Neuroimmune responses following joint mobilisation and manipulation in people with persistent neck pain: a protocol for a randomised placebo-controlled trial

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ABSTRACT
Introduction Joint mobilisation and manipulation often results in immediate pain relief in people with neck pain. However, the biological mechanisms behind pain relief are largely unknown. There is preliminary evidence that joint mobilisation and manipulation lessens the upregulated neuroimmune responses in people with persistent neck pain. Methods and analysis This study protocol describes a randomised placebo-controlled trial to investigate whether joint mobilisation and manipulation influence neuroimmune responses in people with persistent neck pain. People with persistent neck pain (N=100) will be allocated, in a randomised and concealed manner, to the experimental or control group (ratio 3:1). Short-term (ie, baseline, immediately after and 2 hours after the intervention) neuroimmune responses will be assessed, such as inflammatory marker concentrations following in vitro stimulation of whole blood cells, systemic inflammatory marker concentrations directly from blood samples, phenotypic analysis of peripheral blood mononuclear cells and serum cortisol. Participants assigned to the experimental group (N=75) will receive cervical mobilisations targeting the painful and/or restricted cervical segments and a distraction manipulation of the cervicothoracic junction. Participants assigned to the control group (N=25) will receive a placebo mobilisation and placebo manipulation. Using linear mixed models, the short-term neuroimmune responses will be compared (1) between people in the experimental and control group and (2) within the experimental group, between people who experience a good outcome and those with a poor outcome. Furthermore, the association between the short-term neuroimmune responses and pain relief following joint mobilisation and manipulation will be tested in the experimental group. Ethics and dissemination This trial is approved by the Medical Ethics Committee of Amsterdam University Medical Centre, location VUMc (Approval number: 2018.181). Trial registration number NL65748 (trialregister.nl)

INTRODUCTION
The disruption of the bidirectional communication pathways between the central nervous system and the immune system may play an important role in persistent pain.1 Over the last two decades, it has become apparent that neuroimmune crosstalk is present in musculoskeletal pain, and may play a mediating role in the transition from acute to persistent pain.1 For people with persistent neck pain, aberrant neuroimmune responses may be present, such as systemically elevated levels of inflammatory markers.2 3 These increased neuroimmune responses may be relevant to understand and manage persistent spinal pain.3 A growing body of literature suggests that these neuroimmune responses are associated with pain intensity,4–6 disability7 and recovery,8 and can be influenced by musculoskeletal physiotherapy, such as joint mobilisation and manipulation,9–11 nerve mobilisation12 13 and exercise.14–16 Several meta-analyses indicate that musculoskeletal physiotherapy for people with
spinal pain may provide immediately pain relief and improvements in functional activities compared with no treatment, placebo or other treatments.17–19 Nevertheless, unravelling the mechanism of how joint mobilisation and manipulation results in pain relief remains an area for further investigation.20 21 There are various explanations of how joint mobilisation and manipulation might cause pain relief, including neurophysiological,22–25 neuromuscular,26 neuroimmune,27 28 and non-specific responses.29

Recent studies suggest a possible neuroimmune-mediated mechanism of pain relief following joint mobilisation and manipulation.9–11 For example, a reduction in systemic inflammatory marker concentration directly from blood samples9–11 and a reduction in inflammatory marker concentration following in vitro stimulation of whole blood cells10–27 were found immediately following the intervention. These studies have, however, important methodological limitations, such as inclusion of healthy participants,27 modest sample sizes,9–11 a narrow selection of inflammatory markers,9–11 lack of correction for potential confounding variables,9–11 and lack of a placebo-control group.9–11 Therefore, we will conduct an adequately powered, placebo-controlled randomised clinical trial in people with persistent neck pain, which will evaluate a broad range of inflammatory markers. The purpose of this paper is to describe the study protocol to investigate the short-term effects of joint mobilisation and manipulation on neuroimmune responses in people with persistent neck pain.

METHODS
This manuscript followed the guidelines for clinical trial protocols Standard Protocol Items: Recommendations for Interventional Trials,20 (SPIRIT statement) for reporting randomised trials Consolidated Standards of Reporting Trials (CONSORT statement), and for intervention description and replication (TIDieR checklist (Better reporting of interventions: template for intervention description and replication)).31

Aim
The overall aim of this clinical trial is to gain insights in the relation between short-term neuroimmune responses following joint mobilisation and manipulation and pain relief in people with persistent neck pain. The specific aims are: (1) to compare the short-term neuroimmune responses between the experimental and control group; (2) to compare the short-term neuroimmune responses of those in the experimental group with a good outcome (ie, immediately pain relief) with those in the experimental group with a poor outcome and (3) to assess the association between short-term neuroimmune responses and pain relief in the experimental group.

Study design and setting
The study is a placebo-controlled randomised trial with follow-up at three time points: baseline, immediately, and 2 hours and 2 days following the intervention (figure 1). Participants will be recruited from GP clinics, primary care physiotherapy practices and outpatient services (neurology and orthopaedic departments) at secondary care hospitals. Data are anticipated to be collected between February 2019 and January 2022, when data analysis and interpretation are anticipated to commence.

Selection criteria
Individuals meeting the following inclusion criteria are eligible to participate: age: 18–65 years; non-specific neck pain for at least 6 weeks32 with a minimum pain intensity of 40/100 on a Visual Analogue Scale (VAS), and a sufficient spoken and reading level of the Dutch language to complete the study. Exclusion criteria are contraindications for cervical mobilisation or cervicothoracic manipulation,33 34 pregnancy or less than 9 months postpartum, contraindications for venipuncture (eg, phlebitis), treatment for the current neck pain episode during the preceding 2 weeks, use of corticosteroids or cytokine modulatory medication (eg, methotrexate, infliximab) in the preceding 6 weeks, use of botulinum toxin (Botox) injection during the preceding 3 months, non-steroid anti-inflammatory drug medication within the past 7 days (eg, diclofenac, ibuprofen, naproxen), long-distance flight within the past 7 days, ongoing shift work, having a known comorbid condition with immune/endocrine malfunction (eg, ankylosing spondylitis, Crohn’s disease, sarcoidosis, Cushing syndrome, cancer, diabetes), medical red flags suggestive of serious pathology,35 36 and a diagnosed psychological condition (eg, clinical depression).
Consecutive participants who meet all selection criteria and are willing to participate will be admitted to the study. All participants will provide written informed consent prior to participation. Initial screening for eligibility will be conducted via telephone calls.

Randomisation, concealed allocation and blinding
Block randomisation will be used to allocate participants to the experimental or control group with an allocation ratio of 3:1 (experimental:control). A computer random number generator will create block sizes of 4 and 8 participants. To conceal the allocation sequence, an independent person not involved in the study will assign eligible people to the groups on the day the participant will enrol in the study. Blood samples will be coded to blind the research assistant and laboratory investigators to the study groups. The participant, research assistant, and the investigator who includes the participants will be blinded for group assignment. The treating clinicians, research assistant and laboratory investigators will be unaware whether participants experienced a good outcome or not. All laboratory and data analyses will be performed by blinded investigators.

Interventions
Experimental intervention
Spinal mobilisation will consist of low-velocity, low-amplitude mobilisations at the painful cervical segmental levels (figure 2A–C); spinal manipulation will consist of a high-velocity, low-amplitude distraction manipulation at the cervicothoracic junction (figure 2D). These techniques aim to restore motion and reduce pain. They are commonly used and are conform to the Dutch guidelines for musculoskeletal physiotherapy for treating neck pain. All interventions will be performed by two musculoskeletal physiotherapists with more than 5 years of relevant clinical experience.

Cervical mobilisation
Painful and restricted cervical segments will be identified by passive side-bending of the neck targeting each segmental level separately. Reproduction of the participant’s pain will be considered to identify the involved level(s). The intertester reliability for these tests is fair to substantial. Depending on the identified painful or restricted spinal levels, the treating clinician may select from different mobilisation techniques: mobilisation targeting the atlanto-axial joints (figure 2A); segmental zygaphophyseal joint mobilisation (C2–C7) (figure 2B) and occipital-atlanto-axial joint mobilisation (figure 2C). Three series of oscillations (~1 Hz) will be applied for 30 s; with 30 s rest in between the series.

Cervicothoracic junction distraction manipulation
Irrespective of the level of their neck pain, all participants will receive a distraction manipulation of the cervicothoracic junction (figure 2D). If there is no audible
Either group is not achieved, the good outcome group is both the good outcome and poor outcome group. If we anticipate to have a minimum of 25 participants in respectively the best responders and poorest responders. Lutke Schipholt IJ, et al. BMJ Open 2022;12:e055748. doi:10.1136/bmjopen-2021-055748

Points (or an unclear outcome (not fitting the criteria for 20% improvement in pain intensity score at both time points), a poor outcome 50% improvement in pain intensity at both time points), a poor outcome will be categorised (ie, immediately and 2 hours following the intervention; T1, immediately following the intervention; T2, 2 hours following the intervention; T3, 2 days following the intervention; TNF-RII, tumour necrosis factor receptor antagonist 2; TNF-α, tumour necrosis factor-α.

Control (placebo) intervention
The control group will receive a placebo mobilisation and placebo manipulation. Procedures, including the instructions, will be identical as for the experimental intervention, except that the clinician will only apply hand contact and no pressure or movement will occur. Participants will be informed that an audible popping sound may or may not occur, and that this sound is not necessary to restore motion and reduce pain.

The credibility of a control intervention can interact with participant expectations in complex ways. To account for differences in intervention expectations, participants will indicate the extent to which they agree (using a four-point Likert scale) with four statements regarding their intervention expectations (table 1). These statements will be presented before the delivery of the experimental and control intervention.

Based on the short-term changes in pain intensity score (ie, immediately and 2 hours following the intervention), participants in the experimental group will be categorised into those with a good outcome (≥50% improvement in pain intensity at both time points), a poor outcome (≤20% improvement in pain intensity score at both time points) or an unclear outcome (not fitting the criteria for a good or poor outcome). Based on these cut-off scores, we anticipate to have a minimum of 25 participants in both the good outcome and poor outcome group. If our a priori determined minimum of 25 participants in either group is not achieved, the good outcome group and the poor outcome group will be supplemented with respectively the best responders and poorest responders from the uncertain outcome group in order to obtain 25 participants in both groups.

Outcomes
A broad range of neuroimmune responses will be monitored: (1) inflammatory marker concentration following in vitro stimulation of whole blood cells, (2) systemic inflammatory marker concentrations directly from blood samples, (3) phenotypic analysis of peripheral blood mononuclear cells and (4) ex vivo serum cortisol (table 1). To create an inflammatory profile, a range of proinflammatory and anti-inflammatory markers will be used. Ex vivo serum and supernatants after stimulation will be stored at minus 80°C and will be analysed on completion of data collection. The laboratory methodology and sample handling prior to stimulation will be tightly monitored and reported, because inconsistency in interlaboratory methodology and reporting impairs interpretation, comparability and reproducibility.

Primary outcomes
The primary outcomes are the short-term (ie, immediately and 2 hours following the intervention) differences in interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) following in-vitro stimulation of whole blood cells. These cytokines will be determined using Meso Scale Discovery (MSD, Maryland, USA) at baseline, immediately and 2 hours following the intervention. These cytokines are selected because previous research has indicated that these cytokines might play a role in spinal pain.

To induce cytokine production, whole blood cultures will be stimulated for 24 hours with lipopolysaccharide (LPS) from Escherichia coli O55:B5 (Sigma-Aldrich)

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**Table 1 Overview of the neuroimmune responses**

| Domain | Neuroimmune parameters | Timing of measurements |
|--------|------------------------|------------------------|
| Systemic inflammatory marker directly from blood samples* | TNF-α, TNF-RII, IL-1β, IL-1RA, hsCRP† | √ √ √ – |
| Inflammatory marker concentration after in-vitro stimulation of whole blood cells‡ | TNF-α, IL-1β, IL-1RA, IL-4, IL-10, CCL2, CCL3, CCL4 | √ √ √ – |
| Ex vivo serum cortisol§ | Cortisol | √ × – – |
| Phenotypic analysis of peripheral blood mononuclear cells¶ | CD45+, CD3+, CD4+, CD25+, CD8+, CD56+, CD19+, CD14+, HLA-DR, TLR-4 | √ – √ – |

*Measured using multianalyte assay Ella (R&D systems, Minneapolis, USA).
†Cardiac C-Reactive Protein (Latex) High Sensitive using Roche/Hitachi cobas c systems.
‡Stimulated for 24 hours at 37°C in a humidified 5% CO2 incubator, with lipopolysaccharide (LPS) from Escherichia coli O55:B5 at a concentration of 1 ng/mL and 10 μg/mL. Determined using a custom-made U-plex (MSD, Maryland, USA).
§Using conventional electrochemiluminescence immunoassay (ECLIA), Roche (Cobas Cortisol, second generation).
¶Determined by 10-colour flowcytometry (FCM): CD45+=general leucocyte marker; CD3+=T cell marker; CD3 +CD4+=CD4+T-helper marker; CD3 +CD4+CD25 hi=T-regulator cell marker; CD3 +CD8+cytotoxic T-cell marker; CD3-CD56+=natural Killer cell marker; CD19+=B-cell marker; CD14+=monocyte marker; HLA-DR=activation marker for T cells and monocytes; TLR-4=Toll-like receptor four marker.
CCL2, c-c-motif chemokine ligand 2; CCL3, c-c-motif chemokine ligand 3; CCL4, c-c-motif chemokine ligand 4; CD, cluster of differentiation; hsCRP, high sensitive C reactive protein; IL-4, interleukin-4; IL-10, interleukin-10; IL-1RA, interleukin-1 receptor antagonist; IL-1β, Interleukin-1β; T0, baseline; T1, immediately following the intervention; T2, 2 hours following the intervention; T3, 2 days following the intervention; TNF-RII, tumour necrosis factor receptor antagonist 2; TNF-α, tumour necrosis factor-α.
Chemie, Schnelldorf, Germany) at a concentration of 1 nanogram LPS/millilitre whole blood (ng/mL) and 10 µg LPS/mL whole blood (µg/mL) at 37°C in a humidified 5% CO₂ incubator. At baseline (figure 1), blood samples for neuroimmune measurements (one sodium heparin vacutainer without gel and one serum vacutainer without gel for each time point) will be drawn between 8:00 and 9:00 AM. The cytokine levels will be determined using a custom-made U-plex MSD and expressed in pg/mL. The entire blood stimulation procedure and MSD will be performed by an experienced laboratory technician at Amsterdam University Medical Centre, location VUmc, Department of Clinical Chemistry, Medical Immunology Laboratory.

Secondary outcomes
Several additional neuroimmune responses will be quantified as secondary outcomes at various time points (table 1).

- The levels of interleukin-1 receptor antagonist (IL-1RA), interleukin-4 (IL-4), interleukin-10 (IL-10), c-c motif chemokine ligand 2, c-c motif chemokine ligand 3 and c-c motif chemokine ligand 4 will be determined following in-vitro stimulation of whole blood cells.

Systemic inflammatory markers directly from blood samples (TNF-receptor antagonist II, IL-1β and IL-1RA) will be measured using multiplex assay Ella (R&D systems, Minneapolis, United States) and high-sensitive C reactive protein, using Roche/Hitachi cobas c systems (Indianapolis, USA).

To examine a general change in inflammatory marker production, we will calculate in vitro and ex vivo overall inflammatory, proinflammatory, anti-inflammatory and ratio proinflammatory/anti-inflammatory indices. The indices will be calculated as the mean value or the Ln-transformed data in case of non-normality and z-score standardised levels (based on the control group or poor outcome group) of the inflammatory markers (online supplemental appendix A).

Phenotypic analysis of peripheral blood mononuclear cells will be determined. The absolute number of lymphocyte subsets (NK cells, B-cells, CD4 and CD8 T-cells and CD25 regulatory T-cells), monocytes, as well as activation status of these cells, HLA-DR and TLR-4 expression, will be determined by 10-colour flowcytometry (FCM, Gallios Flow Cytometer, Beckman Coulter, Indianapolis, USA; Analyse software: Kaluza). Differences between all groups in serum cortisol concentration will be determined using conventional electrochemiluminescence immunoassay from Roche (Cobas Cortisol, second generation, Indianapolis, USA) in agreement with the manufacturer’s protocol.

Procedures
Once consent is obtained (online supplemental appendix B), baseline measurements will be taken (figure 1). At baseline, participants will undergo physical tests to determine pain characteristics, physical functioning and body composition (tables 2 and 3). After this, participants will complete an electronic survey to collect sociodemographic and clinical information (table 1) and intervention expectations (online supplemental appendix C). Participants will then undergo one venipuncture from the cubital vein to fill two vacutainers which will be used to quantify the neuroimmune responses (table 1). Collection of all baseline data will take 30–45 min and will take place at the Amsterdam University Medical Centre, location VUmc, or at a participating primary care physiotherapy practice, under the supervision of a research assistant.

Participants will then be randomly allocated to the experimental and control group, and treated accordingly. Immediately and 2 hours following the intervention, participants will undergo another venipuncture to fill two vacutainers. Between the immediate and 2 hours follow-up measures, questionnaires will be completed to collect psychosocial information such as sleep, disability and kinesiophobia (table 2).

Immediately and 2 hours following the intervention, participants will undergo physical tests (figure 1) and will rate their pain intensity on a VAS. Two-hours following the intervention, participant will rate their perceived recovery on a 7-point Global Perceived Effect scale (GPE) (table 2). Two-days following the intervention, participants will receive an electronic survey regarding potential adverse events, GPE and pain intensity, figure 1 shows the planned flow of participants through the study.

Sample size
Based on the sample size calculation (longitudinal analysis; three time points (baseline, immediately follow-up, 2 hours follow-up) with 80% power to detect a mean difference of 550 (SD 933) for TNF-α levels with a 0.05 two-sided significance level, correlation of 0.6 among repeated measures, ratio between groups of 0.25, a total sample size of 91 is needed. Allowing for a drop-out rate of ~10%, a total sample size of 100 participants is required.

Statistical analyses
Data will be checked for normality by the Kolmogorov-Smirnov test and visual inspection of Q-Q plots, box plots and histograms. In case of no normality of data, the data will be log transformation. Data will be presented as means with SD unless otherwise noted. For the analyses, statistical significance will be set at p<0.05. Intention-to-treat analyses using mixed models will be performed to analyse differences between the experimental group and control group. Linear mixed model analyses with fixed factor (time), covariate (group) and interaction (time*group) will be used to detect differences between the groups at the three time points (baseline, immediately follow-up, 2 hours follow-up) for TNF-α and IL-β following in-vitro stimulation of whole blood cells. A random intercept will be selected to account for the correlated nature of multiple measurements from the same participant. The regression coefficient (B), p value

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and confidence intervals (95% CI) will be computed for the crude models, as well as for the adjusted models. 28 53

Linear regression analysis will be used to test for differences in phenotypic analysis of peripheral blood mononuclear cells and cortisol between the experimental and control group and of those in the experimental group with a good outcome (ie, immediate pain relief) with those in the experimental group with a poor outcome.

Adverse events

Serious and non-serious adverse events related to the experimental and control intervention, and all other aspects of the study, will be documented. At the three postintervention time points, potential adverse events will be recorded using an online survey. Adverse events will be followed up as needed by an independent clinician. Depending on the nature of the event,
participants may be referred to a GP or a medical specialist, and additional tests or procedures may be proposed. The experimental intervention has been shown to be safe and it is considered unlikely that serious adverse events due to the interventions will occur. Therefore, installing a data monitoring safety board was not requested by the Ethics Committee.

**Patient and public involvement**

A panel of four people with persistent neck pain codeveloped and evaluated the study design, research questions, choice of experimental and control intervention, and burden of study participation for the participants. Two of these people and two representatives from the public reviewed the Patient information letter and their feedback was used to improve the letter.

**Data management and monitoring**

The data will be collected at the Department of Rehabilitation of the Amsterdam University Medical Centre, location VUmc and/or in physiotherapy practices. The collected data will be securely stored at Vrije Universiteit Amsterdam, Faculty of Behavioural and Movement Sciences. All data are deidentified by using unique participant ID numbers in such a way that the data cannot be traced back to the individual participants without the key. The participants code will exist of a random code of three numbers. The electronically key connecting participant names with codes will be kept in a secure location in the principal investigator’s office. The key will be kept for 6 months after the final publication, and will then be destroyed. Data will be stored in a deidentified manner for fifteen years after the final publication.

**Ethics and dissemination**

The results of the study will be published in peer-reviewed journals and disseminated at conferences, in newsletters and social media. The trial is approved by the Medical Ethics Committee of Amsterdam University Medical Centre, location VUmc (Approval number: 2018.181). All procedures will be conducted in accordance with the Declaration of Helsinki. Amendment to this protocol will be submitted for approval to the Medical Ethical Committee and deviations from the protocol will be reported to the trial registration.

**DISCUSSION**

There is considerable debate in the literature regarding the possibility of meaningful neuroimmune-mediated pain relief following joint mobilisation and manipulation. We described a protocol for a randomised placebo-controlled study that will assess potential neuroimmune-mediated pain relief following joint mobilisation and manipulation in people with persistent neck pain. The aim of this study is to gain insights in the relation between changes in neuroimmune responses and pain relief, rather than in the clinical efficacy or effectiveness of joint mobilisation and manipulation for people with persistent neck pain.

Recent data suggest that the production of pro-inflammatory cytokines is higher and production of anti-inflammatory cytokines is lower in patients with persistent neck pain compared with healthy people following in-vitro stimulation of whole blood cells. Additionally, a specific, coordinated inflammatory processes may be important for patient recovery. Contrary to the other studies we are aware of that measured neuroimmune responses following joint mobilisation and manipulation, we will assess a comprehensive range of inflammatory markers. Our approach to measure pro-inflammatory cytokines and their antagonists provides insight into the activation of immunocompetent cells.

We believe the design of our study allows to assess the specific effects of joint mobilisation and manipulation.
on neuroimmune responses. For instance, rather than comparing the joint mobilisation and manipulation with a wait-and-see approach, we will compare responses with a placebo-control intervention that resembles joint mobilisation and manipulation. Additionally, the verbal instructions between the experimental and control groups will be comparable and standardised, which reduces differences in intervention efficacy due to non-specific intervention effects. Differences in verbal instructions have been shown to be associated with differences in endocrine responses following joint manipulation in people with neck pain. Finally, we will record the participant’s intervention expectations and beliefs regarding joint mobilisation and manipulation as a treatment method to alleviate neck pain.

Previous research revealed a non-linearity of the VAS to measure pain intensity, that responsiveness varies along the spectrum of pain intensity and the importance of taking baseline pain into account when evaluating change scores. Consequently, categorising good, unclear and poor outcome using raw data, or change scores in general, are invalid as these will either underestimate or overestimate true change. To overcome this problem, we follow the initiative on methods, measurement and pain assessment in clinical trials recommendation to identify those with a good, poor outcome or unclear outcome.

Besides the strengths, the proposed study has some potential limitations. First, we assume a linear association between neuroimmune responses and musculoskeletal pain. A linear association between neuroimmune responses and musculoskeletal pain is a prerequisite for the justification of the statistics proposed in this protocol. However, one study suggests that an initial threshold of neuroimmune responses might be required, which would suggest a non-linear relationship between neuroimmune responses and musculoskeletal pain. In that study, elevated IL-6 levels were only present in the group of people with pain >40/100 VAS compared with control. Therefore, a minimal pain intensity of 40/100 on the VAS will be a prerequisite for participating in this study.

Another limitation is that only a single session of joint mobilisation and manipulation will be provided together with a short follow-up. While a single session of joint mobilisation and manipulation may induce a pain-relieving effect, the clinical relevance of immediately pain relief is unclear. Nonetheless, our aim is not to examine the efficacy of joint mobilisation and manipulation but rather to understand the biological mechanisms behind pain relief following joint mobilisation and manipulation. In studying the mechanism of action, a short follow-up has the advantage that potential confounding variables can be controlled, such as food intake, stress, physical exercise and health status.

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Contributors All authors contributed to the design of this protocol. ULS, GS-P and MWC initiated the protocol. The protocol was drafted by ULS, GS-P, HB and MWC. Statistical advice was provided by GS-P and MWC. ULS, GS-P and MWC were responsible for ethical board approval. ULS was responsible for drafting the manuscript. All authors contributed to the manuscript and read and approved the final manuscript.

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Data availability statement Individual deidentified participant data that underlie the results will be shared. Investigators whose proposed use of the data had been approved by an independent review committee identified for this purpose can access the data for individual participant data meta-analysis. Data will be available beginning 9 months and ending 36 months following article publication. Proposals may be submitted up to 36 months following article publication. After 36 months the data will be available in our University's data warehouse but without investigator support other than deposited metadata. Information regarding submitting proposals and accessing data may be found at https://research.vu.nl.

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