## Title

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# NEUROPATHIC PAIN SECTION

## *Original Research Article*

# Increased Central Nervous System Interleukin-8 in a Majority Postlaminectomy Syndrome Chronic Pain Population

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**Abstract**

**Background and Objectives. Multiple processes have been identified as potential contributors to chronic pain, with increasing evidence illustrating an association with aberrant levels of neuroimmune mediators. The primary objectives of the present study were to examine central nervous system cytokines, chemokines, and growth factors present in a chronic pain population and to explore patterns of the same mediator molecules over time. Secondary objectives explored the relationship of central and peripheral neuroimmune mediators while examining the levels of anxiety, depression, sleep quality, and perception of pain associated with the chronic pain patient experience.**

**Methods. Cerebrospinal fluid (CSF) from a population of majority postlaminectomy syndrome patients (N = 8) was compared with control CSF samples (N = 30) to assess for significant differences in 10 cytokines, chemokines, and growth factors. The patient population was then followed over time, analyzing CSF, plasma, and psychobehavioral measures.**

**Results. The present observational study is the first to demonstrate increased mean CSF levels of interleukin-8 (IL-8; $P < 0.001$) in a small population**

 

**of majority postlaminectomy syndrome patients, as compared with a control population. Over time in pain patients, CSF levels of IL-8 increased significantly ($P < 0.001$).**

**Conclusions. These data indicate that IL-8 should be further investigated and psychobehavioral components considered in the overall chronic pain paradigm. Future studies examining the interactions between these factors and IL-8 may identify novel targets for treatment of persistent pain states.**

**Key Words. Chronic pain; Cerebrospinal Fluid (CSF); Cytokines; Interleukin-8 (IL-8); Sleep Quality; Anxiety; Depression**

## Introduction

Chronic pain is an increasingly prevalent condition associated with devastating psychological and physical consequences and enormous health care costs. Prior studies have shown that activated glia contribute to the creation and maintenance of persistent pain through production of proinflammatory cytokines, chemokines, and growth factors [1–4]. With longstanding systemic or intrathecal opioid administration, glial-mediated cytokine production increases further, which may lead to opioid tolerance and opioid withdrawal–induced pain enhancement [5–7]. These investigations suggest that glia are not only partially responsible for the maintenance of chronic pain symptoms, but they are also responsible for decreasing the efficacy of one of the most prominent treatments of pain—opioid drugs. Unfortunately most of the previous research is centered on animal models, analysis of cerebrospinal fluid (CSF) is limited to only a few cytokines, and several human studies lack healthy controls.

Interestingly, studies examining anxiety, depression, and sleep quality have also demonstrated altered central inflammatory molecular activation [8–11]. Anxiety, depression, and sleep disturbances are common within chronic pain populations [12,13]; nevertheless, the physiological effects of these comorbidities are not well understood in the context of chronic pain. Many of the previous human studies monitor only peripheral patterns of neuroimmune markers in relation to behavioral and psychological traits, and chronic pain is rarely incorporated. Neuroimmune-driven central nervous system (CNS) inflammation offers one possible mechanism that may link the physiologic and psychobehavioral aspects of the chronic pain paradigm.

Based on this prior research, the objectives of the present study were to examine the central inflammatory cytokines, chemokines, and growth factors present in a chronic pain population compared with a control population and to explore patterns of the same mediator molecules over time in patients with implanted intrathecal pain pumps. The present observational study is one of the first to measure both peripheral and central levels of 10 pro- and anti-inflammatory cytokines, chemokines, and growth factors using multiplex assay analysis. Moreover, the present study is also one of the first to explore changes in the inflammatory molecular response and psychobehavioral aspects of chronic pain over time. Using central neuroinflammation as a paramount factor in the creation and maintenance of chronic pain, this study aimed at investigating and describing the physical and psychobehavioral aspects of chronic pain associated with neuroimmune activation.

## Materials and Methods

### *Subjects*

#### Study Participants

Patients with a chronic pain diagnosis at the Pain Management Center of University of California, Los Angeles (UCLA), who were medically eligible for treatment with an implantable intrathecal analgesic pump between April 2013 and December 2014 were identified for possible participation in the current research study ($N = 14$). Medical exclusion criteria included meningitis, active upper respiratory tract infection/flu or febrile nature, acute medical or psychiatric disorders, altered mental status, pregnancy, and the inability to communicate in English. Inclusion criteria for this study required that participants be age 18 years or older and carry a chronic pain diagnosis for longer than six months. Diagnosis of chronic pain was made on standard clinical criteria, as suggested by the International Association for the Study of Pain (IASP). Study exclusion criteria were the following: a diagnosis of cancer, human immunodeficiency virus (HIV) infection, recent epidural injections for treatment of chronic pain (within six months), drug abuse, history of blood transfusion(s), and palliative pain treatment. Nine patients met inclusion and exclusion criteria. One patient out of nine who were approached to participate in the study declined, making the refusal rate for this study 11%. All eight study participants were instructed to continue their medications and therapies without making any changes prior to or during study involvement. All study procedures were approved by the UCLA Institutional Review Board.

#### Control Donors

Control samples of CSF were obtained from the California NeuroAIDS Tissue Network through the HIV Neurobehavioral Research Center of the University of California, San Diego [14]. Thirty individual control samples of 1 to 2 mL of CSF, collected between the years 2005 and 2012, were utilized as a means to compare baseline levels of central neuroimmunologic mediators. Control samples of CSF were selected by the NeuroAIDS biobank from donors known to be HIV and hepatitis C virus seronegative and with no known history

of recreational drug use, with roughly equal numbers of male and female donors. Age, gender, and date of collection of control samples were the only identifying information obtained from the NeuroAIDS biobank.

## *Procedures*

### Questionnaire and Sample Collection

After informed consent was obtained, study participants underwent a structured interview to compile their demographic information and activity level (measured as metabolic equivalents [METs]). All results were number coded to ensure participant anonymity. Psychological and behavioral instruments (described below) were administered to measure their pain perception, anxiety, depression, and sleep quality. Upon completion of the psychobehavioral questionnaires, study participants were given a $25 Visa gift certificate and parking reimbursement for their participation in the study. A preoperative (baseline) peripheral blood sample was obtained in an EDTA blood collection tube, which was chilled following collection and during transport prior to processing.

Participants then underwent a surgical procedure for a trial placement of an intrathecal analgesic pump. Three mL of baseline CSF was collected from each participant intraoperatively and chilled along with the blood sample for transport; all blood and CSF samples were collected before 1 PM. Chilled samples were transported to the UCLA Cousins Center for Psychoneuroimmunology laboratory for processing.

Participants who had a successful trial (baseline time point) and proceeded to an intrathecal implant were assessed by the same study procedures during return visits for the insertion (implant time point, average time from trial = 45 days) and/or refill (third time point, average time from implant = 156 days) of an implantable intrathecal analgesic pump.

### Psychobehavioral Instruments

To measure the psychological and behavioral variables associated with chronic pain, several tools were selected for this study. Questionnaires were selected for their internal consistency and reliability, their ease of completion, and their use in previous studies [15–18].

*Pain Perception.* The Short Form McGill Pain Questionnaire (SF-MPQ) is a self-reported survey that consists of 15 descriptors known as the pain rating index (PRI), a present pain intensity (PPI) index, and a visual analog scale (VAS). Scores range from 0 to 45 on the PRI, from 0 to 5 on the PPI, and from 0 to 10 cm on the VAS [19].

*Anxiety.* The Hamilton Anxiety Rating Scale (HAM-A) is a survey based on 14 parameters, with each survey item given a score from 0 (not present) to 4 (severe). A total score of less than 17 indicates mild anxiety; 18 to 24 indicates mild-moderate anxiety; and 25 to 30 indicates moderate-severe anxiety levels [20]. The anxiety tool was completed by the practitioner consenting for study involvement.

*Depression.* The Beck Depression Inventory–II (BDI-II) is a self-reported survey that contains 21 questions, each answer being scored on a scale value of 0 to 3. The cutoff values used for this tool were as follows: 0 to 13 indicates minimal depression; 14 to 19 indicates mild depression; 20 to 28 indicates moderate depression; and 29 to 63 indicates severe depression [21].

*Sleep Quality.* The Pittsburgh Sleep Quality Index (PSQI) is a self-reported survey that is comprised of 19 items, grouped into seven components and scored from 0 to 3 for a total score of 0 to 21. Higher scores indicate worse sleep quality, with a score of less than 5 being associated with good sleep quality and higher than 5 being associated with poor sleep quality [16].

*Multiplex Assay Analysis of Plasma and CSF*

All peripheral blood samples were centrifuged at 500 G for 10 minutes at 4°C, and plasma aliquots were stored at –80 °C until batch analysis could be performed. All CSF samples were centrifuged at 2,000 G for 10 minutes at 4°C and stored in multiple small aliquots at –80 °C until batch analysis could be performed. If repeat CSF analyses were required, fresh aliquots were always utilized. To quantify levels of neuroimmunologic cytokines, chemokines, and growth factors in CSF and plasma specimens, the Luminex Performance Human High Sensitivity Cytokine Panel (R&D Systems, Minneapolis, MN, USA) and the Bio-Plex 200 instrument (Luminex) with Bio-Plex Manager software version 5.0 were employed. A total of 10 molecules were assessed using a single multiplex panel that included interleukin-1ß (IL-1ß), IL-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor (TNF)–α, interferon (IFN)-γ, vascular endothelial growth factor (VEGF), and granulocyte-macrophage colony-stimulating factor (GM-CSF), according to the manufacturer's protocol. All plasma and CSF samples were assayed in duplicate at a twofold dilution, per the manufacturer's recommendation for serum/plasma. All CSF or plasma samples from a single study participant were assayed on the same plate; control CSF samples were distributed across multiple plates, in parallel with study participant samples. Any sample with a raw mean fluorescent intensity of less than 10 fluorescent units, or with a calculated concentration of less than 0.1 pg/mL as calculated by the Bio-Plex Manager software for a given analyte, was considered to be below the level of detection of the assay (undetectable) for that analyte. All other calculated concentrations were considered detectable results. The lower limit of detection for each analyte was defined as the lowest detectable concentration observed in either samples or a standard [22]. Samples

with a coefficient of variation (CV%) > 20% between duplicates were evaluated on a case-by-case basis for repeat assays and/or modified data analysis.

*Statistical Analysis*

For both the chronic pain and control groups, descriptive statistics were calculated for demographic and study variables. Neuroimmune markers with detectable levels in less than 20% of either CSF or plasma samples were not analyzed further for that sample type. For markers with more than 50% detectable concentrations, samples with concentrations below the lower limit of detection were assigned the value equivalent to one-half the limit of detection for inclusion in analyses.

Comparisons at baseline between chronic pain and control groups were assessed with *t* tests, Wilcoxon tests, and linear regression models (controlling for age and gender). All three methods yielded the same conclusion.

For inclusion in longitudinal analyses, a participant was required to have at least two time points with viable plasma, CSF, and psychobehavioral data. The longitudinal statistical analysis assessed patterns of the neuroimmune markers across two to three time points (trial, implant, and/or refill of intrathecal pump) in the chronic pain group. Trends over time were assessed using generalized estimating equation (GEE) models with the auto-regressive covariance structure to account for repeated measures over time. Residual analysis was performed to assess model fit, and outcomes were log-transformed as needed. First, models were constructed with only time as the predictor. Next, the *P* values were evaluated after including age and gender in the models.

Correlations were calculated to explore associations between psychobehavioral measures, demographics, and levels of neuroimmune markers using Pearson's *r* or Spearman's Rho correlations.

When assessing differences between groups or within the chronic pain group over time, CSF IL-6 data and plasma IL-1, IL-6, IL-10, and VEGF data were log-transformed after residual analysis showed departures from normality. The threshold for statistical significance was set at a *P* value of less than 0.05 for all analyses. All data are reported as mean ± standard deviation (SD) unless otherwise noted. All analyses were performed using SAS 9.3 (Cary, NC, USA) and SPSS software version 23 (Armonk, NY, USA).

## Results

*Demographic Characteristics*

Of the eight chronic pain patients who participated in this study, five participants were female, and three participants were male. The age range for the study participants was 41 to 74 years, with a mean age of 53.5 ± 10.5 years. Five of eight participants had at least a college education, with half of the participants reporting a socioeconomic status (annual household income) of $100,000 to 149,000. The mean body mass index (BMI) of study participants was 32.4 ± 4.2 kg/m$^2$; six of eight subjects were obese. The mean level of physical activity, based on METs reported by participants, was 3.4 ± 1.5, indicating that participants could complete moderate-intensity activities. The majority of the patients (six of eight) suffered from postlaminectomy syndrome, with a mean length of diagnosis of 9.3 ± 7.8 years. Table 1 summarizes demographic characteristics and analgesic medication use of the chronic pain study participants.

Of the 30 control subjects from whom CSF was obtained, 12 were female and 18 were male. The age range for the control subjects was 19 to 79 years, with a mean age of 46.0 ± 15.7 years. All were known to be HIV and hepatitis C seronegative, with no history of recreational drug use; no additional demographic or clinical data were available.

*Neuroimmune Mediators*

Baseline CSF

CSF from the chronic pain population at study baseline was compared with control samples of CSF to assess for differences in levels of IL-1ß, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-γ, VEGF, and GM-CSF. Eight of the neuroimmune markers (IL-1ß, IL-2, IL-4, IL-10, TNF-α, IFN- γ, VEGF, and GM-CSF) were detectable in less than 20% of CSF samples in both groups and were not analyzed further. Two neuroimmune markers, IL-6 and IL-8, yielded detectable concentrations in at least 50% of CSF samples from patients and controls and were tested for group differences. The frequency of detectable concentrations of CSF IL-6 and IL-8 between the chronic pain and control groups showed no significant differences.

The Wilcoxon test and *t* test demonstrated significantly higher mean IL-8 CSF levels among chronic pain patients compared with controls (*t* test $P < 0.001$), but no significance with regards to IL-6 CSF (*t* test $P = 0.53$). Linear regression models, after adjusting for age and gender, yielded similar results, showing that IL-8 was significantly higher in chronic pain CSF ($P < 0.001$) (Figure 1); IL-6 CSF was not found to be significantly different ($P = 0.56$) (Table 2).

Longitudinal CSF and Plasma in Chronic Pain Patients

CSF and plasma levels of the 10 neuroimmune markers were determined in the chronic pain study population over the trial (baseline time point), insertion (implant time point), and/or refill (third time point) of an implantable

**Table 1** Demographic and clinical characteristics of the chronic pain patients

| Gender | Age, y | Race/Ethnicity | Diagnosis | Length of Diagnosis, y | Pain Medications | Length of Medication, y |
|---|---|---|---|---|---|---|
| F | 41 | Hispanic | S, AS | >0.5 | Cyc, Mor | >0.5 |
| F | 43 | Asian | PLS | 8 | FP, M, O/A | 6 |
| M | 50 | White | PLS | 25 | Dic, Ibu, O/A, Tap | >0.5 |
| F | 52 | White | PLS | >10 | H/A, Mel, Mor, Pre | 2 |
| F | 52 | African American | PLS | 2 | Dil, G, Met, O | 2 |
| M | 54 | White | DPN | >5 | G, O | >1 |
| M | 62 | Hispanic | PLS | 14 | Dul, Esc, FP, G, H/A, Met | 14 |
| F | 74 | African American | PLS | >10 | FP, G, O | >2 |

AS = ankylosing spondylitis; Cyc = cyclobenzaprine; Dic = diclofenac; Dil = dilaudid; DPN = diabetic polyneuropathy; Dul = duloxetine; Esc = escitalopram; FP = fentanyl patch; G = gabapentin; H/A = hydrocodone/acetaminophen; Ibu = ibuprofen; M = methadone; Mel = meloxicam; Met = methocarbamol; Mor = morphine; O = oxycodone; O/A = oxycodone/acetaminophen; PLS = postlaminectomy syndrome; Pre = pregabalin; S = sacroiliitis; Tap = tapentadol.

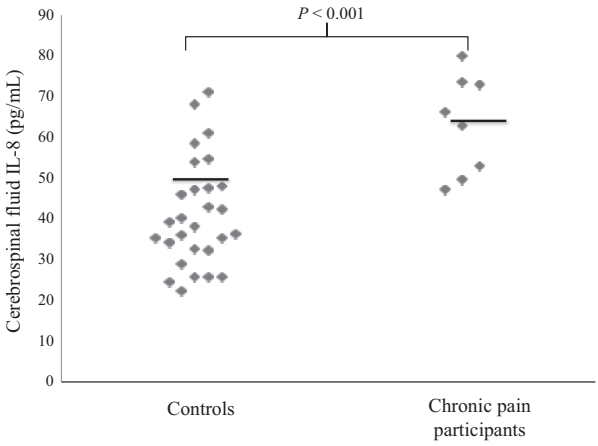


**Figure 1** Cerebrospinal fluid levels of interleukin-8 are increased in patients with chronic pain at baseline compared with control donors. Diamond shapes indicate individual IL-8 levels; horizontal lines indicate group mean IL-8 levels. *P* value shown is for age- and gender-adjusted linear regression.

intrathecal analgesic pump. Two participants were ineligible for longitudinal inclusion due to pump trial failure or removal of the implanted pump. Three participants yielded data from the trial and implant, one participant yielded data from the trial and refill, and two participants completed all three time points. Thus, six of the eight chronic pain participants had biologic samples and psychobehavioral data collected at at least two distinct time points for longitudinal analysis.

Consistent with baseline CSF samples, only IL-6 and IL-8 yielded detectable concentrations in more than 20% of CSF collected during longitudinal follow-up of patients (63% and 100%, respectively). Using GEE and including gender and age in the model, CSF levels of IL-8 were found to significantly change over time ($P < 0.001$) (Table 3), with higher levels at implant and/or refill (Figure 2).

In longitudinal patient plasma samples, four of the neuroimmune markers (IL-2, IL-4, IFN-γ, and GM-CSF) were detectable in less than 20% of samples and were not analyzed further. IL-1ß yielded detectable results in 56% of plasma samples, IL-6 in 94% of plasma samples, and IL-8, IL-10, TNF-α, and VEGF in 100% of plasma samples. Using GEE with gender and age in the model, no significant changes in these six plasma markers over time were found ($P \geq 0.1$) (Table 3).

### *Psychobehavioral Measures*

#### Baseline Psychobehavioral Data

At baseline, the eight chronic pain patients reported a mean PRI of 22.9 ± 7.1, mean PPI of 3.6 ± 1.1, and a mean pain VAS score of 5.9 ± 1.1, indicative of severe pain (Table 4). Moreover, at baseline, mean BDI-II and HAM-A scores indicated mild-moderate depression and anxiety symptomatology. Notably, all subjects reported poor sleep quality (PSQI > 5); the mean PSQI score for the whole group was strikingly high at baseline (15.5 ± 3.2), with all subjects scoring 9 or greater (Table 4).

#### Longitudinal Psychobehavioral Data in Patients

Longitudinal analysis of psychobehavioral measures indicated multiple significant changes. Over time, participants were noted to report increased anxiety ($P = 0.02$) (Table 4). Levels of depression and sleep quality yielded significant changes over time ($P = 0.03$ and $P < 0.01$, respectively) (Table 4); however, because of the biphasic directionality (decrease then increase), the overall scores did not appreciate a significant difference (data not shown). While the PPI score trended toward significance ($P = 0.07$) indicating a decrease in reported pain intensity, no significant difference was noted in subjective pain reports over time (Table 4).

**Table 2** Levels of neuroimmune mediators in CSF of control subjects vs chronic pain patients at baseline

| Neuroimmune Mediator | Lower Limit of Detection, pg/mL | Control CSF (N = 30), % Detectable | Chronic Pain Baseline CSF (N = 8), % Detectable | Control CSF, Mean ± SD, pg/mL | Chronic Pain Baseline CSF, Mean ± SD, pg/mL | *P** |
|---|---|---|---|---|---|---|
| IL-1 | 0.5 | 3 | 0 | na | na | |
| IL-2 | 0.6 | 0 | 0 | na | na | |
| IL-4 | 27.3 | 0 | 0 | na | na | |
| IL-6 | 1.0 | 60 | 50 | 1.7 ± 0.5 | 1.5 ± 0.3 | 0.6 |
| IL-8 | 0.8 | 100 | 100 | 49.5 ± 12.8 | 63.0 ± 12.2 | <0.001 |
| IL-10 | 0.2 | 0 | 0 | na | na | |
| TNF-α | 0.8 | 0 | 0 | na | na | |
| IFN-γ | 0.1 | 0 | 0 | na | na | |
| VEGF | 0.2 | 17 | 13 | na | na | |
| GM-CSF | 0.4 | 0[†] | 0 | na | na | |

GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; na = not analyzed due to low frequency of detectable levels; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.
*Linear regression, adjusting for age and gender.
[†]N = 26 due to missing data.

### Correlational Analysis of Psychobehavioral and Biologic Data

Psychobehavioral data and central (CSF) or peripheral (plasma) neuroimmunologic marker levels, physical activity, and demographic variables (age, gender, race/ethnicity, education level, SES, BMI, diagnosis, length of diagnosis, medications taken, and length of medications taken) were examined for possible correlations, but no significant findings were observed.

## Discussion

A barrage of afferent pain signaling contributes to the maintenance of nociception in chronic pain states. Glial cell activity is implicated as one mechanism [23], with neuroimmune molecules identified as perpetuating pain and rendering current pain management methods ineffective. Potentially culpable molecules secreted by glial cells are neuroinflammatory in nature, specifically proinflammatory cytokines, chemokines, and growth factors. These mediators have been shown to excite pain transmission and experience [24–26] via mechanisms including dorsal horn neuron sensitization [27,28], altered release of neurotransmitters from sensory afferent nerves [29], and alterations in signaling and receptor expression or activity [30–32].

In the present study, the key significant finding was markedly higher CSF levels of IL-8 in chronic pain patients compared with controls, even after controlling for age and gender (Figure 1). Although the majority of our study participants were postlaminectomy syndrome patients, our results are consistent with cohort studies that have shown elevated CSF levels of IL-8 in other chronic pain populations including osteoarthritis [33], postherpetic neuralgia [34,35], sciatica [36], and fibromyalgia [37]. Other studies that utilized postlaminectomy syndrome participants have previously found increases in CSF IL-6 [38] and neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and VEGF [39]. The current study is the first to document increased CSF levels of IL-8 in a majority of postlaminectomy syndrome patients, which was not assessed in these previous studies. It is not clear why we did not also detect increased CSF levels of IL-6 or VEGF, but this may be due to differences in patient populations and/or in the level of detection of the multiplex assays employed in our study.

Interleukin-8, also known as CXCL8, is a chemokine that primarily promotes chemotaxis of T lymphocytes and neutrophils [40]. IL-8's biologic actions are exerted by binding to the IL-8 G-protein-coupled receptors CXCR1 and CXCR2 [41]. In the CNS, CXCR2 have been found on astrocytes, microglia, and neurons [42], and thus IL-8 may be an important factor in intercellular communication between neurons, possibly via modulation of presynaptic excitability [43]. The glial release of proinflammatory cytokines (including IL-8) can be triggered by immune activation, stress, and afferent nociceptive input [44]. In the present study, plasma levels of IL-8 (ranging from 1.6 to 20.0 pg/mL) were found to be much lower than CSF levels in the same individuals with chronic pain, indicating that the IL-8 measured in the CSF may have been produced locally. This is in accordance with results reported by Brisby et al. (2002) [36], Kadetoff et al. (2012) [37], and Lundborg et al. (2010) [33], showing similar levels of CSF IL-8 in chronic pain populations of sciatica and disc herniation, fibromyalgia, and osteoarthritis. Higher central levels of IL-8 may

**Table 3** Alteration of neuroimmune mediator levels over time in chronic pain patients with intrathecal pumps

| | Time Point 1 Baseline, Mean ± SD, pg/mL | Time Point 2 Implant, Mean ± SD, pg/mL | Time Point 3 Refill, Mean ± SD, pg/mL | *P** |
|---|---|---|---|---|
| Cerebrospinal Fluid | | | | |
| IL-6† | 1.5 ± 0.3 | 2.5 ± 1.5 | 1.6 ± 0.1 | 0.4 |
| IL-8 | 63.0 ± 12.2 | 66.0 ± 15.5 | 104.7 ± 35.7 | <0.001 |
| Plasma | | | | |
| IL-1† | 0.9 ± 0.5 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.2 |
| IL-6† | 4.2 ± 2.2 | 4.9 ± 3.4 | 2.2 ± 1.0 | 0.9 |
| IL-8 | 8.9 ± 5.8 | 6.7 ± 3.9 | 10.7 ± 2.1 | 0.1 |
| IL-10† | 2.0 ± 4.3 | 1.0 ± 1.3 | 0.4 ± 0.1 | 0.5 |
| TNF-α | 8.4 ± 2.2 | 9.8 ± 2.1 | 8.2 ± 2.5 | 0.3 |
| VEGF† | 31.4 ± 27.9 | 35.2 ± 28.6 | 42.7 ± 20 | 0.4 |

*Generalized estimating equation models with auto-regressive covariance structure including age and gender in model.
†Log-transformed for analysis.

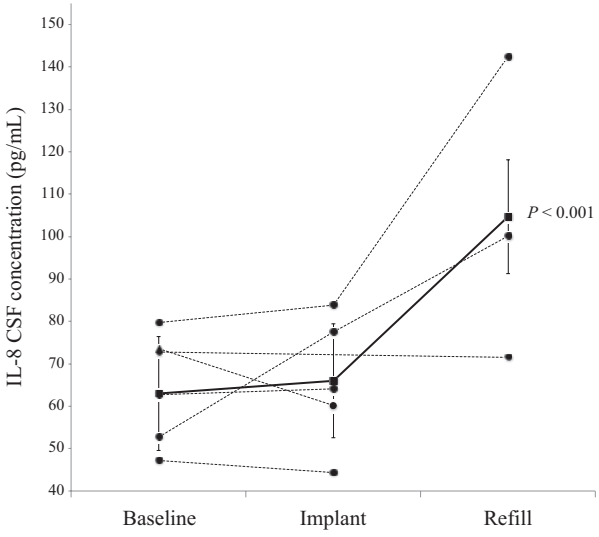


**Figure 2** Longitudinal pattern of cerebrospinal fluid interleukin-8 in six chronic pain patients with assessments at two or more time points. Dashed lines show individual patterns; heavy solid line with error bars indicates group mean values ± standard error. *P* value shown is for generalized estimating equation models including age and gender.

indicate a local production of IL-8 in the CNS in response to long-term nonmalignant pain, and thus may reflect levels of IL-8 produced by glia rather than peripheral macrophages in response to the chronic pain state.

Likewise, a significant longitudinal increase (beginning after the baseline time point through the third time point) in CSF IL-8 was observed over the course of the study in chronic pain patients receiving continuous infusions of opioid intrathecal analgesia for chronic pain therapy (Figure 2). To our knowledge, this is the first time that a longitudinal increase of this specific cytokine has been reported; a finding similar to the longitudinal increase of CSF IL-6 described by Zin et al. (2010) [38] in a study with a similar pain cohort. It is not clear whether the significant increase in IL-8 over time may reflect the surgical implant procedure or other confounding factors within our population, such as demographic factors or medications. Notably, obesity has been shown to be associated with increased plasma IL-8 levels; thus the lack of BMI characterization of the control group constitutes a significant limitation to our study design. Nevertheless, whereas Larsson et al. (2015) [45] recently found that BMI may be associated with CSF levels of a number of cytokines and chemokines, no significant correlation was found for CSF IL-8. In the present study, it is interesting to note that the significant longitudinal increase of CSF IL-8 was not paralleled in the peripheral plasma, and thus may reflect an inflammatory CNS response. CNS inflammation, as noted by increased levels of a variety of proinflammatory cytokines and chemokines, has been reported in multiple chronic pain conditions [46].

As a secondary finding of the present study, psychobehavioral variables were found to appreciate significant changes over time. The average anxiety score reported was shown to significantly increase over time ($P = 0.02$), and both depression ($P = 0.03$) and sleep quality ($P < 0.01$) also changed significantly. While we report that there was no significant correlation of psychobehavioral results to inflammatory markers in the periphery or in the CNS of chronic pain patients, a larger sample size may provide a more robust understanding of these relationships. Previous research has illustrated correlations between peripheral cytokines and anxiety [47,48], depression [49], and sleep disturbances [50,51], but these studies rarely incorporate CNS cytokines or pain patients; this study is one of the first human studies to examine central inflammatory markers and psychobehavioral aspects of chronic pain. Given that pain patients demonstrate anxiety disorders [52], depression [53], and

**Table 4** Longitudinal psychobehavioral data for the chronic pain patients

| | SF-MPQ* | | | | | |
|---|---|---|---|---|---|---|
| | PRI | PPI | VAS | HAM-A | BDI-II | PSQI |
| Time point 1 baseline, mean ± SD | 22.9 ± 7.1 | 3.6 ± 1.1 | 5.9 ± 1.1 | 20.8 ± 11.3 | 19.8 ± 10.5 | 15.5 ± 3.2 |
| Time point 2 implant, mean ± SD | 21.6 ± 8.1 | 3.2 ± 0.8 | 5.7 ± 1.3 | 20.8 ± 7.9 | 11 ± 5.4 | 13.6 ± 4.3 |
| Time point 3 refill, mean ± SD | 16.7 ± 18.5 | 2.7 ± 1.2 | 7.0 ± 0.6 | 26.7 ± 9.3 | 17 ± 5.3 | 14.7 ± 1.5 |
| $P^{\dagger}$ | 0.4 | 0.07 | 0.16 | 0.02 | 0.03 | $<0.01^{\ddagger}$ |

BDI-II = Beck depression inventory-II (range = 0–63); HAM-A = Hamilton anxiety rating scale (range = 0–56); PPI = present pain inventory (range = 0–5); PRI = pain rating index (possible range of scores = 0–45); PSQI = Pittsburgh sleep quality index (range = 0–21); SF-MPQ = Short-Form McGill Pain Questionnaire; VAS = visual analogue scale (range = 0–10).
*Short-Form McGill Pain Questionnaire is comprised of PRI, PPI, and VAS.
†Included age and gender in analysis.
‡Log-transformed for analysis.

sleep disturbances [54,55], the trends of these variables do present clinical comorbidities of chronic pain that warrant additional clinical and research focus.

It cannot be overlooked that all participants in the present study received at least one opioid medication for the treatment of their chronic pain; this is consistent with the general chronic pain population and current national treatment averages [56,57]. To avoid an increased participant burden of CSF collection, participants were selected for this study because their treatment necessitated an intrathecal analgesic pump for pain relief, which facilitated collection of CSF samples. All participants followed over time received an opioid medication through the intrathecal infusion pump, and thus received both long-term systemic and intrathecal opioid analgesia. Intuitively, one must consider the relationship between opioids and inflammatory cytokines.

Multiple preclinical studies have shown that upon long-term administration of opioids to pain-induced rodents, there is an increase in glial cell activity in the CNS [7,58]. Glia have also been shown to upregulate proinflammatory cytokine production and release in the CNS when the organism is treated with long-standing systemic or intrathecal opioids; this increase of neuroimmune marker production and release in the CNS has been shown to contribute to opioid tolerance and withdrawal-induced pain enhancement [5–7]. The literature has repeatedly championed a relationship between central neuroimmune markers and opioid medications, and perhaps it is this relationship that could be exploited for new treatments.

Hutchinson and colleagues (2008) [59] demonstrated in a preclinical study that by arresting cytokine activity, the length of opioid analgesia was significantly increased and the analgesic efficacy of morphine increased. In another preclinical study, Johnston and colleagues indicated, through antagonism of a central cytokine, that the analgesia of an intrathecally administered opioid was enhanced, opioid tolerance was blunted, and both newly developed and established allodynia and hyperalgesia were reduced [5]. Other research has demonstrated that through a blockade of glial cells, or the neuroimmune markers they release, opioid potency can be increased by three to five times [60,61]. The current study replicates and builds upon prior human reports of abnormal neuroimmune mediators in the chronic pain population, but uniquely identifies a pattern of rising CNS levels of IL-8 over time with intrathecal opioid treatment. Clearly, this study identifies an additional neuroimmunologic marker for future inspection and offers the promise of a cytokine to possibly target for future chronic pain therapy.

### *Study Limitations*

As an observational study of patients undergoing medically indicated intrathecal pump implants, the present study's design precludes determination of direction of causality between chronic pain and its comorbidities and central or peripheral levels of neuroimmune mediators. Given the specific criteria and study population, there is a limited ability to generalize the results. Clearly, the small sample size limits our findings. Due to strict inclusion criteria, enrollment of study participants was more difficult than anticipated; moreover, a trend toward increased usage of spinal cord stimulation devices (rather than intrathecal pumps) over the course of the study further complicated recruitment. Our patient sample size is, however, similar to several previous studies evaluating CSF in human pain populations [39,62,63].

While the control CSF samples utilized as the reference population are known to have been collected in the context of a research study and not as part of medical procedures, these samples came only with limited clinical information (HIV and HCV seronegative, with no known history of drug abuse). Aside from age and gender, no demographic information was available on this control population, further highlighting a limitation of this study design. Nevertheless, the presence of an age- and gender-defined control group constitutes a strength in the study as lack of control subjects for comparison

is common in previous studies examining CSF cytokines and neurotrophic factors in chronic pain populations [46].

## Conclusions

In summary, this is the first study to describe elevated CSF levels of IL-8 and dynamic longitudinal increases of IL-8 in a majority postlaminectomy chronic pain population receiving intrathecal opioid treatment. Importantly, this study provides further supportive evidence that there may be an etiological relationship between CNS inflammation and chronic pain states in humans; nevertheless, these results are preliminary and warrant replication in larger studies. Notably, all of the study participants took at least one opioid medication for treatment of their pain, reported depression or poor sleep quality, and on average experienced an increase in anxiety levels over time, illustrating the need to investigate possible mechanisms of association. Moreover, this study highlights the need for further inspection of both physiologic and psychobehavioral attributes of chronic pain. Future longitudinal clinical research, utilizing paradigms of proinflammatory cytokine and/or neurotrophic factor inhibition, may provide further detail to the interactions between neuroimmune mediators and chronic pain mechanisms. Such research may provide a greater understanding of chronic pain pathophysiology and spur development of novel targeted treatments.

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