VROOM: VACCINE RESPONSE ON/OFF METHOTREXATE: DOES TEMPORARILY SUSPENDING METHOTREXATE TREATMENT FOR TWO WEEKS ENHANCE COVID-19 VACCINE RESPONSE? – A RANDOMISED CONTROLLED TRIAL

(Full Scientific Title: A multi-centre randomised controlled trial examining the effects of temporarily suspending low-dose methotrexate treatment for two weeks after SARS-CoV-2 vaccine booster on vaccine response in immunosuppressed adults with inflammatory conditions, including a nested mechanistic sub-study)

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SYNOPSIS

Title	Multi-centre randomised controlled trial examining the effects of temporarily suspending low-dose methotrexate treatment for two weeks after SARS-CoV-2 vaccine booster on vaccine response in immunosuppressed adults with inflammatory conditions, including a nested mechanistic sub-study.
Acronym	VROOM study
Short title	Vaccine Response On/Off Methotrexate Study
Chief Investigator	Professor Abhishek
Objectives	**Clinical study** Primary objective: To assess the effect of a two-week temporary suspension of methotrexate on anti-spike-receptor binding domain (RBD) antibody at four weeks post booster vaccination. Secondary objectives: To assess the effect of a two-week temporary discontinuation of methotrexate on • anti-spike-RBD antibody at 12 weeks post booster vaccination; • underlying disease activity at weeks 2, 4 and 12 post booster vaccination; • underlying disease flare ups and actions taken to deal with the flare during 12 weeks post booster vaccination; • drug treatments including rescue treatments for flare ups or change in disease activity during 12 weeks post booster vaccination; • quality of life at weeks 4 and 12 post booster vaccination. **Mechanistic study (100 participants)** • To assess the effect of a two-week temporary suspension of methotrexate on neutralising antibody response at weeks 4 and 12 post booster vaccination; • To explore association between anti-spike RBD antibody and neutralisation titres pre-booster, and at weeks 4 and 12 post booster vaccination; • To explore the validity of anti-spike-RBD antibody, neutralisation titres, to SARS-Cov-2 booster vaccine in participants adherent to methotrexate at each time-point i.e. pre-vaccination, weeks 4 and 12 post booster vaccination, based on validated biochemical assay.

Trial Configuration	Prospective, parallel group, randomised controlled trial with internal recruitment feasibility assessment, including a nested mechanistic study.
Setting	Secondary care
Sample size estimate	Estimates used were derived from Folegatti et al, which allow the mean (SD) of the anti-spike IgG 28 days after vaccination to be estimated as 191.9 (165.5) ELISA units. The sample size is based on detecting at least a 25% higher antibody response in the methotrexate discontinuation group with 90% statistical power at 2-sided 5% significance level which requires data from 502 participants; leading to 560 after allowing for up to 10% missing data.
Number of participants	560
Eligibility criteria	*Inclusion criteria:* • Age ≥18 years; • Diagnosed with inflammatory conditions such as rheumatoid arthritis, psoriasis with or without arthritis, seronegative spondyloarthritis, reactive arthritis, atopic eczema, polymyalgia rheumatica, systemic lupus erythematosus; o ***Note:*** *This is not an exhaustive list and people with other inflammatory conditions where treatment may be interrupted for two weeks without the risk of substantial increase in disease activity, or organ or life-threatening flare up will also be eligible to participate in the study in order to increase the generalisability of the trial.* • Prescribed oral or subcutaneous methotrexate (≤25 mg/week), +/- hydroxychloroquine, for at least the previous three months; • Able to temporarily suspend methotrexate for two weeks in the opinion of patients' hospital team without the risk of substantial increase in disease activity, or organ or life-threatening flare up; • Able to give informed consent; Eligible for planned booster vaccination for COVID-19 (have received any 2 vaccinations from the original NHS COVID Vaccination Programme 2020/21) and yet to receive a booster vaccination. *Exclusion criteria:* • Diagnosed with inflammatory conditions for which treatment cannot be interrupted safely: such as ANCA associated vasculitis, large vessel vasculitis, myositis, giant cell arteritis, solid organ transplant;

	• Treated with Rituximab drip in the last 18 months or planning to start it; • Concurrent immune suppressive treatments in the last two months specifically: leflunomide, ciclosporin, azathioprine or mercaptopurine, sulfasalazine or other 5-amino-salicylic acid drugs, mycophenolate, apremilast, biologic agents; • Radiotherapy or cancer chemotherapy in last six months; • Prednisolone dose >7.5 mg/day within 30 days of randomisation; • Active solid organ cancer (people with skin cancer or those cured of solid organ cancer are eligible); • Already participating in a CTIMP (clinical trial of an investigational medicinal product).
Description of interventions	Control intervention: To continue on methotrexate treatment as usual irrespective of receiving SARS-CoV-2 booster vaccination. Experimental intervention: To suspend methotrexate treatment for two weeks after* the SARS-CoV-2 booster vaccination then recommence methotrexate treatment. ***Note:*** *The SARS-CoV-2 booster vaccination will be administered via the national NHS booster vaccine rollout. Whether an individual receives the SARS-CoV-2 booster alone or with the influenza vaccine this does not preclude study entry – vaccination delivery will be recorded.* ***** *If the day of the booster is the same day as usual methotrexate administration – methotrexate will be withheld on that day if in the experimental intervention arm. Explicitly the trial aims for those in the experimental intervention group to miss (temporarily suspend) two doses of methotrexate- where a dose can range from 7.5-25 mg/week taken once a week.* ***Note:*** *This is missing 2 weekly doses not a reduction in the dose of methotrexate taken.*
Duration of study	Planned study start date: 01 Sep 2021 Participants will be in the study for 12 weeks post booster vaccination. It is anticipated that the study will take up to 10 months to recruit participants.
Randomisation and blinding	Open label study (participants and site personnel are aware of the randomisation allocation; staff processing lab samples, including the primary outcome measure, are blinded to randomisation allocation) 1:1 individual randomisation, minimised by: • inflammatory conditions (inflammatory rheumatic disease (+/- skin disease) or skin disease alone),

	• age (<40, 40-64, or ≥65 years), and • SARS-CoV-2 vaccine received in primary vaccination cycle (mRNA, vector, or combination).
Outcome measures	• Primary: Anti-spike-RBD antibody level at 4 weeks post booster vaccination • Secondary: Anti-spike RBD antibody level at 12 weeks post booster vaccination; Patient assessments of disease activity: global assessment using a numeric rating scale with one-week recall at baseline, 2, 4 and 12 weeks post booster vaccination, current disease activity level and change since booster, 4 and 12 weeks post booster vaccination; Disease flare-up: self-reported number of flares and actions taken to deal with the flare 4 and 12 weeks post booster vaccination; Quality of life: assessed using EQ-5D-5L at baseline, 4 and 12 weeks post booster vaccination; Adherence with advice to interrupt or continue methotrexate: self-report at 2 and 4 weeks post booster vaccination; COVID-19 neutralising titre against the Wuhan isolate and other circulating variants of concern such as B.1.1.7. *[Mechanistic sub-study samples only];* Adherence with oral methotrexate using biomarker at 4 and 12 weeks *[Mechanistic sub-study only].* Serious Adverse Events (recorded from booster vaccination to 12 weeks post vaccination)
Statistical methods	The principal analysis will be performed on the randomised ("intention to treat") population, analysing participants with available outcome data in their randomised groups, regardless of adherence. The primary objective of the statistical analysis is to identify if a 2 week discontinuation of methotrexate after the booster vaccination against SARS-CoV-2 increases the anti-spike-RBD antibody at 4 weeks post booster vaccination compared to continuing methotrexate. The anti-spike-RBD antibody levels will be summarised descriptively at baseline, 4, and 12-weeks post booster vaccination follow-up. The differences between the study arms will be estimated a using mixed effects regression model, allowing for repeated measures clustered

	within participants. The model will be adjusted for randomisation factors (inflammatory condition, age categories, prime vaccine's platform i.e. mRNA vs. other) and also prior infection status obtained from pre-vaccination anti-nucleocapsid antibodies and type of SARS-CoV-2 vaccine booster received as fixed effects. Adjusted mean differences between the groups will be presented, together with 95% confidence intervals (CI) and p-values. The extent of missing data, and missing data pattern will be described. Consistency of the treatment effects for important prognostic subgroups (methotrexate dose, inflammatory condition type, age group, previous SARS-CoV-2 infection, prime vaccination platform, booster platform, route of administration of methotrexate: subcutaneous versus oral) will be explored. Supplemental analysis will exclude participants who self-report non-adherence to their randomised allocation, and those that were unable to receive their booster vaccines. Continuous secondary outcomes will be analysed using generalised linear models for binary and continuous data, as appropriate, with model adjustment as described above. The number of serious adverse events (SAE) will be presented by treatment arm. Further analyses related to the mechanistic hypotheses will be carried out exclusively on the mechanistic sub-sample including quantifying levels and strength of relationships using appropriate statistical summarises including (mean, SD, range, correlation coefficients etc).

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ABBREVIATIONS

ACR	American College of Rheumatology
AE	Adverse Event
ANCA	Anti-Neutrophilic Cytoplasmic Auto-antibodies
BAFF	B cell activation factor
BritPACT	British Psoriatic Arthritis Consortium
CI	Chief Investigator or Confidence Intervals
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Coronavirus disease
CRF	Case Report Form
CTIMP	Clinical Trial of an Investigational Medicinal Product
DMC	Data Monitoring Committee
ELISA	Enzyme Linked Immunosorbent Analysis
EMR	Experimental Medicine and Rheumatology Group
GCP	Good Clinical Practice
GRAPPA	Group for Research and Assessment of Psoriasis and Psoriatic Arthritis
HAI	Hemagglutination inhibition
ICF	Informed Consent Form
IgG	Immunoglobulin G
INVOLVE	UK's leading public participation charity
ITT	Intention to Treat
ISRCTN	International Standardised Randomised Controlled Trial Number
JCVI	Joint Committee on Vaccination and Immunisation
LC-MS	Liquid Chromatography-Tandem Mass Spectrometry
mRNA	Messenger ribonucleic acid

MSK	Musculoskeletal
MTX	Methotrexate
NHS	National Health Service
NSAID	Non-Steroidal Anti-Inflammatory Drug
OCTRU	Oxford Clinical Trials Research Unit
OMERACT	Outcome Measures in Rheumatoid Arthritis Clinical Trials
OPEN ARMS	Oxford Patient Engagement Network for Arthritis and Musculoskeletal Conditions
PHE	Public Health England
PI	Principal Investigator at a local centre
PIS	Participant Information Sheet
PP	Per Protocol
PPI	Patient and Public Involvement
PsA	Psoriatic Arthritis
QR	Quick Response
RA	Rheumatoid Arthritis
RBD	Receptor Binding Domain
REC	Research Ethics Committee
R&D	Research and Development department
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2 – (name of the virus attributed to the Coronavirus disease attributed by WHO on 11Feb2020)
SD	Standard Deviation
SMS	Short Messaging Service

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TMG	Trial Management Group
TSC	Trial Steering Committee
WHO	World Health Organisation

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STUDY BACKGROUND INFORMATION AND RATIONALE

Inflammatory conditions such as rheumatoid arthritis, psoriasis with or without arthritis, axial spondyloarthritis, and systemic lupus erythematosus affect 3.5% adults in the UK[1-4]. They are often treated with immune suppressing drugs such as low-dose weekly methotrexate, azathioprine, leflunomide, mycophenolate mofetil, and ciclosporin etc [5-7]. Of these, low-dose weekly methotrexate (≤25 mg/week) has emerged as the first line long-term treatment due to its efficacy, tolerability, and relative safety[6,7]. It is also co-prescribed with second line drugs such as biologics e.g. anti-TNFα agents to minimise the risk of anti-drug antibodies[8]. Research using primary-care prescription data from the UK suggests that over 60% of patients with autoimmune rheumatic diseases are treated with oral methotrexate[6].

Methotrexate is the first line treatment for many inflammatory conditions used in the NHS, as it is an immunosuppressant. It slows down the body's immune system and helps reduce inflammation. It acts by inhibiting the enzyme dihydrofolate reductase which is essential for the synthesis of purines and pyrimidines. It is taken at various doses but always once a week, on the same day each week. For some doses an individual may have to take up to 10 tablets to make up the dose their clinician prescribes. (Methotrexate pill packets and patient cards often tell a patient on which day to take the medicine). In the UK the following brand names of methotrexate are used currently Jylamvo, Maxtrex, Methofill, Metoject, Nordimet, Zlatal.

Adults with inflammatory diseases are at an increased risk of hospitalisation and death due to COVID-19 with odds ratio (95% confidence interval (CI)) 1.19 (1.05-1.35) as reported in a recent systematic review and meta-analysis[9]. The excess risk was present when studies with mean age 65 years or above were excluded[9]. Studies using routine UK healthcare data also report that inflammatory conditions increase the risk of hospitalisation (adjusted hazard ratio (95% CI) 1.35 (1.17-1.56) and 1.30 (1.07-1.57) in women and men respectively)[10] and death from COVID-19 (adjusted hazard ratio (95% CI) 1.19 (1.11-1.27))[11]. In anticipation of this risk, people with inflammatory conditions treated with immune suppressing medicines were advised to shield[12], and have been prioritised for vaccination.

Whether COVID-19 vaccines are as effective in immunosuppressed adults as in the general population is not fully understood. In an observational study undertaken by co-applicant Valdes using the COVID Symptom Study app, individuals with at least one comorbidity, including those on immunosuppressive therapy had 15% lower risk reduction for COVID-19 with the SARS-CoV-2 vaccines (-54% [95%CI: -59%, -48%]) compared to those without any comorbidity (-69% [95%CI: -71%, -68%])[13]. SARS-CoV-2 vaccines are not live and are safe to use in the context of immune suppressing treatment. However, there is considerable uncertainty in the clinical and patient communities on whether immune suppressing therapies reduce the efficacy of these vaccines, and whether they should temporarily be discontinued peri-vaccination. Direct evidence for the impact of immune suppressive therapies on the immunogenicity of these vaccines is lacking. However, low-dose weekly oral methotrexate reduces the antibody response to pneumococcal polysaccharide and inactivated influenza vaccines with odds ratio (95% CI) 0.33 (0.20-0.54) and 0.35 (0.18-0.66) respectively[14]. Anti-TNFα agents, the most commonly used biologic, and azathioprine, another commonly used immune suppressing drug, does not inhibit the serological response to these vaccines[14-18]. Similarly, combinations of other biologics/targeted therapies (e.g. tofacitinib, tocilizumab) and methotrexate, but not the targeted therapies alone, associate with impaired immune response to these vaccines[15]. Of

interest, withholding methotrexate for two-weeks post inactivated influenza vaccine increased the proportion of participants achieving protective hemagglutination inhibition (HAI) antibody titre[19-20].

These issues were 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, typically methotrexate, 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 potential consequences caused by a two-week pause is likely to be low and is something that often occurs when patients on long-term methotrexate become ill. The biggest consequence that may be seen is that participants may get a flare up of their condition – if this occurs participants are able to use all rescue therapies such as corticosteroids that they usually have available to them.

Recently, the American College of Rheumatology (ACR) advised patients with stable disease to discontinue methotrexate for one-week post SARS-CoV-2 vaccine while the American Academy of Dermatology advised continuation of treatment but monitoring of post-vaccination serology, further confusing the issue[21,22]. Of relevance to this study, the ACR guidance advises patients to continue on other immune suppressing drugs peri-vaccination and the advice to hold treatment is specifically for methotrexate, further highlighting the need for this study [21].

Immunological response to SARS-CoV-2 infection and vaccination:

Two structural proteins, the nucleocapsid and spike proteins of SARS-CoV-2 are used to detect antibodies in the serum of individuals who have been infected. The spike protein contains the receptor binding domain (RBD) which interacts with the cellular receptor angiotensin converting enzyme 2, resulting in conformational changes in the spike. This interaction ultimately leads to delivery of the virus into the cytoplasm where replication of the viral genome and production of new viral proteins occurs. Neutralising antibodies, produced by B-cells, target the spike protein and prevent viral entry[23]. T cell responses provide help to B cell responses and act against SARS-CoV-2; the two mechanisms can be discordant[23].

In the UK, three main vaccines against COVID-19 have been approved at the time of writing this protocol, Pfizer-BioNTech BNT162b2, Moderna mRNA-1273, and Oxford-AstraZeneca ChAdOx1 nCoV-19 (AZD1222). All generate immunity to spike protein, and the resulting anti-spike antibody titres correlate closely with functional neutralising activity[24-26]. Among diverse immune mechanisms, neutralising antibodies are strongly implicated as a correlate of protection and modelling studies suggest that 20-28.5% of the neutralisation titres observed in convalescent sera might be sufficient to protect from COVID-19 [27]. Following two doses of BNT162b2, high titres of antibodies that neutralise SARS-CoV-2 and helper T cell responses were observed by week 2 in animal model of COVID-19 vaccination[28]. Furthermore, after challenge with the USA-WA1/2020 strain of SARS-CoV-2, the immunised animals showed no viral replication in the lung and no clinical signs of disease[28]. The production of neutralising antibodies relies on the stimulation of B cells by CD4+ T-helper cells[29].

Effect of methotrexate on T cells and B cells:
Methotrexate reduces B cells and CD8+IFNγ T-cells[30-32]. Helper T-cell depletion can result in lower efficacy of antibody production by B-cells. Additionally, methotrexate also causes humoral suppression by interacting with the B cell activation factor (BAFF) and increasing immunosuppressive adenosine and regulatory B cells[33,34]. These are the key mechanisms by which vaccines are effective and if both B-cell and T-cell responses are dampened by methotrexate, vaccinated individuals will be unlikely to mount a strong immune response to fight the SARS-CoV-2 infection and vaccination would therefore be less effective in preventing infection or severe disease in such individuals.

Neutralisation responses elicited by SARS-CoV-2 vaccines in people on immune-suppressive treatments such as methotrexate are poorly understood. Thus, generating additional data on post-vaccine antibody responses in immunocompromised individuals, and the impact of treatment discontinuation is particularly important. As the half-life of antibody detected in serum can be short, it is important to understand measures of specific immunity in the B and T cells responsible for rapid, responsive antibody to any future exposures. Thus, the overall purpose of this study is to examine the effect of temporarily discontinuing low-dose methotrexate treatment for two-weeks after SARS-CoV-2 booster vaccination on immunogenicity of the booster.

It was impossible to test whether such interruption in treatment will improve the immunogenicity of SARS-CoV-2 vaccines in the current wave of vaccinations due to the speed of rollout of the vaccination programme in the UK. However, the emergence of SARS-CoV-2 variants and, uncertainty about the durability of protection from the initial doses of the vaccine suggests that booster doses are likely to be utilised in the winter of 2021 and regularly thereafter[35].

Study hypothesis:
We hypothesize that given the effects of methotrexate individuals treated with methotrexate at the time of vaccination against SARS-CoV-2 will have an impaired immune response to the vaccine, and therefore lower production of anti-spike and neutralising antibodies, and that a two-week temporary suspension in methotrexate treatment will improve these responses without significant worsening of underlying inflammatory disease.

Proof of concept studies:
There are no trials examining the impact of temporary interruption in immune suppressing drug treatments on immunogenicity of SARS-CoV-2 vaccines. However, two previous studies ("pilot" and follow-on definitive study) together demonstrated that a 2-week interruption of weekly low-dose methotrexate treatment post inactivated influenza vaccine significantly increased the serological response [19-20].

Previously published pilot study[19]:
This was a randomised parallel-group trial in which 199 patients with rheumatoid arthritis on stable doses of methotrexate were randomised 1:1:1:1 to:

- Group 1 - continue methotrexate;
- Group 2 - suspend methotrexate for four weeks pre-influenza-vaccination;

- Group 3 - suspend methotrexate for two weeks pre and two weeks post--influenza-vaccination;
- Group 4 - suspend methotrexate for four weeks post--influenza-vaccination.

There was no difference in proportion of participants achieving a ≥4-fold increase in HAI antibody titre 4 weeks post-vaccination between groups 1 and 2, while groups 3 and 4 were numerically more likely to have such an increase. Similarly, participants in groups 3 and 4 (but not in group 2) had significantly greater fold change in pre-vaccination antibody titres to two of three strains compared to group 1.

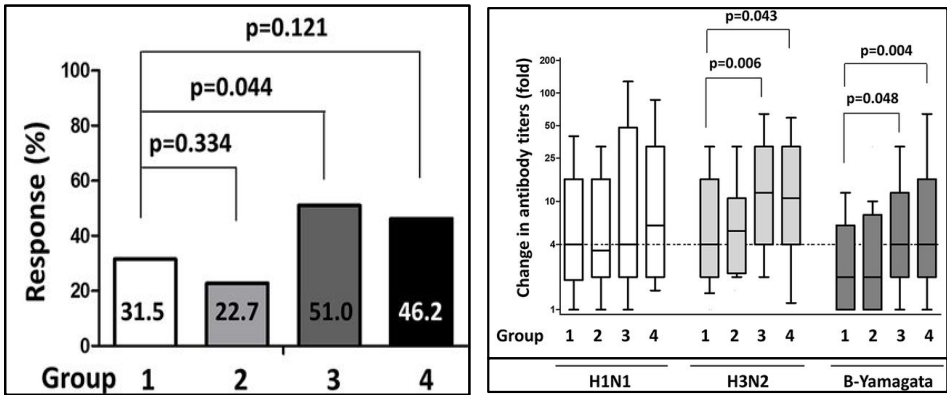


Figure 1: Proportion of participants achieving HAI antibody titre of ≥ 1: 40 (Left panel), and the fold change in antibodies (Right panel). Taken from Park et al, 2019 [33]

However, 34% participants in groups 2 to 4 reported rheumatoid arthritis flare, compared to 24% in Group 1. Based on these results, the authors concluded that interrupting methotrexate before vaccination with the inactivated influenza vaccine has no effect on its immunogenicity, and hypothesized that methotrexate discontinuation for two-weeks post-vaccination may be sufficient to optimise the immune-response and carry a smaller flare-up risk.

Previously published definitive study[20]:

In a randomised parallel-group trial (n=316), participants with rheumatoid arthritis on stable doses of methotrexate were randomised 1:1 to either continue or to suspend methotrexate for two weeks post quadrivalent influenza vaccine. Significantly more participants randomised to 2-week methotrexate discontinuation met the primary and secondary outcomes (Figure 2). Participants in treatment hold group also had significantly greater fold change of HAI antibody titres against all strains.

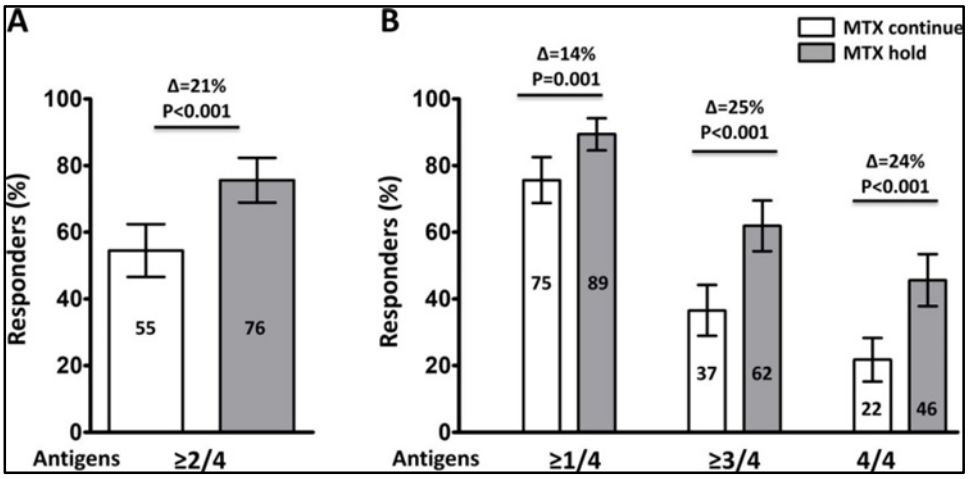


Figure 2: Frequency of vaccination responses to the influenza vaccine. Satisfactory vaccine response, defined as ≥ 4-fold increase in HAI antibody titre at 4 weeks after vaccination, against ≥2 of 4 vaccine strains (primary outcome) (A); and against ≥1, ≥3 and 4 of 4 vaccine strains (secondary outcomes) (B).

In the treatment interruption group, 10.6% participants self-reported disease flare compared to 5.1% in the continued treatment group. However, rescue treatment was required in comparable numbers in both groups (i.e. 6.3% vs. 4.5%).

In a post hoc analysis[36], there was no association between HAI antibody titres and the number of days between the date of the last weekly methotrexate dose and the date of vaccination

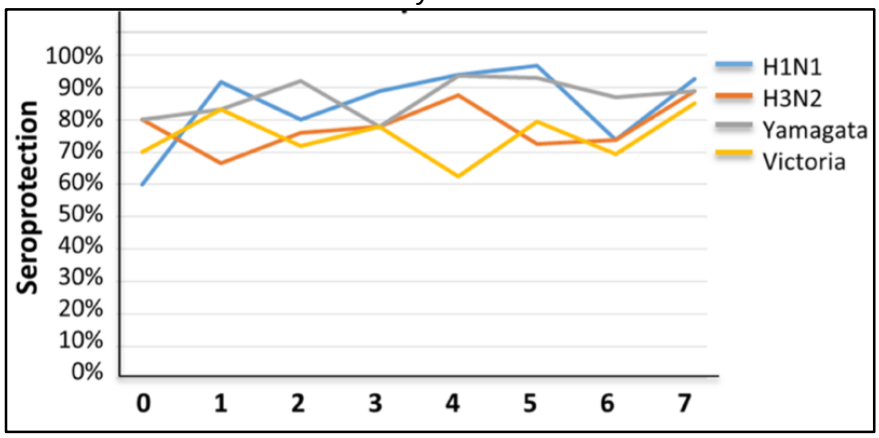


Figure 3: Number of days between methotrexate dose and seroprotection in two-week methotrexate hold group.

This further supports that a period of methotrexate suspension pre-vaccination is not necessary.

Other studies:

The role of methotrexate as an effective "immunological poison" that inhibits immunogenicity against foreign antigens is further highlighted by its efficacy in reducing antibody formation against anti-TNF α drugs, and against pegloticase (a recombinant uricase used to treat refractory gout), thereby increasing their efficacy, minimising side-effects and increasing tolerability[8,37]. Thus, whether temporarily interrupting methotrexate treatment will improve immunogenicity of the SARS-CoV-2 vaccines merits further investigation.

Strategy of literature search:
PubMed, CENTRAL and EPISTOMONIKOS were searched on the 15th March 2021, using key words and their related terms: “Vaccination” and either “rheumatoid arthritis” or “psoriasis” or “ankylosing spondylitis” or “lupus” to identify studies reporting on association between immune suppressing drugs and vaccine response. Systematic reviews and randomised controlled trials were identified and utilised for providing the study, rationale and the proof-of-concept.

Competing studies:
A review of the WHO clinical trials database, ISRCTN and Clinicaltrials.gov on 15th March 2021 did not identify any study addressing the research question we are proposing to answer. An observational study (OCTAVE study) funded by the Medical Research Council is examining the duration of immune-response from the first two doses of the SARS-CoV-2 vaccines in people with cancer, inflammatory arthritis, chronic kidney or liver diseases or those having a stem cell transplant. Researchers will compare results from participants with a variety of immune suppressing conditions against control groups of healthy people, without these underlying diseases, who also received SARS-CoV-2 vaccines. The OCTAVE study will not answer the question of whether a 2 week temporary suspension of methotrexate improves vaccine response, and, the VROOM study us will complement the results of the OCTAVE study, and will provide evidence-based strategy for optimising immune response to booster SARS-CoV-2 vaccines.

STUDY OBJECTIVES AND PURPOSE

The main aim of this study is to assess whether a temporary two-week suspension of low-dose weekly methotrexate treatment post SARS-CoV-2 vaccine booster improves the immune response in people with inflammatory conditions, with key secondary outcomes looking at disease control.

An additional mechanistic aim is to explore the efficiency of the serological response in terms of neutralisation and the underpinning cellular mechanisms.

A sensitivity analysis of participants’ adherence to methotrexate based on a validated bioassay will be undertaken to improve the internal validity of the study[38].

Purpose (Hypothesis)

- **Clinical:** A two-week temporary suspension in weekly low-dose methotrexate treatment after SARS-CoV-2 vaccine boosters will improve the anti-spike-receptor binding domain (RBD) response.
- **Mechanistic (in a subset of 100 participants):** The neutralising antibody response will correlate with the anti-spike-RBD antibody in this immune-suppressed population as in other healthy populations.

Primary Objective

To assess the effectiveness of a two-week temporary suspension of methotrexate treatment on anti-spike-RBD antibody levels at 4 weeks post SARS-CoV-2 vaccination booster vaccination.

Secondary Objectives

To assess the effectiveness of a two-week temporary suspension of methotrexate treatment on:

- anti-spike-RBD antibody levels at 12 weeks post booster vaccination;
- disease activity at weeks 2, 4 and 12 post booster vaccination;
- disease flare ups and actions taken to deal with them during the 12 weeks post booster vaccination;
- quality of life at weeks 4 and 12 weeks post booster vaccination.

Embedded mechanistic sub-study (100 participants)

- To assess the effect of a two-week temporary suspension of methotrexate treatment on neutralising antibody responses at weeks 4 and 12 post booster vaccination;
- To explore association between anti-spike-RBD antibody and neutralisation titres pre-booster, and at weeks 4 and 12 post booster vaccination;
- To explore the validity of anti-spike-RBD antibody, neutralisation titres, to SARS-CoV-2 booster vaccine in participants' adherence to methotrexate at each time-point i.e. pre-vaccination, weeks 4 and 12 post booster vaccination, based on a validated biochemical assay[37].

DETAILS OF PRODUCT(S)

This study aims to research the impact of a temporary medication suspension (break) for those in the intervention group of two weeks of taking their previously prescribed and ongoing methotrexate treatment regime. This trial does not have any part in the provision of the methotrexate including route of administration, dosage or any issues arising from the medication.

The VROOM study will not impact on when and which booster vaccination an individual receives – booster vaccinations will only be delivered as part of the NHS COVID-19 vaccination programme. The study will sit alongside the national programme.

STUDY DESIGN

A 2-arm parallel group, multicentre, superiority randomised trial performed in two continuous phases: a "pilot" phase with evaluation of stop-go criteria at 4-months after the randomisation of the first participant, with pre-specified progression criteria, followed by a main trial phase.

There will be no break in recruitment as the trial proceeds from the pilot phase to the main phase unless the pre-specified progression criteria are not met.

This trial will be conducted in approximately 25 secondary care hospitals or sites delivering NHS provided care randomising 560 participants. Participants will be advised to either temporarily suspend for 2 weeks or continue on weekly low-dose methotrexate treatment (+/- hydroxychloroquine) post SARS-CoV-2 vaccine booster delivered by the national vaccination programme either in vaccination centres or in GP surgeries.

We anticipate the use of either messenger Ribonucleic Acid (mRNA) or protein subunit technologies in the booster vaccination programme.

Participants will be followed up for 12 weeks from their booster vaccination.

Experimental intervention: To suspend methotrexate for two weeks immediately after receiving the SARS-CoV-2 booster vaccination.

Control intervention: To continue on the same dose of methotrexate as usual after SARS-CoV-2 booster vaccination.

Participants will be advised to only interrupt or continue methotrexate. If they are on other treatments for their condition e.g. hydroxychloroquine, oral prednisolone, topical treatments etc. these should not be discontinued.

Primary outcome

- Anti-spike RBD antibody level at 4 weeks post booster vaccination.

Secondary outcomes

- Level of anti-spike RBD antibody at 12 weeks post booster vaccination.
- Patient assessments of disease activity: global assessment using a numeric rating scale with one-week recall at baseline, 2, 4 and 12 weeks post booster vaccination, current disease activity level and change since booster, 4 and 12 weeks post booster vaccination.
- Disease flare-up and actions taken to deal with them at 4 and 12 weeks post booster vaccination.
- Effect on quality of life (assessed using EQ-5D-5L) at 4 and 12 weeks post booster vaccination.
- Adherence with advice to interrupt or continue methotrexate: self-report at 2 and 4 weeks post booster vaccination.
- COVID-19 neutralising titre (mechanistic sub-study only) at 4 and 12 weeks post booster vaccination.
- Adherence to methotrexate allocation at 4 and 12 weeks post booster vaccination (*mechanistic sub-study only*).

Safety outcomes

- Serious Adverse Events (recorded from booster vaccination to 12 weeks post vaccination).

Stopping rules and discontinuation

The pilot phase has explicit stop-go criteria listed in Table 1 at 4-months after the randomisation of the first participant.

	Black	Red	Amber	Green
% of expected recruitment	<25%	26-50%	51-75%	>90%
Self-reported adherence to intervention	<40%	41-60%	61-80%	>80%
Action	Stop	Continue – major action needed in discussion with EME programme; protocol review, assess and resolve barriers, assess feasibility of improvement	Continue – action needed; assess and resolve barriers to recruitment/ adherence	Continue – no action needed

Table 1: Stop/go criteria

A single interim analysis is planned to take place approximately at eight months after the start of recruitment (once primary outcome data are available for 250 participants).

Decisions for stopping the trial early for benefit or futility will be based on a Haybittle-Peto stopping boundary ($p \leq 0.001$ for the primary endpoint at 28 days), but also taking account of the representativeness of the study population, magnitude of estimated effect, sufficient participants having been recruited into the important subgroups, sufficient data being available for the mechanistic components of the study, and attrition.

The Independent Data Monitoring Committee will be responsible for making a recommendation to the Trial Steering Committee. All meeting regarding decisions to continue or stop the trial will be minuted and held in the Trial Master File.

Randomisation and Blinding

Eligible patients will be randomised when they receive a date for their booster vaccination. Participants will be asked to contact the CTU and randomisation will be performed using the centralised validated computer randomisation program through a secure (encrypted) web-based service, REDCap and RRAMP (https://rramp.octru.ox.ac.uk), provided by the Oxford Clinical Trials Research Unit (OCTRU).

Participants will be asked to confirm that their health circumstances and consent given since their baseline visit have not changed. If no changes are reported they will be randomised. If they report a change the participants will be referred back to the recruiting site – to decide if

they are still suitable for inclusion in the study. If the participant is still eligible to be randomised then the site will log into the randomisation system to randomise the participant.

The randomisation system has a minimisation algorithm to ensure balanced allocation across treatment groups, stratified in a 1:1 ratio to either continuing their methotrexate taking as usual or to have a 2 week temporary suspension immediately following their COVID-19 booster vaccination.

The trial will use the minimisation factors of:

- inflammatory condition type (inflammatory rheumatic disease (+/- skin disease) or skin disease alone),
- age group (<40 years, 40-64 years, ≥65 years), and
- previous vaccination platform received (mRNA or vector or combination).

Randomisation is being minimised on the above factors as the magnitude of immune-response differs between mRNA and other SARS-CoV-2 vaccine that have been used in the UK in the first cycle of vaccinations, and younger age increases the immune response to vaccines[40]. We chose not to minimise on past COVID-19 infection even though it is a strong modifier of serological response to SARS-CoV-2 vaccines[39,41,42], as it is difficult to ascertain this reliably from participant self-report at the time of randomisation. We will however obtain the past infection status using anti-nucleocapsid antibodies and account for this in any analyses as required. This is likely to be more reliable than participant self-report based on constellation of symptoms, recall of physician diagnosis, and test results. Given the shielding recommendations in the UK and evidence now emerging that shielding was high in the UK for rheumatology patients, we anticipate the proportion of participants previously infected with the SARS-CoV-2 virus to be less than 10%[54].

To ensure the unpredictability of treatment allocation the minimisation algorithm will include a probabilistic element which entails a small number of initial participants randomised by simple randomisation.

The mechanistic study sub-sample will be 100 randomised participants who have given samples at baseline, 4 and 12 weeks post COVID-19 booster vaccination.

Maintenance of randomisation codes and procedures for breaking code

Due to the nature of the study intervention, the participants and the clinical team will not be blind to the allocated arm of the study. However, those analysing the study samples will be blinded to the participants' allocation.

STUDY MANAGEMENT

The Chief Investigator has overall responsibility for the study and shall oversee all study management. The data custodian will be the Chief Investigator.

Study Committees

Data Monitoring Committee (DMC)

The DMC is a group of independent experts external to the study who assess the progress, conduct and critical outcomes of the study. The study DMC will adopt a DAMOCLES based charter, which defines its terms of reference and operation in relation to the oversight of the study. The DMC will meet regularly throughout the study at time-points agreed by the Chair of the Committee and the CI. At a minimum this will be on an annual basis. The DMC will review the safety data generated, including all serious adverse events, and make recommendations as to whether the protocol should be amended to protect patient safety. Recommendations of the DMC will be discussed between the CI, TSC, CTU and the Sponsor.

Study Steering Committee (SSC also known as the TSC)

The role of the Study Steering Committee (SSC) is to provide the overall supervision of the study. They will monitor the study's progress and conduct, and will advise on scientific credibility. The committee will consider and act, as appropriate, upon the recommendations of the DMC and ultimately carries the responsibility for deciding whether the study needs to be stopped on grounds of safety or efficacy. The committee includes independent members and members of the research team, and provides overall supervision of the study on behalf of the funder. Its terms of reference will be agreed and will be recorded in a TSC charter.

Trial Management Group (TMG)

The Trial Management Group (TMG) consists of those individuals responsible for the operational management of the study such as the chief and lead-co-investigator, EMR operational lead, the trial manager and the trial statistician. Other specialities/ individuals will be invited as required for specific items/issues.

The TMG will meet usually at least once a month throughout the lifetime of the study and will:

- Supervise the conduct and progress of the study, and adherence to the study protocol;
- Assess the safety as compiled by the study team and assessed by the DMC;
- Evaluate the quality of the study data;
- Review relevant information from other sources (e.g. related studies);
- Escalate any issues for concern to OCTRU, specifically where the issue could compromise patient safety or the integrity of the study or quality of the study data.

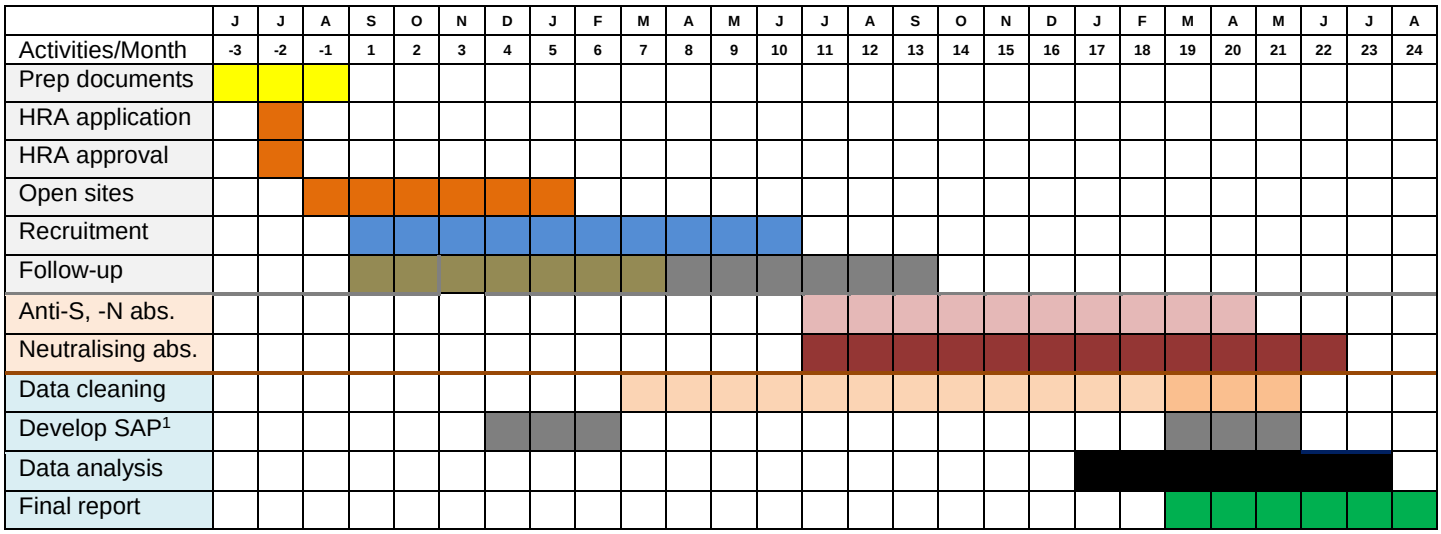
Duration of the Study and Participant Involvement

Study Duration

The study is funded for a period of 24 months, with 3 months proposed for set up, 10 months recruitment, 3 months follow-up, 12 months for laboratory analysis, 9 months for data analysis and write-up.

Some of the activities will be concurrent and data cleaning and data chasing will be a continuous process throughout the study. The study flow is also heavily dependent on the national booster vaccination programme roll-out – if this gets delayed this would impact this study. Also if the vaccination programme is put into abeyance then so would this study.

Study Gantt Chart



[1]statistical analysis plan

Figure 4: Study overview Gantt Chart

Participant Duration:

Participants will be in the study for 12 weeks following the receipt of their booster vaccination. However, participants will physically be registered in the study from when they express their interest in participating. If the booster vaccination programme is rapid it may mean that from an individual expressing their interest in the study, being consented, randomised and data collected in the 12 weeks following the receipt of a booster vaccination – this may be a period of approximately 16 weeks. However, the VROOM study has no control over when booster vaccinations will be offered to participants.

End of the Study

The end of study is the point at which all the clinical data has been entered and queries resolved in the study REDCap database, and all laboratory outcomes have been analysed and any queries also resolved.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

Participants will be recruited from participating secondary care rheumatology and dermatology clinics where lists are held for clinical care purposes of those on long term subcutaneous or oral methotrexate therapy.

Sites will be requested to review recent clinic or clinical databases lists to identify potentially eligible participants i.e. those on methotrexate monotherapy +/- hydroxychloroquine. The initial approach will be via a study recruitment pack that participating sites will distribute to patients who in the previous 12 months have been listed as being prescribed low dose (<25mg/week) methotrexate. The pack will include a generic study introductory letter from

the local clinical team, Freepost return envelope, patient information sheet (PIS) and instructions on how to get more information about the study.

Posters will also be displayed in the clinic and participants may also be approached about the study by their usual clinical care team during scheduled routine remote or face-to-face consultations and given one of the study recruitment packs.

Individuals wishing to take part will then have the option to express their interest in the study either by:

- scanning a QR code on the introductory letter;
- entering into a web browser the study url included on the introductory letter;
- calling the VROOM study team on the number included on the introductory letter;
- completing the reply slip in the study recruitment pack and posting to study team via the included Freepost envelope.

The study introductory pack will include documentation only in English. There will be reference made in the pack that the PIS is also available in Urdu, Punjabi and Polish – which if the study office is contacted a version of this will be sent out. If needed, the usual hospital interpreter and translator services should be contacted to assist with discussion of the study if required, however the consent form and study questionnaires will not be provided in other languages.

It will be explained to the potential participant in the PIS that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. Participants will be made aware of importance of the data and blood samples they provide; including how valuable their continued follow-up will be to make the study results as representative as possible, even if they cannot/ did not adhere to their randomised allocation, or after they missed an appointment or response. It will also be explained that they can withdraw from their randomised intervention or follow-up at any time. In the event of their withdrawal it will be explained that their data collected so far cannot be erased, nor will their samples be destroyed.

Eligibility criteria

When an individual expresses their interest in potentially taking part in the study, they will be asked the following questions to determine if they are potentially eligible. It is envisaged that the majority of those individuals who express their interest will do so electronically and the questions will be asked electronically via the study's secure REDCap instance. Those who phone the study team, or send back the reply slip, will be contacted as they deem on their reply slip to be asked to answer the below questions:

- Are you 18 years old or older?
- Have you been prescribed methotrexate for at least the last 3 months?
- Have you had the first two COVID vaccinations?
- Have you been treated with Rituximab drip in the last 18 months or are you planning on starting it?
- Have you been diagnosed with vasculitis?
- Do you have cancer affecting your internal organs? (Please select no if your cancer has been cured, or if you have skin cancer)

- Have you received chemotherapy or radiotherapy (radiation treatment) for cancer in the previous six months? (Please select no if you are taking hormone treatment for cancer)
- Have you received any of the following treatments within the last two months? Leflunomide, ciclosporin, azathioprine, sulfasalazine, mycophenolate, apremilast, biologic agents.

Depending upon the answers to the above – if the potential participant is deemed potentially suitable – the database system will flag up that they meet these inclusion criteria and none of the exclusion criteria – they will be asked to give their name, address, date of birth, NHS or CHI number, phone number(s) and email address to the central study co-ordinating team. The hospital that sent them the introductory pack will be informed that they are potentially suitable for the study and have shown an interest in participating.

Potential participants will then to asked to attend for a study visit at the hospital – eligibility will be confirmed, for those eligible written consent will be sought and baseline data and bloods taken (7ml).

Inclusion criteria

- Age ≥18 years;
- Diagnosed with inflammatory conditions such as rheumatoid arthritis, psoriasis with or without arthritis, seronegative spondyloarthritis, reactive arthritis, atopic eczema, polymyalgia rheumatica, systemic lupus erythematosus;

Note: *This is not an exhaustive list and people with other inflammatory conditions where treatment may be interrupted for two weeks without the risk of substantial increase in disease activity, or organ or life-threatening flare up will also be eligible to participate in the study in order to increase the generalisability of the study.*

- Prescribed with oral or subcutaneous methotrexate (≤25 mg/week) +/- hydroxychloroquine for at least the previous three months;
- Able to temporarily suspend methotrexate for two weeks in the opinion of patients' hospital team without the risk of substantial increase in disease activity, or organ or life-threatening flare up;
- Able to give informed consent;
- Eligible for planned booster vaccination for COVID-19 (have received any 2 vaccinations from the original NHS COVID Vaccination Programme 2020/21) and yet to receive a booster vaccination.

Exclusion criteria

- Diagnosed with inflammatory conditions for which treatment cannot be interrupted safely: such as ANCA associated vasculitis, large vessel vasculitis, myositis, giant cell arteritis, solid organ transplant;
- Treated with Rituximab drip in the last 18 months or planning to start it;
- Concurrent immune suppressive treatments in the last two months specifically: leflunomide, ciclosporin, azathioprine or mercaptopurine, sulfasalazine or other 5-amino-salicylic acid drugs, mycophenolate, apremilast, biologic agents;

- Radiotherapy or cancer chemotherapy in last six months;
- Prednisolone dose >7.5 mg/day within 30 days of randomisation;
- Active solid organ cancer (people with skin cancer or those cured of solid organ cancer are eligible);
- Already participating in a CTIMP (clinical trial of an investigational medicinal product) or planning to participate in a CTIMP during the 12 week study period.

Expected duration of participant participation

Study participants will be participating in the study for 12 weeks after receiving their COVID-19 booster vaccination.

Removal of participants from therapy or assessments/Participant Withdrawal

During the course of the study a participant may choose to withdraw from the study investigation/ follow-up at any time. This may happen for several reasons, including but not limited to:

- The occurrence of what the participant perceives as an intolerable SAE;
- Inability to comply with study procedures;
- Another reason.

The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data and samples collected to date cannot be erased/destroyed and may still be used in the final analysis.

In addition, the Investigator may discontinue a participant from the study investigation at any time if the Investigator considers it necessary for any reason including, but not limited to:

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with the investigation or study requirements
- Clinical decision including a decision that it is unsafe to interrupt methotrexate dosing

Withdrawn participants will not be replaced. The type of withdrawal and reason for withdrawal will be recorded in the CRF.

Informed consent

Informed consent will be obtained by means of a completed eConsent form, by electronic completion in clinic on a tablet device with a member of the local study team. All participants need to provide written informed consent by means of a participant dated electronic signature before they enter the study. The signature will be either achieved by a finger tracing across a tablet device, or using an electronic stylus on a tablet device or using a mouse dragging the cursor across the screen – all methods are to be used as if signing with a traditional pen. The Investigator will explain the details of the study in addition to the already presented Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. A member of the site research team (authorised to do so on the delegation log) will answer any questions that the participant has concerning study participation then add their own dated electronic signature to the consent form.

Informed consent will be collected by a member of the study team listed on the delegation log from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of the consent will be emailed to the participant or a nominee of their choice if they do not have an email address and the site will be able to download a copy of the consent form from the study's electronic system.

Consent for inclusion in the VROOM study will be obtained on the day of the baseline samples and baseline data collection.

The person who obtains the consent must be suitably qualified and experienced and have been authorised to take consent by the site's Principal Investigator.

VROOM aims to be a paperless study, but if necessary, a paper Consent form can be used.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the study, continuing consent will be obtained using an amended Consent form which will be signed by the participant.

STUDY TREATMENT AND REGIMEN

This study has no study treatment delivered per se, it is about temporarily suspending a commonly used medication for inflammatory conditions – methotrexate.

Figure 5 shows the patient flow through the study and Table 2 the schedule of participant interactions for those who decide to participate in the study.

All interactions for this study are all research exposures, due to the fast nature of the roll out of the initial COVID-19 vaccination programme and expected roll out of the booster programme it is imperative that participants are recruited prior to receiving their booster hence all visits are undertaken outside of routine out-patient clinics.

After registering their interest in the study with the central study team in Oxford. The following information will be recorded on a secure web-based form in the study database system (REDCap) by the attending clinician, participant or delegate including a member of research team to enable follow-up:

- Patient details e.g. name, Hospital number, address, NHS/CHI number, date of birth, telephone number, email address, GP name and GP address.

The GP details are required to allow the central trial team to send a letter to the patient's GP informing them of their VROOM participation. The email address will enable a copy of the completed consent form to be sent to the patient or at their request a different individual for safekeeping. Depending upon patient preference the email /postal address and/or telephone may be utilised for follow up questionnaires, reminders/text messages, results and thank you acknowledgements.

The details of the individuals are passed to the local hospital from where the study approach letter pack will have been sent from. The hospital (care centre) will then further screen the potential participants and invite those potentially suitable into the hospital (care centre) if the individual is suitable consent will be requested and baseline data and a baseline blood sample taken (2 tubes of 3.5ml in Serum Stabilisation Tubes). All of the baseline data being

requested will be asked of the participant, however their medical notes will be reviewed and data extracted for the participants latest levels of serum creatinine and serum albumin measured as part of the routine care for their inflammatory condition, as this data will be used to classify the clinical status of participants with regard to their ability to interact with methotrexate – as methotrexate is protein bound, and creatinine levels are to report on any kidney disease – this is purely to describe the patient population – there are no levels that would be recorded that could exclude an individual..

Individuals will then be asked to contact the central study team in Oxford when they receive their booster date.

To remind participants of the need to notify the study team, reminder texts will be sent out on a weekly basis to those participants who indicate on their consent form that they would be happy to receive booster date reminder texts. The wording of the text will say:

'A message from the VROOM study team. Thank you for agreeing to take part in the VROOM study. We are contacting you to find out if you have received a date for your COVID-19 booster vaccination yet. If you have please could you click this link to take you to the VROOM web portal to enter your vaccination date, or alternatively please could you call the VROOM study team on 0808 196 2101or by emailing vroom@ndorms.ox.ac.uk.'

Alternatively for those who agree to be telephoned or emailed by the study office, calls or emails will be made to see if they have had a booster vaccination on a weekly basis.

On receiving a date, if the participant notifies through the study database they will be directly taken to the randomisation system for the study. On entry of a Booster date into the VROOM study database participants will be asked to confirm that their health circumstances and consent given since their baseline visit have not changed, if nothing has changed they will immediately be randomised. If they report a change the participants will be referred back to the recruiting site – to decide if they are still suitable for inclusion in the study. If the participant is still eligible to be randomised then the site will log into the randomisation system to randomise the participant.

Where an individual does not notify the central study team through the study database, the database will be accessed by a member of the central study team on receipt of a communication from a participant and the individual will be randomised by a member of the central study team and the participant informed of their allocation.

The randomisation system will allocate a participant to either the control or experimental (treatment) group.

If another vaccine is also being given at the same time such as for seasonal influenza this is not an exclusion and data will be recorded on co-administration of any other vaccines.

Treatment Regime:
Control group – nothing changes for the participant in their methotrexate taking – individuals will be asked not to alter how, when and how much methotrexate they take as part of their standard inflammatory condition care.

Experimental group – individuals in this group will be asked to suspend their methotrexate treatment for two weeks immediately after they receive their SARS-CoV-2 booster vaccination then recommence methotrexate treatment after 2 weeks of methotrexate doses have been missed.

If the participant is due to take their methotrexate on the day they are to receive the booster – they should refrain from taking their methotrexate on that day and then also the dose due a week later, therefore for these individuals it will strictly be temporarily suspending one dose on the same date as the SARS-CoV-2 vaccine booster and one post vaccination. . In all other cases, the two doses due immediately after receiving the booster vaccination are the ones to be missed – therefore there will be a range of 1-6 days since last methotrexate dose when vaccines are received.

Explicitly the study aims for those in the experimental intervention group to miss (temporarily suspend) two doses of methotrexate- where a dose can range from 7.5-25mg/week always taken on the same day of the week. This is 2 weekly doses not a reduction in the dose of methotrexate taken. For the majority of individuals in this group this will mean that the 2 doses of methotrexate scheduled to be taken in the weeks after their vaccination will be missed, for those who receive their booster vaccination on the day they usually take their methotrexate – this dose is missed and the one the following week – this still results in 2 suspended doses.

The study only requires participants to temporarily suspend their methotrexate for 2 weeks if they are assigned to the treatment arm – any other associated treatments that they take can be continued such as folic acid (however if participants decide to stop taking their folic acid during the 2 week suspension of methotrexate this is acceptable and should not be considered a protocol deviation).

The study database systems will send out reminder texts /electronic links about allocation during the 2 weeks following the booster date, this will include collecting data on what methotrexate has been taken over the 2 weeks and how a participant's inflammatory condition has affected them over the preceding 7 days. (Depending upon an individuals preferences recorded on their consent – these checks will be by text, email or short phone calls).

The recruiting site will be notified when one of their participants has a booster vaccination date scheduled. After this date has passed the site will be in touch with the participant to check that the vaccination has occurred and then to arrange their subsequent 4 and 12 week visits.

If the participants are happy to receive the patient reported outcome measure questionnaires at 4 and 12 weeks to be completed remotely – these can be sent electronically by the central CTU team by emails to reduce the time taken at an individual's hospital visit and result in timely completion of these instruments, and also help in reducing missing data if participants fail to attend follow up visits.

Where a participant fails to attend a study visit – the central and site study team will attempt to collect the data due at these points using the contact methods agreed to via the consent form.

At the 4 and 12 week visits – data will be collected and repeat blood samples (2 tubes of 3.5ml in Serum Stabilisation Tubes) will be taken as at the baseline.

After the 4 and 12 week visits have been completed, each time a postcard will be sent from the study office to thank a participant for their continued involvement in the study.

At the end of the study, participants will receive a copy of the results and a small token of appreciation for their continued involvement in the study.

Data to be collected:

Baseline Data
Completed at hospital by local study team member with participant

- Date of baseline visit
- Participant demographics
    - Ethnicity
    - Smoking status
    - Serum creatinine, serum albumin (Latest available in hospital records)
    - Diagnosed inflammatory diseases – rheumatoid arthritis, psoriasis with arthritis, psoriasis without arthritis, seronegative spondyloarthritis, reactive arthritis, atopic eczema, polymyalgia rheumatica, systemic lupus erythematosus, other.
    - Comorbidities – diabetes including diet-controlled diabetes, hypertension, ischaemic heart disease, congestive cardiac failure, asthma, COPD, high cholesterol, stroke including transient ischaemic attack.
    - Biological sex
    - Height/weight
    - Concomitant systemic medications – specifically corticosteroids, hydroxychloroquine, anti-diabetic drugs and folic acid
    - Usual residence (home or residential care)
- COVID-19 Disease and Vaccination history
    - First COVID-19 vaccination – date
    - First COVID-19 vaccination – which vaccination was given?
    - Second COVID-19 vaccination – date
    - Second COVID-19 vaccination – which vaccination was given?
    - Any previous COVID-19 positive PCR tests, previous hospitalisations due to COVID-19, COVID-19 instances not requiring hospitalisations
- Methotrexate and associated drug administration
    - Usual day of the week Methotrexate is taken
    - Dose of Methotrexate prescribed
    - Usual method of administration of Methotrexate
    - Usual dose of hydroxychloroquine (if taken)
- Patient global assessment of disease activity: assessed on a 0-10 numeric rating scale for past week – specifically 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?

Poor	0	1	2	3	4	5	6	7	8	9	10	Excellent

- Quality of life (assessed using EQ-5D-5L)

Booster appointment booked
Completed remotely either by participant in study system or by contacting study team to arrange for data to be captured

- Date of planned booster appointment

2 weeks post booster (+ 5 days)
Completed remotely either by participant in study system or by contacting study team to arrange for data to be captured

- Check on protocol compliance – has methotrexate been temporarily suspended or continued as per protocol?
- Patient global assessment of disease activity: assessed on a numeric rating scale 0-10 for past week – specifically 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?

Poor	0	1	2	3	4	5	6	7	8	9	10	Excellent

4 weeks post booster vaccination (+/- 10 days)
Completed at hospital by member of local study team and participant
Note: If a patient is unable to attend this information could be collected remotely but this would mean the blood sample is missed

- Date of visit
- Confirmation of date of booster vaccination date
- Name/brand of COVID-19 booster vaccination received
- Were any other vaccinations given at the same time e.g. influenza?
- Confirmation of protocol compliance – was methotrexate temporarily suspended or continued for 2 weeks as per the protocol?
- What doses of Methotrexate were taken in weeks 3 and 4?
- Dose of Methotrexate currently prescribed
- Route of methotrexate administration
- Concomitant systemic medications – specifically corticosteroids, hydroxychloroquine, anti-diabetic drugs and folic acid any changes from baseline
- VROOM Condition and flare questionnaire
- Any serious adverse events
- Quality of life: EQ-5D-5L questionnaire

12 weeks post booster vaccination (+/- 10 days)
Completed at hospital by member of local study team and participant
Note: If a patient is unable to attend this information could be collected remotely but this would mean the blood sample is missed

- Date of visit
- Dose of Methotrexate currently prescribed
- Route of methotrexate administration
- Concomitant systemic medications – specifically corticosteroids, hydroxychloroquine, anti-diabetic drugs and folic acid any changes from baseline
- VROOM Condition and flare questionnaire
- Any serious adverse events
- Quality of life: EQ-5D-5L questionnaire

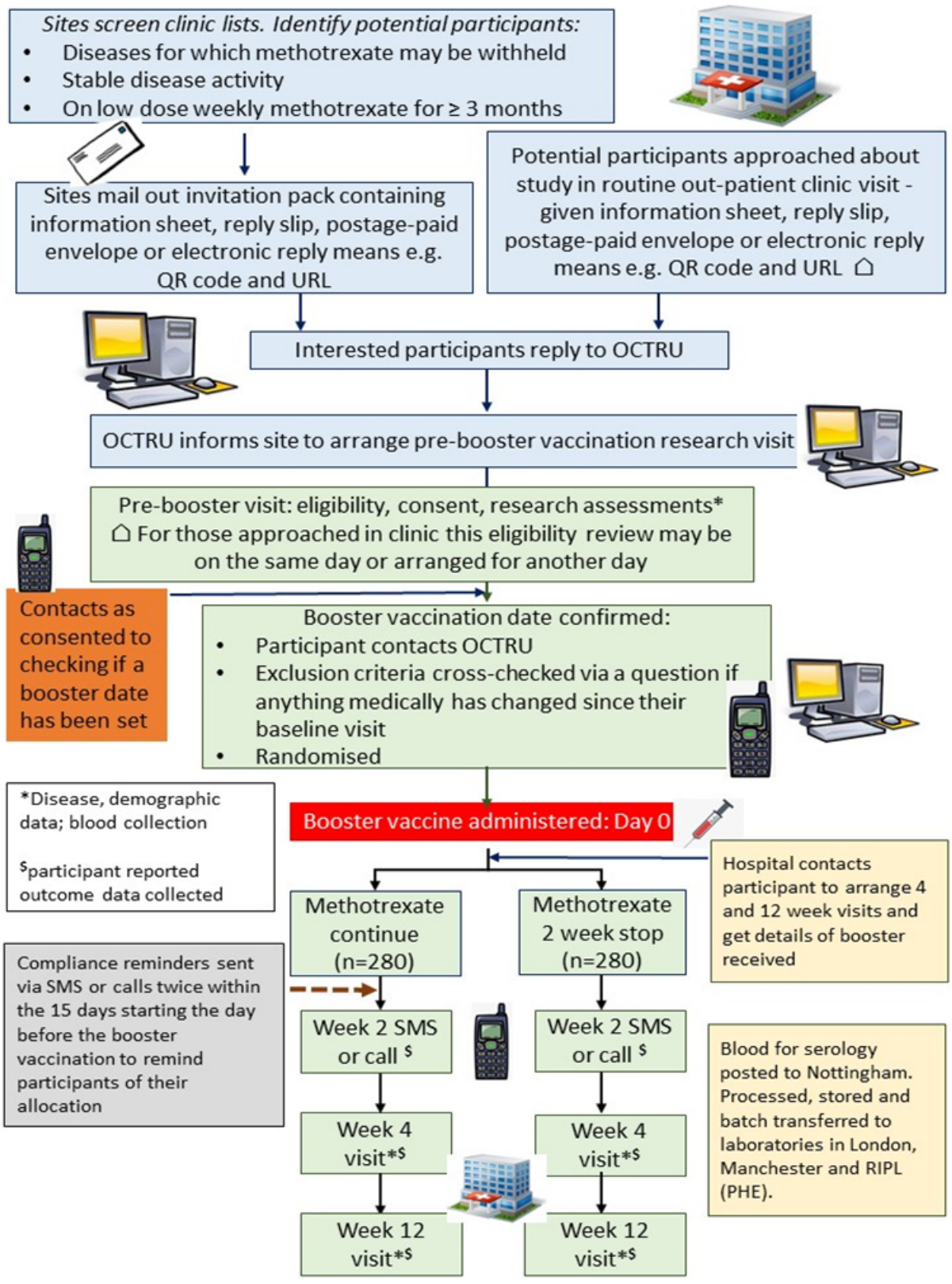


Figure 5: VROOM study flow

Assessments	Pre-booster (Baseline)[1]	Booster vaccine date known[2]	In the week after the booster date [2]	2 weeks post booster[2]	4 weeks post booster[1]	12 weeks post booster[1]
Clinical study						
Demographic	+					
Height/weight	+					
Current Medication	+				+	+
Comorbidities	+					
Past SARS-CoV-2 vaccine	+					
Blood sample taken for anti-spike-RBD antibody	+				+	+
Disease activity	+			+	+	+
Quality of life	+				+	+
Disease flare up					+	+
Reminder of allocation and to continue or withhold methotrexate			+			
Adherence to intervention				+	+	
Thank you note sent after visit	+				+	+
Safety				+	+	+
Date of vaccination known		+				
Randomisation		+				
Details of vaccination					+	
Mechanistic						
Blood sample taken for neutralisation assay[3]	+				+	+
Blood sample taken for methotrexate adherence bioassay[3]	+				+	+

Notes: [1]Face to face in clinic [2]Remote via text, email or phone call [3] In a subset of 100 participants

Table 2: Schedule of participant interactions

Adherence

SMS (text) messages where a participant consented to receiving these will be sent out during the 15 days around the participant's booster date.

Participants always take their methotrexate on the same day and this is recorded at baseline – and the date of booster vaccination will be recorded prospectively - this data will be used to schedule the contact

For those who do not reply to the 2 week questions, adherence to their allocation will be checked at the 4 week visit as some participants may not respond or be able to respond to the 2 week check.

Where an individual does not have access to a mobile phone, emails will be sent or phone calls made instead. All communications with participants will be as per the contact methods they give to the Central Study Team and to which they indicate in their contact preferences.

Criteria for terminating study

See section of Stopping rules and discontinuation for details.

Transport and Storage of the Blood Samples

Participants should have a 7ml whole blood sample collected at each visit in a 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. Tubes will be labelled with participants study ID and date of blood draw and initials.

On receipt of samples they will be centrifuged and the serum aliquoted into separate 0.5ml vials and stored in -80°C freezers.

They will be batch transferred on dry ice with all shipments containing a complete inventory of all samples, along with the name of the person responsible for sending the samples to the respective laboratories:

- For assessing anti-spike-RBD and anti-nucleocapsid antibodies to the laboratories at Porton Down, PHE (one vial);
- For assessing neutralisation titres to the laboratories at Queen Mary University, London (one vial);
- For assessing methotrexate levels to the laboratories at Manchester NHS Foundation Trust (one vial).

Remaining vials will be stored for future research and as a backup for this study in case any analyses need to be repeated.

Once analysis has taken place, samples will be stored within the Research Tissue Bank for future research (DI William Dunn- Licence Number 12265) if participants are agreeable and sign the optional clause on the consent form.

Where participants do not agree to the future use of the samples they will be destroyed in accordance with the Human Tissue Act, 2004.

Laboratory Analyses

A sample handling manual will be produced for the study, this will list all how samples will be processed and all the data to be generated from the study samples and how this should be interpreted.

Assessment of immunity and biomarkers:

[1] Anti-spike-RBD and nucleocapsid antibodies:
Antibody measurements will be undertaken at the Rare & Imported Pathogens Laboratory at Public Health England (PHE) using validated commercial assay: the Euroimmun anti-SARS-CoV-2 enzyme-linked immunosorbent assay (ELISA) targeting IgG specific for S1, and the Roche Elecsys Anti-SARS-CoV-2 electrochemiluminescence immunoassay that detects antibodies (including IgG) for nucleocapsid protein[46,47]. The reported assay sensitivity (92.3% and 96.2%-100% for Roche and Euroimmune respectively) and specificities (100%) are high.

[2] Neutralising antibody titres:
Co-applicant McKnight and her team at Queen Mary University London have expertise in this field and their neutralisation assays with authentic live virus (Wuhan Hu-1 SARS-CoV-2) have been previously described in detail[23]. All experiments will be conducted in duplicate and absorbance readings will be standardised against positive and negative controls, and averaged. Neutralisation curves will be plotted, with the percentage neutralisation modelled as a logistic function of the serum dilution factor (log10). A non-linear regression (curve fit) method will be used to determine the dilution fold that neutralised 50% (IC50) of samples.

[3] Methotrexate biomarker:
This biochemical assay was developed and validated by co-applicant Bluett[38] and subsequently optimised in Manchester NHS Foundation Trust. It uses liquid chromatography–tandem mass spectrometry (LC-MS) performed on a Waters TQ-S micro Triple Quadrupole Mass Spectrometry. It provides an objective measurement of adherence and has been developed with drug concentration limits according to methotrexate dose and detects methotrexate partial omission or delayed ingestion. Samples are spiked with an internal standard and a calibration curve with quality control samples is undertaken during each run. Adherence, defined as ingestion of methotrexate in the past 6 days is dichotomised.

STATISTICS

Statistical Analysis Plan (SAP)

Full details of the statistical analysis will be detailed in a separate statistical analysis plan (SAP) which will be drafted early in the study and finalised prior to the interim analysis data lock, and will receive review and input from the TSC and DMC. Stata (StataCorp LP) or other appropriate validated statistical software such as R (R Core Team) will be used for analysis. A summary of the planned statistical analysis is included here.

Methods

The principal analysis will be performed on the as randomised ("intention to treat") population, analysing participants with available outcome data in their randomised groups, regardless of adherence. A full statistical analysis plan will be agreed before the start of the analysis, and analyses will be performed in Stata. The study will be reported in line with Consolidated Standards of Reporting Trials (CONSORT) guidelines.

The primary objective of the statistical analysis is to identify if a temporary two week suspension of methotrexate after the booster vaccination against SARS-CoV-2 increases the anti-spike-RBD antibody at 4 weeks post booster vaccination compared to continuing methotrexate. The anti-spike-RBD antibody levels will be summarised descriptively at baseline and four, and 12-weeks post booster vaccination follow-up. The differences between the study arms will be estimated a using multi-level mixed effects regression model, allowing for repeated measures clustered within participants. The model will be adjusted for randomisation factors (inflammatory condition, age categories, prime vaccine's platform i.e. mRNA vs. other) and also prior infection status obtained from pre-vaccination anti-nucleocapsid antibodies and type of SARS-Cov-19 vaccine booster received as fixed effects. We anticipate the use of mRNA or protein subunit technologies for booster vaccination. A treatment by time interaction will be included. The model is anticipated to use an unstructured covariance matrix, and maximum likelihood estimation. Data will be log-transformed prior to analysis, if appropriate. Model diagnostics, including approximate normality of the residuals, will be assessed. Adjusted mean differences between the groups will be presented, together with 95% CI and p-values.

Consistency of the treatment effects for important prognostic subgroups (methotrexate dose, inflammatory condition type, age group, previous SARS-CoV-2 infection, prime vaccination platform, booster platform, route of administration of methotrexate: subcutaneous versus oral) will be explored with 95% CIs. The subgroup effects will be obtained from linear regression models for the 4-week primary outcome, adjusted in line with the above model specifications, and an interaction between randomised treatment and subgroup. We will also explore the time effect of delay between the original vaccination and the booster. Findings will be presented graphically and viewed as exploratory. Supplemental analysis will exclude participants who self-report non-adherence to their randomised allocation, and those that were unable to receive their booster vaccines. The frequency of deviations from the randomised intervention, with reasons where available, will be presented. Similar analyses will be performed for week 12 data.

Continuous secondary outcomes will be analysed using generalised linear models for binary and continuous data, as appropriate, with model adjustment as described above.

The number of serious adverse events (SAE) will be presented by treatment arm. The proportion of participants with at least one SAE will be compared. Details of the events, including expectedness and relatedness of the SAEs will be presented, together with information on the timing of the events.

Further analyses related to the mechanistic hypotheses will be carried out exclusively on the mechanistic sub-sample including quantifying levels and strength of relationships using appropriate statistical summarises including (mean, SD, range, correlation coefficients etc). Neutralising antibody titres will be compared between the two groups at different time points using parametric or non-parametric tests depending on data distribution. Additionally, the proportion of samples with IC50 greater than 49 will be compared between the two groups as this has been shown to prevent clinical infection in Rhesus macaques[25]. Other analyses will look at proportion with titres above 200. Sensitivity analysis for primary and key secondary

outcomes will be conducted in participants' adherent with methotrexate using validated biochemical measurement[38]. In our statistical analysis, we will explore the effect of non-compliance to the randomised intervention using per-protocol and complier-average causal effects analyses.

Timing of analyses

The final unblinded (to the study's non-statistician investigators) statistical analysis will take place after all follow-up has been completed, and sufficient time has been allowed for data collection and cleaning.

A single interim analysis is planned to take place approximately at eight months after the start of recruitment (once primary outcome data are available for 250 participants).

Analysis will be conducted on University of Oxford computers and all data will be held and backed up on University of Oxford servers.

Sample size and justification

Main trial:

A total of 560 participants will be randomised into this study. The sample size estimates were derived from Folegatti et al, which allow the mean (SD) of the anti-spike IgG 28 days after vaccination to be estimated as 191.9(165.5) ELISA units[24,48,49]. The sample size is based on detecting at least a 25% higher antibody response in the methotrexate discontinuation group with 90% statistical power at 2-sided 5% significance level which requires data form 502 participants; leading to 560 after allowing for up to 10% missing data. This calculation was performed using the Stata version 15.1 'power twomeans' command.

Initial studies indicate that anti-spike-RBD antibody will soon emerge as a correlate of protection[44] from COVID-19 as it correlates strongly with viral neutralisation titres[24-29,43]. Methotrexate reduces B cell (CD19+) count by 15% and its effect on antibody production may be greater given the other effects on B cell[30-33]. Interrupting methotrexate for two weeks improved the titre of antibodies against H1N1, H3N2, B-Yamagata, and B-Victoria strains in the quadrivalent influenza vaccine by 59%, 92%, 50% and 68% respectively[20]. Taking these two into account, and given the lack of certainty around the serological correlate of protection from COVID-19 alongside a higher risk of serious complications including death than with seasonal influenza, we have powered the study for detecting a much smaller difference. Our team does not think a <25% difference in anti-spike-RBD antibody will be of immediate clinical relevance per se as the vaccines and infections elicit a broad range of antibody responses, and, most vaccines have very high level of protection from SARS-CoV-2 infection and complications[23-26, 50-52].

However, a 25% lower anti-spike-RBD titre at baseline will result in a shorter time during which the individual is protected from infection or severe COVID-19. The thresholds for 50% protection from both of these outcomes have been derived by Khoury and coworkers[27] . They estimated that the neutralization level for 50% protection against detectable SARS-CoV-2 infection to be 20.2% of the mean convalescent level. Using the half life estimated Dan et al [53] of 108 days and an exponential decay curve we find that a 25% reduction in initial antibody titres from vaccination will result in 45 days shorter span at which an individual will have titres corresponding to protection from infection or protection from severe COVID-19.

Mechanistic sub-study:
The mechanistic study sub-sample will be 100 randomised participants who have given samples at baseline, 4 and 12 weeks post COVID-19 booster vaccination. This will enable detection of a difference between treatment arms of 0.6 standard deviation (SD) with 80% power, 5% significance level and allowing for 10% loss to follow-up; this means this sample will enable detection of increases in the methotrexate interruption arm compared to the control arm of 54% (48.2) for neutralisation assay using pseudo-viruses, based on an observed mean (SD) of 91.0 (81.6) [24].

These differences are in the same order of magnitude as the increase in HAI antibody titres with the quadrivalent influenza vaccine and we expect to see similar changes in our study[20]. Our mechanistic study will have a larger sample size (45 participants per arm after accounting for loss to follow-up), which may result in decreased variances and hence additional power for the corresponding analyses.

Decision points

The pilot phase has explicit stop-go criteria listed below at 4-months after the randomisation of the first participant as defined in the Stopping rules and discontinuation section of this protocol.

Stopping rules

An independent DMC will review the accumulating data at regular intervals and may recommend pausing or stopping the study in the event of futility concerns, and in line with the planned interim analysis.

Procedures for missing, unused and spurious data

The procedure for handling spurious or missing data will be described in the Statistical Analysis Plan, and the Data Monitoring and Sharing Plan. The study will attempt to collect data as completely as possible.

Missing data will be minimised by careful data management, information provided to participants and training of study staff. Missing data will be described with reasons given where available; the number and percentage of individuals in the missing category will be presented by intervention arm. All data collected on the database will be used, since only essential data items will be collected. No data will be considered spurious in the analysis since all data will be checked and cleaned before analysis.

The nature and mechanism for missing variables and outcomes will be investigated, and if appropriate multiple imputation will be used. Sensitivity analyses will be undertaken assessing the underlying missing data assumptions. Any imputation techniques will be fully described in the Statistical Analysis Plan. The effect of deviations from the missing at random assumption made in the primary analysis will be explored by considering a range of plausible missing not at random scenarios, whereby participants with missing outcomes will be assumed to have worse outcomes than participants with available data. These sensitivity analyses will be implemented using pattern mixture models using Stata's 'rctmiss' command or similar.

Procedures for Reporting any Deviation(s) from the Original Statistical Plan

A detailed statistical analysis plan will be drawn up early in the study with review and appropriate sign-off following OCTRU SOPs. Any changes to the statistical analysis plan during the study will be subject to the same review and sign-off procedure with details of changes being included in the new version. Any changes/deviations from the original SAP will be described and justified in protocol and/or in the final report, as appropriate should these occur.

Definition of populations analysed

The principal analysis will be performed on the as randomised (ITT) population, whereby participants will be analysed according to their randomisation allocation, irrespective of compliance with the protocol. If appropriate, additional analysis population, such as a per protocol (PP) population, will be defined in the SAP. A PP population may exclude participants who deviate from specific aspects of the protocol.

ADVERSE EVENTS

Definitions

An adverse event is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.

An AE does include a / an:
1. exacerbation of a pre-existing illness.
2. increase in frequency or intensity of a pre-existing episodic event or condition.
3. condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.
4. continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE does not include a / an:
1. medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that lead to the procedure is an AE.
2. pre-existing disease or conditions present or detected at the start of the study that did not worsen.
3. situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).
4. disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.
5. overdose of concurrent medication without any signs or symptoms.

A Serious Adverse Event (SAE) is any adverse event occurring following study mandated procedures, having received the treatment or intervention that results in any of the following outcomes:
1. Death
2. A life-threatening adverse event
3. Inpatient hospitalisation or prolongation of existing hospitalisation
4. A disability / incapacity
5. A congenital anomaly in the offspring of a participant

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

All serious adverse events will be assessed for seriousness, expectedness and causality:

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

Causality

Not related or improbable: a clinical event including laboratory test abnormality with temporal relationship to study treatment / intervention administration which makes a causal relationship incompatible or for which other treatments, chemicals or disease provide a plausible explanation**.** This will be counted as "unrelated" for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, but which could also be explained by other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to study treatment / intervention administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as "related" for notification purposes.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

Reporting of serious adverse events

SAEs will be captured at the 4 and 12 week data points. Temporarily suspending methotrexate may cause a disease flare – which will be recorded via the VROOM Condition and Flare Questionnaire, the vast majority of which should not result in the definition of a reportable SAE. Only serious adverse events that are possibly, probably or definitely related to the study intervention will be reported to the CTU on behalf of the Sponsor and closely monitored until resolution, stabilisation, or until it has been shown that the study intervention is not the cause.

Sites will report SAEs on REDCap, and the PI will make a full assessment of expectedness and causality of the SAE.

The Chief Investigator shall be informed immediately of any serious adverse events via OCTRU and the database alerting both to the SAE report. The CI shall assess the information in conjunction with any treating medical practitioners, and confirm causality and expectedness. If in doubt, the CI will raise queries with the treating medical practitioner.

All intervention related serious adverse events will be recorded and reported to the REC as part of the annual reports. Unexpected serious adverse events related to the intervention/study procedures will be reported within the timeframes to the REC as stated below. The CTU shall be responsible for all adverse event reporting.

All intervention related serious adverse events will be recorded and reported to the REC as part of the annual reports. Unexpected serious adverse events related to the intervention/study procedures will be reported within the timeframes to the REC as stated below. The Chief Investigator shall be responsible for all adverse event reporting.

Study Intervention Related SAEs

A serious adverse event that is unexpected in its severity and seriousness *and* deemed directly related to or suspected to be related to the study intervention (withdrawal of methotrexate) shall be reported to the ethics committee that gave a favourable opinion as stated below.

The Chief Investigator who will also take the role of nominated person will:

- Assess the event for seriousness, expectedness and relatedness to the study intervention;
- Take appropriate medical action, which may include halting the study and inform the Sponsor of such action;
- If the event is deemed related to the study intervention shall inform the REC using the reporting form found on the HRA web page within 7 days of knowledge of the event;
- Shall, within a further eight days send any follow-up information and reports to the REC;
- Make any amendments as required to the study protocol and inform the REC as required

Participant removal from the study due to serious adverse events

Any participant who experiences a serious adverse event may be withdrawn from the study at the discretion of the Investigator.

PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g. consent process or administration of study intervention) or from GCP or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and recorded in the study database.

Waivers from the inclusion/exclusion criteria are not allowed.

SERIOUS BREACHES

A ‘serious breach’ is a breach of the protocol, or of the conditions or principles of GCP, which is likely to affect to a significant degree:

(a) the safety or physical or mental integrity of the study subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

ETHICAL AND REGULATORY ASPECTS

Ethics Committee and Regulatory Approvals

The study will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Research Ethics Committee (REC), the respective National Health Service (NHS) or other healthcare provider’s Research & Development (R&D) department, and the Health Research Authority (HRA) if required. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, and the UK Department of Health Policy Framework for Health and Social Care, 2017.

Informed Consent and Participant Information

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in study database system (REDCap). A second copy will be filed in the participant’s medical notes and a signed and dated note made in the notes that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasise to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

Records

Case Report Forms

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). The baseline CRF may collect data on COVID-19 history that has not necessarily been documented elsewhere. All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent and follow-up contact details, the participant will be referred to by the study participant number not by name.

All data will be recorded either directly by local study teams, by the central study co-ordinating office or by participants directly into the study validated REDCap instance hosted at the University of Oxford.

Each participant will be assigned a study participant number, allocated at consent if appropriate, for use on CRFs, other study documents and the electronic database.

eCRFs will be treated as confidential documents and held securely in accordance with regulations. eCRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Study Delegation Log.'

CRFs are used to record clinical study data and are an integral part of the study and subsequent reports.

Laboratory data will be produced by the three laboratories listed in this protocol. All data will be sent directly either to the central trial team and entered into the study's REDCap database, or entered directly by the laboratory team into the database.

Sample Labelling

Each participant will be assigned a study identity code number for use on the samples, consent forms and other study documents and the electronic database.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also

completely serve as its own source data. Only study staff as listed on the Delegation Log shall have access to study documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities (e.g. Department of Health, Human Tissue Authority).

Direct access will be granted to authorised representatives from the Sponsor, OCTRU staff and host institution to permit study-related monitoring, audits and inspections.

Where data is submitted directly to the study office, contemporaneous access by local research teams to the online database will enable the local research teams at sites to download copies of their participants' data.

Data Protection

All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the Data Protection Act, 2018. The CRFs will only collect the minimum required information for the purposes of the study. Electronic CRFs are being used and the data will be held securely on University of Oxford managed servers. Access to the information will be limited to the study staff and investigators and relevant regulatory authorities (see above). Computer held data including the study database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).

Information about the study in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

The data will be stored and used in compliance with the relevant, current data protection laws (Data Protection Act 2018; General Data Protection Regulation 2018). The study data (including data for SAEs) and e-consent forms will be entered during the study onto a validated REDCap study database developed and maintained by OCTRU and which can only be accessed by authorised users via the application. The application resides on a webserver hosted and managed by Oxford University's Information Technology (IT) Services division. The server is on the university's backbone network and is backed up nightly to a secure off-site location.

After closure of the study and data analyses, the data will be made publicly available at the time of publication. The Trial Master File will be archived for at least seven years from the publication of the study. The Investigators will maintain appropriate medical and research records for this study, in compliance with the principles of GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The Chief Investigator, site teams and central study team will have access to records. The Investigators will permit authorised representatives of the Sponsor and OCTRU staff, as well as ethical and regulatory agencies to examine (and when required by applicable law, to copy) clinical

records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

It is aimed for all study data to be captured directly in the study's instance of REDCap or the study instance of their randomisation system RRAMP. There are no paper CRFs or worksheets to be completed by sites, however participants who wish to complete the follow up data via paper will be sent paper CRFs/questionnaires to be returned to the study office and then entered into the study's instance of REDCap. At the end of the study post analysis any paper documents received will be retained as source and sent to the University of Nottingham for storage and archiving.

Identifiable information will be recorded on a secure web-based form in the study randomisation system (RRAMP) by the attending clinician or delegate including a member of research team to enable follow-up:

The Investigator and/or Sponsor must retain copies of the essential electronic documents for a minimum of 7 years following the publication of the study. Site investigators will always have contemporaneous access to all data entered into the system for patients from their site including any direct patient completed questionnaires.

The Investigator will inform the Sponsor of the storage location of the essential documents and of any changes in the storage location should they occur. The Investigator must contact OCTRU and the Sponsor for approval before disposing of any documentation. The Investigator should take measures to prevent accidental or premature destruction of these documents.

QUALITY ASSURANCE & AUDIT

The study may be monitored or audited in accordance with the current approved protocol, GCP, relevant regulations and CTU standard operating procedures. This research will be coordinated by the Experimental Medicine and Rheumatology Group (EMR), which falls under the Oxford Clinical Trials Research Unit (OCTRU) and EMR personnel work according to OCTRU SOPs. The OCTRU SOPs and related quality assurance and control procedures will be used by EMR staff to ensure that the study procedures are assessed and carried out as defined in this protocol.

Risk assessment

A risk assessment will be conducted according to OCTRU's process and a monitoring plan will be drafted to include all central monitoring activities. The study will be conducted in accordance with the current approved protocol, Principles of GCP, relevant regulations and OCTRU standard operating procedures.

Study monitoring

Regular monitoring will be performed according to the study specific Monitoring Plan. Monitoring will be limited to central monitoring activities – there will be no site monitoring, missing data will be queried with sites where mandatory. Monitoring of the data will occur remotely via reports after data has been entered into the database.

Quality assurance

The Sponsor or its designated representative will assess each study site to verify the qualifications of each Investigator and the site staff and to ensure that the site has all of the required equipment. A virtual study Initiation meeting will occur where among other things the Investigator will be informed of their responsibilities and procedures for ensuring adequate and correct study documentation.

Insurance and indemnity

Insurance and indemnity for study participants and study staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but study participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff with both public liability insurance and clinical trials insurance in respect of claims made by research subjects.

Study Conduct

Study conduct may be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the study; Study Delegation Log; CVs of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; accountability of study materials and equipment calibration logs.

Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

Transparency in Research

Prior to the recruitment of the first participant, the study will have been registered on a publicly accessible database.

Where the study has been registered on multiple public platforms, the study information will be kept up to date during the study, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the study declaration.

CTU Involvement

This study is being coordinated by the UKCRC registered OCTRU at the University of Oxford, the affiliated Experimental Medicine and Rheumatology Group in OCTRU will lead the study on a day to day basis.

STUDY DATA

Monitoring of study data shall include confirmation of informed consent; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation.
Study data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

Record retention and archiving

In compliance with the principles of ICH GCP, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and study documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. Whilst the study is conducted the TMF and all study documents will be held on University of Oxford servers and securely transferred at the end of the study.

This archive shall include all study databases and associated meta-data encryption codes. OCTRU will also retain copies of the documentation for the retention period specified.

Discontinuation of the study by the Sponsor

The Sponsor reserves the right to discontinue this study at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

Statement of Confidentiality

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above.
Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.
Data generated as a result of this study will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

This study aims to find out whether methotrexate should be discontinued for a two-week period after SARSCov-2 booster vaccination to optimise the immune response. It may also tell us the mechanism by which methotrexate impairs the immune-response. It may result in lasting policy change in the UK and also globally. The study and the embedded mechanistic study will build multidisciplinary cross-specialty collaborative research networks and relationships that will be utilised for future multi-disciplinary translational research. We will inform and engage patients and the wider community by disseminating our results.

Patient partners will assist in the interpretation of overall study findings, and how any potential predictors of booster response may be communicated to the general public and positioned for further investigation. Where appropriate, patient advisors will be co-authors on publications. As a NIHR funded project, the standard monograph will be produced, however we will work with our PPI collaborators to ensure any plain English parts of the monograph are written in truly plain English. We also plan to produce an infographic to explain the findings.

The study will be publicised to research, clinical and patient communities and other important stakeholders, such as self-help groups. Once the study is completed, in addition to the final report for the NIHR EME Programme, we aim to publish the study results in peer-reviewed high impact journals such as the BMJ or the Lancet and present at national and international meetings to ensure maximum impact and rapid dissemination. Additionally, we will seek to disseminate findings through publication in other journals, such as Pulse, newsletters to British Society for Rheumatology, British Association of Dermatology, and Royal College of General Practitioners. We will engage with patients; primary care clinicians; Royal College of General Practitioners. We will ensure that the study results are disseminated to the guideline writing groups.

The results of this study will also provide the Joint Committee on Vaccination and Immunisation (JCVI) and specialist societies with the requisite evidence base to recommend continuing or temporarily suspend methotrexate after SARS-CoV-2 vaccine boosters.

We will organise a patient forum and listening exercise at the end of the study to report study findings and explore with patients how/where to take the research findings forward into clinical practice. We will engage with traditional and online media to disseminate the study findings.

The study's results will be shared with the British Society for Rheumatology and British Association of Dermatology COVID-19 working groups, both of which supported the premise of this study. We will work with them to influence national guidelines. We will engage with international rheumatology and dermatology societies and disseminate our results widely to change health policy at international level. Such changes may either support or discourage the discontinuation of methotrexate post-vaccination depending on the study findings. Globally, the results may be used to guide primary vaccination in countries with slow vaccine rollout.

The study has been prospectively registered, prior to ethics approval, on the International Standard Randomised Controlled Trial Number register. The study protocol will be published

in an open-access peer-reviewed journal in accordance with the Standard Protocol Items: Recommendations for Interventional Trials statement (SPIRIT, www.spirit-statement.org/).

The study results will be published in an open-access journal, in accordance with the NIHR's policy on open-access research. The study will be reported following the Consolidated Standards of Reporting Trials guideline (CONSORT, www.consort-statement.org). The Template for Intervention Description and Replication (TIDieR) statement will be used for reporting the intervention, ensuring that replication is possible.

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by National Institute for Health Research – Efficacy Mechanism Evaluation Programme. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

All study materials will be made freely available via the study website.

At study recruitment, participants will be asked if they wish to receive a copy of the study results and also at the end of the study if they want to know what happened with their antibody (RBD) levels and the summary levels from each of the groups and an explanation to aid their interpretation.

As of now the implications of anti-RBD antibody concentration on the level of immunity from COVID-19 is not understood. It is anticipated that this knowledge will emerge over the next few months. We will convey results to the participants in the context of current knowledge at the time of such disclosure, potentially in early 2023. If the serologic correlates of protection are not understood even till 2023, we will explain that the implications of such results are not yet known and that they should not change their behaviour based on these results. We will also explain that antibody levels only reflect part of the immunity to COVID-19, and the protection provided by cells has not been measured in this study, furthermore the tests were conducted as part of a research study and shouldn't be relied on for clinical decision making. Our discussions with participants with low levels of COVID-19 antibodies will be tempered by the state of knowledge at that time point. If serological correlates of protection are well understood and defined, we will advise participants to discuss any concerns with their GP and specialists. However, if this is still not well understood, we will advise the participant accordingly and in writing. We will encourage participants to discuss these findings with their GP and/or specialist. We are keen to provide this feedback to participants as this was suggested by our PPI input.

The results of this study will impact on the wellbeing of patients with inflammatory conditions treated with low-dose weekly methotrexate in the UK and globally. If the study shows that temporarily suspending methotrexate improves response to the SARS-CoV-2 booster vaccine, patients will be advised to interrupt treatment by their clinicians. On the contrary, if this study does not demonstrate such an effect, it will reduce inappropriate treatment discontinuations, and the risk of disease flares and patient concern.
We will update participants on study progress and findings using emails and/or post depending on their preference. A webpage hosted by the University of Oxford will publicise progress, participants' stories and staff blogs to generate interest in the study.

The study's PPI volunteers will advise on the content of all public facing content for dissemination. We will engage and partner with the National Rheumatoid Arthritis Society, Versus Arthritis, Psoriasis Association for sharing articles and findings.

USER AND PUBLIC INVOLVEMENT

User groups both at the University of Oxford and University of Nottingham helped with the development of this study and the PPI co-applicant will steer the study management group in delivery of the study and its results. 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, inflammatory bowel disease) 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 two weeks if they were in the study because the study question needed answering.

Results from the flu vaccine studies were presented and all PPI volunteers agreed that a two-week pause in methotrexate offered the best balance of potential benefit without risking a disease flare that could happen with a longer four-week treatment pause. Many had already experienced pausing methotrexate for two 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.

The PPI volunteers advised that participants should be given the option of whether to continue or pause their folic acid depending on their preference.

This study involves three extra visits to the hospital for blood tests around the booster vaccine dose. The PPI volunteers felt that this may be a turn-off 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 risk of attending 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.

Finger prick blood collection kit (e.g. Mitra) were discussed to avoid hospital visits for the blood collection so that participants could have used them 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 as much good information as a usual blood sample taken from veins. So we decided not to use this method of sampling.

Picking the best study outcomes was also discussed with our patient research partners. They supported the use of levels of proteins in the blood-stream 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 things 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. During the study, the PPI co-applicant Ms. Yvonne Hurt will expand this by reaching out to further local patient representatives to form a PPI advisory group.

The study PPI advisory group will include people from a mix of different age, gender, ethnic and socioeconomic backgrounds with a mix of inflammatory conditions, drawn from the PPI community coordinated by Oxford NIHR-BRC, Nottingham NIHR-BRC and Versus Arthritis Pain Centre. Individuals who agree to contribute to project management will meet with Dr Coates (PPI lead) and the Clinical Trials Unit (CTU) team to gain understanding of their previous involvement in research (if any). Appropriate training will be offered and tailored as needed. The PPI lead (Coates) has worked with patient partners within national and international societies such as BritPACT, GRAPPA, OMERACT, and individual studies, including as official liaison with patient partners for these groups. Training and support will be offered through the Oxford Patient Engagement Network for Arthritis and Musculoskeletal Conditions (OPEN ARMS) with a regular PPI meeting and use of INVOLVE and local patient group materials.

As well as providing a patient perspective on all aspects of the programme and its execution, the patient research partners will help with decisions on best ways of recruiting, questionnaire burden, and design of patient materials. Patients will be involved in writing and reviewing of the patient information leaflet, and what information to put on the study website. Additional PPI work and initiatives may also be undertaken as the study progresses.

With our patient co-applicant and the PPI groups, communication for patients/carers and the public will be developed, mindful of the need to reach out to diverse communities. Newsletters, Facebook, Twitter etc. will be used to ensure the results of the study are communicated to the wider community. In addition, the study team will follow the Public Involvement Impact Assessment Framework to maximise PPI in the study. Financial support of the patient partners has been costed appropriately for the entire study duration according to INVOLVE.

Anonymised patient level data will be available at the end of the study once the study has been published for a period of 2 years post publication by request to the Chief Investigator.

STUDY FINANCES

Funding source

The study is supported by the National Institute for Health Research – Efficacy Mechanism Evaluation programme under the reference NIHR134607. The views expressed in this

document are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

Participant stipends and payments

Travel expenses will be offered for any hospital visits as per the expenses processes of the University of Nottingham. Participants will receive a thank you text message after each visit to maintain engagement with the study, and a thank you card at the end of their three research visits with a small token of appreciation – a £25 voucher.

ARCHIVING

During the clinical study and after study closure the Investigator will maintain adequate and accurate records to enable the conduct of the clinical study and the quality of the research data to be evaluated and verified. All essential documents will be stored in such a way that ensures that they are readily available, upon request for the minimum period required by national legislation or for longer if needed. The medical files of study subjects will be retained in accordance with applicable national legislation and the host institution policy.

It is the University of Nottingham's policy to store data for a minimum of 7 years from publication. Investigators may not archive or destroy study essential documents or samples without written instruction from the study office.

SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: Professor Abhishek Abhishek

Signature:_____

Date: _____

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