppressing treatments soon after vaccination against COVID-19 would change the immune response in terms of antibody, T-cell and memory B-cell responses, all of whom are key correlates of protection. Further research in the field should also evaluate whether a shorter hold in methotrexate, for example 1 week hold in treatment as opposed to a 2-week hold in treatment, would improve the immune response elicited by vaccination against COVID-19.

Conclusions

A 2-week interruption of methotrexate treatment immediately after COVID-19 booster vaccination enhanced the S1-RBD antibody response for patients with a range of IMIDs. The increased antibody response was maintained at 26 weeks. The effect was present across a range of prognostic factors including prior SARS-CoV-2 infection, an exclusion criterion in previous studies, thereby indicating wide applicability Subgroup analysis suggested a larger effect on S1-RBD antibody response from interrupting treatment in those on higher doses of methotrexate and the S1-RBD antibody level in the suspend methotrexate group at 26 weeks was numerically greater than that in the continue methotrexate group at 4 weeks. The neutralising capacity was numerically higher for the ancestral Wuhan Hu-1 strain at weeks 4 and 26 and for the Omicron BA.1 VOC at week 4. This suggests improved protection against COVID-19 including severe disease, though of an uncertain magnitude. SARS-CoV-2 infections were very low during the evaluation period and none of the participants experienced severe COVID-19 requiring hospitalisation or leading to death, therefore precluding any direct assessment in this study. This low level of severe impact likely reflects the health of the participants, the stage of the COVID-19 pandemic, the length of the follow-up and prior receipt of COVID-19 vaccination.

Offsetting the potential gain in improved immune response was a short-term deterioration (up to 12 weeks) in inflammatory control and self-reported flare-up. The majority of these flares were self-managed with no indication of an increased need for healthcare input. No

impact was detected from interrupting methotrexate for 2 weeks on QoL, general health or patient assessment of inflammatory disease.

In summary, there was a sustained increase in binding S1-RBD antibody on interruption of methotrexate treatment for 2 weeks immediately after vaccination against COVID-19 for patients with IMIDs. This is likely to indicate some benefit in immunity to infection. Interruption of methotrexate medication also led to a short-term increase in risk of inflammatory disease flare-ups which were typically self-managed. A 2-week break in methotrexate treatment immediately after vaccination provides a simple, low-cost, easy-to-implement and effective intervention. It should be considered for patients with stable IMID. However, there exists a trade-off of improved immune response versus increased risk of a flare-up in the short term following vaccination if medication is interrupted for 2 weeks. Furthermore, any benefit from an interruption in methotrexate medication for future vaccination is reliant upon the effectiveness of the vaccine(s) being used and should be considered in light of this. Individualised decision-making may be appropriate when deciding whether methotrexate medication is to be interrupted following vaccination.

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Acknowledgements

The coauthors would like to acknowledge the contribution of PPI volunteers in Oxford and Nottingham for their help in designing this study and members of OCTRU and the University of Nottingham sponsor office who enabled the rapid set-up of this study and have provided ongoing support. The design, development and execution of T-cell and memory B-cell laboratory work were funded solely by the DuRaCoV study (UKRI ref MR/W020610/1).

Data-sharing statement

The authors will make available relevant anonymised patient-level data to bona fide researchers upon reasonable request. Data requests should be directed to the corresponding author at abhishek.abhishek@nottingham.ac.uk.

Ethics statement

This study was approved on 20 August 2021 by Leeds West Research Ethics Committee and Health Research Authority (REC Reference: 21/YH/0209, HRA COVID-19 fast-track reference: 21/HRA/3483, IRAS: 303827). Written informed consent was obtained from participants before participating in the study.

Information governance statement

Under the Data Protection legislation University of Oxford is the Data Processor; University of Nottingham is the Data Controller and we process personal data in accordance with their instructions. You can find out more about how we handle personal data, including how to exercise your individual rights and the contact details for University of Nottingham's Data Protection Officer at www.nottingham.ac.uk/utilities/privacy.aspx.

Disclosure of interests

Full disclosure of interests: Completed ICMJE forms for all authors, including all related interests, are available in the toolkit on the NIHR Journals Library report publication page https://doi.org/10.3310/KYTK6537.

Primary conflicts of interest: The institutions of the authors received funding from the NIHR-MRC-EME programme (Award number NIHR134607) towards conducting this research. Abhishek Abhishek reports personal payments from UpToDate (royalty), Springer (royalty), Cadila Pharmaceuticals (lecture fees), NGM Bio (consulting), Limbic (consulting), and Inflazome (consulting) unrelated to the work and was on the HTA Prioritisation Committee B 2022–2026. Duncan Appelbe reports NIHR HTA grants to the University of Oxford. Daniel M Altmann has received honoraria for consultancy work with Novavax, Pfizer and AstraZeneca. James Bluett reports research grants from Pfizer and travel/conference fees from Fresenius Kabi, UCB, Pfizer and Eli Lilly. Laura C Coates is funded by a National Institute for Health Research Clinician Scientist award and has received grants/research support from AbbVie, Amgen, Celgene, Eli Lilly, Janssen, Novartis, Pfizer and UCB; worked as a paid consultant for AbbVie, Amgen, Bristol Myers Squibb, Celgene, Eli Lilly, Gilead, Galapagos, Janssen, Moonlake, Novartis, Pfizer and UCB; has been paid as a speaker for AbbVie, Amgen, Biogen, Celgene, Eli Lilly, Galapagos, Gilead, GSK, Janssen, Medac, Novartis, Pfizer and UCB in the past 36 months; and was on the HTA general committee 2022–2023. Ines Rombach reports NIHR and the Medical Research Council (MRC) partnership award (NIHR134607). Amanda Semper reports grant/contract from WHO (UKHSA; coinvestigator; SARS-CoV-2 serology), support for attending Wellcome Attendance at R&D Roadmap meetings. Jonathan S Nguyen-Van-Tam was seconded to the Department of Health and Social Care, England (DHSC) until 31st March 2022. Subsequent to that date, he has received one-off lecture fees from AstraZeneca and Sanofi Pasteur and performed consulting for Gilead all unrelated to the presented work. He began general paid consulting for Moderna in May 2023. Hywel C Williams worked for the National Institute of Health Research between 2015 and 2021 and has been a member of a number of NIHR Committees from 2008 to present. He played no part in the funding decision for this study. The views expressed in this manuscript are those of its authors and not necessarily those of the NHS, NIHR, DHSC or the JCVI.

Department of Health and Social Care disclaimer

This publication presents independent research commissioned by the National Institute for Health and Care Research (NIHR). The views and opinions expressed by authors in this publication are those of the authors and do not necessarily reflect those of the NHS, the NIHR, MRC, NIHR Coordinating Centre, the Efficacy and Mechanism Evaluation programme or the Department of Health and Social Care.

This synopsis was published based on current knowledge at the time and date of publication. NIHR is committed to being inclusive and will continually monitor best practice and guidance in relation to terminology and language to ensure that we remain relevant to our stakeholders.

Publications

Abhishek A, Boyton RJ, McKnight Á, Coates L, Bluett J, Barber VS, *et al.* Effects of temporarily suspending low-dose methotrexate treatment for 2 weeks after SARS-CoV-2 vaccine booster on vaccine response in immunosuppressed adults with inflammatory conditions: protocol for a multicentre randomised controlled trial and nested mechanistic substudy [Vaccine Response On/Off Methotrexate (VROOM) study]. *BMJ Open* 2022;**12**:e062599. https://doi.org/10.1136/bmjopen-2022-062599

Abhishek A, Boyton RJ, Peckham N, McKnight A, Coates LC, Bluett J, *et al.* Effect of a 2-week interruption in methotrexate treatment versus continued treatment on COVID-19 booster vaccine immunity in adults with inflammatory conditions (VROOM study): a randomised, open label, superiority trial. *Lancet Respir Med* 2022;**10**:840–50. https://doi.org/10.1016/s2213-2600(22)00186-2

Abhishek A, Peckham N, Pade C, Gibbons J, Cureton L, Francis A, *et al.* Two-week break in methotrexate treatment and COVID-19 vaccine response. Results of the Vaccine Response on off Methotrexate (VROOM) Study, an Open Label, Prospective, Two-arm Parallel-group, Multi-center, Superiority, Randomized Controlled Trial [abstract]. *Arthritis Rheumatol* 2023;**75**. URL: https://acrabstracts.org/abstract/two-week-break-in-methotrexate-treatment-and-covid-19-vaccine-response-results-of-the-vaccine-response-on-off-methotrexate-vroom-study-an-open-label-prospective-two-arm-parallel-group-multi-cen/ (accessed 1 November 2023).

Abhishek A, Peckham N, Pade C, Gibbons J, Cureton L, Francis A, *et al.* Two-week break in methotrexate treatment and COVID-19 vaccine response: a prospective, open label, two-arm parallel-group, multicentre, superiority, randomised controlled trial. *Lancet Rheumatol* 2024;**6**:e92–e104.

Study registration

Current Controlled Trials ISRCTN11442263.

Funding

This synopsis presents independent research funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation programme as award number NIHR134607.

This synopsis provided an overview of the research award Vaccine Response On/off Methotrexate (VROOM): does temporarily suspending methotrexate treatment for two weeks enhance COVID-19 vaccine response?- A Randomised Controlled Trial. For more information about this research, please view the award page (https://fundingawards.nihr.ac.uk/award/NIHR134607).

About this synopsis

The contractual start date for this research was in September 2021. This article began editorial review in December 2023 and was accepted for publication in August 2024. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The Efficacy and Mechanism Evaluation editors and publisher have tried to ensure the accuracy of the authors' article and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this article.

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List of abbreviations

ACR	American College of Rheumatology
BMI	body mass index
BMJ	*British Medical Journal*
DMEM	Dulbecco's Modified Eagle Medium
EQ-5D-5L	EuroQol-5 Dimensions, five-level version

FCS	fetal calf serum
FFU	focus forming unit
GMR	geometric mean ratio
HRA	Health Research Authority
IBD	inflammatory bowel disease
IC50	inhibitory concentration half-maximal
IMID	immune-mediated inflammatory disease
JCVI	Joint Committee on Vaccination and Immunisation
MTX	methotrexate
NSAIDs	non-steroidal anti-inflammatory drug
OCTRU	Oxford Clinical Trials Research Unit
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PI	principal investigators
PIS	patient information sheet
PPI	patient and public involvement
QoL	quality of life
RA	rheumatoid arthritis
REC	Research Ethics Committee
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
S1-RBD	spike protein of the receptor binding domain
TSC	Trial Steering Committee
UKHSA	United Kingdom Health Security Agency (prev. Public Health England)
VOC	variant of concern
VROOM	Vaccine Response On/Off Methotrexate

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This synopsis should be referenced as follows:
Abhishek A, Peckham N, Pade C, Gibbons JM, Cureton L, Reynolds CJ, *et al*. Temporary 2-week suspension of methotrexate treatment to enhance COVID-19 vaccine response in people with immune-mediated inflammatory diseases: the VROOM RCT. [published online ahead of print April 30 2025]. *Efficacy Mech Eval* 2025. https://doi.org/10.3310/KYTK6537

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Appendix 1 Wuhan Hu-1 SARS-CoV-2 and B.1.1.529 variants micro-neutralisation assay

Virus isolates SARS-CoV-2 strain 2019-nCoV/BavPat1/2020 (Wuhan Hu-1) and Omicron variant (B.1.1.529) authentic virus cell culture supernatants were purchased from the European Virus Archive Global.

Preparation of viral stocks: VeroE6 were seeded in 75 cm^2 cell culture flasks 24 hours before inoculation with virus cell culture supernatant containing 2.2 × 106 plaque-forming unit in a volume of 10 ml Dulbecco's Modified Eagle Medium (DMEM) 10% fetal bovine serum. Flasks were observed daily, and virus-containing cell culture medium was harvested when > 80% of cells showed cytopathic effect. Supernatant was centrifuged at 500 × g for 5 minutes to clear cell debris and aliquots stored at −80°C.

Titration of viral stocks to determine the titre of SARS-CoV-2 virus stocks, VeroE6 cells were seeded at 3 × 104 cells per well in 48-well plates. After 24 hours, adherent cell monolayers were challenged with serial 1 in 10 duplicate dilutions of virus and titre was assessed after 20 hours by in situ intracellular staining to identify foci of infection. Cells were washed in phosphate-buffered saline (PBS), fixed in ice-cold methanol:acetone (50 : 50) and virus antigen was stained using sera from convalescent individuals diluted 1 in 2000 in PBS 1% fetal calf serum (FCS) for 1 hour at 37°C. Cells were washed a further three times in PBS and incubated with goat anti-human immunoglobulin G β-galactosidase-conjugated antibody (#2040-06, Southern Biotech) diluted 1 in 400 in PBS 1% FCS for 1 hour at 37°C. After three further PBS washes, 300 µl of 0.5 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside chromogenic substrate (X-gal) in PBS containing 3 mM potassium ferricyanide, 3 mM potassium ferrocyanide and 1 mM magnesium chloride was added to each well. Infected cells incubated at 37°C stained blue within 1 and 4 hours after addition of substrate and clusters of blue cells were counted as foci of infection to determine the virus titre defined as focus forming units (FFU) per ml.

Virus variant stocks were confirmed by sequencing.

Table of spike mutations in Wuhan Hu-1 and B.1.1.529 viral isolates used in this study.

Lineage	Spike mutations (mutations in RBD)
Wuhan Hu-1	*S247R*
B.1.1.529	*A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211I, L212I, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F*

Virus neutralisation assays[22]:

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VeroE6 cells were seeded at 1.75 × 10^4 cells per well in a clear, flat-bottom 96-well tissue culture plate 24 hours before infection. Participant serum was heat-inactivated for 30 minutes at 56°C to remove complement activity. Serum dilutions in DMEM were performed in duplicate in clear u-bottom 96-well plates with a starting dilution of 1 in 20 and consecutive twofold dilutions in a total volume of 50 µl per well. 3 × 10^4 FFU of SARS-CoV-2 virus (TCID100) were added to each serum dilution and incubated at 37°C for 1 hour. After incubation, serum/virus preparations were transferred into cell culture plates containing semi-confluent VeroE6 monolayers. Each plate had eight control wells with virus and cells only (virus control) and another eight wells with cells only (background only). Plates were incubated (37°C and 5% CO_2) for 72 hours, after which supernatants were removed and wells washed with PBS. Cells were fixed with 100 µl 3.7% (vol/vol) formaldehyde for 1 hour. After two further PBS washes, cells were stained with 0.1% (wt/vol) crystal violet solution for 10 minutes. Plates were washed four times in distilled water to remove excess crystal violet and left to air dry. Crystal violet stain was re-solubilised by addition of 100 µl 1% (wt/vol) sodium dodecyl sulphate solution to each well and incubated at 37°C for 10 minutes. Absorbance readings were taken at 570 nm using a CLARIOStar Plate Reader (BMG Labtech). Negative controls of pooled pre-pandemic sera, collected prior to 2008, and serum from a neutralisation-positive SARS-CoV-2 convalescent individual were spaced throughout the plates. Absorbance readings for each well were standardised against technical positive (virus control) and negative (cells only) controls on each plate to determine a percentage neutralisation value. An average neutralisation was calculated across the two sample replicates for each serum dilution. Neutralisation curves for each serum tested were plotted, with the percentage neutralisation modelled as a logistic function of the serum dilution factor (log10). A non-linear regression (curve fit) method was used to determine the dilution fold that neutralised 50% (IC50). We classified positive samples as those with an IC50 > 49. SARS-CoV-2 is classified as a hazard group 3 pathogen and therefore all authentic SARS-CoV-2 propagation and micro-neutralisation assays were performed in a containment level 3 facility.

Appendix 2 Methotrexate bioassay

The liquid chromatography-tandem mass spectrometry methotrexate assay was performed in the clinical biochemistry department, Wythen-shawe Hospital.

Sample preparation: A 7 ml whole blood sample was collected in two 3.5 ml serum stabilisation tubes at baseline, 4 and 12 weeks' post booster vaccination and posted to The University of Nottingham after each visit using Royal Mail next-day delivery safe boxes. On receipt of samples, they were centrifuged and the serum aliquoted and stored in −80°C freezers. One hundred samples of patients prescribed oral methotrexate were batch-transferred on dry ice to Manchester NHS Foundation Trust for measurement of methotrexate levels and biochemical adherence. Details of the methodology are published in McTaggart *et al.*[23]

Adherence measurement: methotrexate (MTX) adherence cut-offs developed from a population PK model previously published were used to determine biochemical adherence.[24] Using real-world self-reported samples, the adherence cut-offs have a sensitivity of 95%. Biochemical adherence was dichotomised.

TABLE 7 Oral MTX dose (mg/week) and MTX adherence limit (nM) with > 80% proportion of subjects who are adherent according to the 1000 hypothetical subjects ingesting MTX 168 hours prior to blood sampling

MTX dose (mg/week)	Adherence limit (nM)
5	0.1
7.5	0.15
10	0.2
12.5	0.25
15	0.25
17.5	0.25
20	0.25
22.5	0.5
25	0.5

Adapted from Bluett *et al.*[24]

Appendix 3 Peripheral blood mononuclear cell assay methodology

Memory B-cell assay: Prior to B-cell enzyme-linked immunosorbent spot (ELISpot) assays, PBMCs were cultured for 5 days in medium containing TLR7/8 agonist R848 plus recombinant human IL-2. After 4-day PBMC stimulation ELISpot PVDF plates were coated with PBS, purified anti-human IgG MT91/145, Wuhan Hu-1 or B.1.1.529 SARS-CoV-2 S1 spike proteins and incubated overnight. Plates were washed and blocked with FCS-supplemented RPMI1640. Pre-stimulated PBMCs were washed before seeding at 15,000–7500 cells/well for anti-human IgG coated wells and 150,000–15,000 cells/well for SARS-CoV-2 spike-coated wells and incubated for 20 hours. Assays were run in duplicate. For ELISpot development, plates were washed and incubated with biotinylated anti-human IgG MT78/145 followed by Streptavidin-ALP. ELISpot plates were developed and analysed by adding BCIP/NBT substrate and using an AID classic ELISpot plate reader. Spot numbers were adjusted for cell numbers seeded and the average of PBS-only coated wells subtracted from antigen-coated wells. Number of SARS-CoV-2 spike antigen-specific Ab secreting cells (ASC) was expressed as a per cent of the total number of IgG ASC.

T-cell ELISpot assay: PBMCs were isolated from heparinised blood samples. To stimulate PBMCs, megapools of spike peptides covering the whole sequence of spike were used. Assay PBMCs were cultured in supplemented RPMI medium. Pre-coated ELISpot plates were washed and blocked for 1 hour. Two hundred thousand PBMCs were used per well and stimulated for 20 hours with peptide megapools. Internal plate controls were R10 alone (without cells) and anti-CD3. Plates were developed using human biotinylated IFNγ detection Ab directly conjugated to alkaline phosphatase for 2 hours followed by sterile BCIP/NBT-plus phosphatase substrate. Plates were washed and read on an AID-ELISpot plate reader. The average of two R10 wells was subtracted from peptide-stimulated wells and responses that were < 2 standard deviation of the sample-specific control wells were not considered peptide-specific. Results were expressed as difference in (delta) spot forming cells/106 PBMC between the negative control and peptide stimulation. Results were excluded if negative control wells had > 100 SFU/106 PBMC or if positive control wells were negative.

Results were plotted using Prism v8.0 for Mac OS (GraphPad, GraphPad Software Inc., San Diego, CA, USA).

Appendix 4 Supplementary results

TABLE 8 List of investigators

Site	Name	Role[a]
Aneurin Bevan UHB	Dr Gwenan Huws	PI
Chesterfield Royal Hospital NHS Foundation Trust	Dr Stamatios Oikonomou	Co-investigator
Chesterfield Royal Hospital NHS Foundation Trust	Dr Rengi Mathew	PI
Gateshead Health NHS Foundation Trust	Dr Alaa Mustafa	Co-investigator
Gateshead Health NHS Foundation Trust	Dr Vadivelu Saravanan	PI
Great Western Hospitals NHS Foundation Trust	Dr Lindsay Whittam	Associate PI
Great Western Hospitals NHS Foundation Trust	Dr Elizabeth Price	PI
Harrogate and District NHS Foundation Trust	Prof Alison Layton	Co-investigator
Harrogate and District NHS Foundation Trust	Dr Gui Tran	PI
Imperial College Healthcare NHS Trust	Dr Taryn Youngstein	PI
Lancashire and South Cumbria NHS Foundation Trust	Dr Ayesha Madan	Associate PI
Lancashire and South Cumbria NHS Foundation Trust	Dr Sarah Horton	PI
Midlands Partnership Foundation Trust	Prof Samantha Hider	PI
Newcastle upon Tyne Hospitals NHS Foundation Trust	Dr Arthur Pratt	PI
Newcastle upon Tyne Hospitals NHS Foundation Trust	Prof Nick J Reynolds	Co-investigator
Norfolk and Norwich University Hospitals NHS Foundation Trust	Dr Agnieszka Lapin	Co-investigator
Norfolk and Norwich University Hospitals NHS Foundation Trust	Dr Sarah Bingham	Co-investigator
Norfolk and Norwich University Hospitals NHS Foundation Trust	Prof Karl Gaffney	PI
North Cumbria Integrated Care NHS Foundation Trust	Dr Alaa Hassan	PI
North West Anglia NHS Foundation Trust	Dr John Pradeep	PI
Nottingham University Hospitals NHS Trust	Dr Ting Seng Tang	Co-investigator
Nottingham University Hospitals NHS Trust	Dr Ira Pande	PI
Oxford University Hospitals NHS Foundation Trust	Dr Anushka Soni	PI
Royal Glamorgan Cwm Taf Morgannwg University Health Board	Dr Ceril Rhys-Dillon	PI
Royal Glamorgan Cwm Taf Morgannwg University Health Board	Dr Catrin Jones	Co-investigator
Sherwood Forest Hospitals NHS Foundation Trust	Dr Theresa Joseph	Co-investigator
Sherwood Forest Hospitals NHS Foundation Trust	Prof David Walsh	PI
The Dudley Group NHS Foundation Trust	Dr Gen Nen Ho	Co-investigator
The Dudley Group NHS Foundation Trust	Dr Karen Douglas	PI
The Dudley Group NHS Foundation Trust	Dr Kirsty Levasseur	Co-investigator
The Royal Wolverhampton NHS Trust	Dr Srinivasan Venkatachalam	PI

continued

TABLE 8 List of investigators (*continued*)

Site	Name	Role[a]
Torbay and South Devon NHS Foundation Trust	Dr Catherine Gwynne	PI
Torbay and South Devon NHS Foundation Trust	Dr Rory Crowder	Associate PI
University Hospital Southampton NHS Foundation Trust	Dr Chris Holroyd	PI
University Hospital Southampton NHS Foundation Trust	Dr May Lwin	Co-investigator
University Hospital Southampton NHS Foundation Trust	Dr Salema Khalid	Associate PI
University Hospitals Coventry and Warwickshire NHS Trust	Dr Nicola Gullick	PI
University Hospitals Sussex NHS Foundation Trust	Dr Cristina Tacu	PI
University Hospitals Sussex NHS Foundation Trust	Dr Thomas Batty	Associate PI
Wirral University Teaching Hospital NHS Foundation Trust	Dr Emmanuel George	PI
York and Scarborough Teaching Hospitals NHS Foundation Trust	Dr Mike Green	PI
York and Scarborough Teaching Hospitals NHS Foundation Trust	Dr Laura Hunt	Co-investigator
York and Scarborough Teaching Hospitals NHS Foundation Trust	Dr Nicola Alcorn	Co-investigator
York and Scarborough Teaching Hospitals NHS Foundation Trust	Dr Rob Ellis	Co-investigator

a Role in the study could be principal investigator (PI), co-investigator, or associate PI.

Note
From 24 hospitals that randomised at least one participant. The study was stopped early. Consequently, two additional hospitals where the trial was open for recruitment did not recruit any participants.

TABLE 9 Summary of reasons for withdrawal from follow-up before 12 weeks by study arms

Reason for withdrawal	Continue methotrexate *N* = 192	Suspend methotrexate *N* = 191	Total *N* = 383
Participant taken off methotrexate by dermatologist and participant chose to withdraw from study	1	0	1
Patient had vaccines prior to enrolment	0	1	1
Personal reasons	0	1	1
Participant felt too ill to continue	2	0	2
Unable to contact patient	0	3	3
No reason given	1	2	3

Note
Of the 11 participants that withdrew from the trial by week 12, 10 participants withdrew before their 4-week visit, with 1 participant in the continue methotrexate arm withdrawing after week 4. One participant in the suspend methotrexate arm initially reported being too ill to continue with the trial but was then not contactable and is recorded as not being contactable.

TABLE 10 Self-reported adherence with intervention by study arms

	Continue methotrexate *N* = 192	Suspend methotrexate *N* = 191
	n (%)	*n* (%)
Compliant[a]	187 (97.4)	184[b] (96.3)
Non-compliant[c]	4 (2.1)	1 (0.5)
Missing text-message data[d]	1 (0.5)	6 (3.1)
Methotrexate doses taken		
0 doses	0	184
1 dose	4	1
2 doses	187	0
Missing data[d]	1	6

a Participants in continue with methotrexate arm should have taken two doses of methotrexate and are deemed compliant if they self-report to have taken two doses.
b Three participants in the methotrexate suspend arm did not take their methotrexate dose prior to their vaccination and therefore missed three weekly doses of methotrexate. One further participant in the methotrexate suspend arm did not take their methotrexate dose for 5 weeks post COVID-19 vaccination, and then restarted it.
c Participants in the suspend methotrexate arm should have taken zero doses of methotrexate and are deemed compliant if they self-report to have taken zero doses.
d Seven participants withdrew before replying to text messages on adherence.

TABLE 11 Adherence with methotrexate treatment by study arms using bioassay for a sample (*N* = 102) of participants taking oral methotrexate

Reason for withdrawal	Continue methotrexate *N* = 51	Suspend methotrexate *N* = 51	Total *N* = 102
Baseline			
Adherent	48 (94.1)	48 (94.1)	96 (94.1)
Non-adherent	3 (5.9)	3 (5.9)	6 (5.9)
4 weeks			
Adherent	46 (90.2)	42 (82.4)	88 (86.3)
Non-adherent	3 (5.9)	9 (17.6)	12 (11.8)
Missing	2 (3.9)	0 (0.0)	2 (2.0)
12 weeks			
Adherent	47 (92.2)	45 (88.2)	93 (91.2)
Non-adherent	3 (5.9)	6 (11.8)	9 (8.8)
Missing	1 (2.0)	0 (0.0)	1 (1.0)

Note
Patients treated with subcutaneous methotrexate were excluded as the bioassay is not validated for assessing adherence to methotrexate treatment by this route. For details, see *Appendix 2*, *Table 7*.

TABLE 12 Sensitivity analyses for primary outcome [anti-S1-RBD titre (U/mL)] at 4, 12 and 26 weeks

	Continue methotrexate (*n*)	Suspend methotrexate (*n*)	Model: GMR (95% CI)[a]
Analysis of covariance model [adjusted by baseline value, stratification factors (age, inflammatory condition, vaccine platform)]			
4 weeks	187	180	2.12 (1.78 to 2.51)
12 weeks	184	179	1.93 (1.58 to 2.36)
26 weeks	151	137	1.55 (1.16 to 2.07)
Per-protocol population (analysis of covariance model adjustments as above)			
4 weeks	178	175	2.02 (1.76 to 2.32)
12 weeks	173	174	1.92 (1.64 to 2.25)
26 weeks	145	133	1.64 (1.31 to 2.05)
Model including time (days) between original vaccination and booster (post hoc)[b]			
4 weeks	187	180	1.32 (0.62 to 2.77)
12 weeks	184	179	1.19 (0.50 to 2.82)
26 weeks	151	137	1.45 (0.41 to 5.14)
Interaction term (1 day extra between previous vaccine dose and booster)			1.00 (0.99 to 1.00)
Model including methotrexate dose at baseline as covariate (post hoc)[b]			
4 weeks	187	180	2.01 (1.76 to 2.29)
12 weeks	184	179	1.92 (1.65 to 2.23)
26 weeks	151	137	1.62 (1.30 to 2.01)
Model excluding participants with an additional COVID vaccine between 12 and 26 weeks (post hoc)[b]			
26 weeks	117	97	1.56 (1.10 to 2.22)

a Linear regression model, adjusted by baseline value, stratification factors (age, inflammatory condition, vaccine platform).
b Analyses not pre-specified in the SAP are labelled 'post hoc'.

TABLE 13 Subgroup analyses for primary outcome [anti-S1-RBD titre (U/mL)] at 4, 12 and 26 weeks

	Continue methotrexate: GM (95% CI)	Suspend methotrexate: GM (95% CI)	Total: GM (95% CI)	*N*	Linear regression: GMR (95% CI)[a]
Subgroup analyses at 4 weeks					
Methotrexate dose					
≤ 15 mg/week	15,882 (12,433 to 20,288)	24,544 (19,649 to 30,658)	19,368 (16,350 to 22,943)	136	1.68 (1.27 to 2.23)
> 15 mg/week	9731 (7896 to 11,992)	26,010 (21,797 to 31,037)	16,237 (13,973 to 18,867)	217	2.49 (2.00 to 3.10)
Interaction effect (> 15 mg/week vs. ≤ 15 mg/week)					1.48 (1.04 to 2.12)

TABLE 13 Subgroup analyses for primary outcome [anti-S1-RBD titre (U/mL)] at 4, 12 and 26 weeks (*continued*)

	Continue methotrexate: GM (95% CI)	Suspend methotrexate: GM (95% CI)	Total: GM (95% CI)	*N*	Linear regression: GMR (95% CI)[a]
Methotrexate route of administration					
Oral	13,252 (10,668 to 16,462)	28,156 (23,275 to 34,061)	19,169 (16,449 to 22,338)	196	2.12 (1.67 to 2.68)
Subcutaneous injection	10,424 (8169 to 13,301)	22,569 (18,495 to 27,540)	15,376 (13,011 to 18,170)	157	2.12 (1.64 to 2.75)
Interaction effect (injection vs. oral)					1.00 (0.71 to 1.42)
Disease type					
Rheumatic (±skin) disease	11,546 (9636 to 13,835)	24,786 (21,240 to 28,924)	16,783 (14,786 to 19,048)	289	2.16 (1.78 to 2.62)
Skin disease alone	13,926 (9638 to 20,121)	28,575 (20,881 to 39,104)	20,282 (15,793 to 26,048)	64	1.92 (1.28 to 2.90)
Interaction effect (skin disease alone) vs. rheumatic (±skin) disease					0.90 (0.57 to 1.41)
Age group					
< 40 years	19,727 (13,115 to 29,673)	26,088 (16,469 to 41,325)	22,686 (16,965 to 30,335)	26	1.60 (0.86 to 3.00)
40–64 years	10,454 (8409 to 12,997)	21,752 (18,085 to 26,164)	14,852 (12,760 to 17,286)	192	2.02 (1.60 to 2.56)
≥ 65 years	13,215 (10,009 to 17,447)	31,233 (24,865 to 39,231)	20,642 (17,055 to 24,983)	135	2.39 (1.80 to 3.17)
Interaction effect < 40 vs. ≥ 65 years					1.49 (0.75 to 2.960)
Interaction effect 40–64 vs. ≥ 65 years					1.18 (0.82 to 1.71)
Previous SARS-CoV-2 infection					
No	10,737 (9000 to 12,809)	22,383 (19,309 to 25,945)	15,463 (13,683 to 17,473)	286	2.02 (1.67 to 2.45)
Yes	18,528 (12,572 to 27,306)	44,472 (32,558 to 60,745)	28,512 (21,846 to 37,213)	65	2.63 (1.74 to 3.96)
Interaction effect (infection vs. no)					1.30 (0.83 to 2.05)
Primary COVID-19 vaccine type					
AstraZeneca AZD1222	16,302 (12,562 to 21,157)	29,122 (22,982 to 36,903)	21,789 (18,186 to 26,106)	215	1.77 (1.33 to 2.35)
mRNA (Pfizer-BioNTech BNT162b2 or Moderna mRNA-1273)	9799 (8009 to 11,989)	23,785 (20,124 to 28,113)	15,142 (13,120 to 17,476)	135	2.38 (1.91 to 2.97)
Interaction effect [mRNA (BNT162b2 or mRNA-1273) vs. AZD1222]					1.35 (0.94 to 1.94)

continued

TABLE 13 Subgroup analyses for primary outcome [anti-S1-RBD titre (U/mL)] at 4, 12 and 26 weeks (*continued*)

	Continue methotrexate: GM (95% CI)	Suspend methotrexate: GM (95% CI)	Total: GM (95% CI)	*N*	Linear regression: GMR (95% CI)[a]
COVID-19 booster brand					
Pfizer-BioNTech BNT162b2	11,692 (9727 to 14,054)	23,575 (20,283 to 27,402)	16,560 (14,607 to 18,774)	272	1.90 (1.57 to 2.32)
AstraZeneca AZD1222	9097 (3303 to 25,057)	32,074 (4089 to 251,566)	14,385 (6106 to 33,888)	11	4.36 (1.59 to 11.93)
Moderna mRNA-1273	13,673 (9263 to 20,184)	35,242 (24,956 to 49,768)	21,952 (16,617 to 28,998)	68	2.93 (1.97 to 4.37)
Interaction effect (AZD1222 vs. BNT162b2)					2.29 (0.82 to 6.38)
Interaction effect (mRNA-1273 vs. BNT162b2)					1.54 (0.99 to 2.40)
Subgroup analyses at 12 weeks					
Methotrexate dose					
≤ 15 mg/week	11,738 (8769 to 15,711)	16,701 (13,503 to 20,656)	13,815 (11,479 to 16,627)	132	1.49 (1.07 to 2.06)
> 15 mg/week	7132 (5594 to 9093)	17,452 (14,388 to 21,169)	11,415 (9690 to 13,446)	215	2.32 (1.80 to 2.99)
Interaction effect (> 15 mg/week vs. ≤ 15 mg/week)					1.56 (1.03 to 2.37)
Methotrexate route of administration					
Oral	9280 (7291 to 11,812)	17,924 (14,542 to 22,093)	12,831 (10,876 to 15,138)	193	1.92 (1.46 to 2.52)
Subcutaneous injection	8103 (5974 to 10,990)	16,336 (13,375 to 19,953)	11,610 (9631 to 13,997)	154	1.95 (1.44 to 2.63)
Interaction effect (injection vs. oral)					1.02 (0.68 to 1.52)
Disease type					
Rheumatic (±skin) disease	8160 (6602 to 10,085)	16,472 (13,945 to 19,456)	11,564 (10,050 to 13,307)	283	2.03 (1.62 to 2.54)
Skin disease alone	12,048 (8005 to 18,133)	20,461 (15,361 to 27,253)	15,894 (12,403 to 20,366)	64	1.55 (0.97 to 2.47)
Interaction effect (skin disease alone) vs. rheumatic (±skin) disease					0.77 (0.45 to 1.29)
Age group					
< 40 years	15,364 (8981 to 26,284)	18,002 (11,300 to 28,678)	16,684 (12,021 to 23,156)	25	1.46 (0.70 to 3.04)
40–64 years	8017 (6254 to 10,276)	13,932 (11,422 to 16,995)	10,492 (8910 to 12,354)	189	1.73 (1.31 to 2.27)
≥ 65 years	8990 (6434 to 12,561)	22,534 (17,929 to 28,320)	14,481 (11,709 to 17,909)	133	2.39 (1.73 to 3.31)
Interaction effect < 40 vs. ≥ 65 years					1.64 (0.73 to 3.65)
Interaction effect 40–64 vs. ≥ 65 years					1.39 (0.91 to 2.12)

TABLE 13 Subgroup analyses for primary outcome [anti-S1-RBD titre (U/mL)] at 4, 12 and 26 weeks (*continued*)

	Continue methotrexate: GM (95% CI)	Suspend methotrexate: GM (95% CI)	Total: GM (95% CI)	*N*	Linear regression: GMR (95% CI)[a]
Previous SARS-CoV-2 infection					
No	7976 (6427 to 9898)	15,730 (13,522 to 18,298)	11,215 (9780 to 12,859)	279	1.85 (1.48 to 2.31)
Yes	12,844 (8804 to 18,738)	27,165 (18,584 to 39,710)	18,572 (14,083 to 24,491)	65	2.34 (1.46 to 3.75)
Interaction effect (Infection yes vs. no)					1.27 (0.75 to 2.14)
Primary COVID-19 vaccine type					
AstraZeneca AZD1222	11,145 (7993 to 15,540)	21,668 (16,995 to 27,627)	15,700 (12,734 to 19,357)	131	1.81 (1.30 to 2.52)
mRNA (Pfizer-BioNTech BNT162b2 or Moderna mRNA-1273)	7603 (6046 to 9560)	15,074 (12,686 to 17,913)	10,637 (9153 to 12,362)	213	2.06 (1.58 to 2.70)
Interaction effect [mRNA (BNT162b2 or mRNA-1273) vs. AZD1222]					1.11 (0.73 to 1.68)
COVID-19 booster brand					
Pfizer-BioNTech BNT162b2	8187 (6674 to 10,043)	16,714 (14,237 to 19,622)	11,667 (10,181 to 13,370)	268	1.95 (1.55 to 2.45)
AstraZeneca AZD1222	4876 (1620 to 14,682)	21,011 (2556 to 172,732)	8294 (3269 to 21,046)	11	4.78 (1.49 to 15.28)
Moderna mRNA-1273	13,337 (7904 to 22,506)	19,938 (13,923 to 28,552)	16,507 (12,153 to 22,421)	66	1.61 (1.01 to 2.56)
Interaction effect (AZD1222 vs. BNT162b2)					2.45 (0.75 to 8.02)
Interaction effect (mRNA-1273 vs. BNT162b2)					0.82 (0.49 to 1.38)
Subgroup analyses at 26 weeks					
Methotrexate dose					
≤ 15 mg/week	12,382 (8557 to 17,917)	15,914 (11,329 to 22,355)	13,857 (10,775 to 17,822)	107	1.38 (0.86 to 2.21)
> 15 mg/week	8803 (6667 to 11,622)	15,645 (11,871 to 20,619)	11,716 (9605 to 14,291)	171	1.70 (1.17 to 2.46)
Interaction effect (> 15 mg/week vs. ≤ 15 mg/week)					1.23 (0.67 to 2.25)
Methotrexate route of administration					
Oral	10,173 (7595 to 13,625)	14,123 (10,423 to 19,135)	11,887 (9637 to 14,663)	158	1.40 (0.95 to 2.07)
Subcutaneous injection	10,035 (7064 to 14,255)	18,114 (13,508 to 24,292)	13,350 (10,574 to 16,856)	120	1.76 (1.13 to 2.73)
Interaction effect (injection vs. oral)					1.26 (0.70 to 2.26)

continued

TABLE 13 Subgroup analyses for primary outcome [anti-S1-RBD titre (U/mL)] at 4, 12 and 26 weeks (*continued*)

	Continue methotrexate: GM (95% CI)	Suspend methotrexate: GM (95% CI)	Total: GM (95% CI)	*N*	Linear regression: GMR (95% CI)[a]
Disease type					
Rheumatic (±skin) disease	10,057 (7777 to 13,006)	14,828 (11,623 to 18,917)	12,108 (10,132 to 14,469)	227	1.52 (1.10 to 2.09)
Skin disease alone	10,362 (6741 to 15,929)	20,382 (13,313 to 31,203)	14,345 (10,548 to 19,509)	51	1.72 (0.87 to 3.43)
Interaction effect [skin disease alone vs. rheumatic (±skin)] disease					1.14 (0.53 to 2.44)
Age group					
< 40 years	8577 (4576 to 16,075)	11,444 (7405 to 17,686)	9659 (6624 to 14,084)	17	1.96 (0.59 to 6.55)
40–64 years	10,364 (7763 to 13,837)	11,665 (8690 to 15,659)	10,938 (8915 to 13,421)	147	1.11 (0.75 to 1.65)
≥ 65 years	10,056 (6688 to 15,121)	22,977 (16,628 to 31,750)	15,423 (11,824 to 20,116)	114	2.32 (1.48 to 3.64)
Interaction effect < 40 vs. ≥ 65 years					1.18 (0.33 to 4.27)
Interaction effect 40–64 vs. ≥ 65 years					2.09 (1.15 to 3.82)
Previous SARS-CoV-2 infection					
No	9604 (7473 to 12,344)	15,360 (12,179 to 19,370)	11,976 (10,070 to 14,244)	234	1.54 (1.13 to 2.12)
Yes	13,721 (9135 to 20,611)	21,015 (12,256 to 36,032)	16,981 (12,209 to 23,617)	42	1.59 (0.74 to 3.39)
Interaction effect (infection yes vs. no)					1.03 (0.45 to 2.34)
Primary COVID-19 vaccine type					
AstraZeneca AZD1222	10,975 (7712 to 15,620)	22,064 (15,978 to 30,468)	15,413 (12,062 to 19,695)	109	1.99 (1.25 to 3.16)
mRNA (Pfizer-BioNTech BNT162b2 or Moderna mRNA-1273)	9606 (7185 to 12,844)	12,409 (9406 to 16,371)	10,835 (8867 to 13,241)	168	1.32 (0.90 to 1.91)
Interaction effect [mRNA (BNT162b2 or mRNA-1273) vs. AZD1222]					0.67 (0.37 to 1.21)
COVID-19 booster brand					
Pfizer-BioNTech BNT162b2	9707 (7686 to 12,259)	15,134 (11,878 to 19,283)	11,970 (10,105 to 14,181)	214	1.57 (1.13 to 2.18)
AstraZeneca AZD1222	5237 (1258 to 21,801)	31,859 (2720 to 373,156)	9560 (3046 to 30,010)	9	5.78 (1.08 to 30.87)
Moderna mRNA-1273	14,073 (7062 to 28,045)	18,055 (10,697 to 30,475)	15,977 (10,526 to 24,253)	53	1.22 (0.63 to 2.37)
Interaction effect (AZD1222 vs. BNT162b2)					3.69 (0.67 to 20.34)
Interaction effect (mRNA-1273 vs. BNT162b2)					0.78 (0.37 to 1.64)

a Linear regression model, adjusted by baseline value, stratification factors (age, inflammatory condition, vaccine platform), prior infection, booster platform, with treatment by subgroup interaction.

TABLE 14 Self-reported disease activity by study arms

	Continue methotrexate	Suspend methotrexate	Total
	n (%)	*n* (%)	*n* (%)
Disease activity			
4 weeks	*N* = 192	*N* = 191	*N* = 383
None (inactive)	35 (18.2)	20 (10.5)	55 (14.4)
Mild activity	94 (49.0)	93 (48.7)	187 (48.8)
Moderate activity	52 (27.1)	49 (25.7)	101 (26.4)
Severe activity	5 (2.6)	19 (9.9)	24 (6.3)
Very severe activity	0 (0.0)	0 (0.0)	0 (0.0)
Missing data	6 (3.1)	10 (5.2)	16 (4.2)
Ordinal logistic regression OR (95% CI)[a]	1.569 (1.056 to 2.331)		
12 weeks			
None (inactive)	38 (19.8)	19 (9.9)	57 (14.9)
Mild activity	92 (47.9)	95 (49.7)	187 (48.8)
Moderate activity	45 (23.4)	52 (27.2)	97 (25.3)
Severe activity	13 (6.8)	15 (7.9)	28 (7.3)
Very severe activity	0 (0.0)	1 (0.5)	1 (0.3)
Missing data	4 (2.1)	9 (4.7)	13 (3.4)
Ordinal logistic regression OR (95% CI)[a]	1.494 (1.010 to 2.211)		
26 weeks			
None (inactive)	32 (16.7)	20 (10.5)	52 (13.6)
Mild activity	75 (39.1)	81 (42.4)	156 (40.7)
Moderate activity	42 (21.9)	33 (17.3)	75 (19.6)
Severe activity	5 (2.6)	8 (4.2)	13 (3.4)
Very severe activity	0 (0.0)	0 (0.0)	0 (0.0)
Missing data	38 (19.8)	49 (25.7)	87 (22.7)
Ordinal logistic regression OR (95% CI)[a]	1.186 (0.764 to 1.842)		

a Ordinal logistic regression model adjusted by baseline value, stratification factors (age, inflammatory condition, vaccine platform), prior infection, booster platform.

TABLE 15 Self-reported disease description by study arms

	Continue methotrexate	Suspend methotrexate	Total
	n (%)	*n* (%)	*n* (%)
Self-reported disease description			
4 weeks	*N = 192*	*N = 191*	*N = 383*
Much better	1 (0.5)	2 (1.0)	3 (0.8)
Somewhat better	8 (4.2)	6 (3.1)	14 (3.7)
About the same	163 (84.9)	125 (65.4)	288 (75.2)
Somewhat worse	12 (6.3)	39 (20.4)	51 (13.3)
Much worse	2 (1.0)	9 (4.7)	11 (2.9)
Missing data	6 (3.1)	10 (5.2)	16 (4.2)
Ordinal logistic regression OR (95% CI)[a]	3.328 (1.908, 5.804)		
12 weeks			
Much better	3 (1.6)	3 (1.6)	6 (1.6)
Somewhat better	7 (3.6)	11 (5.8)	18 (4.7)
About the same	156 (81.3)	140 (73.3)	296 (77.3)
Somewhat worse	20 (10.4)	24 (12.6)	44 (11.5)
Much worse	2 (1.0)	4 (2.1)	6 (1.6)
Missing data	4 (2.1)	9 (4.7)	13 (3.4)
Ordinal logistic regression OR (95% CI)[a]	0.693 (0.399, 1.203)		

a Ordinal logistic regression model adjusted by baseline value, stratification factors (age, inflammatory condition, vaccine platform), prior infection, booster platform.

TABLE 16 Safety, flare outcomes and their treatment by study arms

	Continue methotrexate	Suspend methotrexate	Total
	n (%)	*n* (%)	*n* (%)
SAEs and disease flare-up			
Number of participants with at least one event (out of total randomised to each allocation)	*n* = 192	*n* = 191	*n* = 383
SAEs related to intervention	0	0	0
SAEs unrelated to intervention	1 (0.8)	2 (1.6)	3 (1.2)
Any self-reported flare-up by 4 weeks	63 (32.8)	102 (53.4)	165 (43.1)
Any self-reported flare-up by 12 weeks	89 (46.4)	124 (64.9)	213 (55.6)
Any self-reported flare-up by 26 weeks	117 (60.9)	132 (69.1)	249 (65.0)
Logistic regression analysis – participants with at least one flare-up	Odds ratio[a]	(95% CI)[a]	
0–4 weeks	2.53	(1.65 to 3.87)	
0–12 weeks	2.28	(1.49 to 3.50)	
0–26 weeks	1.47	(0.94 to 2.29)	
0–4 weeks	***n = 192***	***n = 191***	***n = 383***

TABLE 16 Safety, flare outcomes and their treatment by study arms (*continued*)

	Continue methotrexate	Suspend methotrexate	Total
	n (%)	*n* (%)	*n* (%)
Number of separate self-reported disease flare-ups			
0	129 (67.2)	89 (46.6)	218 (56.9)
1	30 (15.6)	46 (24.1)	76 (19.8)
2	18 (9.4)	24 (12.6)	42 (11.0)
3	10 (5.2)	13 (6.8)	23 (6)
4	2 (1.0)	5 (2.6)	7 (1.8)
5	0 (0.0)	4 (2.1)	4 (1.0)
6 +	3 (1.6)	10 (5.2)	13 (3.4)
Medical or nursing help sought to treat disease flare-ups[b]	**8 (4.2)**	**12 (6.3)**	**20 (5.2)**
Hospital helpline	0 (0.0)	3 (1.6)	3 (0.8)
GP/practice nurse	4 (2.1)	6 (3.1)	10 (2.6)
Hospital outpatient (telephone or in-person)	4 (2.1)	3 (1.6)	7 (1.8)
Hospital accident and emergency	1 (0.5)	0 (0.0)	1 (0.3)
Other	1 (0.5)	0 (0.0)	1 (0.3)
*Painkillers/*NSAIDs *used to treat disease flare-ups*			
Yes	60 (31.3)	76 (39.8)	136 (35.5)
No	92 (47.9)	78 (40.8)	170 (44.4)
Unknown[c]	32 (16.7)	30 (15.7)	62 (16.2)
Glucocorticoid used to treat disease flare-ups			
Yes	12 (6.3)	21 (11)	33 (8.6)
No	148 (77.1)	140 (73.3)	288 (75.2)
Unknown[c]	32 (16.7)	30 (15.7)	62 (16.2)
Cream used to treat flare-up of skin condition			
Yes	30 (15.6)	36 (18.8)	66 (17.2)
No	99 (51.6)	97 (50.8)	196 (51.2)
Unknown[c]	32 (16.7)	30 (15.7)	62 (16.2)
0–12 weeks			
Number of separate self-reported disease flare-ups			
0	103 (53.6)	67 (35.1)	170 (44.4)
1	29 (15.1)	27 (14.1)	56 (14.6)
2	19 (9.9)	31 (16.2)	50 (13.1)
3	11 (5.7)	12 (6.3)	23 (6)
4	6 (3.1)	14 (7.3)	20 (5.2)
5	6 (3.1)	12 (6.3)	18 (4.7)
6 +	18 (9.4)	28 (14.7)	46 (12.0)

continued

TABLE 16 Safety, flare outcomes and their treatment by study arms (*continued*)

	Continue methotrexate	Suspend methotrexate	Total
	n (%)	*n* (%)	*n* (%)
Medical or nursing help sought to treat disease flare-ups[b]	**25 (13.0)**	**25 (13.1)**	**50 (13.1)**
Hospital helpline	8 (4.2)	7 (3.7)	15 (3.9)
GP/practice nurse	6 (3.1)	10 (5.2)	16 (4.2)
Hospital outpatient (telephone or in-person)	11 (5.7)	12 (6.3)	23 (6.0)
Hospitalisation	0 (0.0)	1 (0.5)	1 (0.3)
Hospital accident and emergency	1 (0.5)	0 (0.0)	1 (0.3)
Other	2 (1.0)	2 (1.0)	4 (1.0)
*Painkillers/*NSAIDs *used to treat disease flare-ups*			
Yes	81 (42.2)	88 (46.1)	169 (44.1)
Glucocorticoid used to treat disease flare-ups			
Yes	22 (11.5)	34 (17.8)	56 (14.6)
Cream used to treat flare-up of skin condition			
Yes	38 (19.8)	54 (28.3)	92 (24.0)
0–26 weeks			
Medical or nursing help sought to treat disease flare-ups[b]	**39 (20.3)**	**32 (16.8)**	**71 (18.5)**
Hospital helpline	13 (6.8)	11 (5.8)	24 (6.3)
GP/practice nurse	12 (6.3)	12 (6.3)	24 (6.3)
Hospital outpatient (telephone or in-person)	17 (8.9)	15 (7.9)	32 (8.4)
Hospitalisation	0 (0.0)	1 (0.5)	1 (0.3)
Hospital accident and emergency	1 (0.5)	0 (0.0)	1 (0.3)
Other	5 (2.6)	4 (2.1)	9 (2.3)

a Logistic regression model adjusted by stratification factors (age, inflammatory condition, vaccine platform), prior infection, booster platform.
b Participants can seek help from more than one source.
c Participants did not provide answer for this question.

TABLE 17 Severe acute respiratory syndrome coronavirus 2 infections at each time point and booster vaccination prior to week 26 visit

	Continue methotrexate	Suspend methotrexate	Total
	n = 192 (%)	*n* = 191 (%)	*n* = 383 (%)
New SARS-CoV-2 infection at each time point[a] (out of total numbers of blood samples analysed)			
Baseline	36 (18.8)	36 (18.9)	72 (18.8)
0–4 weeks	8 (4.3)	10 (5.6)	18 (4.9)
5–12 weeks	16 (8.7)	18 (10.1)	34 (9.4)
13–26 weeks	24 (15.9)	30 (21.9)	54 (18.8)
Further COVID-19 vaccination ≥ 26 weeks after booster vaccine immediately after entering the VROOM study[b]	**35 (22.3)**	**40 (28.0)**	**75 (25.0)**
Pfizer	17 (10.8)	20 (14.0)	47 (15.7)
Moderna	17 (10.8)	19 (13.3)	46 (15.3)
100 mg	5 (3.2)	4 (2.8)	9 (3.0)
50 mg	4 (2.5)	3 (2.1)	7 (2.3)
0.1 mg/0.5 ml	2 (1.3)	1 (0.7)	3 (1.0)
Dose unknown	6 (3.8)	11 (7.7)	17 (5.7)
Spikevax bivalent	1 (0.6)	0 (0.0)	1 (0.3)
Unknown	0 (0.0)	1 (0.7)	1 (0.3)

a Number of new SARS-CoV-2 infections at each time point as assessed by *N*-serology.
b Vulnerable adults in the UK were offered an additional booster vaccination against COVID-19 at least 26 weeks after the booster with which they entered the VROOM study. For some participants, their week 26 visit was delayed for reasons outside our control, for example, patient unavailability, site capacity for clinic space, staffing issues at sites, etc. The UK COVID-19 vaccination programme offered additional booster vaccinations against COVID-19 from 26 weeks after the previous vaccination. So as not to disadvantage the study participants' health and well-being they were not restricted from accessing these vaccinations before attending for their week 26 study visit in the VROOM study.

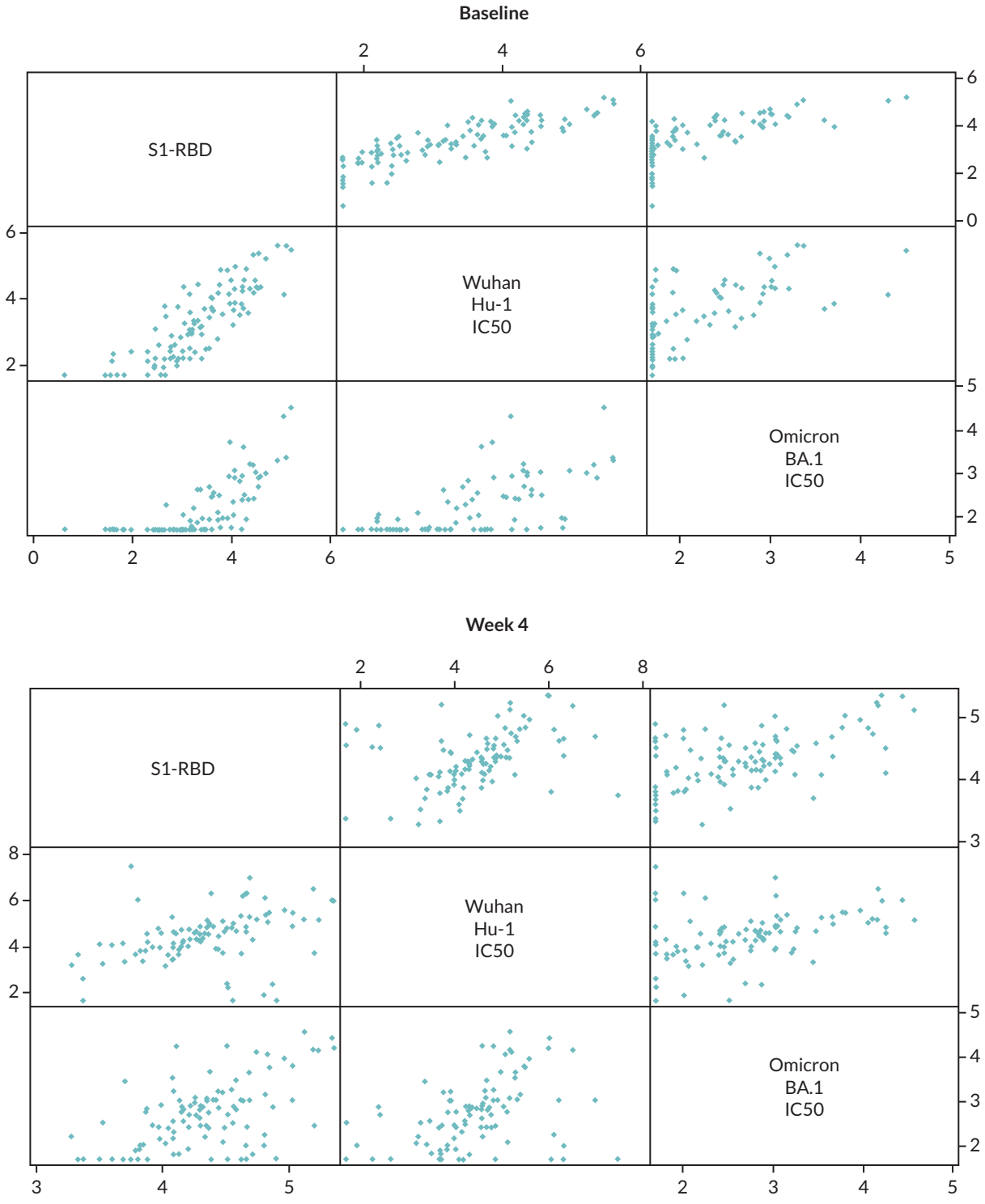

FIGURE 6 Correlations between Wuhan and Omicron neutralisation titres to S1-RBD.

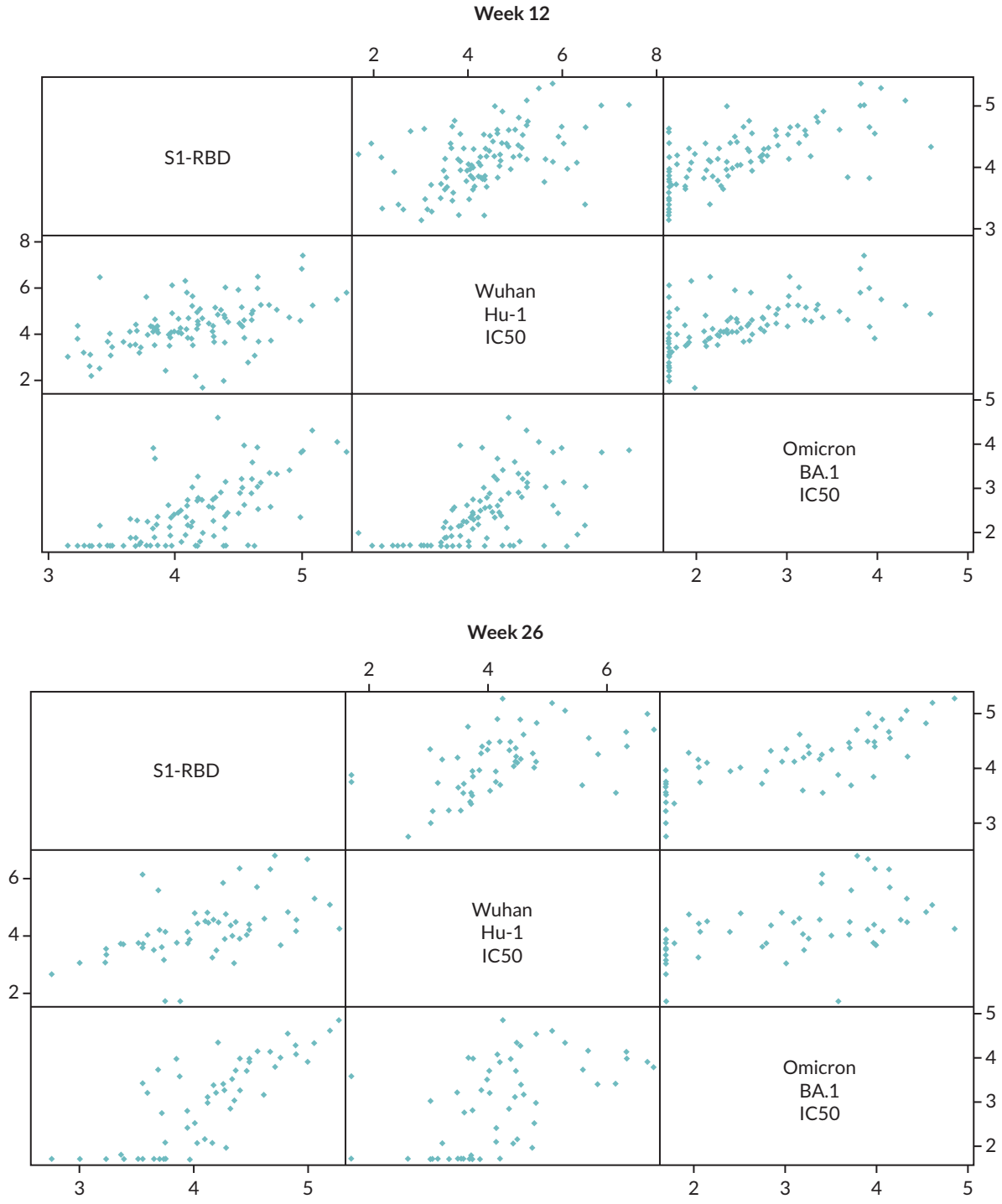

FIGURE 6 (*continued*)

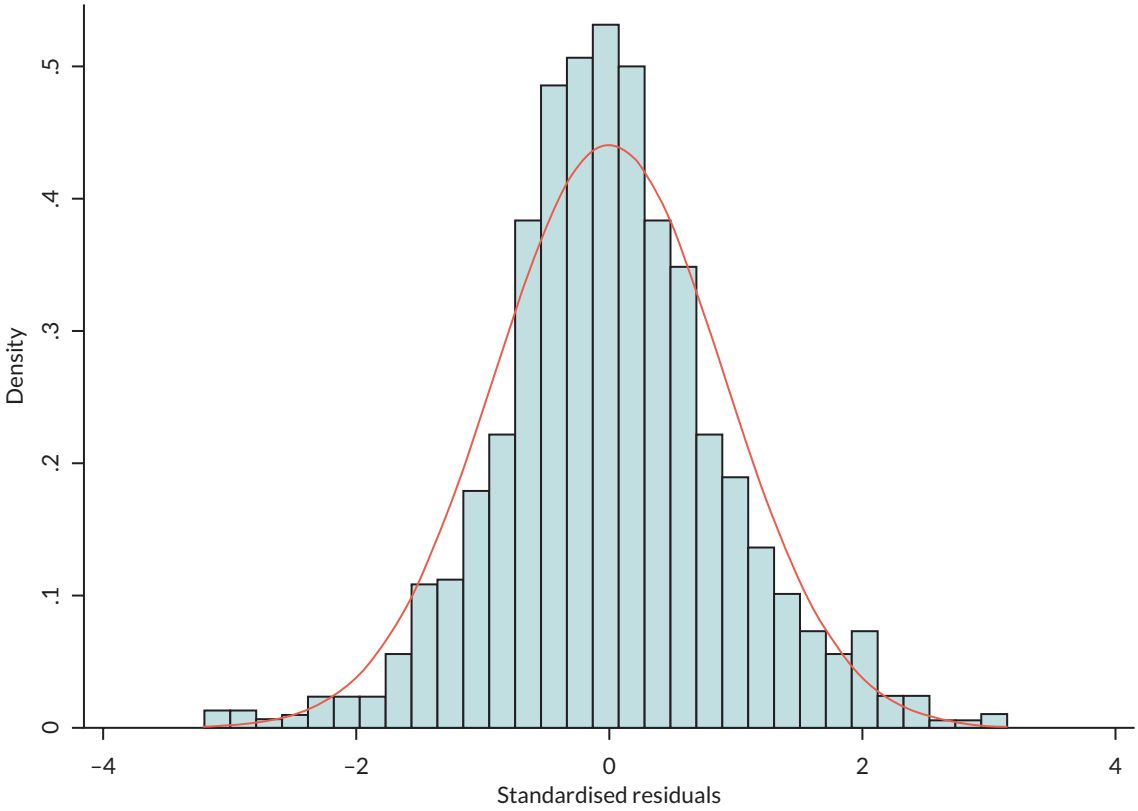

FIGURE 7 Histogram of the standardised residuals from the primary model at 4 weeks (primary model diagnostics – normal approximation).

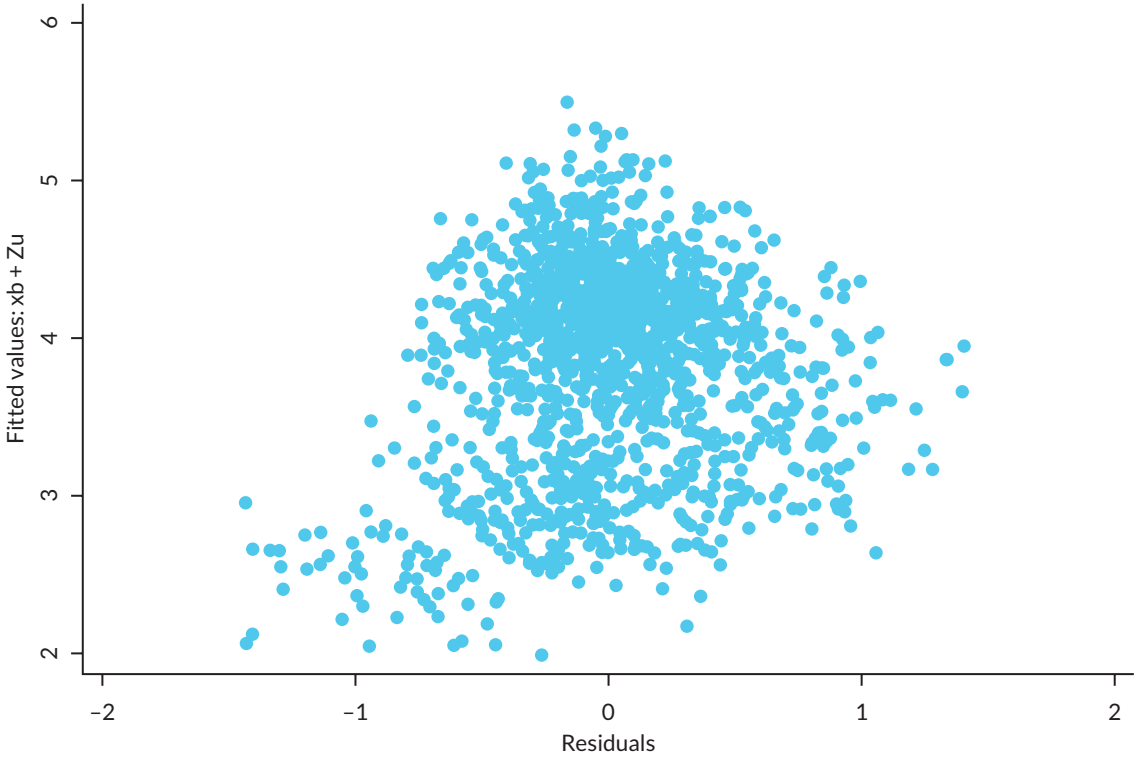

FIGURE 8 Scatterplot of the fitted values against the residuals from the primary model at 4 weeks (primary model diagnostics – residual diagnostics).