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Differential expression of cerebrospinal fluid neuroinflammatory mediators depending on osteoarthritis pain phenotype

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Abstract

Neuroinflammation is implicated in the development and maintenance of persistent pain states, but there are limited data linking cerebrospinal fluid (CSF) inflammatory mediators with neurophysiological pain processes in humans. In a prospective observational study, CSF inflammatory mediators were compared between patients with osteoarthritis (OA) who were undergoing total hip arthroplasty due to disabling pain symptoms ($n = 52$) and pain-free comparison controls ($n = 30$). In OA patients only, detailed clinical examination and quantitative sensory testing were completed. Cerebrospinal fluid samples were analyzed for 10 proinflammatory mediators using Meso Scale Discovery platform. Compared to controls, OA patients had higher CSF levels of interleukin 8 (IL-8) ($P = 0.002$), intercellular adhesion molecule 1 ($P = 0.007$), and vascular cell adhesion molecule 1 ($P = 0.006$). Osteoarthritis patients with central sensitization possibly indicated by arm pressure pain detection threshold <250 kPa showed significantly higher CSF levels of Fms-related tyrosine kinase 1 (Flt-1) ($P = 0.044$) and interferon gamma-induced protein 10 (IP-10) ($P = 0.024$), as compared to subjects with PPDT above that threshold. In patients reporting pain numerical rating scale score $\geq 3/10$ during peripheral venous cannulation, Flt-1 was elevated ($P = 0.025$), and in patients with punctate stimulus wind-up ratio ≥ 2 , CSF monocyte chemoattractant protein 1 was higher ($P = 0.011$). Multiple logistic regression models showed that increased Flt-1 was associated with central sensitization, assessed by remote-site PPDT and peripheral venous cannulation pain, and monocyte chemoattractant protein-1 with temporal summation in the area of maximum pain. Multiple proinflammatory mediators measured in CSF are associated with persistent hip OA-related pain. Pain phenotype may be influenced by specific CSF neuroinflammatory profiles.

Keywords: Persistent pain, Neuroinflammation, Proinflammatory mediators, Cerebrospinal fluid, Quantitative sensory testing, Central sensitization, Osteoarthritis

1. Introduction

Persistent pain and pain-related conditions are leading global causes of disability.³² In addition to the distress and suffering associated with pain itself, long-term pain impacts humans through negative mental health effects, sleep disturbance, and increased morbidity.^{28,67} Despite substantial research efforts, treatment options remain limited, only partially effective, and associated with side effects.^{29,84}

Over the past 3 decades, neuroinflammation has emerged as a central feature of persistent pain states.^{34,46} Upon peripheral

nerve injury or inflammation, spinal cord microglia and astrocytes are activated to produce proinflammatory cytokines, chemokines, and growth factors, which amplify central nervous system (CNS) immunoactivation.^{46,65} Central neuroimmune activation is well established in animal models of inflammation and neuropathic pain.³⁴ For example, intrathecal (IT) administration of proinflammatory cytokines such as interleukin (IL) 1 β , IL-8, monocyte chemoattractant protein 1 (MCP-1), and TNF- α induces allodynia and hyperalgesia, indicative of central sensitization (CS).^{2,63,73,83} Conversely, upregulation or administration of

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the anti-inflammatory cytokine IL-10, or IT treatment with an IL-4/IL-10 fusion protein, inhibits neuroinflammation in the dorsal root ganglia (DRG) and spinal cord, with subsequent decrease of nociceptive behavior.^{27,66} Knockout animals lacking IL-1 β , IL-1 receptor 1, or MCP-1 demonstrate reduced or abrogated pain behavior in peripheral nerve injury models, and inhibition of IL-8 decreased pain in a rodent chronic low back pain model.^{1,20,54} Attenuation of neuroinflammation, through inhibition of microglial activation, targeting of specific microglial genes, or selective depletion of spinal microglia, relatively consistently reverses pain hypersensitivity.^{25,35}

In contrast to preclinical findings that clearly indicate the importance of neuroimmune signaling for initiation and maintenance of persistent pain, studies in humans are limited. Emerging evidence from observational studies shows that proinflammatory mediators and glial activation may be important for modulation of pain perception in clinical persistent pain conditions, but numerous methodological considerations prevent firm conclusions.^{10,57} Moreover, research evaluating whether cerebrospinal fluid (CSF) inflammation is related to objective measures of pain, eg, quantitative sensory testing (QST), has remained largely absent. Investigation of links between CSF neuroinflammatory patterns and neurophysiological pain phenotypes, such as high levels of CS, may increase our understanding of pain mechanisms and guide identification of novel treatment targets.

In this study, we therefore prospectively examined associations between CSF proinflammatory mediators and advanced characteristics of pain, sleep, and neurophysiology in patients with disabling osteoarthritis (OA)-related pain undergoing total hip arthroplasty (THA). Specifically, to examine whether there are coherent changes in CSF inflammation associated with clinically relevant categorical objective measures of persistent pain, we predefined subgroups according to degree of sensitization. In addition, we extended these within-subjects analyses, and cross-sectionally evaluated differences in CSF concentrations of inflammatory mediators between OA subjects and well-characterized pain-free controls. Our main hypotheses were that CNS pain processes would be differentially regulated by inflammatory mechanisms such that persistent pain patients would have changes in CSF levels of inflammation, and that there would be a dose-response relationship in which increasing pain is associated with differential changes of CSF mediators.

2. Methods

2.1. Study design and overview

The current report presents data from a prospective, observational study termed neuroPSI (neuro pain sleep immunology). The study was conducted in the setting of a fast-track, state-of-the-art orthopedic surgical center (Department of Orthopedics, Håssleholm Hospital, Sweden), which has conducted the highest annual rate of THAs in Sweden since 2002. Preoperatively, a cross-sectional comparison of OA pain subjects and age- and sex-matched, pain-free healthy controls was conducted. In OA subjects only, repeated follow-up assessments after THA were conducted over the course of 6 months.

All procedures were approved by the Regional Ethics Committee (Dnr 2018/396 and Dnr 2008/290). Written informed consent was obtained from each study participant before study activities.

2.2. Participants

Patients scheduled to undergo fast-track THA were consecutively screened preoperatively through chart review and structured interview. Inclusion criteria were: age \geq 18 years, persistent

OA-related pain \geq 12 months, average pain numerical rating scale (NRS) score \geq 4 and/or movement-related pain score \geq 4 after 5 minutes walking, and primary THA conducted under spinal anesthesia. Exclusion criteria were: acute illness (eg, infectious disease), malignancy, immunomodulating treatment, neurological disorder, severe psychiatric disorder (eg, severe major depression, bipolar disorder, and psychotic disorders), contraindications for lumbar puncture, American Society of Anesthesiology (ASA) physical status classification $>$ 3, substance abuse during the past 12 months, poor Swedish-language fluency, or inability to provide informed consent.

A total of 79 patients were screened for participation; 16 were deemed ineligible (low-pain OA $n = 9$, cognitive impairment $n = 2$, severe psychiatric comorbidity $n = 1$, systemic steroid treatment $n = 1$, acute infectious disease $n = 1$, leg ulcer $n = 1$, and non-Swedish speaking $n = 1$). Of the remaining 63 patients, 10 declined to participate, and for one patient anesthesia modality was changed from spinal anesthesia to general anesthesia on the day of surgery, resulting in 52 subjects being included. Study participants were included in September 2018 to November 2018, and follow-up assessments were concluded in May 2019. The subjects received no payment or other incentives for participating in the study.

The healthy controls consisted of 30 volunteers separately identified from a prior study on CSF inflammatory markers in Parkinson disease.³⁶ The most important exclusion criteria for healthy control participants were: acute illness, contraindications to lumbar puncture, neurological disorder, cancer, substance or alcohol abuse, and inadequate Swedish-language fluency. In addition to demographic and clinical variables, information regarding pain and sleep status was obtained through a customized questionnaire. No control participant had a diagnosis associated with persistent pain. All control participants denied having experienced any significant acute pain for 7 days before enrollment, as confirmed by a negative response to the first question of the brief pain inventory short form (BPI-sf), modified to cover the past week.

2.3. Study procedures

2.3.1. Anesthesia, surgery, and perioperative care in the osteoarthritis patients

Spinal anesthesia was administered to all patients through IT injection of hyperbaric bupivacaine. The surgery was performed or supervised by 1 of the 5 consultant surgeons doing $>$ 50 THAs per year. A posterior approach was used in all cases and, according to the surgeon preference, patients had a cemented, hybrid, or uncemented THA. The stems used were a cemented Exeter V40 stem (Stryker Orthopaedics, Mahwah, NJ) or an uncemented Accolade II stem (Stryker Orthopaedics). The acetabular cups used were a cemented Exeter X3 RimFit socket (Stryker Orthopaedics) or an uncemented Trident Acetabular System (Stryker Orthopaedics).

Key elements of the perioperative care associated with fast-track THA at Håssleholm Hospital, aiming to reduce surgical stress and facilitate postoperative recovery, include early mobilization on the day of surgery and multimodal pain control (celecoxib 200 mg twice daily until postoperative day [POD] 3; paracetamol 1 g 3 times daily until POD7; and oxycodone 5 mg as needed).

2.3.2. Quantitative sensory testing in the osteoarthritis subjects

The QST protocol was based on protocols that have previously been validated for evaluation of somatosensory abnormalities in

hip OA patients,^{50,51} and designed to comply with recommendations made by the German Research Network on Neuropathic Pain.⁷⁴ All OA subjects had been given instructions to refrain from taking analgesic medications 24 hours before QST.

The QST was performed in a quiet, air-conditioned (20–24°C) room, approximately 60 minutes before surgery. All testing was conducted by one physician with extensive QST experience (M.F.B.). Throughout the QST, the participants were asked to keep their eyes closed to enable complete focus on the evoked sensations. To acclimate the participant to the different testing modalities, brief demonstrations of the procedures were performed on the nondominant forearm, before initiation of the QST protocol.

For all testing modalities, 3 body areas were examined: (1) the area of maximum pain on the OA-affected side, (2) the corresponding contralateral area, and (3) a proximal, volar area on the dominant forearm.

2.3.2.1. Pressure pain detection and pressure pain tolerance thresholds

To determine the pressure pain detection threshold (PPDT) and pressure pain tolerance threshold (PPTT), a digital algometer (SBMEDIC Electronics, Solna, Sweden) with a 1-cm² probe area was applied to the skin at a constant rate of 20 kPa/second. The subject was instructed to indicate (verbally and/or through pushing a button) when the stimulus “first feels painful” (PPDT) or when the pain was no longer tolerable (PPTT). Three trials with a 30-second interstimulus interval were completed per area for the pressure pain detection; the PPDT was calculated as the average value. To assess PPTTs, only one trial was performed in each area. To avoid potential harm, pressure was not applied beyond 588 kPa.

2.3.2.2. Temporal summation

To evaluate CNS enhancement of mechanical pain caused by repeated painful stimulation (temporal summation, TS), a monofilament calibrated to deliver 588 mN of force (Aesthesio; DanMic Global, San Jose, CA) was used. After rating the pain response to a single punctate stimulus (0–100; 0 = no pain, 100 = worst pain imaginable), a sequence of 10 repeated stimuli (1 Hz) was administered within an area of 1 cm²; participants rated the peak pain experience during the sequence. The wind-up ratio (WUR) was calculated as: peak pain rating/pain rating of single stimulus. In addition, dynamic mechanical allodynia was assessed through light tactile stimulation with a cotton swab applied for 60 seconds (2 Hz) over a 2-cm² area. Aftersensations (eg, paresthesias, numbness, and pain) lasting beyond 30 seconds after completion of the TS testing were recorded.

2.3.2.3. Conditioned pain modulation

The capacity of endogenous descending pain inhibitory systems was assessed through a QST paradigm comprising an occlusion cuff conditioning stimulus and a pressure pain test stimulus. After having assessed the PPDT, a 12-cm wide occlusion cuff was placed approximately 2 cm proximal to the cubital fossa on the dominant arm. The cuff was manually inflated and for each 10 to 15 mm Hg increase of pressure, the participant was asked to provide a pressure pain NRS grading 0 to 10 (0 = no pain, 10 = worst imaginable pain), until a pain NRS 5 was achieved. An arm occlusion cuff conditioning pain intensity level of 5/10 has previously been shown sufficient to induce a CPM effect.^{78,90} The pressure pain test stimulus was then applied to assess PPDT while the conditioning stimulus was maintained. During testing, if the conditioning stimulus produced an increasing pain level

exceeding NRS 5, the cuff was deflated, and a 5-minute pause was implemented, after which the sequence could be repeated. The procedure was conducted in all 3 areas (area of maximum pain, contralateral site, and nondominant forearm). The CPM effect (%) for each area was calculated as: $((PPDT_{conditioning} - PPDT_{baseline})/PPDT_{baseline}) \times 100$.

2.3.2.4. Pain assessment during peripheral venous cannulation

The subjects were asked to rate the maximum pain intensity during peripheral venous cannulation (1.1 mm inner diameter; Becton Dickinson Venflon, Franklin Lakes, NJ) on a pain NRS 0 to 10. The cannulation site was standardized (back of the hand) and conducted by one physician (M.F.B.).

2.3.3. Cerebrospinal fluid collection

Before IT administration of local anesthetic, 10 mL of CSF was carefully aspirated using 2-mL syringes. The CSF was immediately transferred to 5-mL Protein LoBind Tubes (Eppendorf AG, Hamburg, Germany) and chilled for transport. To minimize circadian variation of biomarkers, all samples were collected between 8 AM and 12 PM.

2.3.4. Questionnaires

To provide a comprehensive assessment of pain, sleep, psychopathology, health-related quality of life, OA-related symptoms, and physical activity, validated Swedish versions of the following questionnaires were completed preoperatively, on the day before surgery: BPI-sf,¹⁷ douleur neuropathique 4 (DN4),¹² pain catastrophizing scale (PCS),⁸⁰ Pittsburgh sleep quality index (PSQI),¹⁴ insomnia severity index (ISI),⁶ hospital anxiety and depression scale (HADS),⁹² European quality of life-5 dimensions (EQ-5D),³⁸ Western Ontario and McMaster Universities osteoarthritis index (WOMAC OA),⁷ and the international physical activity questionnaire short form (IPAQ-sf).¹⁹ In addition, the quality of recovery 15 (QoR-15)⁷⁹ questionnaire was administered to assess postoperative recovery throughout the follow-up period.

2.3.4.1. Pain perception

The BPI-sf yields 2 domain scores: a mean pain severity score, and a pain interference score. The scores range from 0 to 10 (0 = no pain, 10 = worst imaginable pain; 0 = no pain interference, 10 = complete pain interference).

The DN4 consists of 10 items: 7 related to pain quality and nonpainful symptoms, and 3 based on clinical examination, which yields a score 0 to 10. Scores ≥ 4 indicate that the pain is likely to be neuropathic in origin.

2.3.4.2. Pain catastrophizing

The PCS contains 13 items, each scored 0 to 4, yielding a total score 0 to 52, with higher scores indicating more pain catastrophizing thoughts. A total PCS score ≥ 30 suggests clinically relevant levels of catastrophizing.

2.3.4.3. Sleep

The PSQI is composed of 19 items, each scored 0 (no difficulty) to 3 (severe difficulty). Component scores are summed to a global score, ranging from 0 to 21. Higher scores indicate worse sleep quality; scores >5 indicate poor sleep quality.¹⁵

The ISI consists of 7 questions, each scored 0 to 4, yielding a total score of 0 to 28 (0–7: no clinically significant insomnia;

8-14: subthreshold insomnia; 15-21: clinical insomnia [moderate severity]; 22 to 28: clinical insomnia [severe]).

2.3.4.4. Anxiety and depression

The HADS consists of 14 items, 7 related to anxiety and 7 related to depression. Each item is scored 0 to 3, which yields a score 0 to 21 for either anxiety or depression. The scores can be interpreted as: 0 to 7 = normal, 8 to 10 = borderline abnormal, and 11 to 21 = abnormal.

2.3.4.5. Health-related quality of life

The EQ-5D has 5 dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression), each with 5 response levels. The EQ-5D also comprises a visual analogue scale, which represents the subject's self-rated health on a scale of 0 to 100 (0 = the worst health you can imagine, 100 = the best health you can imagine). Single EQ-5D index values were obtained through the EQ-5D-5L index value calculator⁸⁵ using value sets from Denmark.

2.3.4.6. Osteoarthritis-related symptoms

The WOMAC OA consists of 24 items regarding OA-related symptoms, each scored 0 to 4 (0 = none, 1 = slight, 2 = moderate, 3 = severe, and 4 = extreme). Three subscores are generated: pain (0-20), stiffness (0-8), and physical function (0-68); higher scores indicate worse symptomatology.

2.3.4.7. Physical activity

The IPAQ-sf was used to assess activity level. Separate scores are generated for walking, moderate-intensity, and vigorous-intensity activities. Both categorical (inactive, minimally active, health-enhancing physical activity active) and continuous (median MET-minutes per week; MET = multiple of the resting metabolic rate) measures of physical activity can be calculated. Kilocalories can be computed from MET-minutes: MET-minutes \times (weight in kilograms/60 kg).

2.3.4.8. Postoperative recovery

The QoR-15 consists of 15 questions covering important domains related to recovery after surgery (eg, pain, sleep, physical comfort, physical independence, psychological support, and emotional state). Each question is scored 0 to 10, which generates a total score of 0 to 150 (0 = very poor recovery, 150 = excellent recovery).

2.3.5. Multiplex assay analysis of cerebrospinal fluid

All CSF samples were centrifuged at 2000 G for 10 minutes at 4°C and stored in 1-mL aliquots at -80°C until batch analysis could be performed. Cerebrospinal fluid concentrations of the neuro-inflammatory and cerebrovascular biomarkers (Fms-related tyrosine kinase 1 [Flt-1], granulocyte-macrophage colony-stimulating factor, intercellular adhesion molecule 1 [ICAM-1], IL-6, IL-8, IL-15, interferon gamma-induced protein 10 [IP-10], MCP-1, phosphatidylinositol-glycan biosynthesis class F protein, vascular cell adhesion molecule 1 [VCAM-1], and vascular endothelial growth factor [VEGF]) were analyzed using ultrasensitive Mesoscale Discovery immunoassay and a customized V-PLEX kit according to the manufacturer's recommendations. Biomarkers were selected based on their known or potential relevance to pain processes and our previous findings regarding detectability. All inflammatory markers were evaluated in the

same assay batch. All samples were analyzed in duplicates and randomized according to diagnosis across plates/runs to minimize the effects of run-to-run variation. The lower limits of detection and interassay coefficients of variance (CV) are shown in Appendix 1 (available online as supplemental digital content at <http://links.lww.com/PAIN/B18>). Most granulocyte-macrophage colony-stimulating factor values (65%) were below the lower limits of detection and consequently excluded from further analyses. With the exception of VEGF, all interassay CV were low (6.6% for VEGF, and 2.0%-3.6% for the other biomarkers). Interassay CV for the VEGF assay were between 20% and 30% for 4 samples.

2.3.6. Postoperative follow-up assessments

Four postoperative follow-up assessments were conducted: POD1, POD7, 3 months postoperative, and 6 months postoperative. On POD1, subjects were assessed in the orthopedic ward before discharge from the hospital; on this occasion, the QoR-15 was completed. Information regarding opioid consumption during the first 24 hours after surgery was extracted from the subject's electronic medical chart. On POD7, all subjects were contacted through telephone to acquire information regarding postoperative analgesic consumption (mg oxycodone). In addition, the QoR-15, BPI-sf, and a modified seven-day version of the PSQI were administered. Three and 6 months postoperatively, subjects were contacted through telephone to complete the following questionnaires: QoR-15, BPI-sf, PSQI, and WOMAC OA. All postoperative assessments were conducted by one physician (M.F.B.), before any data analyses.

2.4. Statistical analysis

The Mann-Whitney *U* test was used for nonparametric comparisons between groups. Bivariate correlations were evaluated using Spearman rho. Longitudinal clinical data were examined through repeated-measures analysis of variance (Bonferroni adjustment was applied for multiple pairwise comparisons). Logistic regression models were used to examine the association between CSF biomarkers and dichotomized outcomes (tested with and without adjustment for covariates, including age and sex). The following subgroup analyses were predetermined: CSF proinflammatory mediator subgroup analyses of subjects with (1) signs of CS as suspected by arm PPDT <250 kPa vs \geq 250 kPa (<250 kPa cutoff as previously used,⁵⁸ based on a level 2 SDs below the mean PPDT in a healthy, non-OA population⁸⁹), (2) sensitization in the area of maximum pain according to WUR \geq 2 vs WUR <2 (above/below median in the current data set because no relevant a priori cutoff was available), and (3) maximum pain NRS score during peripheral venous cannulation (PVC) <3 vs \geq 3 (cutoff level based on PVC pain NRS score \geq 3/10 being associated with increased postoperative pain intensity and opioid consumption⁷¹). There was almost no missing longitudinal follow-up data (0.2%). All data were explored and examined through numerical and graphical methods to ensure that test and model assumptions were fulfilled for the analyses. Data are presented as mean \pm SD unless otherwise stated. Variables were log-transformed (natural log) as needed. Significance was accepted for $P < 0.05$. Analyses were performed in SPSS version 25 (IBM Corp, Armonk, NY).

2.4.1. Sample size calculation

Sample size power calculations were based on the hypothesis of biomarker differences between persistent pain patients and

controls, and previously reported CSF IL-8 group differences (hip/knee OA subjects vs controls).⁵⁹ Detection of a significant CSF IL-8 between-group difference, with a two-sided significance level of 5% and power of 90%, yielded an estimation of 30 pain subjects and 30 control participants. Given the a priori determined subgroup analyses, we aimed to recruit 50 persistent pain patients.

3. Results

3.1. Demographic data

The THA and control groups did not differ on mean age (70.4 ± 8.3 vs 67.8 ± 6.6 years, $P = 0.21$) or sex distribution (female:male 31:21 vs 16:14, $P = 0.65$), but the THA subjects had slightly higher body mass index (BMI) (28.0 ± 3.7 vs 25.3 ± 2.9 kg/m², $P = 0.002$). All participants were Caucasian. Current smoking was rare (OA subjects 5.8%, controls 6.7%). Education level was lower among OA subjects compared to controls (7–9 years: 42.3 vs 25.0%; 10–12 years: 34.6 vs 21.4%; >12 years: 23.1 vs 53.6%, $P = 0.02$).

3.2. Clinical and questionnaire data

3.2.1. Comorbidities and medications

Most OA subjects were classified as ASA physical status 1 or 2; only 3 subjects were ASA 3. The most common medical comorbidities in the OA group were hypertension ($n = 25$) and obesity grade 1 (BMI 30–35, $n = 12$). The most commonly used medications were angiotensin-converting enzyme-inhibitors ($n = 17$) and beta-blockers ($n = 12$). A complete description of comorbidities and medications is shown in Appendix 2 (available online as supplemental digital content at <http://links.lww.com/PAIN/B18>). Control participants had received few medical diagnoses—only 3/30 exhibited any relevant clinical condition (localized lichen ruber, synovial chondromatosis of the temporomandibular joint, and unspecified joint problems). None of the 3 subjects reported current pain symptoms or used analgesic/anti-inflammatory medications.

3.2.2. Preoperative pain

Previous, but no longer ongoing, long-term pain (duration ≥ 3 months), besides the present OA-related pain, was reported by 55.8% ($n = 29$). The most common diagnoses were low back pain, knee OA, and contralateral hip OA. The mean duration of current hip OA-related pain was 2.4 ± 2.2 years. Although use of paracetamol and nonsteroidal anti-inflammatory drugs was common, preoperative opioid treatment was relatively rare (codeine [$n = 6$], morphine [$n = 4$], and oxycodone [$n = 2$]), and only 2 subjects were taking gabapentinoids (Appendix 2, available online as supplemental digital content at <http://links.lww.com/PAIN/B18>). Preoperative pain scores can be found in Table 1. The OA cohort had high pain levels with major impact on activities of daily life. According to the DN4, at least 2 neuropathic pain features were exhibited by 61.5%; a DN4 score ≥ 4 , indicative of neuropathic pain, was detected in 15.4%. Clinically relevant pain catastrophizing was found among 9.6%. No participant in the control group had ongoing pain problems by subjective report; 23/30 never used analgesics and 7/30 reported infrequent use of nonopioid analgesics on a nondaily basis during the past week.

3.2.3. Preoperative sleep status

Significant past sleep problems lasting more than 3 months were reported by 44.2% of the pain subjects. The duration of past

sleep problems was 7.3 ± 8.8 years. Most OA subjects (73.1%) had poor preoperative sleep quality, as demonstrated by PSQI scores > 5 . Baseline PSQI and ISI scores are found in Table 1. Subjective sleep duration was 6.2 ± 1.4 hours (range, 3.0–9.0 hours). More than half of the subjects (61.5%) reported sleep problems ≥ 3 times/week due to night-time pain. Sleep medications such as zolpidem and zopiclone were intermittently used by 23.5%. The control participants reported very good (22.2%) or fairly good (63.0%) sleep quality during the past month; only 14.8% had fairly bad sleep quality and no controls reported very bad sleep quality.

3.2.4. Psychopathology, health-related quality of life, and physical activity

The prevalence of psychopathologies was low (Table 1). One OA subject showed evidence of depression, and 3 subjects had abnormal anxiety levels according to the HADS. As expected, HADS scores were low also for the control group (anxiety 3.7 ± 4.5 ; depression 2.3 ± 3.2). Only one control subject was taking an antidepressant medication (citalopram). For OA subjects, EQ-5D index scores indicated relatively poor quality of life, and as anticipated for a cohort awaiting THA, physical activity level was generally low (Table 1).

3.3. Quantitative sensory testing

PPDTs and PPTTs are shown in Figure 1. More than half (61.5%, $n = 32$) of the OA subjects showed evidence of CS according to arm PPDT < 250 kPa.^{58,89} All QST results, including pressure pain, punctate pain, WUR, conditioned pain modulation, and pain

Table 1
Preoperative baseline questionnaire variables for the hip OA pain subjects ($n = 52$).

Clinical variables	Results
Pain	
BPI-sf pain average (0-10)	5.5 ± 1.4 (2-8)
BPI-sf pain best (0-10)	3.2 ± 1.8 (0-7)
BPI-sf pain worst (0-10)	7.3 ± 1.3 (4-10)
BPI-sf pain severity score (0-10)	5.4 ± 1.3 (2.3-8.0)
BPI-sf pain interference score (0-10)	5.9 ± 1.8 (2.0-8.4)
WOMAC OA pain score (0-20)	11.2 ± 3.3 (2-18)
DN4 score (0-10)	2.0 ± 1.5 (0-7)
PCS total score (0-52)	15.8 ± 9.7 (1-39)
Sleep	
PSQI global score (0-21)	9.2 ± 5.0 (1-18)
ISI score (0-28)	10.2 ± 7.0 (0-21)
Psychopathology	
HADS anxiety score (0-21)	4.7 ± 3.8 (0-19)
HADS depression score (0-21)	3.7 ± 3.0 (0-13)
Health-related quality of life	
EQ-5D index score	0.527 ± 0.139 (0.102-0.859)
Physical activity and function	
IPAQ MET-min/week	1983.0 ± 1982.5 (0-8958)
IPAQ category (n): inactive/minimally active/ HEPA active	17/21/14
WOMAC OA physical function score (0-68)	37.2 ± 10.9 (5-57)

Data are mean \pm SD (range).

BPI-sf, brief pain inventory short form; DN4, douleur neuropathique; EQ5D, European quality of life-5 dimensions; HADS, hospital anxiety and depression scale; HEPA, health-enhancing physical activity; IPAQ, international physical activity questionnaire; ISI, insomnia severity index; MET, multiple of the resting metabolic rate; OA, osteoarthritis; PCS, pain catastrophizing scale; PSQI, Pittsburgh sleep quality index; WOMAC OA, Western Ontario and McMaster Universities osteoarthritis index.

response to PVC, can be found in **Table 2**. Two subjects exhibited mechanical allodynia; no subject exhibited wind-up due to cotton brush stimulation. Approximately one-third of subjects ($n = 15$, 28.8%) showed evidence of impaired pain inhibitory capacity, as demonstrated by negative CPM% when the test stimulus was applied to the arm.

3.4. Cross-sectional comparison of cerebrospinal fluid inflammatory mediators: osteoarthritis subjects vs pain-free controls

Between-group comparisons of CSF proinflammatory mediators are shown in **Table 3** and **Figure 2**. Mean concentrations of IL-8, ICAM-1, and VCAM-1 were significantly higher in the OA pain group compared to controls. Notably, there were also relatively large numerical differences between groups regarding Flt-1 and IP-10, with higher levels of the markers in the pain group. Excluding the VEGF samples with high interassay CV did not affect the results. Several biomarkers were significantly associated with each other in both the OA and control groups (results for the OA group are shown in **Table 4**).

3.5. Cerebrospinal fluid inflammation related to objective measures of pain in the osteoarthritis subjects

3.5.1. Central sensitization as suspected by pressure pain threshold

Comparison of CSF biomarkers between subjects with PPDT possibly indicating CS (PPDT arm <250 kPa; $n = 32$) vs subjects with PPDT above the defined threshold (PPDT ≥ 250 kPa indicating moderate-low sensitization; $n = 20$) showed significantly higher mean levels of Flt-1 (40.9 ± 12.0 vs 34.4 ± 11.9 pg/mL, $P = 0.044$) and IP-10 (366.3 ± 219.1 vs 239.6 ± 86.1 pg/mL, $P = 0.024$) in the CS group (**Fig. 3**). Age and BMI did not differ between groups with low vs high PPDT, but there were significantly more females in the CS group compared to the moderate-low sensitization group (25 vs 6 females, $\chi^2 P = 0.001$). There were no significant between-sex differences for the whole group regarding mean Flt-1 or IP-10.

Using logistic regression models, IP-10 predicted the binary sensitization outcome variable (ln IP-10 unadjusted odds ratio [OR] 3.9 [95% confidence interval 1.04-14.5], $P = 0.044$), but the effect was attenuated when sex was added to the model (sex OR 8.3 [2.1-32.7], $P = 0.002$; ln IP-10 OR 4.6 [0.98-21.5], $P = 0.052$). By contrast, sex and Flt-1 independently predicted the CS outcome (sex OR 10.6 [2.6-43.0], $P = 0.001$; ln Flt-1 OR 12.8 [1.2-130.6], $P = 0.032$).

3.5.2. High sensitization defined by wind-up ratio

Subjects with WUR ≥ 2 ($n = 29$) in the area of maximum pain had significantly higher levels of CSF MCP-1 compared to those with WUR <2 ($n = 22$) (341.9 ± 94.7 vs 277.2 ± 88.2 pg/mL, $P = 0.011$) (**Fig. 4**). Comparison of these 2 subgroups showed no significant differences regarding age, BMI, or sex. Multiple logistic regression showed that MCP-1 predicted WUR ≥ 2 (ln MCP-1 adjusted OR 10.6 [1.3-88.7], $P = 0.029$; model adjusted for sex, age, and BMI).

3.5.3. High pain response to peripheral venous cannulation

Subjects who reported pain NRS score ≥ 3 ($n = 28$) vs pain NRS score <3 ($n = 24$) during PVC had significantly higher CSF levels of Flt-1 (40.9 ± 10.4 vs 35.4 ± 13.7 pg/mL, $P = 0.025$) (**Fig. 5**).

There were no demographic differences between the 2 subgroups. There were several significant associations between pain NRS during PVC and QST outcomes: punctate pain arm ($\rho = 0.476$, $P < 0.0001$), punctate pain contralateral area ($\rho = 0.314$, $P = 0.023$), PPTT arm ($\rho = -0.296$, $P = 0.033$), and PPTT contralateral area ($\rho = -0.301$, $P = 0.030$). In a multiple logistic regression model adjusting for sex and age, Flt-1 predicted the categorical PVC pain outcome (ln Flt-1 adjusted OR 8.4 [1.1-65.4], $P = 0.043$).

3.6. Cerebrospinal fluid inflammation related to self-report measures of pain in the osteoarthritis subjects: explorative analyses

Analyses of baseline and follow-up subjective pain measures related to the 3 predefined objective pain phenotype categories revealed multiple significant findings.

The subjects with possible indications of CS according to arm PPDT, with elevated mean levels of Flt-1 and IP-10, had more pain-related symptoms, as shown by BPI-sf pain interference scores (POD7 4.7 ± 1.8 vs 3.7 ± 1.8 , $P = 0.016$; 3 months postoperative 1.4 ± 2.0 vs 0.6 ± 1.5 , $P = 0.028$; 6 months postoperative 2.1 ± 2.6 vs 0.7 ± 1.8 , $P = 0.052$), BPI-sf pain severity scores (3 months postoperative 1.2 ± 1.2 vs 0.6 ± 1.2 , $P = 0.012$; 6 months postoperative 2.0 ± 1.8 vs 0.9 ± 1.2 , $P = 0.042$), and QoR-15 pain scores (3 months postoperative 17.5 ± 2.6 vs 18.9 ± 2.8 , $P = 0.005$; 6 months postoperative 16.8 ± 4.2 vs 18.6 ± 2.5 , $P = 0.059$). Notably, although only 4 subjects received intravenous oxycodone during the first 24 hours after surgery, all these subjects belonged to the CS group.

Compared to the group with WUR <2 in the area of maximum pain, subjects with WUR ≥ 2 , with higher mean CSF MCP-1

Table 2

Preoperative quantitative sensory testing results for the OA pain cohort ($n = 52$).

QST variables	Results
Pressure pain thresholds (kPa)	
PPDT arm	250.5 \pm 102.7 (104.3 to 588.0)
PPTT arm	413.1 \pm 122.2 (205.0 to 588.0)
PPDT max pain	250.7 \pm 87.2 (128.7 to 493.5)
PPTT max pain	432.0 \pm 126.2 (172.0 to 588.0)
PPDT contralat	296.9 \pm 115.8 (125.3 to 575.0)
PPTT contralat	458.3 \pm 122.1 (195.0 to 588.0)
Punctate pain (0-100)	
PunctP arm	7.5 \pm 9.8 (0 to 40)
PunctP max pain	13.0 \pm 14.8 (0 to 60)
PunctP contralat	7.0 \pm 9.7 (0 to 35)
Wind-up ratio (0-100)	
WUR arm	7.0 \pm 11.4 (0 to 50)
WUR max pain	9.3 \pm 14.4 (0 to 60)
WUR contralat	9.2 \pm 12.3 (0 to 40)
Conditioned pain modulation (%)	
CPM arm	11.7 \pm 33.6 (-42.0 to 121.1)
CPM max pain	24.5 \pm 31.3 (-38.1 to 118.8)
CPM contralat	17.9 \pm 32.0 (-28.0 to 128.1)
CPM average (arm, max pain, contralat)	18.0 \pm 25.5 (-28.6 to 88.9)
Pain response to PVC (0-10)	
PVC pain	2.9 \pm 1.3 (1 to 6)

Data are mean \pm SD (range).

CPM, conditioned pain modulation; contralat, contralateral area (to the area of maximum pain); max pain, area of maximum pain; OA, osteoarthritis; PPDT, pressure pain detection threshold; PPTT, pressure pain tolerance threshold; PunctP, punctate pain; PVC, peripheral venous cannulation; QST, quantitative sensory testing; WUR, wind-up ratio.

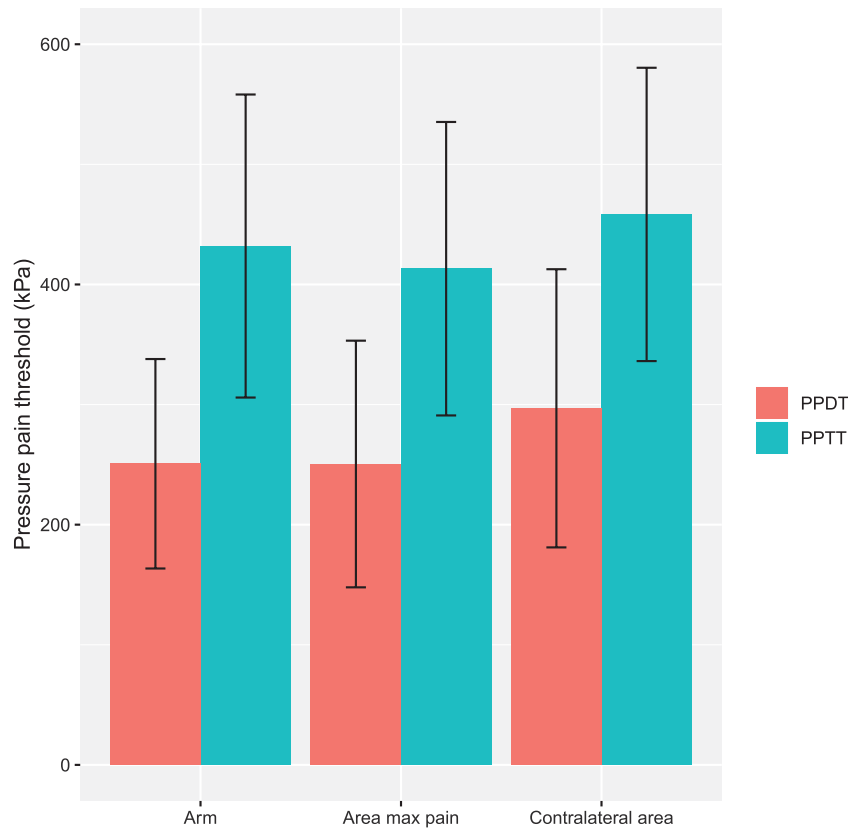


Figure 1. Pressure pain detection and pressure pain tolerance thresholds for the hip OA pain cohort ($n = 52$). Area max pain, area of maximum pain; OA, osteoarthritis; PPDT, pressure pain detection threshold; PPTT, pressure pain tolerance threshold.

levels, had more baseline pain (BPI-sf pain severity 5.7 ± 1.2 vs 4.9 ± 1.4 , $P = 0.042$; WOMAC pain score 12.0 ± 2.7 vs 10.1 ± 3.8 , $P = 0.044$; EQ5D pain score 3.7 ± 0.5 vs 3.3 ± 0.6 , $P = 0.018$).

High pain response during PVC, associated with elevated CSF Flt-1 concentrations, correlated with poorer postoperative pain control on POD7 (BPI-sf pain severity $\rho = 0.388$, $P = 0.004$; pain

interference $\rho = 0.286$, $P = 0.040$), more pain-related sleep problems on POD7 (PSQI subscore $\rho = 0.326$, $P = 0.018$), and higher pain levels 6 months after surgery (BPI-sf pain severity $\rho = 0.308$, $P = 0.026$).

Overall, there were few other clear patterns of significant bivariate correlations between IL-8, ICAM-1, VCAM-1, Flt-1, IP-10, and demographic, clinical, or neurophysiological baseline or follow-up variables in the whole pain cohort, or among males and females analyzed separately.

Table 3

Cerebrospinal fluid multiplex analyses of proinflammatory mediators: OA pain group vs pain-free controls.

Mediator	OA pain group ($n = 52$)	Control group ($n = 30$)	<i>P</i>
Flt-1	38.4 ± 12.2	33.7 ± 7.5	0.154
ICAM-1	2703.6 ± 758.3	2229.9 ± 697.1	0.007
IL-15	2.4 ± 0.6	2.2 ± 0.6	0.308
IL-6	0.7 ± 0.3	0.6 ± 0.2	0.294
IL-8	35.2 ± 9.2	29.9 ± 7.8	0.002
IP-10	319.1 ± 190.4	248.0 ± 102.5	0.151
MCP-1	314.0 ± 96.6	306.7 ± 90.5	0.597
PIGF	12.3 ± 4.8	10.5 ± 3.7	0.157
VCAM-1	7833.1 ± 2135.4	6496.5 ± 1721.9	0.006
VEGF	3.7 ± 1.6	3.5 ± 1.2	0.885

Data are mean \pm SD (pg/mL). All *P*-values are from Mann-Whitney *U*-test. Flt-1, Fms-related tyrosine kinase 1; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; OA, osteoarthritis; PIGF, phosphatidylinositol-glycan biosynthesis class F protein; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

4. Discussion

4.1. Main findings

This study provides an assessment of neuroinflammatory mediators and neurophysiological pain profiles in OA subjects undergoing THA due to disabling pain. To the best of our knowledge, differential expression of multiple CSF proinflammatory mediators depending on objective characteristics of pain in humans has previously not been reported. Osteoarthritis subjects with possible evidence of CS according to the predefined pressure pain threshold cutoff level had significantly elevated CSF concentrations of Flt-1 and IP-10. Flt-1 was also increased among subjects reporting high PVC pain, a clinically relevant evoked measure of CS. Moreover, subjects who exhibited increased mechanical TS, that is, enhancement of pain during repeated noxious stimulation, had elevated CSF levels of MCP-1. Cross-sectional comparison of CSF inflammatory mediators between OA subjects and pain-free controls showed that IL-8, ICAM-1, and VCAM-1 were higher among pain subjects, which strengthens and expands the

Table 4
Bivariate correlations between CSF proinflammatory mediators in the hip OA pain cohort (n = 52).

	Fit-1	ICAM-1	IL-15	IL-6	IL-8	IP-10	MCP-1	PIGF	VCAM-1	VEGF
Fit-1	—	0.228	0.445†	-0.261	0.176	0.154	0.099	-0.241	0.253	-0.418†
ICAM-1	0.228	—	0.503‡	0.169	0.487‡	0.383†	0.289*	0.305*	0.787‡	0.410†
IL-15	0.445†	0.503‡	—	0.175	0.569‡	0.405†	0.269	0.321*	0.572‡	0.081
IL-6	-0.261	0.169	0.175	—	0.394†	0.203	0.354*	0.189	0.114	0.378†
IL-8	0.176	0.487‡	0.569‡	0.394†	—	0.467†	0.077	0.251	0.605‡	0.257
IP-10	0.154	0.383†	0.405†	0.203	0.467†	—	0.078	0.245	0.416†	0.019
MCP-1	0.099	0.289*	0.269	0.354*	0.077	0.078	—	0.043	0.037	0.024
PIGF	-0.241	0.305*	0.321*	0.189	0.251	0.245	0.043	—	0.230	0.521‡
VCAM-1	0.253	0.787‡	0.572‡	0.114	0.605‡	0.416†	0.037	0.230	—	0.400†
VEGF	-0.418†	0.410	0.081	0.378†	0.257	0.019	0.024	0.521‡	0.400†	—

Spearman's ρ ; bold coefficients represent statistical significance.

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.001$.

CSF, cerebrospinal fluid; ICAM, intercellular adhesion molecule; MCP-1, monocyte chemoattractant protein 1; PIGF, phosphatidylinositol-glycan biosynthesis class F protein; VCAM, vascular cell adhesion molecule; OA, osteoarthritis.

evidence implicating neuroinflammation, as characterized by multiple markers, is associated with long-term pain.

4.2. Pain mechanisms underlying persistent osteoarthritis pain

Pain is the most disabling symptom of OA.^{42,69} In the periphery, pain is generated due to tissue injury, inflammation, angiogenesis, and ectopic growth of sensory and sympathetic nerve fibers in the affected joint.⁶² Immune cells and nonneuronal cells increase the excitability of primary sensory neurons (peripheral sensitization).¹⁸ In the CNS, CS may contribute to the pain experience. Indeed, the correlation between structural joint affection and presence of pain is only weak-moderate,⁴² 7% to 23% of hip OA patients continue to experience long-term pain after joint replacement,⁸ and meta-analyses show decreased PPDTs at remote sites in OA subjects compared to healthy controls.^{56,81} In OA rodent models, mechanisms underlying CS include macrophage infiltration of the DRG, activation of spinal cord glial cells, and increased production of proinflammatory mediators.^{43,45,48,61,72,81} Congruent with our results, impairment

of endogenous descending pain inhibition is common in OA populations.⁵⁶ Features of neuropathic pain are prevalent among patients with hip OA,³⁰ and increasing evidence demonstrates an association between neuroinflammation and persistent neuropathic pain in humans.^{5,34} Comorbidities to OA, particularly sleep disturbance and affective disorders, are also related to proinflammatory changes in the CNS.⁴⁴

4.3. The role of IL-8, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in pain processes

We and others have previously reported elevated CSF levels of IL-8 in persistent pain populations such as fibromyalgia,⁴⁷ failed back surgery syndrome,³³ lumbar disk herniation/radiculopathy,^{54,68,70} hip/knee OA,^{52,59} and postherpetic neuralgia.^{49,53} IL-8 (also known as CXCL8) exerts its actions through binding to the receptors CXCR1 and CXCR2.²⁰ In the human CNS, CXCR2 is expressed on neurons, microglia, and astrocytes; glial release of IL-8 is stimulated by immune activation and afferent nociceptive input.^{39,65} Pain-promoting effects of IL-8 include activation of CXCR2 on nociceptors, leading to upregulation of

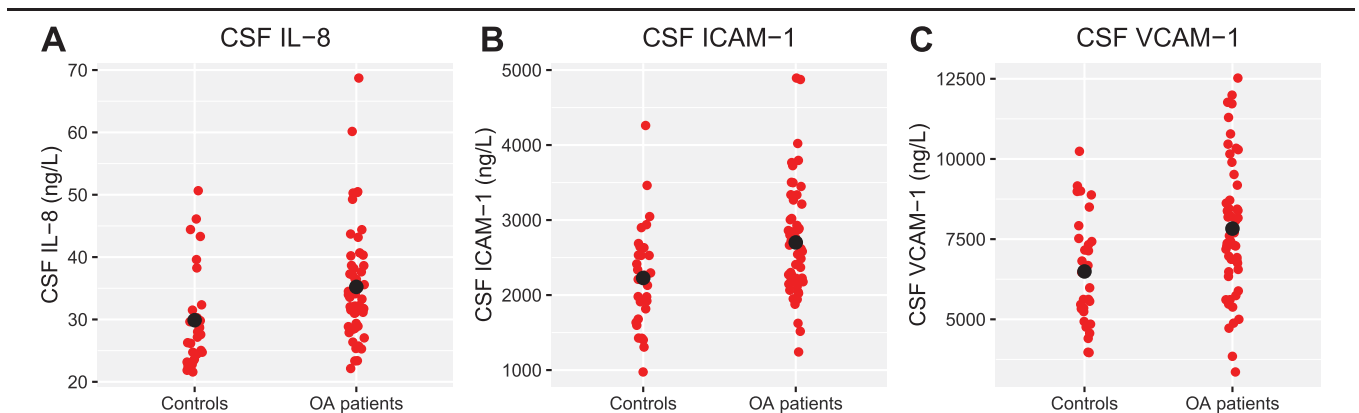


Figure 2. Cerebrospinal fluid IL-8, ICAM-1, and VCAM-1: OA pain cohort (n = 52) vs healthy controls (n = 30). Between-group comparison P -value (Mann-Whitney): (A) $P = 0.002$, (B) $P = 0.007$, (C) $P = 0.006$. Black dots represent the mean value. CSF, cerebrospinal fluid; ICAM1, intercellular adhesion molecule 1; IL-8, interleukin 8; OA, osteoarthritis; VCAM1, vascular cell adhesion molecule 1.

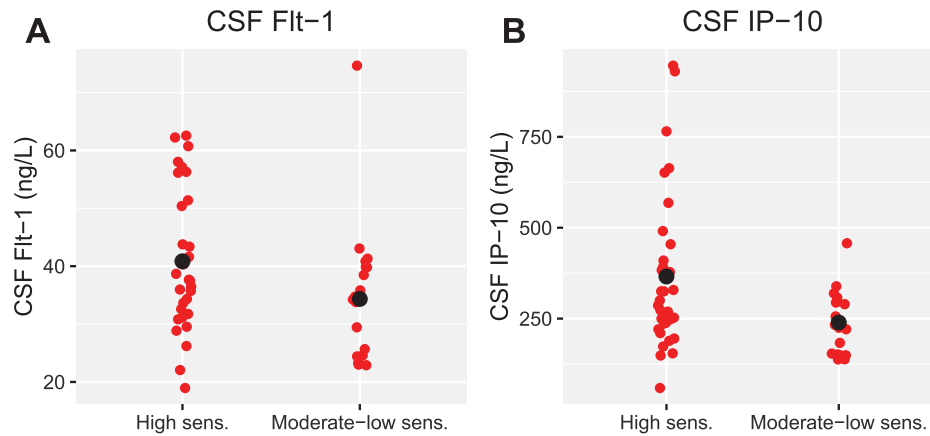


Figure 3. Subgroup analyses within the OA pain cohort: Flt-1 and IP-10 in patients with arm PPDT <250 kPa (indicative of central sensitization; n = 32) vs arm PPDT \geq 250 kPa (moderate-low sensitization; n = 20). Between-group comparison *P*-value (Mann–Whitney): (A) *P* = 0.044, (B) *P* = 0.024. Black dots represent the mean value. Flt-1, Fms-related tyrosine kinase 1; IP-10, interferon gamma-induced protein 10; OA, osteoarthritis; PPDT, pressure pain detection threshold; sens, sensitization.

sodium channels,⁸⁷ and enhanced transient receptor potential vanilloid 1 (TRPV1) channel function,²² increasing neuronal excitability. Importantly, IL-8 drives inflammation through chemotactic effects on T lymphocytes and neutrophils.⁷⁵ Although activated glia cells at multiple levels of the CNS may be the main source of IL-8,^{3,4,57} disruption of the blood–spinal cord barrier, through nerve injury or circulating cytokines, can facilitate transmission of inflammatory mediators and leukocytes into the CNS.^{25,40,55,82} Recently, reparixin, a CXCR1/2 inhibitor, was found to attenuate nocifensive behavior in a mouse model of chronic low back pain.⁵⁴

In contrast to IL-8, less is known about the role of ICAM-1 and VCAM-1 in pain populations, with few studies in humans. Intercellular adhesion molecule-1 and VCAM-1 mediate interactions between vascular endothelium and leukocytes,

promoting recruitment of leukocytes to inflammatory foci. Inflammatory cytokines trigger increases of ICAM-1 on the luminal surface of endothelial cells and multiple other cell types.²³ In a peripheral inflammatory pain model, upregulation of ICAM-1 in the endothelium was associated with enhanced migration of β -endorphin-containing immune cells, and decreased pain behavior.⁶⁰ In another study, inflammatory pain increased ICAM-1 expression at the blood–brain barrier, and induced region-specific ICAM-1 expression in cerebral microvessels correlated with microglia activation in brain areas involved with pain modulation.⁴¹ Interestingly, in a population-based study, baseline blood levels of soluble VCAM-1 independently predicted the 15-year probability of knee and hip joint replacement in OA patients.⁷⁶

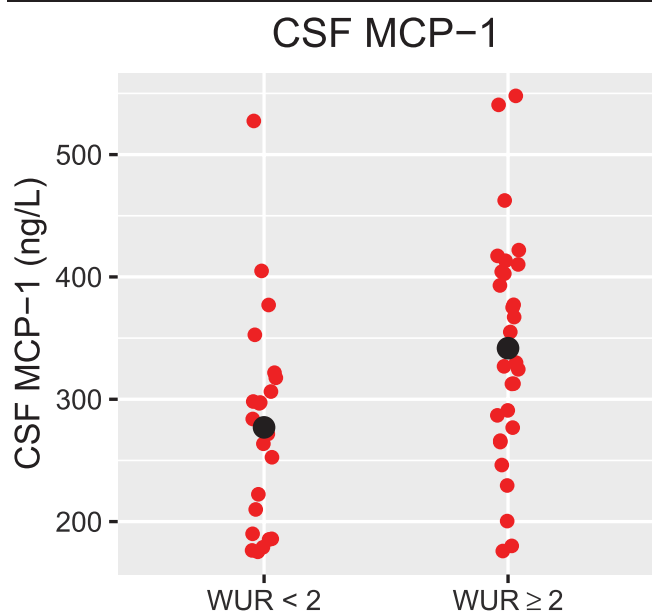


Figure 4. Subgroup analysis within the OA pain cohort: MCP-1 in subjects with WUR \geq 2 (n = 29) vs WUR <2 (n = 22) in the area of maximum pain. Between-group comparison *P*-value (Mann–Whitney): *P* = 0.011. Black dots represent the mean value. MCP-1, monocyte chemoattractant protein 1; OA, osteoarthritis; WUR, wind-up ratio.

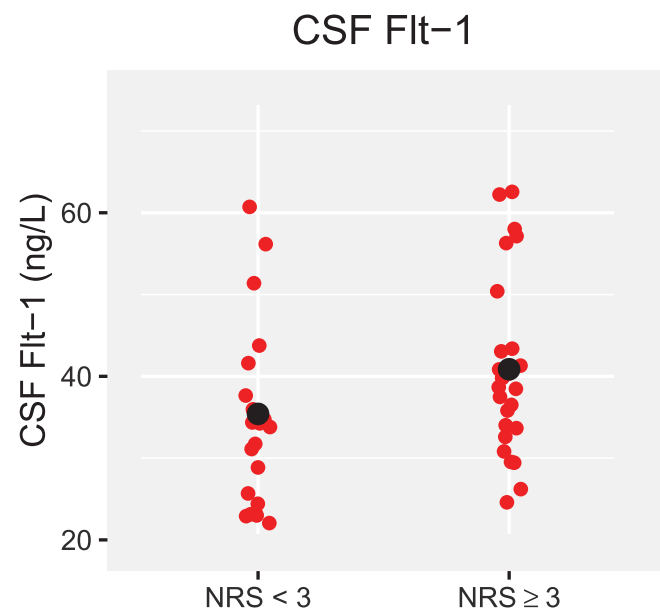


Figure 5. Subgroup analysis within the OA pain cohort: Flt-1 in subjects stratified according to high (NRS score \geq 3) or low (NRS score <3) pain response to peripheral venous cannulation. Between-group comparison *P*-value (Mann–Whitney): *P* = 0.025. Black dots represent the mean value. Flt-1, Fms-related tyrosine kinase 1; NRS, numerical rating scale (0 = no pain, 10 = worst imaginable pain); OA, osteoarthritis.

4.4. Links between cerebrospinal fluid neuroinflammatory mediators and pain phenotype

To the best of our knowledge, no previous study has incorporated both comprehensive QST and CSF proinflammatory mediator assessment in a pain population. Two studies have, however, included pressure pain testing in remote body sites in conjunction with examination of CSF markers.^{52,70} In a recent study, subgroup analysis showed a negative correlation between CSF IL-8 and spinal pressure pain threshold in male lumbar disk herniation subjects.⁷⁰ By contrast, another study reported that increased CSF IL-8 levels were associated with reduced pressure pain sensitivity, albeit for only 1 out of 3 pressure pain measures.⁵² Importantly, in both of these studies, pressure pain testing was conducted “within a week from surgery”, whereas CSF was sampled immediately preoperative. Our QST protocol was completed approximately one hour before CSF acquisition, which optimizes interpretation of associations between pain phenotype and neuroimmune profile.

Cerebrospinal fluid Flt-1 was elevated in OA subjects demonstrating signs of CS as determined by PPDT and PVC pain response in 2 different body sites remote from the affected joint. Notably, although the overlap between the groups with CS according to low PPDT or high PVC-pain was low (18/52 = 34.6%), CSF Flt-1 was still coherently increased in both groups. Flt-1 (or VEGF receptor 1) is 1 of 3 types of receptor kinases that binds members of the VEGF family of glycoproteins. VEGF conveys multiple functions, including angiogenesis and chemotaxis. Aberrant VEGF signaling in OA tissues correlates with disease severity and pain.³⁷ Pronociceptive mechanisms of Flt-1 signaling include indirect effects through stimulation of sensory nerve ingrowth, and direct pain sensitizing actions on neurons. Selvaraj and colleagues recently showed that Flt-1 is upregulated in sensory neurons in human and mouse cancer models, and that activation of Flt-1 leads to sensitization of TRPV1.⁷⁷ Notably, systemic anti-Flt-1 antibody treatment reversed mechanical and thermal hyperalgesia.⁷⁷ In a mouse knee OA model, IT inhibition of Flt-1 reduced mechanical hyperalgesia,²¹ indicating a CNS role of Flt-1 in pain transmission.

In addition to Flt-1, IP-10 was increased among subjects with PPDT possibly indicating CS. IP-10 is a chemokine that mediates leukocyte entry into the CNS. Although the role of IP-10 is established in several neuroinflammatory diseases,⁶⁴ little is known about CNS IP-10 in human pain populations. In rodent models of neuropathic pain, mechanical allodynia and thermal hyperalgesia are associated with increased expression of IP-10, and its receptor CXCR3, in the DRG and spinal cord.^{9,16} The induced pain hypersensitivity is attenuated by IT injection of a CXCR3 inhibitor.¹⁶ In a metastatic cancer bone pain model, blockade of IP-10/CXCR3 decreased pain behavior and microglial activation.¹³

We also found that subjects with increased TS had elevated CSF MCP-1. Interestingly, the high-TS subgroup showed low overlap with both high-CS subgroups (low arm PPDT 17/52 = 32.7%; high PVC pain 15/52 = 28.8%). TS arises in part due to sensitization of second-order dorsal horn C fibers in the spinal cord.²⁶ N-methyl-D-aspartate-receptor activation is central for TS, and MCP-1 potentiates N-methyl-D-aspartate-induced currents.³¹ Ample preclinical evidence implicates MCP-1 in processes underlying CS. For example, IT MCP-1 causes mechanical and thermal hypersensitivity, and conversely, IT inhibition of MCP-1 attenuates neuropathic pain sensitization.^{31,83} Monocyte chemoattractant protein-1 is a chemokine that promotes infiltration of leukocytes through the BSCB, and

increases BSCB permeability after nerve injury.²⁴ Monocyte chemoattractant protein-1 is released in the periphery by multiple cell types and primary afferent neurons,⁸⁶ and in the CNS by activated BSCB endothelial cells,⁸⁸ and spinal cord astrocytes.³¹ After peripheral nerve injury, MCP-1 is an important factor for microglial activation in the spinal cord, and stimulates proliferation and differentiation of macrophages/monocytes to microglia.^{83,91}

4.5. Methodological considerations

First, the QST protocol and full set of questionnaires were only applied to the pain cohort. Although the study focused on within-group analyses among OA subjects, more detailed characterization of the control group, including neurophysiological examination, could have increased our understanding of the investigated pain processes. Second, the arm PPDT threshold <250 kPa, indicating CS, is not an established cutoff level, but was chosen based on statistics and previous research^{58,89}; results should be interpreted accordingly. Third, no objective sleep measures were collected. For future studies, we encourage inclusion of actigraphy for acquisition of objective sleep continuity data, to enable detailed evaluation of links between perioperative sleep and pain.¹¹ Finally, besides our predetermined group and subgroup analyses, our correlation analyses were not adjusted for multiple comparisons and should be regarded as explorative.

4.6. Conclusions

In conclusion, our results suggest that persistent pain phenotype may be associated with distinct CSF neuroinflammatory profiles in hip OA patients. Although there is no gold standard for determination of CS, our findings indicate a connection between Flt-1, IP-10, MCP-1, and measures of sensitization. Elevated CSF levels of IL-8, ICAM-1, and VCAM-1 in pain subjects compared to pain-free controls strengthens the evidence of neuroinflammation contributing to long-term pain. Given the salient links between CNS inflammation and pain perception, future mechanistic studies assessing neuroimmune mediators and QST measures of pain are encouraged. Targeting of mediators and pathways underlying neuroinflammation and glia activation may provide improved pain control for patients suffering from persistent pain.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Appendix A. Supplemental digital content

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