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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 concentration 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 NL6575 (trialregister.nl

Strengths and limitations of this study

- ► This study provides insight in the interplay between joint mobilisation and manipulation, neuroimmune responses, and pain relief in people with persistent neck pain.
- ► By adding a placebo-control group, possible working mechanisms of joint mobilisation and manipulation on neuroimmune responses may be revealed.
- ► The interventions will be delivered by two musculoskeletal physiotherapists, which may limit the generalisability.
- ► Due to the small control group, it is not feasible to divide the control participants according to outcome.
- ► Inflammatory indices will be calculated that combine overall inflammatory, proinflammatory, anti-inflammatory and ratio pro/anti-inflammatory markers.

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] disability[7] and recovery,[8] and can be influenced by musculoskeletal physiotherapy, such as joint mobilisation and manipulation,[9–11] nerve mobilisation[12 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 23] neuromuscular,[20] neuroimmune[24 25] and non-specific responses.[26]

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 samples[9 11] and a reduction in inflammatory marker concentration following in vitro stimulation of whole blood cells[10 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 10] a narrow selection of inflammatory markers,[9–11] lack of correction for potential confounding variables,[9 10 28] and lack of a placebo-control group.[9 10] 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,[29](SPIRIT statement) for reporting randomised trials Consolidated Standards of Reporting Trials (CONSORT statement),[30] 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.

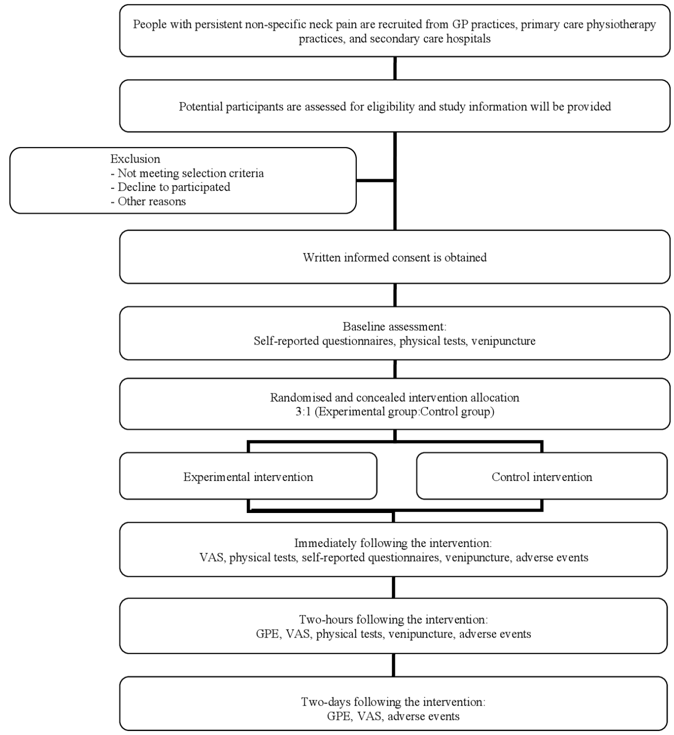

Figure 1 Anticipatedco flow of the study. GPE, global perceived effect; VAS, Visual Analogue Scale.

Selection criteria

Individuals meeting the following inclusion criteria are eligible to participate: age: 18–65 years; non-specific neck pain for at least 6 weeks[32] with a minimum pain intensity of 40/100 on a Visual Analogue Scale (VAS), and a sufficient speaking 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, taken 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).

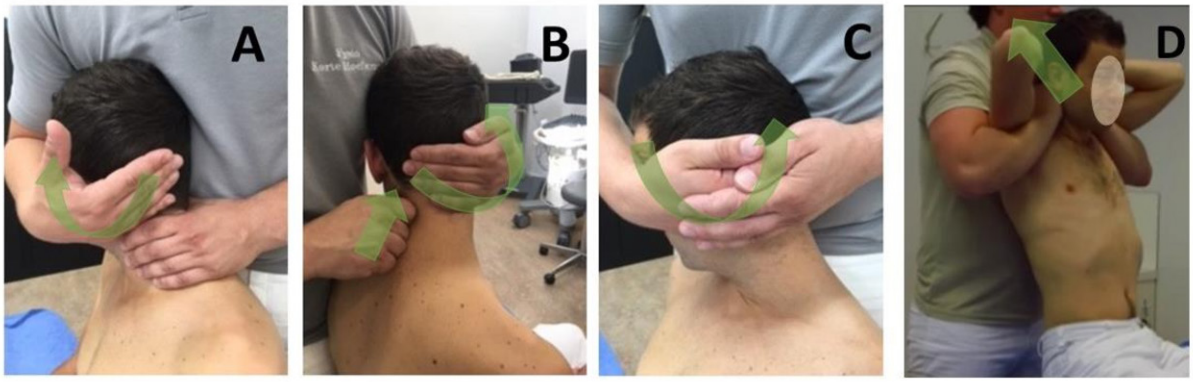

Figure 2 Spinal mobilisation and manipulation techniques. Depending on the identified painful segmental levels, the clinician can select from different cervical mobilisation techniques (A–C); for techniques A-C, the participant will be seated on a chair, leaning against the upper leg or shoulder of the clinician. (A) Mobilisation targeting the atlanto-axial joints. The cervical segments below the second cervical vertebrae are submaximal rotated and lateroflexed. With the clinician's hypothenar region of the hand over the structures overlying the arcus of the first vertebrae, the clinician moved the head further in rotation.[40] (B) Segmental zygapophyseal joint mobilisation (C2–C7; the image shows the technique for C3–C4). first, the occipital-atlanto-axial joint is maximally rotated in the direction of the facet joint being mobilised. Subsequently, the head is moved to extension, ipsilateral lateroflexion and rotation until pressure from the thumb is felt. This technique is repeated on the lower level until the painful cervical segment is reached (C3–C4). Next, on the painful cervical segment, pressure will be given in a cranio-ventral direction.[40] (C) Mobilisation technique targeting the occipital-atlanto-axial joints. The clinician's hypothenar region is placed against the mastoid process. C2–C7 are submaximally locked in flexion, rotation and lateroflexion. The head is then moved in a mediocaudal direction.[40] (D) Spinal manipulation technique targeting the cervicothoracic junction. The participant will be seated on a treatment table. The height of the table will be adjusted to the level of the clinician's abdomen. The participant's hands will be placed on the back of their head (with one hand placed over the other hand, rather than with interlocking fingers), and with the shoulders slightly retracted. The clinician's hands will be placed over the hands of the participant, with the clinician's forearms ventral to the shoulder of the participant. Then, a high-velocity, low-amplitude movement will be applied in a dorsal-cranial direction.[40]Green arrows represent the direction of the mobilisation (A–C) or manipulation (D).

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).[37] 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.[35] 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.[38] 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.[38 39]

Depending on the identified painful or restricted spinal levels, the treating clinician may select from different mobilisation techniques: mobilisation targeting the atlanto-axial segment (figure 2A); segmental zygapophyseal 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).[40] If there is no audible

Table 1 Overview of the neuroimmune responses

Domain	Neuroimmune parameters	Timing of measurements			
		T0	T1	T2	T3
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¶	$CD45^+$, $CD3^+$, $CD4^+$, $CD25^{hi}$,$CD8^+$,$CD56^+$, $CD19^+$, $CD14^+$, 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% CO_2 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.

cavitation sound during the first attempt, the manipulation will be repeated once.

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.[41] 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.[42]

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).[43] 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,[44] 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.[45]

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 those cytokines might play a role in spinal pain.[11 27 46–48]

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

Chemie, Schnelldorg, Germany) at a concentration of 1 nanogram LPS/millilitre whole blood (ng/mL) and 10 µg LPS/mLe whole blood (µg/mL) at 37°C in a humidified 5% CO_2 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.[49] 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 multianalyte 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.[50 51] 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 $CD25^{hi}$ 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[52] (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.[27] 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

Table 2 Self-reported questionnaires and physical tests

Domain	Self-reported questionnaires	Timing of measurements T0	T1	T2	T3
Disability	Neck Disability Index (NDI)*	–	√	–	–
Perceived effect	Global Perceived Effect (GPE)†	–	–	√	√
Fear of movement	Tampa Scale of Kinesiophobia‡	–	√	–	–
Type of pain	PAIN Detect Questionnaire (PDQ)§	–	√	–	–
Type of pain	Central Sensitisation Inventory (CSI)¶	–	√	–	–
Depression, Anxiety, Stress	Depression Anxiety Stress Scale (DASS21)**	–	√	–	–
Physical activity	International Physical Activity Questionnaire (IPAQ)††	–	√	–	–
Catastrophising	Pain Catastrophising Scale (PCS)‡‡	–	√	–	–
Sleep Quality	Pittsburgh Sleep Quality Index (PSQI)§§	–	√	–	–
Pain Intensity	Visual Analogue Scale (VAS)¶¶	√	√	√	√
Mental health	Mental health inventory (MHI-5)***	√	–	–	–
Domain	**Physical tests**	**Timing of measurement T0**	**T1**	**T2**	**T3**
Range of motion	Cervical Range of Motion (CROM)†††	√	√	√	–
Pain intensity	CROM-VAS test‡‡‡	–	√	√	–
Quantitative sensory testing	Pressure Pain Threshold (PPT)§§§	√	√	√	–
Quantitative sensory testing	Wind-up ratio¶¶¶	√	√	√	–

*The Dutch version of the NDI is a valid and responsive measure of disability.[64]
†The GPE is a validated and reliable tool to assess health transitions in patients with musculoskeletal disorders.[65]
‡Preferred self-administrated questionnaire to asses fear of movement in musculoskeletal pain.[66]
§Persistent pain will be categorised in two-mechanism based groups: nociceptive and neuropathic pain using the PDQ. The PD-Q is a reliable screening tool with high specificity.[67]
¶The Dutch Central Sensitisation Inventory (CSI) has good internal consistency, good discriminative power and excellent test–retest reliability. A cut-off score of 40/100 provides a sensitivity of 81% and specificity of 75%.[68]
**Preferred self-administrated questionnaire to assess depression, anxiety and stress in musculoskeletal pain.[66 69]
††Expressed in 1000 metabolic equivalent minutes per week (Dutch-language version).[70] The IPAQ has good reliability (intraclass correlation coefficient (ICC)=0.70–0.96) and moderate validity (r=0.36–0.49) of the IPAQ compared with an accelerometer.[71]
‡‡Preferred self-administrated questionnaire to assess pain catastrophising in musculoskeletal pain.[66]
§§Score above 5 yield a sensitivity of 89.6% and specificity of 86.5% in distinguishing good and poor sleepers.[72]
¶¶The reliability and validity of the VAS as a measure of pain for neck pain patients is good.[73]
***General psychological status will be assessed using the MHI-5.[74] A higher score indicates better mental health. Cronbach's alpha for the MHI-5 scale is 0.85.[75]
†††The CROM is a clinically reliable tool to measure active cervical range of motion people with neck pain and healthy participants.[76]
‡‡‡This novel test consists of two parts. In part 1, the participant is asked to perform maximal active right and left cervical rotation and the degrees of rotation are reordered using the CROM device. In this position, the pain intensity is measured with the VAS following intervention. After the intervention, part 2 of the test is performed . The participant is again asked to actively rotate (left and right) to the same position as in part 1 and the pain intensity is recorded. The difference on VAS scores is the outcome of the CROM-VAS test.
§§§Pressure algometry over the cervical spine has shown excellent intrarater and good-to-excellent inter-rater reliability in individuals with acute neck pain.[77] This study reported that the MDC for PPT over the cervical spine and tibialis anterior muscle in patients with acute neck pain was 47.2 and 97.9 kPa, respectively.[77] To determine changes in widespread pressure pain sensitivity, PPTs will be assessed bilaterally over the mid-point trapezius (pars descendens), second metacarpal and tibialis anterior muscle.
¶¶¶Using a pinprick 256 mN wind up ratio will be calculated bilaterally over the midpoint trapezius (pars descendens) and tibiales anterior muscle.[78]
T0, baseline; T1, immediately following the intervention; T2, 2 hours following the intervention; T3, 2 days following the intervention; VAS, Visual Analogue Scale.

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,

Table 3 Potential confounding variables that will be assessed

Potential confounding variables	
Comorbidities	Number of comorbidities
Alcohol use	Non-drinker
	Moderate drinker
	(Women: 1–14 glasses/week)
	(Men: 1–21 glasses/week)
	Heavy drinker
	(Women:>14 glasses/week)
	(Men:>21 glasses/week)
Smoking	Never smoked
	Former smoker
	Current smoker
Body mass index (BMI)	BMI calculated by dividing body weight (kg) by height (m²)
Medication use	Type and number of medications used
Drugs use	Recreational drugs use
	Yes
	No
Visceral Adipose Tissue[79 80]	Linear distance between abdominal peritoneum and ventral aspect of vertebrae will be assessed using ultrasonography
Physical activity	International Physical Activity Questionnaire, expressed in 1000 metabolic equivalent minutes per week (Dutch version)
Menstrual cycle[81]	Regular menstrual cycle (yes/no), whether women are in the luteal or follicular stage (yes/no), menopause (yes/no) and post menopause (yes/no)
Season[82]	Timing of experiment (summer, autumn, spring or winter)
Age	Age in years
Psychological status[74]	Mental health inventory-5
Intervention expectations[42]	The extent to which they agree (using a four-point Likert scale) with four statements (online supplemental appendix 3)

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[11 27] 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.[54] 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.[2 7 55 56] 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 proinflammatory cytokines is higher and production of anti-inflammatory cytokines is lower in patients with persistent-pain compared with healthy people following in-vitro stimulation of whole blood cells.[44] Additionally, a specific, coordinated inflammatory processes may be important for patient recovery.[3] Contrary to the other studies we are aware of that measured neuroimmune responses following joint mobilisation and manipulation,[7 44 57] we will assess a comprehensive range of inflammatory markers. Our approach to measure proinflammatory cytokines and their antagonists provides insight into the activation of immunocompetent cells.[58]

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.[59] Differences in verbal instructions have been shown to be associated with differences in endocrine responses following joint manipulation in people with neck pain.[60] Finally, we will record the participant's intervention expectations and beliefs regarding joint mobilisation and manipulation as a treatment method to alleviate neck pain.[42]

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.[43 61 62] 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.[62] 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.[43]

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.[63] In that study, elevated IL-6 levels were only present in the group of people with pain >40/100 VAS compared with control.[63] 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,[17] 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. IJLS, GS-P and MWC initiated the protocol. The protocol was drafted by IJLS, GS-P, HB and MWC. Statistical advice was provided by GS-P and MWC. IJLS, GS-P and MWC were responsible for ethical board approval. IJLS was responsible for drafting the manuscript. All authors contributed to the manuscript and read and approved the final manuscript.

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