Title
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Permalink
https://escholarship.org/uc/item/0d00933p

Journal
Pain, 161(9)

ISSN
0304-3959

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Publication Date
2020-05-06

DOI
10.1097/j.pain.0000000000001903

Peer reviewed
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 ≥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. 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. Despite substantial research efforts, treatment options remain limited, only partially effective, and associated with side effects. Over the past 3 decades, neuroinflammation has emerged as a central feature of persistent pain states. 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. Central neuroimmune modulation is well established in animal models of inflammation and neurogenic pain. For example, intrathecal (IT) administration of proinflammatory cytokines such as interleukin (IL) (IL-1β, IL-8), monocyte chemoattractant protein 1 (MCP-1), and TNF-α induces allodynia and hyperalgesia, indicative of central sensitization (CS). Conversely, upregulation or administration of...
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. Knockout animals lacking IL-1β, IL-1 receptor 1, or MCP-1 demonstrate reduced or abrogated pain behavior in peripheral nerve injury models, and inhibition of IL-6 decreased pain in a rodent chronic low back pain model. Attenuation of neuroinflammation, through inhibition of microglial activation, targeting of specific microglial genes, or selective depletion of spinal microglia, relatively consistently reverses pain hypersensitivity.

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. 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 proinflammatory 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, Hässleholm 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 ≥18 years, persistent OA-related pain ≥12 months, average pain numerical rating scale (NRS) score ≥4 and/or movement-related pain score ≥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), contra-indications 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. 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 Hässleholm 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 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 and designed to comply with recommendations made by the German Research Network on Neuropathic Pain. 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 PPTT 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 alldynia 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. 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}) × 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-stf, 12 pain catastrophizing scale (PCS), 80 Pittsburgh sleep quality index (PSQI), 14 insomnia severity index (ISI), 6 hospital anxiety and depression scale (HADS), 32 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). 15 In addition, the quality of recovery 15 (QoR-15) questionnaire was administered to assess postoperative recovery throughout the follow-up period.

2.3.4.1. Pain perception

The BPI-stf 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.

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 calculator 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, active) and continuous (metabolic rate) measures of physical activity can be calculated. Kilocalories can be computed from MET-minutes: MET-minutes X (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 neuroinflammatory 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 ≥250 kPa (<250 kPa cutoff as previously used, based on a level 2 SDs below the mean PPDT in a healthy, non-OA population), (2) sensitization in the area of maximum pain according to WUR ≥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 ≥3 (cutoff level based on PVC pain NRS score ≥3/10 being associated with increased postoperative pain intensity and opioid consumption). 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 ± 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), 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 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 = 9], 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. 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 = 32).

| 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 ± 1.6 (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 ± SD (range).

BPI-sf, brief pain inventory short form; DN4, douleur neuropathique; EQSD, 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, 28.6%) 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 PPDT 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 PPDT (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 PPDT or IP-10.

Using logistic regression models, IP-10 predicted the binary sensitization outcome variable (IP-10 unadjusted odds ratio [OR] 3.9 [95% confidence interval 1.04-14.5], $P = 0.023$), PPDT arm (OR = 10.6 [2.6-43.0], $P = 0.016$), and PPDT contralateral (OR = 10.4 vs 35.4 $P = 0.024$). In a multiple logistic regression model adjusting for sex and age, Flt-1 predicted the categorical PVC pain outcome (Flt-1 adjusted OR 8.4 [1.1-65.4], $P = 0.043$).

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 (OR = 0.476, $P < 0.0001$), punctate pain contralateral area (OR = 0.314, $P = 0.023$), PPTT arm (OR = -0.296, $P = 0.033$), and PPTT contralateral area (OR = -0.301, $P = 0.030$). In a multiple logistic regression model adjusting for sex and age, Flit-1 predicted the categorical PVC pain outcome (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). |
| OST variables | Results |
| Pressure pain thresholds (kPa) | |
| PPDT arm | 250.5 ± 102.7 (104.3 to 588.0) |
| PPDT max pain | 250.7 ± 87.2 (128.7 to 493.5) |
| PPTT arm | 413.1 ± 122.2 (205.0 to 588.0) |
| PPTT max pain | 432.0 ± 128.2 (172.0 to 588.0) |
| PPTT contralateral | 296.9 ± 115.8 (125.3 to 575.0) |
| PPTT contralateral | 458.3 ± 122.1 (195.0 to 588.0) |
| Punctate pain (0-100) | |
| PunctP arm | 7.5 ± 9.8 (0 to 40) |
| PunctP max pain | 13.0 ± 14.8 (0 to 60) |
| PunctP contralateral | 7.0 ± 9.7 (0 to 35) |
| Wind-up ratio (0-100) | |
| WUR arm | 7.0 ± 11.4 (0 to 50) |
| WUR max pain | 9.3 ± 14.4 (0 to 60) |
| WUR contralateral | 9.2 ± 12.3 (0 to 40) |
| Conditioned pain modulation (%) | |
| CPM arm | 11.7 ± 33.6 (−42.0 to 121.1) |
| CPM max pain | 24.5 ± 31.3 (−28.1 to 118.8) |
| CPM contralateral | 17.9 ± 32.0 (−28.0 to 128.1) |
| CPM average (arm, max pain, contralateral) | 18.0 ± 25.5 (−28.6 to 88.9) |
| Pain response to PVC (0-10) | |
| PVC pain | 2.9 ± 1.3 (1 to 6) |

Data are mean ± 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.
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 \( r = 0.388, P = 0.004 \); pain interference \( r = 0.286, P = 0.040 \), more pain-related sleep problems on POD7 (PSQI subscore \( r = 0.326, P = 0.018 \)), and higher pain levels 6 months after surgery (BPI-sf pain severity \( r = 0.308, P = 0.026 \)).

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.

### 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 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) | \( P \) |
|------------|-----------------------|------------------------|--------|
| 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 ± 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.
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. 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. Immune cells and nonneuronal cells increase the excitability of primary sensory neurons (peripheral sensitization). 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. To 23% of hip OA patients continue to experience long-term pain after joint replacement, and meta-analyses show decreased PPDTs at remote sites in OA subjects compared to healthy controls. 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. Congruent with our results, impairment of endogenous descending pain inhibition is common in OA populations. Features of neuropathic pain are prevalent among patients with hip OA, and increasing evidence demonstrates an association between neuroinflammation and persistent neuropathic pain in humans. Comorbidities to OA, particularly sleep disturbance and affective disorders, are also related to proinflammatory changes in the CNS.

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, failed back surgery syndrome, lumbar disk herniation/radiculopathy, hip/knee OA, and postherpetic neuralgia. IL-8 (also known as CXCL8) exerts its actions through binding to the receptors CXCR1 and CXCR2. Pain-promoting effects of IL-8 include activation of CXCR2 on nociceptors, leading to upregulation of

Table 4

|        | Flt-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 | 0.298* | 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 r; 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.

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

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. 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. 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. 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.

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 ≥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.

Figure 4. Subgroup analysis within the OA pain cohort: MCP-1 in subjects with WUR ≥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 ≥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. 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. 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. Importantly, in both of these studies, pressure pain testing was conducted “within a week from surgery”, whereas CSF was sampled immediately preoperatively. 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. 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. Notably, systemic anti-Flt-1 antibody treatment reversed mechanical and thermal hyperalgesia. In a mouse knee OA model, IT inhibition of Flt-1 reduced mechanical hyperalgesia, 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, 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. The induced pain hypersensitivity is attenuated by IT injection of a CXCR3 inhibitor. In a metastatic cancer bone pain model, blockade of IP-10/CXCR3 decreased pain behavior and microglial activation.

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. N-methyl-D-aspartate-receptor activation is central for TS, and MCP-1 potentiates N-methyl-D-aspartate-induced currents. 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.

Monocyte chemoattractant protein-1 is a chemokine that promotes infiltration of leukocytes through the BSCB, and increases BSCB permeability after nerve injury. Monocyte chemoattractant protein-1 is released in the periphery by multiple cell types and primary afferent neurons, and in the CNS by activated BSCB endothelial cells, and spinal cord astrocytes. 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.

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; 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. 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.

Acknowledgments

Work at the authors’ laboratory was supported by Stiftelsen Olle Engkvist Byggmästare, the Skåne University Hospital Foundation, the Wallenberg Center for Molecular Medicine, The Knut and Alice Wallenberg Foundation, The Medical Faculty at Lund University, Region Skåne, the European Research Council, the Swedish Research Council, the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson’s disease) at Lund University, the Swedish Alzheimer Foundation, the Swedish Brain Foundation, the Torsten Söderberg Foundation, the Parkinson foundation of Sweden, The Parkinson Research Foundation, the Swedish Medical Association, the Konung Gustaf V:s och Drottning Victoria Frimurarestiftelse, and the Swedish federal government under the ALF agreement. O. Hansson has acquired research support (for the institution) from Roche, Pfizer, GE Healthcare, Biogen, AVID Radiopharmaceuticals, and...
Eurandom. In the past 2 years, O. Hansson has received consultancy/speaker fees (paid to the institution) from Biogen and Roche. The authors thank all anesthesiologists, orthopedic surgeons, and nurse anesthetists at the Department of Orthopedics, Hässleholm Hospital, who contributed to the data collection of the study. The authors also thank Maria Jönsson for excellent logistical assistance and technical help.

Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B18.

Article history:
Received 17 January 2020
Received in revised form 19 April 2020
Accepted 21 April 2020
Available online 6 May 2020

References

[1] Abbadie C, Lindia JA, Curniskey AM, Peterson LB, Mudgett JS, Bayne EK, DeMartino JA, Macintyre DE, Forrest MJ. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc Natl Acad Sci U S A 2003;100:7947–52.
[2] Ahn DK, Lee KR, Lee HJ, Kim SK, Choi HS, Lim EJ, Park JS. Intracisternal administration of chemokines facilitated formalin-induced behavioral responses in the orofacial area of freely moving rats. Brain Res Bull 2005;66:50–8.
[3] Albrecht DS, Ahmed SU, Kettner NW, Borra RJH, Cohen-Adad J, Deng H, Houle TT, Opalacz A, Roth SA, Melo MFV, Chen L, Mao J, Hooker JM, Lloggia ML, Zhang Y. Neuroinflammation of the spinal cord and nerve root in chronic pain patients. PAIN 2018;159:968–77.
[4] Albrecht DS, Forsberg A, Sandstrom A, Bergan C, Kadelott D, Protensko E, Lampia J, Lee YC, Hoglund CO, Catana C, Cervenka S, Akeju O, Lekander M, Cohen G, Haldin C, Taylor N, Kim M, Hooker JM, Edwards RR, Napadow V, Kosek E, Lloggia ML. Brain gial activation in fibromyalgia—a multi-site positron emission tomography investigation. Brain Behav Immun 2019;75:72–89.
[5] Backroyd E, Lind AL, Thulun M, Larson A, Gerde B, Gorth D. High levels of cerebrospinal fluid chemokines point to the presence of neuroinflammation in peripheral neuropathic pain: a cross-sectional study of 2 cohorts of patients compared with healthy controls. PAIN 2017;158:2487–95.
[6] Bastien CH, Vallieres A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. Sleep Med 2002;1:297–307.
[7] Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt L. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes following total hip or knee arthroplasty in osteoarthritis. J Orthopaaedic Rheumatol 1988;1:96–108.
[8] Beswick AD, Wylde V, Gooberman-Hill R, Blom A, Dieppe P. What constitutes success in chronic pain? a comprehensive review. Pain Pract 2016;16:183–203.
[9] Bjurstrom MF, Irwin MR. Perioperative pharmacological sleep-promotion and pain control: a systematic review. Pain Pract 2019;19:552–69.
[10] Bouhassira D, Attal N, Alchaar H, Boureuff F, Brochet B, Bruxelle J, Curin G, Ferrannian J, Gneiss P, Grun-Overdzyking A, Hafard-Stuelpnagel J, Lanteri-Minet M, Laurent B, Minet M, Royaert S, Seo Y, Valade D, Vicaut E. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). PAIN 2005;114:29–36.
[11] Bu H, Shu B, Gao F, Liu C, Guan X, Ke C, Cao F, Hinton AO Jr, Xiang H, Yang H, Tian X, Tian Y. Spinal IFN-gamma-induced protein-10 (CXCL10) mediates metastatic breast cancer-induced bone pain by activation of microglia in rat models. Breast Cancer Res Treat 2014;143:255–63.
[12] Buyssse DJ, Reynolds CF III, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193–213.
[13] Buyssse DJ, Reynolds CF III, Monk TH, Hoeh CC, Yeager AL, Kupfer DJ. Quantification of subjective sleep quality in healthy elderly men and women using the Pittsburgh Sleep Quality Index (PSQI). Sleep 1991;14:331–8.
[14] Chen Y, Yin D, Fan Z, Zou X, Chen Q, Li Y, Zhou S, Lu R, Xu Z. Chemokine CXCL10/CXCR3 signaling contributes to neuropathic pain in spinal cord and dorsal root ganglia after chronic constrictor injury in rats. Neurosci Lett 2019;694:20–8.
[15] Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. Ann Acad Med Singapore 1994;23:129–38.
[16] Conaghan PG, Cook AD, Hamilton JA, Tak PP. Therapeutic options for targeting inflammatory osteoarthritis pain. Nat Rev Rheumatol 2019;15:355–63.
[17] Craig CL, Marshall AL, Spitsom M, Baumane AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Saliss JF, Oja P. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35:1381–95.
[18] Cui GB, An JZ, Zhang N, Zhao MG, Liu SB, Yi J. Elevated interleukin-8 enhances prefrONTAL synaptic transmission in mice with persistent inflammatory pain. Mol Pain 2012;8:11.
[19] Das V, Kr P, Li X, O-Sullivan I, van Wijnen AJ, Kross JS, Pytowski B, Applegate DT, Votta-Velis G, Rippler RL, Park TJ, Him HJ. Blockade of vascular endothelial growth factor receptor-1 (Flt-1), reveals a novel analgesic for osteoarthritis-induced joint pain. Gene Rep 2018;11:94–100.
[20] Dong F, Du YR, Xie W, Strong J, He XJ, Zhang JM. Increased function of the TRPV1 channel in small sensory neurons after local inflammation or in vitro exposure to the pro-inflammatory cytokine GRO/KC. Neurosci Bull 2012;28:155–64.
[21] DosSantos MF, Holanda-Afonso RC, Lima RL, DaSilva AF, Moura-Neto V. The role of the blood-brain barrier in the development and treatment of migraine and other pain disorders. Front Cell Neurosci 2011;5:302.
[22] Echeyverri S, Shi QX, Rives S, Zhang J. Peripheral nerve injury alters the neuronal cord functionality and molecular integrity through a selective inflammatory pathway. J Neurosci 2011;31:10819–28.
[23] Echeyverri S, Shi QX, Yang M, Huang H, Wu Y, Lorenzo LE, Perez-Sanchez J, Bonin RP, De Konincy Y, Zhang J. Spinal microglia are required for long-term maintenance of neuropathic pain. PAIN 2017;158:1792–801.
[24] Edie PK, Wind-up and the NMDA receptor complex from a clinical perspective. Eur J Pain 2000;4:5–15.
[25] Ejikkelkamp N, Steen-Louws C, Hartgringen SA, Willerem HL, Prado J, Lafeber FP, Heijnjen CJ, Hack CE, van Roon JA, Kavelaars A. IL-4 long-term fusion protein is a novel drug to treat persistent inflammatory pain. J Neurosci 2016;36:7353–63.
[26] Finnerup NB, Attal N, Haroutounian S, McNicoll E, Baron R, Dworkin RH, Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH. Long-term consequences of chronic pain: mounting evidence for pain as a neurological disease and parallels with other chronic disease states. Pain Med 2011;12:996–1004.
[27] Finnerup NB, Atal N, Haroutounian S, McNicoll E, Baron R, Dworkin RH, Gilron I, Haanpaa M, Hansson P, Jensen TS, Kamenar PM, Lund K, Moore A, Raja SN, Risberg AM, Rowbotham M, Sense S, Siddall P, Smith BH, Wallace M. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet Neurol 2015;14:162–73.
[28] French HP, Smart KM, Doyle F. Prevalence of neuropathic pain in knee or hip osteoarthritis: a systematic review and meta-analysis. Semin Arthritis Rheum 2017;47:1–8.
[29] Gao YJ, Zhang L, Samad QA, Suter MR, Yasuhiko K, Xu ZZ, Park YJ, Lind AL, Wu Q, Ji RR. JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. J Neurosci 2009;29:4096–108.
[30] GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390:1211–50.
[31] Giron SE, Bjurstrom MF, Griffs CA, Ferrante FM, Wu IL, Nicoll AL, Grogan TR, Burkard JF, Irwin MR, Breec EN. Increased central nervous system interleukin-8 in a majority postmastectomy syndrome chronic pain population. Pain Med 2018;19:1033–43.
[32] Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol 2014;14:217–31.
[33] Haight ES, Forman TE, Cordonnier SA, James ML, Tawfik VL. Microglial activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35:1381–95.
[34] Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol 2014;14:217–31.
[35] Haight ES, Forman TE, Cordonnier SA, James ML, Tawfik VL. Microglial modulation as a target for chronic pain: from the bench to the bedside and back. Anesth Analg 2019;128:737–46.

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Krock E, Millecamps M, Anderson KM, Srivastava A, Reihsen TE, Hari P, Kotani N, Kushikata T, Hashimoto H, Kimura F, Muraoka M, Yodono M, Kosek E, Finn A, Ultenius C, Hugo A, Svensson C, Ahmed AS. Differences in somatosensory perception in patients with chronic low back pain: upregulation in human cerebrospinal fluid and preclinical studies. J Neuroimmunol 2018;4:247–67.

Koike K, Ono Y, Tsukui T, Suzuki Y, Hara Y, Ioka M, Nishida M, Yamaoka Y, Hori T, Nishida A, et al. Blood-nerve barrier dysfunction contributes to the generation of neuropathic pain and allows targeting of injured nerves for pain relief. Eur J Pain 2013;17:1476–87.

Kohno K, Sugimoto K, Ishii H, Yasuda K, Okano H, Ikeda Y, et al. Vascular cell adhesion molecule-1 expression is a predictor of severe osteoarthritis of the hip and knee joints. Arthritis Rheum 2009;60:2381–9.

Kohno K, Watanabe Y, Enomoto T, Hasegawa R, Ito T, Furuyama Y, et al. Cytokines and their mediators in pain. Rheumatology (Oxford) 2018;57:429–40.

Kogure K, Hayakawa M, Ogata K, et al. Correlation between interleukin-6 levels and spinal response to an experimental osteoarthritis pain model. Anesthesiology 2000;92:247–67.

Kohno K, Iida T, et al. An experimental osteoarthritis pain model. Arthritis Res Ther 2012;14:R173.

Kohno K, Hasegawa T, et al. A functional role for VEGFR1 in peripheral nerve injury-induced leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. PAIN 2002;100:163–70.

Kohno K, Kiyosue T, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. PAIN 2006;123:231–43.

Kohno K, Iida T, et al. An experimental osteoarthritis pain model. Arthritis Res Ther 2012;14:R173.

Kohno K, Hasegawa T, et al. A functional role for VEGFR1 in peripheral nerve injury-induced leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. PAIN 2002;100:163–70.

Kohno K, Kiyosue T, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. PAIN 2006;123:231–43.

Kohno K, Iida T, et al. An experimental osteoarthritis pain model. Arthritis Res Ther 2012;14:R173.

Kohno K, Hasegawa T, et al. A functional role for VEGFR1 in peripheral nerve injury-induced leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. PAIN 2002;100:163–70.

Kohno K, Iida T, et al. An experimental osteoarthritis pain model. Arthritis Res Ther 2012;14:R173.

Kohno K, Hasegawa T, et al. A functional role for VEGFR1 in peripheral nerve injury-induced leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. PAIN 2002;100:163–70.

Kohno K, Iida T, et al. An experimental osteoarthritis pain model. Arthritis Res Ther 2012;14:R173.

Kohno K, Hasegawa T, et al. A functional role for VEGFR1 in peripheral nerve injury-induced leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. PAIN 2002;100:163–70.
[84] Turk DC, Wilson HD, Cahana A. Treatment of chronic non-cancer pain. Lancet 2011;377:2226–35.

[85] van Hout B, Janissen MF, Feng YS, Kohlmann T, Busschbach J, Golicki D, Lloyd A, Scalone L, Kind P, Pickard AS. Interim scoring for the EQ-5D-5L: mapping the EQ-5D-5L to EQ-5D-3L value sets. Value Health 2012;15:708–15.

[86] Van Steenwinckel J, Reaux-Le Goazigo A, Pommier B, Mauborgne A, Dansereau MA, Kitabgi P, Sarret P, Pohl M, Melik Parsadaniantz S. CCL2 released from neuronal synaptic vesicles in the spinal cord is a major mediator of local inflammation and pain after peripheral nerve injury. J Neurosci 2011;31:5865–75.

[87] Wang JG, Strong JA, Xie W, Yang RH, Coyle DE, Wick DM, Dorsey ED, Zhang JM. The chemokine CXCL1/growth related oncogene increases sodium currents and neuronal excitability in small diameter sensory neurons. Mol Pain 2008;4:38.

[88] Wu F, Liu L, Zhou H. Endothelial cell activation in central nervous system inflammation. J Leukoc Biol 2017;101:1119–32.

[89] Wylde V, Palmer S, Learmonth ID, Dieppe P. Test-retest reliability of Quantitative Sensory Testing in knee osteoarthritis and healthy participants. Osteoarthritis Cartilage 2011;19:655–8.

[90] Yamitsky D, Bouhassira D, Drewes AM, Fillingim RB, Granot M, Hansson P, Landau R, Matre D, Nilsson KB, Stubhaug A, Treede RD, Wilder-Smith OH. Recommendations on practice of conditioned pain modulation (CPM) testing. Eur J Pain 2015;19:805–6.

[91] Zhang J, Shi XQ, Echeverry S, Mogil JS, De Koninck Y, Rivest S. Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. J Neurosci 2007;27:12396–406.

[92] Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361–70.